INSIGHTS IN PEDIATRIC NEUROLOGY: 2021

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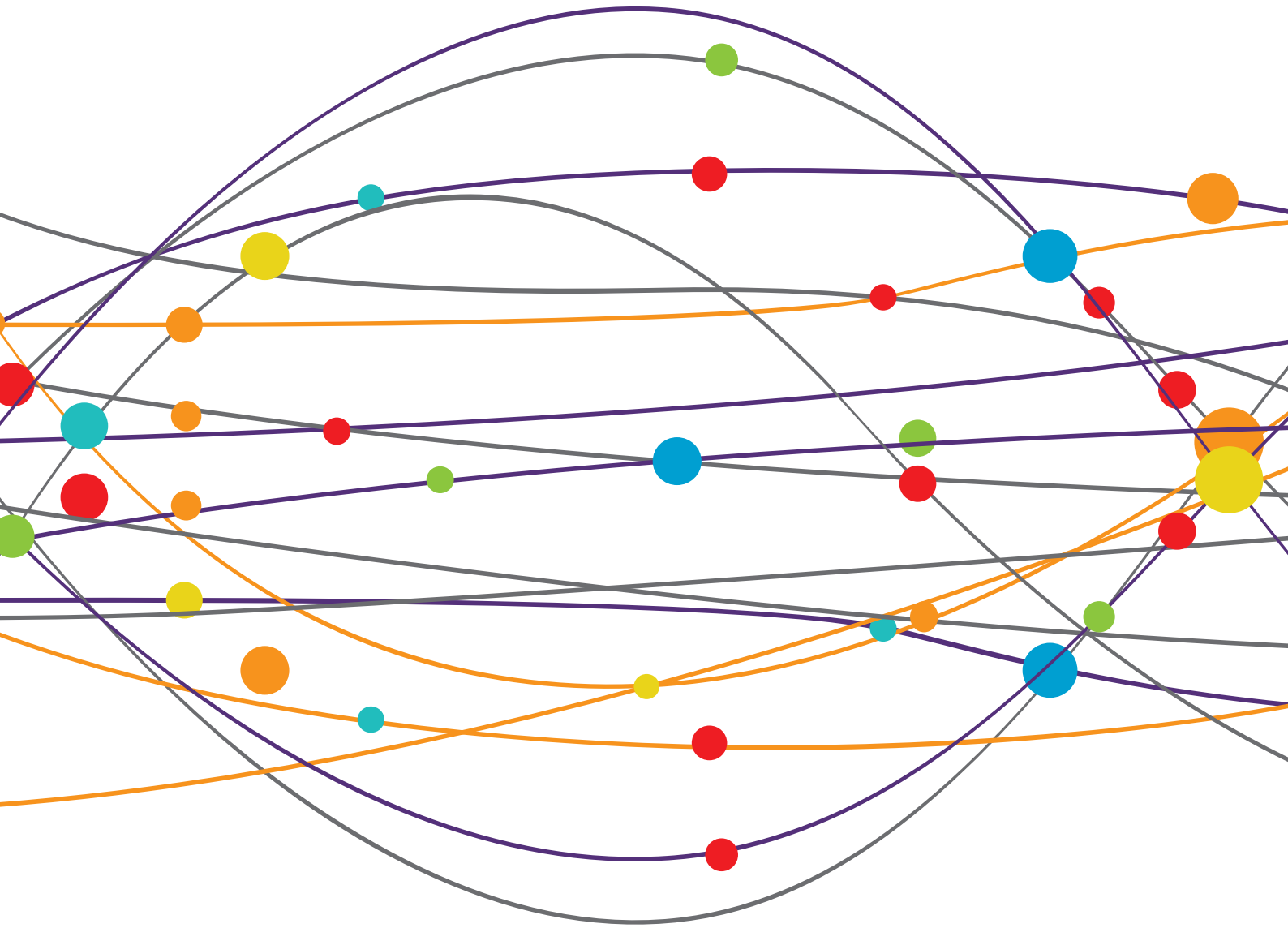

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INSIGHTS IN PEDIATRIC NEUROLOGY: 2021

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Editorial: Insights in pediatric neurology: 2021

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pediatric neurology, multiple center clinical trial, CDKL5, COVID Infection, epilepsy

Editorial on the Research Topic
Insights in pediatric neurology: 2021

Over the past few years, scientists have made exceptional achievements, resulting in major advancements in the rapidly evolving field of pediatric neurology. This Research Topic contains 19 articles (including 15 original research articles, one brief research report, two case reports, and one review), with contributions from 209 authors from 13 countries. The theme focuses on the most recent discoveries, latest advances, ongoing challenges, and future perspectives in the field of pediatric neurology. Diverse topics are covered inclusive of the international consensus recommendations for CDKL5 deficiency disorder, and the neurological and psychological presentation in children, and young populations with COVID Infection. The collection includes new findings and scales in pediatric psychiatry and psychotherapy and the diagnosis and intervention of different pediatric neurological disorders.

Multinational and multi-center cooperative research is the highlight of this topic. The CDKL5 kinase gene encoding mutations rank amongst the most common genetic childhood epilepsies and can manifest as the severe neurodevelopmental condition CDD (*CDKL5* deficiency disorder) (1). One contribution to this Research Topic by Amin et al. involved 18 pediatric medical and research institutions from the United Kingdom, Spain, France, the United States of America, Australia, and Italy, aiming to provide international expert consensus on recommendations for *CDKL5* Deficiency Disorder that will aid approaches to standardize and improve care for individuals with CDD. As a rare subacute complication of intrathecal or high-dose Methotrexate (MTX) administration stroke-like syndrome (SLS) can occur. Santangelo et al. from 12 Italian clinical and research institutes retrospectively described patients diagnosed with SLS at four major referral centers for Pediatric Hematology-Oncology. The results supported previous findings but in addition, found a linear correlation between age and disease severity.

Epilepsy is one of the most common pediatric neurological disorders. The antiseizure effect of the ketogenic diet (KD) is the focus of recent pediatric neuroclinical and basic fields (2–7), but there is still a need for multicenter studies in other pediatric neurological diseases.

Fang et al. reported on children with tuberous sclerosis complex (TSC) with drug-resistant epilepsy (DRE) and cognitive impairment, from 10 major city hospitals in China, for the efficacy and safety of KD to manage these co-morbidities. They found that KD could reduce seizure frequency and potentially improve cognition and behavior for this group. Yuan et al. analyzed the interictal discharges (IID) from pre-operative surface-electrode electroencephalograms (EEG) and compared the IID pattern changes post surgical excision of epileptogenic tubers in preschool children with TSC-related epilepsy. Those with post-operative seizure freedom were more likely to have non-IIDs vs. those with new focal IIDs were less likely to have seizure freedom at 3-year follow-up. Baker et al. investigated patients with infantile spasms (IS) for their longitudinal health outcomes post prednisolone (PRED) compared to Adrenocorticotropic Hormone (ACTH) treatment characterized using a phenome-wide association study. The findings were similar, across neurological and non-neurological outcomes. In addition, Makridis et al. retrospectively evaluated the outcome of 16 children with epilepsy who were treated with cenobamate, a drug for the treatment of adults with focal-onset epilepsy. The agent was effective and well-tolerated, suggesting that it could be a novel treatment for pediatric patients.

Yu et al. presented a Chinese patient with mild developmental delay who was found to have a *de novo* truncating variation in *SATB1*. Their study enhances the knowledge gap on the prognosis and treatment of rare neurological developmental disorders caused by gene mutations.

The current series on the neurological impact of COVID-19 on children has focused on severe multisystem inflammatory syndrome (MIS-C) with neurologic symptoms or other rare neurologic sequelae. Riva et al. describe a large group of children who had been infected by COVID-19, discussing their neurological complications and investigating these findings in relation to disease severity and population demographics. Except for headaches, the group found that neurological manifestations are an unusual presenting feature, and disease severity was not related to the pre-existing medical state. Guido et al. assessed the long-term outcome psychological consequences of COVID-19 infection on children and found that some symptoms were still present 3–5 months after infection. The data demonstrate that long-COVID presents psychological and ongoing cognitive issues, requiring intervention to avoid compromise on the quality of life of children and adolescents.

Learning and memory impairments have been the focus of pediatric neuropsychiatry and psychology due to the association between reading achievement and socioeconomic status (8, 9). An exploration of brain volume in patients with dyslexia by Ligges et al. found that reading deficits in those affected have gray matter volume variances in the reading network compared to unaffected readers. Behavioral improvement in reading skills was identified in different brain anatomical patterns, supporting the notion that dyslexia has lifelong consequences requiring consistent support in educational and professional career pathways. Stubberud et al. demonstrated that following pediatric acquired brain injury (pABI), diverse medical factors are associated with functional school outcomes. The study supported reintroduction to school with personalized programs tailored to the child's specific needs. Early identification and intervention of children at risk of learning disorders (LD) may improve outcomes. This tool is lacking in mainland China. The Preschool Learning Skills Scale was adapted by Yao et al., who created a Chinese version. This adapted version was then investigated for its validity and reliability and found to have good reliability and validity. In addition, a review by Melillo et al. explores the debate on whether Autism Spectrum Disorder (ASD) may be related to interregional brain functional disconnectivity as part of maturational delays in brain networks, in particular, the role of the inhibition of retained primitive reflexes (RPRs).

The early predictive value of novel parameters and markers in the diagnosis and prognosis of childhood neurological diseases has also been a key area of research in recent years (10). In pediatric Guillain-Barré syndrome (GBS) Jin et al. explored cerebrospinal fluid neurofilament light chain (CSF-NfL) levels as a potential prognostic biomarker and found that high CSF-NfL levels predict worse motor function and poor short-term prognosis of pediatric GBS. Pizzo et al. evaluated the incidence and prognostic value of brain MRI lesions and increased cerebrospinal fluid protein in children with Guillain-Barré syndrome. The results suggest a correlation between the MRI score, CSF protein, and prognosis. Du et al. retrospectively analyzed manifestations of cerebral paragonimiasis in children through neuroimaging. They found that lesions were mostly located in the cerebral parenchyma plus involved adjacent meninges, which could be of diagnostic value. Ma et al. found that independent ambulation as a milestone combined with the reading-frame rule significantly improved the early diagnosis of Duchenne muscular dystrophy (DMD).

The application of new technologies and methods in clinical diagnosis and treatment is also reflected in this special topic. Liu et al. investigated novel biomarkers and mechanisms related to Friedreich's ataxia (FRDA) progression. The results demonstrated that *CD28*, *FAS*, and *IFIT5* downregulation may be associated with disease progression. For patients with FRDA, pathogenesis may be related to the RNA regulatory pathway driven by NEAT1-hsa-miR-24-3p-CD28. Vališ et al. using the Czech National Registry for multiple sclerosis reported on affected children treated with disease-modifying drugs in 2013–2020.

In conclusion, the present clinical studies shed light on progress in Pediatric Neurology and its future challenges. We hope that the information gathered from this Research Topic will inspire, update and provide guidance to researchers in the field.

Author contributions

JW is the leader of the Research Topic. HN wrote the draft. JW and PS reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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ORIGINAL RESEARCH
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Downregulation of Three Immune-Specific Core Genes and the Regulatory Pathways in Children and Adult Friedreich's Ataxia: A Comprehensive Analysis Based on Microarray

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Background: Friedreich's ataxia (FRDA) is a familial hereditary disorder that lacks available therapy. Therefore, the identification of novel biomarkers and key mechanisms related to FRDA progression is urgently required.

Methods: We identified the up-regulated and down-regulated differentially expressed genes (DEGs) in children and adult FRDA from the GSE11204 dataset and intersected them to determine the co-expressed DEGs (co-DEGs). Enrichment analysis was conducted and a protein-protein interaction (PPI) network was constructed to identify key pathways and hub genes. The potential diagnostic biomarkers were validated using the GSE30933 dataset. Cytoscape was applied to construct interaction and competitive endogenous RNA (ceRNA) networks.

Results: Gene Set Enrichment Analysis (GSEA) indicated that the genes in both the child and adult samples were primarily enriched in their immune-related functions. We identified 88 co-DEGs between child and adult FRDA samples. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome enrichment analysis suggested that these co-DEGs were primarily enriched in immune response, inflammatory reaction, and necroptosis. Immune infiltration analysis showed remarkable differences in the proportions of immune cell subtype between FRDA and healthy samples. In addition, ten core genes and one gene cluster module were screened out based on the PPI network. We verified eight immune-specific core genes using a validation dataset and found CD28, FAS, and ITIF5 have high diagnostic significance in FRDA. Finally, NEAT1-hsa-miR-24-3p-CD28 was identified as a key regulatory pathway of child and adult FRDA.

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Conclusions: Downregulation of three immune-specific hub genes, CD28, FAS, and IFIT5, may be associated with the progression of child and adult FRDA. Furthermore, NEAT1-hsa-miR-24-3p-CD28 may be the potential RNA regulatory pathway related to the pathogenesis of child and adult FRDA.

Keywords: Friedreich's ataxia, biomarker, hub genes, bioinformatics, RNA regulatory pathways

INTRODUCTION

Friedreich's ataxia (FRDA) is a familial hereditary disorder involving the spinal cord and cerebellum, which is mainly caused by repeat amplification of homozygous guanine–adenine–adenine (GAA) triplet located in the frataxin gene (1). Such repeat amplification and mutation eventually lead to a decrease in the expression level of functional Frataxin. Frataxin deficiency can promote the activation of oxidative stress and ferroptosis, resulting in mitochondrial dysregulation (2, 3). Children often manifest with initial symptoms including disturbance of balance and progressive ataxia. With the progress of disease and age, patients may gradually develop dysarthria and loss of tendon reflex, and in many patients this is accompanied by myocardial injury and diabetes (4). At present, no effective therapies have been proven to prevent FRDA progression, most of which are symptomatic treatment (5). Therefore, further elucidating the underlying pathogenesis and developing more valid treatment strategies is an urgent demand. Presently, some serum biomarkers have been reported as potential key signatures in the pathogenesis of FRDA. For example, the levels of neurofilament light chain and heavy chain are enhanced significantly in Friedreich's ataxia patients and decrease with age (6, 7). In addition, serum hsTnT, NT-proBNP, and miRNAs have also been demonstrated to be associated with the progression of cardiomyopathy in adult FRDA (8, 9). However, the effectiveness of these biomarkers has either not been validated in prospective cohorts, or the clinical correlation between them is barely understood. Moreover, these biomarkers have not yet been used in clinical diagnosis of FRDA. Therefore, identifying additional biomarkers may provide critical insights into the diagnosis and treatment of FRDA.

Currently, bioinformatics analysis has been extensively applied in a variety of diseases, including cancer, cardiac disease, and neurodegenerative disease, to identify key biomarkers closely related to the prognosis of the disease (10–12). Additionally, competitive endogenous RNA (ceRNA) networks will help to clarify the novel mechanism of transcriptional regulatory networks in advancing disease progression (13). Although recent studies have concentrated on FRDA-induced transcriptome changes, only a few studies have explored the association between differentially expressed genes (DEGs) in children and adult FRDA.

In this study, we identified co-expressed differentially expressed genes (co-DEGs) by intersecting the up-regulated and down-regulated DEGs in child and adult FRDA samples (GSE11204). Next, we performed various enrichment analysis and constructed a PPI network to ascertain the key pathways and hub genes related to the progression of FRDA in children and adults. In addition, we predicted target miRNAs of hub genes and validated the diagnostic significance of selected hub genes using the GSE30933 dataset, Finally, we constructed FRDA-related ceRNA networks based on mRNAs-miRNAs-long noncoding RNAs (lncRNAs) interactions. Our study provides a novel perspective for revealing the pathophysiological mechanism of FRDA progression at the transcriptome level and investigates potential targets for the diagnosis and treatment of FRDA in children and adults.

MATERIALS

Microarray Data Acquisition

All microarray datasets were downloaded from Gene Expression Omnibus (GEO) (www.ncbi.nlm.nih.gov/geo/) (14). GSE11204 dataset (GPL887 platform) (15), including whole gene expression profiles of peripheral blood from 10 healthy children, 28 children with FRDA, 15 healthy adults, and 14 adults with FRDA, were selected as the test set. The GSE30933 dataset (GPL6255 platform) (16), which included whole gene expression profiles of peripheral blood from 40 healthy and 34 FRDA samples, was selected as the validation set.

Data Processing

The whole gene expression profiles obtained from the GEO database have been pre-processed and normalized by the robust multi-array average (RMA) method according to the "affy" package (http://www.bioconductor.org/packages/release/bioc/html/affy.html) (version 1.70.0) of R software. The "limma" package (http://www.bioconductor.org/packages/release/bioc/html/limma.html) (version 3.48.1) was performed to analyze the differentially expressed genes (DEGs). Original p-values were adjusted by the Benjamini-Hochberg method, and the fold-changes (FC) were calculated based on the false detection rate (FDR) procedure. Genes expression values of $|\log2\ FC| > 1$ and $p < 0.05$ were considered to be statistically significant. In order to visualize the identified DEGs, the R packages of

Abbreviations: FRDA, Friedreich's ataxia; GEO, Gene Expression Omnibus; DEGs, Differentially expressed genes; FDR, false detection rate; co-DEGs, co-expressed Differentially expressed genes; PPI, Protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ceRNA, Competitive endogenous RNA; GSEA, Gene Set Enrichment Analysis; RMA, Robust Multiarray Average; lncRNAs, long noncoding RNAs; FC, fold-changes; NES, normalized enrichment score; DAVID, Database for Annotation, Visualization, and Integrated Discovery; BP, biological process; CC, cell composition; MF, molecular function; MCODE, Minimal Common Oncology Data Elements; MNC, Maximum Neighborhood Component; ROC, Receiver operating characteristic; AUC, Area under the ROC curve.

"ggpubr" and "pheatmap" were conducted to make the volcano plots and heatmaps, respectively. The online tool Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was conducted to generate Venn diagrams of co-DEGs.

Enrichment Analysis

To identify the distribution trend of overall genes between the FRDA and the control groups, the "clusterProfiler" (http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html) (version 4.0.2) and "msigdbr" (https://cran.r-project.org/web/packages/msigdbr/index.html) (version 7.4.1) packages were conducted to make Gene Set Enrichment Analysis (GSEA) enrichment analysis. In brief, the gene symbols with corresponding FC were imported, and the c5: GO:BP gene sets (c5.go.bp.v7.4.symbols) were then applied for functional enrichment analysis. Gene sets with $p < 0.05$, $Q < 0.25$, and $|$ normalized enrichment score (NES)$| > 1.5$ were defined as significantly enriched gene sets.

Next, Gene Ontology (GO) enrichment analysis of co-DEGs was carried out based on the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/summary.jsp) (17). The biological process (BP), cell composition (CC), and molecular function (MF) of co-DEGs were then identified. Enriched GO terms (BP) with FDR < 0.05 were defined as significant and visualized using the "GOplot" package (https://wencke.github.io/) (version 1.0.2).

In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome enrichment analysis of co-DEGs were conducted with the "clusterProfile" package (18). A value of adjusted $p < 0.05$ was considered as significantly enriched functions and pathways. The top 5 KEGG and Reactome enrichment pathways were exhibited in a bubble plot.

Protein-Protein Interaction Network Analysis

The PPI network of co-DEGs was established using the online database STRING (https://string-db.org/) based on the Screening criteria: combined score > 0.4 (19). Afterward, the protein-protein interaction information was imported into the Cytoscape software (3.8.2) to realize the visualization of the PPI network. Then, the Minimal Common Oncology Data Elements (MCODE) plugin was applied for identifying key gene clusters with the default parameters. CytoHubba plugin was carried out to screen out hub genes based on the PPI network (20). The first 20 hub genes were calculated using the five algorithms: Degree, Stress, Maximum Neighborhood Component (MNC), Closeness, and Radiality (21, 22). Finally, a total of 10 hub genes were screened out by intersecting all the results.

Gene-miRNA Analysis

Gene-miRNA interactions were identified using the online database miRWalk 3.0 (http://mirwalk.umm.uni-heidelberg.de/) (23). The target miRNAs of selected genes were predicted using the miRWalk and miRDB databases with the default parameters ($p < 0.05$, seed sequence lengths more than 7 mer, the target gene-binding regions: 3′ UTR). The miRNAs were selected by intersecting all the results. Finally, mRNA-miRNA interaction network was constructed and visualized using Cytoscape software.

Functional Analysis of Target miRNAs

All the predicted target miRNAs were uploaded to the Funrich software (3.1.2), the molecular functions and biological pathways of target miRNAs were then identified. $p < 0.05$ was defined as markedly enriched functions and pathways.

Construction of ceRNA Networks

The target lncRNAs interacting with the selected miRNAs were predicted using the online database StarBase 3.0 (24). We selected the lncRNAs with most of the cross-linked miRNAs as our predicted lncRNAs according to the following screening criteria: mammalian, human h19 genome, CLIP-Data more than 5, and with or without degradome data. CeRNA networks based on the mRNAs-miRNAs-lncRNAs interactions were constructed and visualized using Cytoscape software.

Immune Infiltration Analysis

CIBERSORT is an analytic algorithm based on the gene expression profiles of 547 genes. The CIBERSORT could calculate the compositions of different immune cell subtypes using the deconvolution precisely on the algorithm, thus exhibiting the signature of each immune cell subtype (25). The gene expression profiles of all genes or co-DEGs including Control and FRDA samples were imported to perform the immune infiltration analysis using the CIBERSORT algorithm through R software. Student's *T*-tests were conducted to analyze the difference in the proportion of each immune cell subtype between Control and FRDA samples. A value of $p < 0.05$ was considered statistically significant. Finally, the "ggplot" (https://cran.r-project.org/src/contrib/Archive/ggplot/) (version 0.4.2) and "ggplot2" (http://had.co.nz/ggplot2/) (version 3.3.5) packages were performed to visualize the results.

Statistics Analysis

The "ggpubr" package (https://cran.r-project.org/web/packages/ggpubr/index.html) version 0.4.0) was applied to perform statistical analyses, the "ggplot2" and "ggplot" packages were conducted to draw boxplots and bar plots, respectively. The "GOplot" package was used to draw a chord plot. Student's *t*-test was performed to analyze the differences between the two groups.

RESULTS

Identification of DEGs

The dataset GSE11204, which included 10 healthy children, 28 children with FRDA, 15 healthy adults, and 14 adults with FRDA, were selected to analyze and identify the DEGs. We screened out a total of 530 (177 up-regulated and 353 down-regulated) DEGs in child samples and a total of 857 (483 up-regulated and 374 down-regulated) DEGs in adult samples. The volcano plots exhibited the number of DEGs identified from child and adult samples, respectively. The heatmaps displayed the expression of the top 25 up-regulated and down-regulated DEGs in child

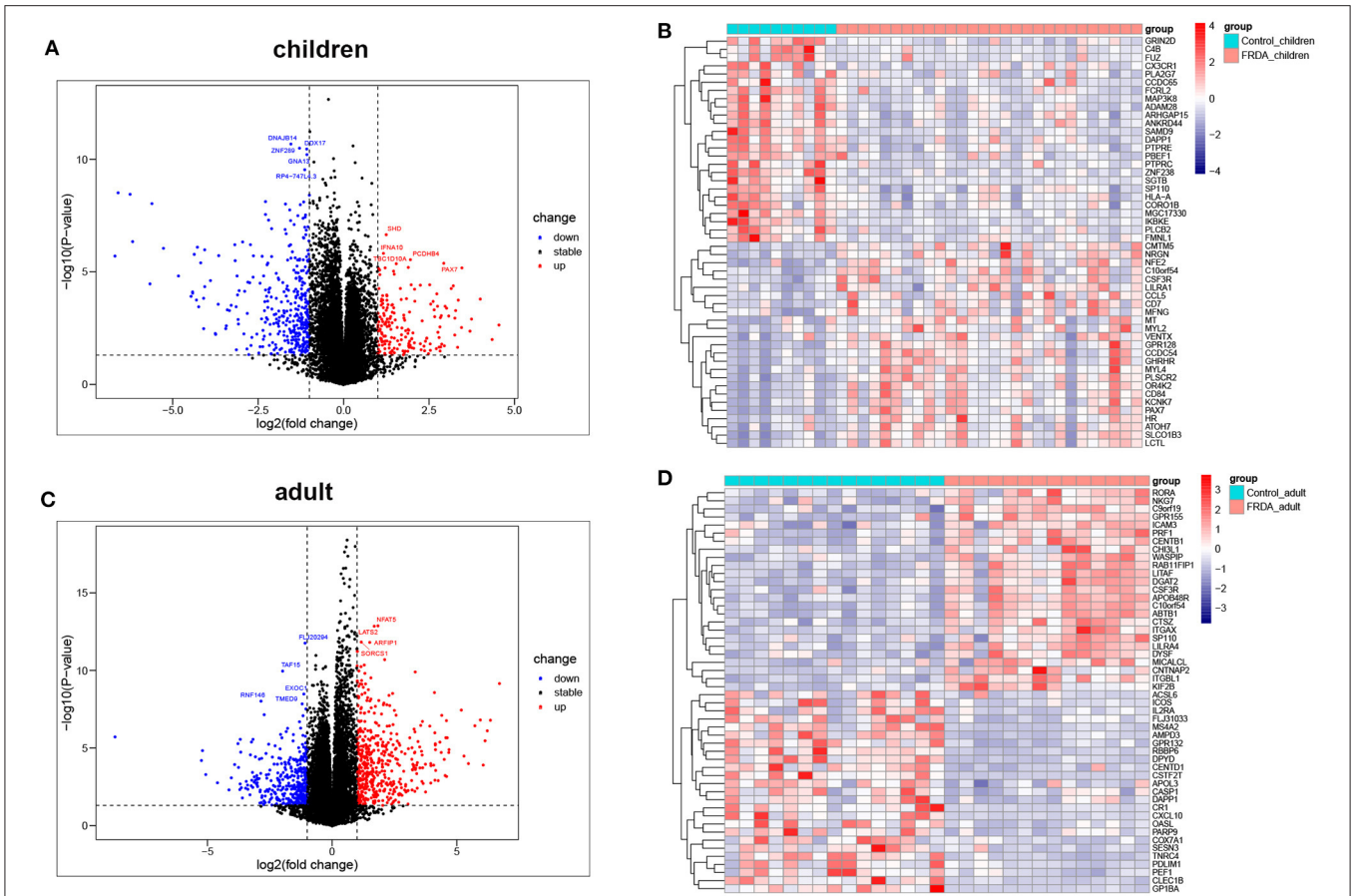

FIGURE 1 | Identification of DEGs between Control and FRDA samples. **(A)** The volcano plot of the genes in children samples. **(B)** Representative heatmap of DEGs in children samples. The top 25 up-regulated and down-regulated DEGs are shown, respectively. **(C)** The volcano plot of the genes in adult samples. **(D)** Representative heatmap of DEGs in adult samples. The top 25 up-regulated and down-regulated DEGs are exhibited, respectively. Red points: up-regulated DEGs, blue points: down-regulated DEGs, gray points: no changed genes; Red rectangles: high expression, blue rectangles: low expression.

and adult samples, respectively (**Figures 1A–D**). The expression levels of all DEGs in child and adult samples were also visualized in heatmaps (**Supplementary Figures 1A,B**).

GSEA and Immune Infiltration Analysis

In order to clarify the biological process and the immune cell subtype involved in the child and adult samples, respectively, we firstly performed the GSEA and found the genes in children were significantly enriched in antigen-receptor mediated signaling pathway, defense response to virus, NF-κB signaling, regulation of immune response signaling pathways, and T cell receptor signaling pathways (**Figure 2A**). In adults, the enriched gene sets were mainly involved in defense response to virus, regulation of T cell differentiation, response to interferon-gamma, response to virus, and viral gene expression (**Figure 2B**). Subsequently, we applied immune infiltration analysis and found the proportions of immune cell subtype distinct between groups (**Figures 2C,D**). Compared with the Control_children group, the FRDA_children group contained a greater number of resting memory CD4+ T cells and Neutrophils (**Figure 2E**). In addition, the FRDA_adult group markedly elevated the number of CD8+ T cells and activated NK cells, whereas the number of memory B cells, resting memory CD4+ T cells, activated memory CD4+ T cells, M1 Macrophages, resting Dendritic cells, activated Dendritic cells, and resting Mast cells decreased when compared with the Control_adult group (**Figure 2F**).

Identification of Co-DEGs and Functional Enrichment Analysis

The DEGs from the child and adult datasets were intersected and eventually identified 29 co-up-regulated and 59 co-down-regulated DEGs (**Figures 3A,B**). Therefore, we screened out a total of 88 co-DEGs. To further explore the enrichment pathways involved in these co-DEGs, we firstly performed GO enrichment analysis using the DAVID website. These co-DEGs were primarily involved in the biological processes including the immune effector process, defense response to virus, immune system process, defense response to other organisms, and hemopoiesis (**Table 1** and **Figures 3C,D**). In addition, KEGG pathway enrichment analysis suggested that these co-DEGs were mainly involved in the intestinal immune network for IgA production, autoimmune thyroid disease, measles, lysosome,

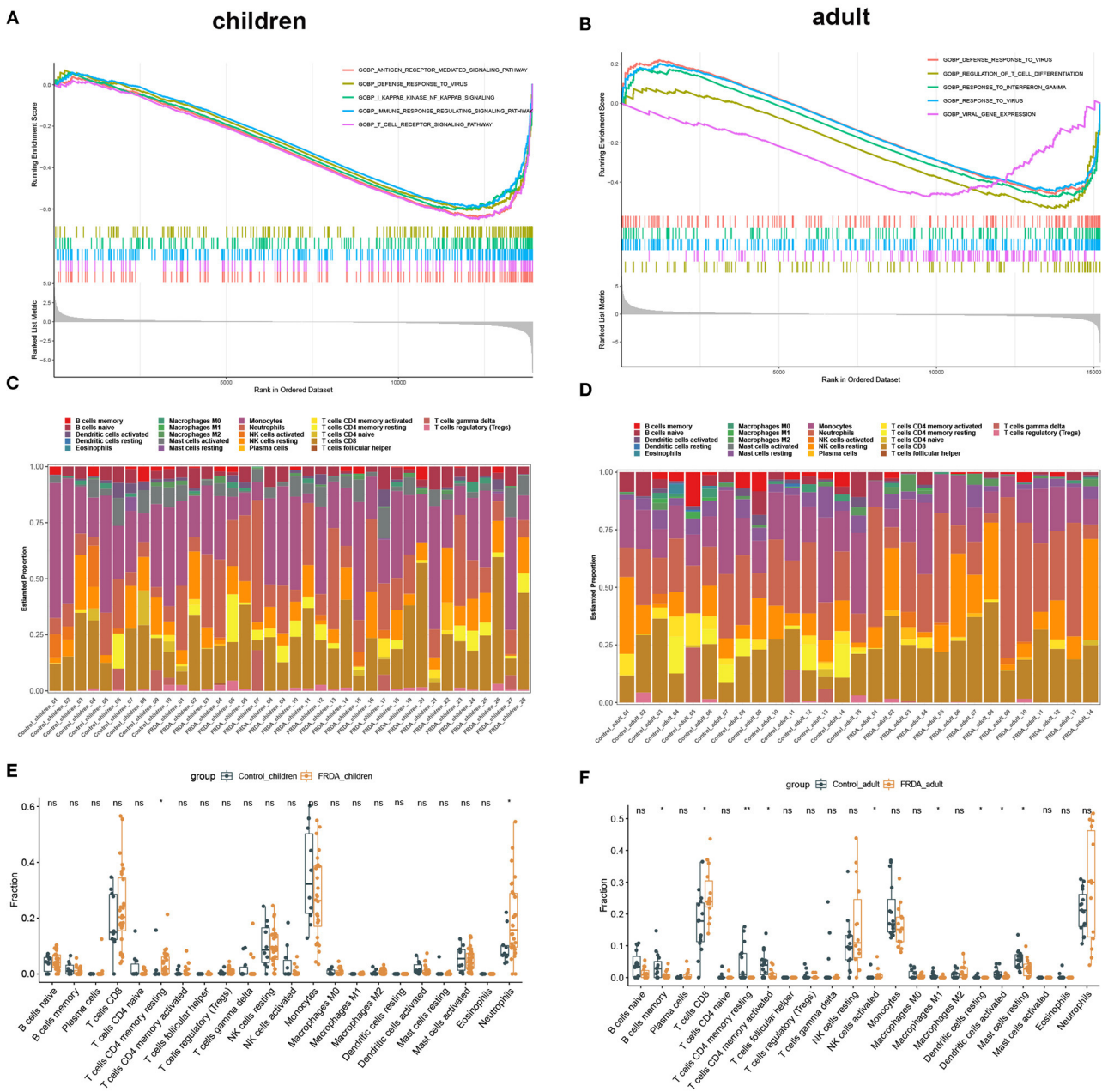

FIGURE 2 | GSEA and Immune infiltration analysis in children and adult samples. **(A,B)** GSEA between Control and FRDA group in children **(A)** and adult **(B)**. The top 5 gene sets were exhibited. **(C,D)** The landscape of all 22 types of immune cell components in children **(C)** and adults **(D)**. **(E,F)** Boxplots of the differentially infiltrated immune cells between Control and FRDA group in children **(E)** and adult (F). $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with the Control group.

necroptosis, and influenza A (**Figure 3E**). Reactome enrichment analysis indicated that these co-DEGs were primarily enriched in the immune system, inflammatory reaction, necrosis, and signal transduction (**Figure 3F**).

Next, we perform the immune infiltration analysis of co-DEGs in child and adult datasets, respectively. The obvious proportions of immune cell subtypes were exhibited in different groups (**Figures 4A,B**). The number of Plasma cells, M0 Macrophages, and Neutrophils were significantly increased, while the number of resting memory CD4+ T cells, activated memory CD4+ T cells, Monocytes, M1 Macrophages, and M2 Macrophages were remarkably attenuated in the FRDA _children group (**Figure 4C**). Additionally, the FRDA _adult group notably elevated the number of naïve B cells, Plasma cells, M0 Macrophages, and Neutrophils, whereas markedly decreased the number of naïve T cells CD4, M1 Macrophages, M2 Macrophages, and resting Mast cells when compared with the Control_adult group (**Figure 4D**).

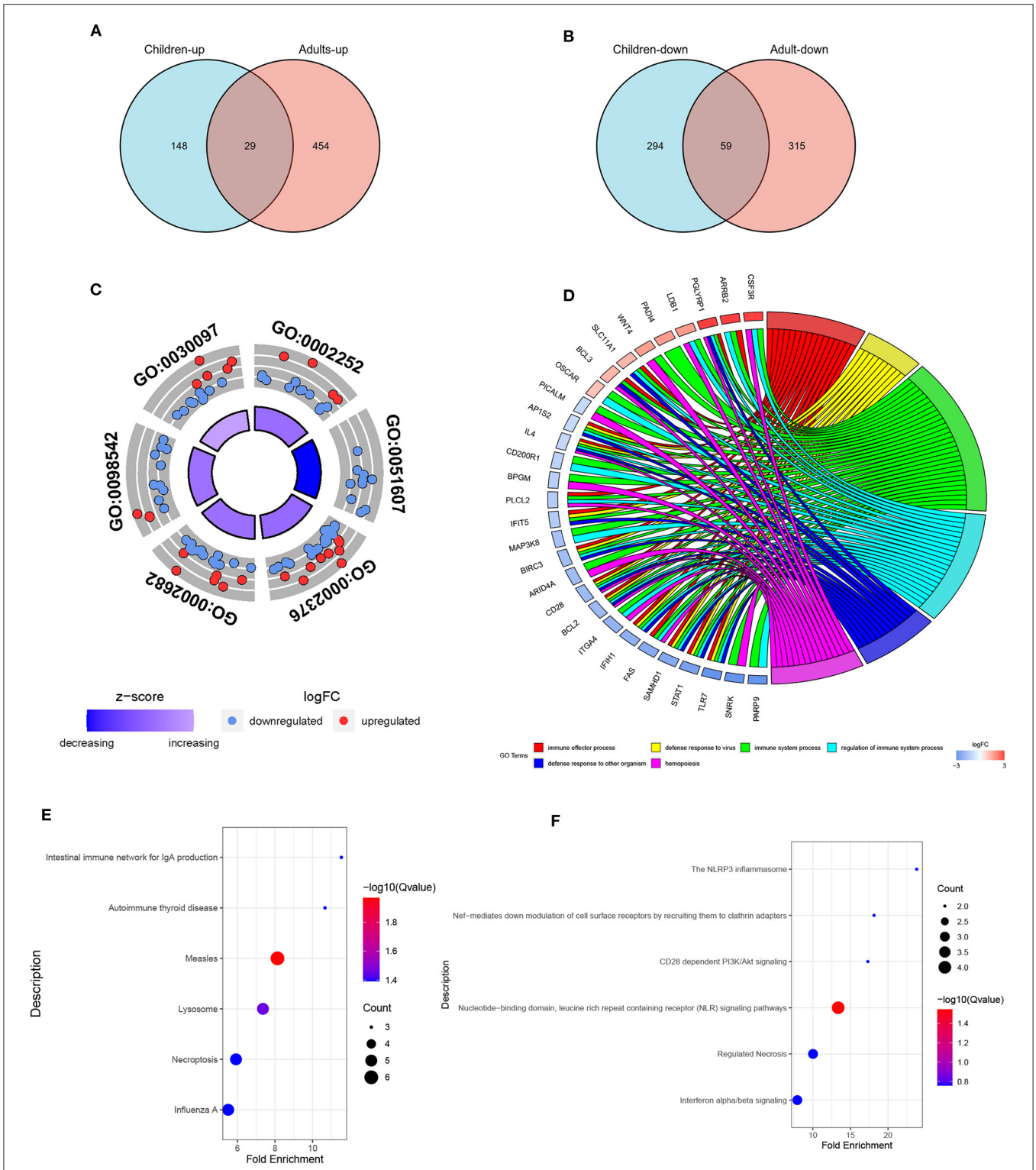

FIGURE 3 | The intersections of DEGs between groups and functional enrichment analysis of co-DEGs. **(A,B)** Venn diagram of common up-regulated and down-regulated DEGs between children **(A)** and adults **(B)**. **(C)** Top 6 GO terms (BP) of the 88 co-DEGs with the DAVID analysis. **(D)** Representative chord plot of the top 6 GO terms (BP) of co-DEGs. **(E,F)** Representative bubble plot of the top 5 enriched KEGG (E) and Reactome **(F)** pathways of co-DEGs.

TABLE 1 | Top 6 GO terms (BP) of the 88 co-DEGs with the DAVID analysis.

ID	Term	Count	Genes	Fold enrichment	FDR
GO:0002252	immune effector process	16	STAT1, SLC11A1, PLCL2, IFIT5, ARRB2, SAMHD1, IFIH1, IL4, BCL3, AP1S2, CD28, BCL2, FAS, TLR7, PGLYRP1, BIRC3	4.82789	6.64E-04
GO:0051607	defense response to virus	10	IFIH1, IL4, STAT1, BCL2, AP1S2, IFIT5, CD28, TLR7, SAMHD1, BIRC3	9.77700	6.64E-04
GO:0002376	immune system process	29	CSF3R, LDB1, IFIT5, ARID4A, ARRB2, SAMHD1, IFIH1, AP1S2, MAP3K8, PGLYRP1, WNT4, ITGA4, STAT1, SLC11A1, PLCL2, BPGM, PARP9, OSCAR, IL4, SNRK, CD200R1, BCL3, BCL2, CD28, FAS, TLR7, PADI4, BIRC3, PICALM	2.63404	6.64E-04
GO:0002682	regulation of immune system process	21	CSF3R, ITGA4, LDB1, STAT1, SLC11A1, PLCL2, ARRB2, SAMHD1, PARP9, OSCAR, IFIH1, IL4, CD200R1, AP1S2, CD28, BCL2, FAS, MAP3K8, TLR7, PGLYRP1, BIRC3	3.41202	6.64E-04
GO:0098542	defense response to other organism	13	STAT1, SLC11A1, IFIT5, SAMHD1, IFIH1, IL4, BCL3, AP1S2, CD28, BCL2, TLR7, PGLYRP1, BIRC3	5.89193	6.64E-04
GO:0030097	hemopoiesis	15	CSF3R, ITGA4, LDB1, PLCL2, ARID4A, BPGM, IL4, SNRK, BCL3, CD28, BCL2, FAS, PGLYRP1, PICALM, WNT4	4.54452	0.00131

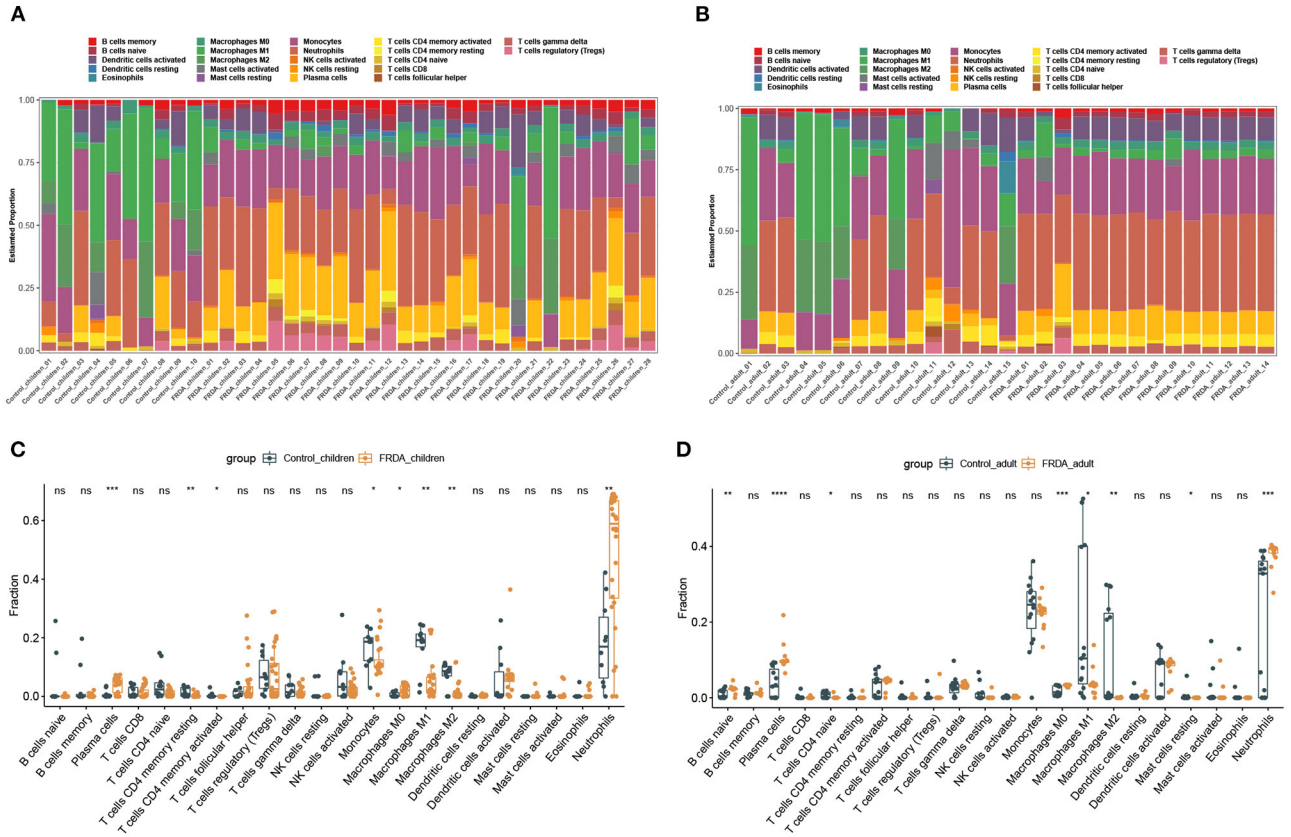

FIGURE 4 | Immune infiltration analysis of co-DEGs in children and adult samples. **(A,B)** The landscape of all 22 types of immune cell components in children **(A)** and adults **(B)**. **(C,D)** Boxplots of the differentially infiltrated immune cells between the Control and FRDA group in children **(C)** and adults **(D)**. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ when compared with the Control group.

PPI Network and Cluster Modules Analysis, Hub Genes Identification

The PPI network of co-DEGs, including 45 nodes and 56 edges, was constructed using the STRING website and visualized by Cytoscape software (**Figure 5A**). Next, we screened out a cluster module containing 6 down-regulated genes using the MCODE plugin (**Figure 5B**). Subsequently, a total of 10 hub genes were identified by intersecting the results of five algorithms from the cytoHubba plugin (Degree, MNC, Closeness, Stress, and Radiality) (**Figure 5C**). These identified hub genes were

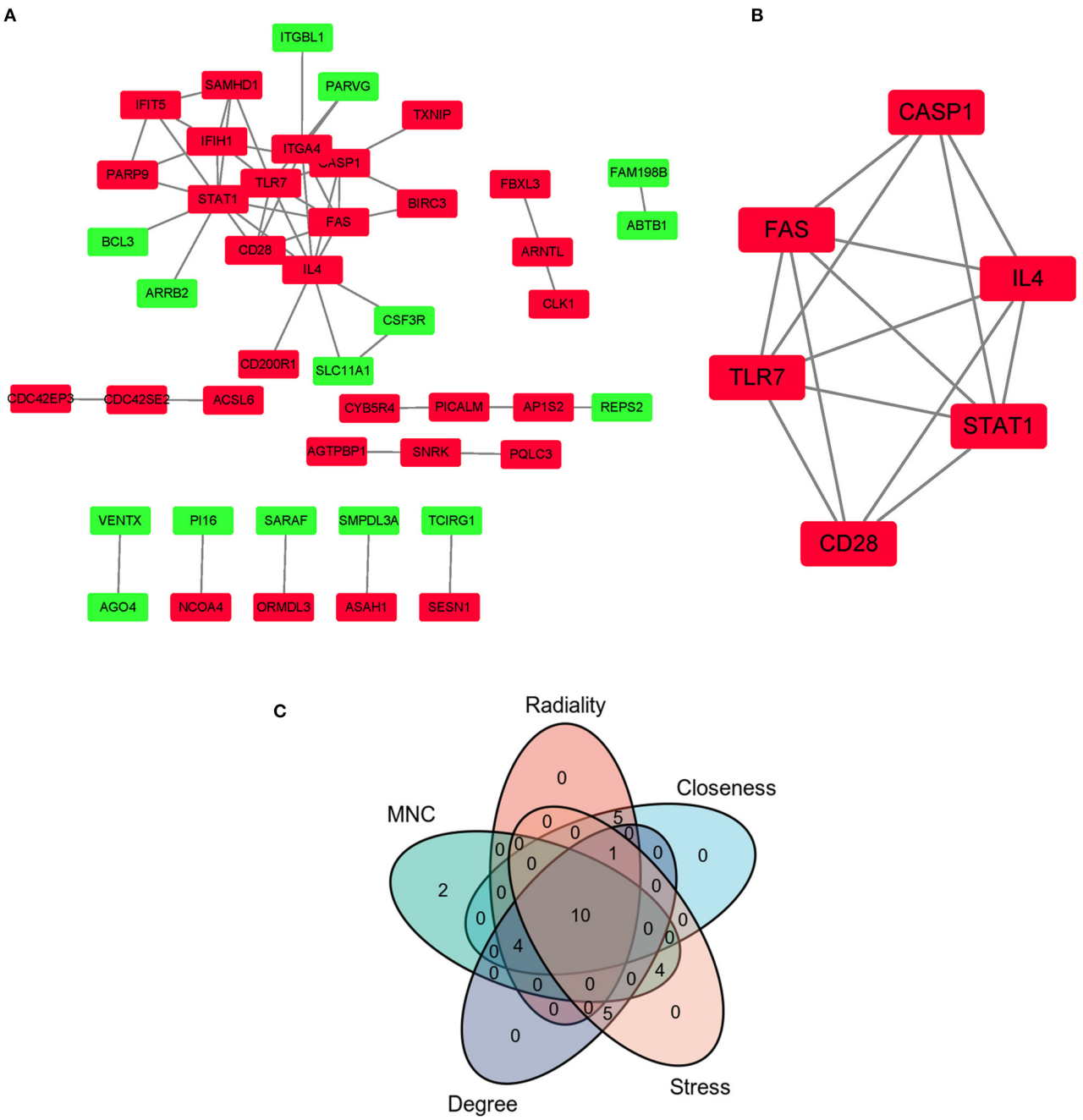

FIGURE 5 | PPI network of co-DEGs and hub gene selection. **(A)** PPI network of co-DEGs was comprised of 45 nodes and 56 edges. Red rectangles: down-regulated genes; Green rectangles: up-regulated genes. **(B)** The key cluster module identified was by the MCODE plugin. **(C)** Hub genes were screened out by intersecting the first 20 genes in the five algorithms of cytoHubba.

all significantly down-regulated in FRDA samples and were primarily enriched in the immune system process and response to other organisms (**Table 2**). These results evidence the major role of the declined expression of these hub genes in the pathogenesis of FRDA.

Target miRNAs Mining, Construction of the Interaction Network, and Functional Enrichment Analysis of Target miRNAs

miRNAs play a vital role in inducing gene degradation by binding the 3'UTR of mRNAs, thus exerting a negative regulation

mechanism. We obtained a total of 150 target miRNAs of 8 identified hub genes and ascertained 156 mRNA-miRNA pairs. In addition, based on the prediction results, a mRNA-miRNA interaction network with 158 nodes and 156 edges was constructed and visualized by the Cytoscape software (**Figure 6**). The miRNAs with a greater number of cross-linked genes (≥2) were identified (**Table 3**).

In addition, the results of miRNAs functional analysis indicated that the molecular functions were markedly enriched in protein serine/threonine kinase activity, transcription factor activity, GTPase activity, ubiquitin-specific protease activity, receptor binding, and receptor signaling protein serine/threonine kinase activity. The main biological pathways

TABLE 2 | A total of 10 hub genes were identified by intersecting the results of five algorithms from the cytoHubba plugin.

Gene	log2FC		*p*-value		Regulation
	Children	Adult	Children	Adult	
Immune system process					
IFIH1	−1.33255	−2.19049	0.00744	0.00090	down
STAT1	−1.23078	−2.652	0.002283	2.26E-05	down
IL4	−1.63090	−1.09967	0.00020	0.03174	down
IFIT5	−1.51727	−1.40953	0.01086	0.01804	down
TLR7	−1.91344	−3.00512	0.00574	0.00090	down
FAS	−1.32193	−1.12326	0.00177	0.00011	down
CD28	−1.09838	−1.89092	0.02392	0.00018	down
ITGA4	−2.11557	−2.41976	0.00013	0.00000	down
SAMHD1	−3.10336	−1.40975	0.00011	0.01618	down
Response to other organisms					
CASP1	−2.82706	−3.38080	0.02651	0.00458	down

TABLE 3 | Predicted miRNAs and genes targeted by miRNAs.

miRNA	Genes targeted by miRNA	Gene count
hsa-miR-6843-3p	TLR7, CD28	2
hsa-miR-6811-3p	SAMHD1, CD28	2
hsa-miR-5580-5p	STAT1, CD28	2
hsa-miR-4768-3p	TLR7, IFIT5	2
hsa-miR-4713-5p	SAMHD1, IFIT5	2
hsa-miR-10393-5p	SAMHD1, FAS	2

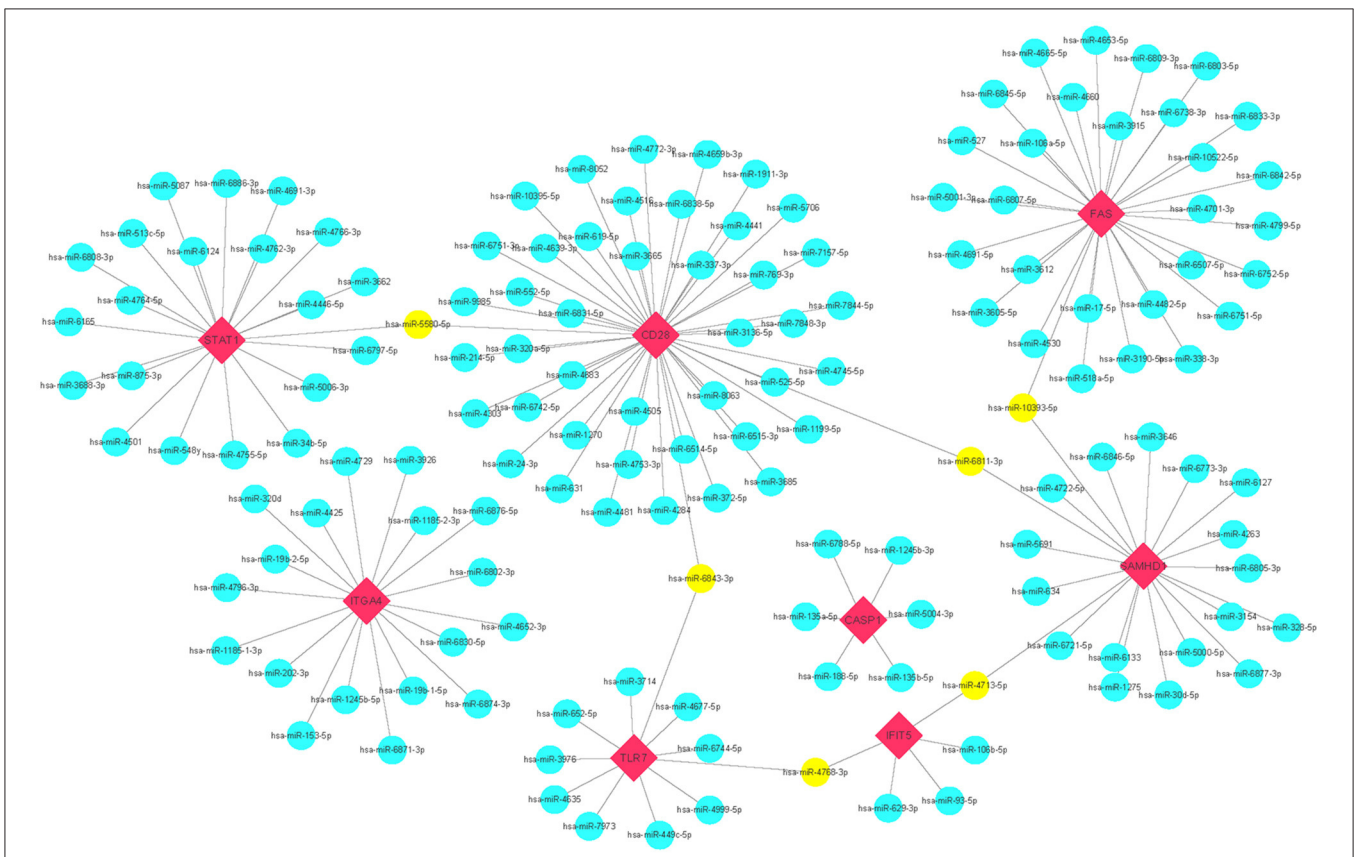

FIGURE 6 | Predicted interactions among 8 hub genes and their target miRNAs. mRNA-miRNA co-expressed network was comprised of 158 nodes and 156 edges. Red diamonds: hub genes; Cyan circles: miRNAs; Yellow circles: miRNAs with more than 2 cross-linked genes.

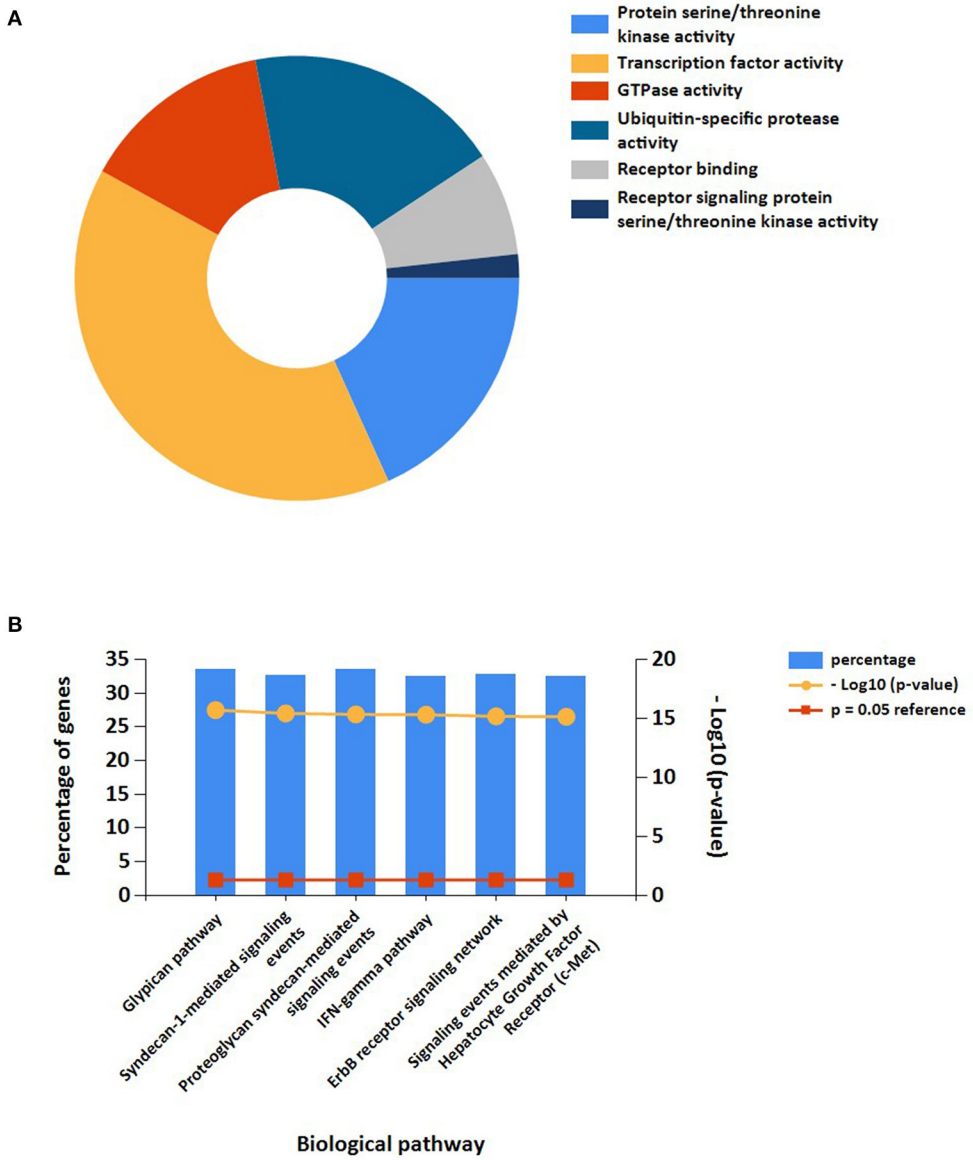

FIGURE 7 | Functional enrichment analysis of target miRNAs. **(A)** Representative Pie chart of molecular functions of target miRNAs. **(B)** Representative Bar chart of Biological pathways of target miRNAs.

involved were glypican pathway, syndecan-1-mediated signaling, proteoglycan syndecan-mediated signaling, IFN-gamma pathway, ErbB receptor signaling, and c-Met-mediated signaling (**Figures 7A,B**).

Validation of the 8 Hub Genes Expression in the GSE30933 Dataset

The GSE30933 dataset, which included 40 healthy and 68 FRDA samples, was applied for validating the expression of 8 hub genes that interacted with target miRNAs. We found the mRNA expression of CD28, FAS, and IFIT5 were significantly attenuated in the FRDA group when compared with the Control group (**Figure 8**). Next, we performed SPSS software to analyze the expression profiles of 8 hub genes in healthy and FRDA samples and draw the ROC curves. CD28 (AUC = 0.818), FAS (AUC = 0.659), and IFIT5 (AUC = 0.701) genes all had the ability to differentiate FRDA from normal samples, and CD28 had the highest diagnostic value in FRDA samples (**Figures 9A–H**). Therefore, combined with the expression levels of these hub genes in the GSE30933 dataset, we assume that the downregulation of CD28, FAS, and IFIT5 might be potential diagnostic biomarkers for FRDA progression.

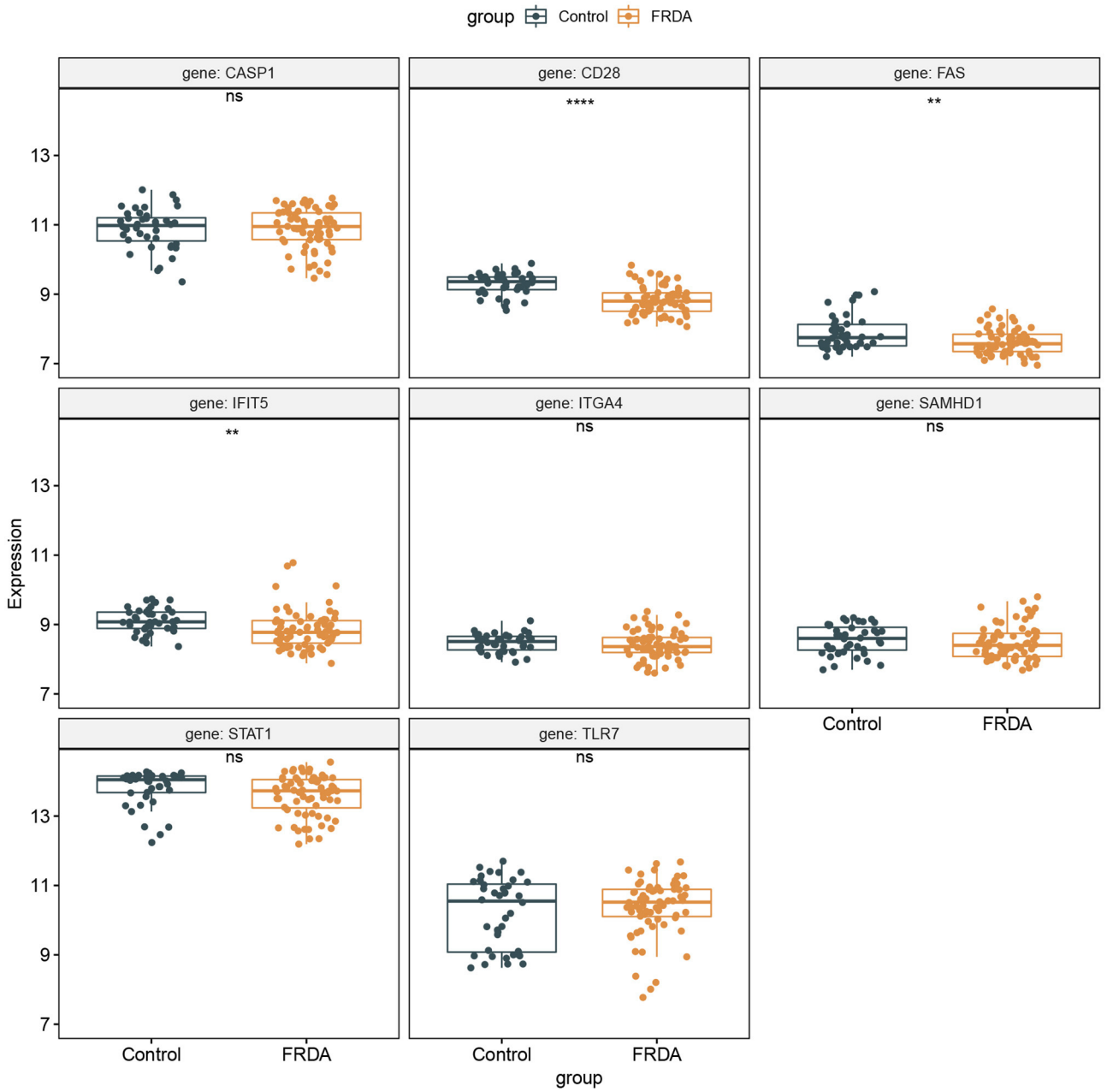

FIGURE 8 | Validation of the 8 specifically expressed core genes using the GSE30933 dataset. $^{**}p < 0.01$, $^{****}p < 0.0001$ when compared with the Control group.

Target lncRNAs Prediction and Construction of ceRNA Networks

As the upstream molecules of miRNAs, lncRNAs could regulate the biological function of miRNAs. Therefore, we predicted the target lncRNAs of the miRNAs interacting with CD28, FAS, and IFIT5 genes. A total of 5 target lncRNAs were obtained in the CD28-miRNA interaction network, 3 and 12 target lncRNAs were identified in FAS-miRNA and IFIT5-miRNA interaction networks, respectively. Three ceRNA networks were established and visualized by Cytoscape software (**Figures 10A–C**). Subsequently, we performed a literature search and only found miR-24-3p had been reported in FRDA. Therefore, we proposed that NEAT1-hsa-miR-24-3p-CD28 may be the potential RNA regulatory pathway involved in the progression of child and adult FRDA (**Figure 10D**).

DISCUSSION

FRDA is an autosomal recessive genetic disease with multiple system damage. In recent years, bioinformatics analysis has

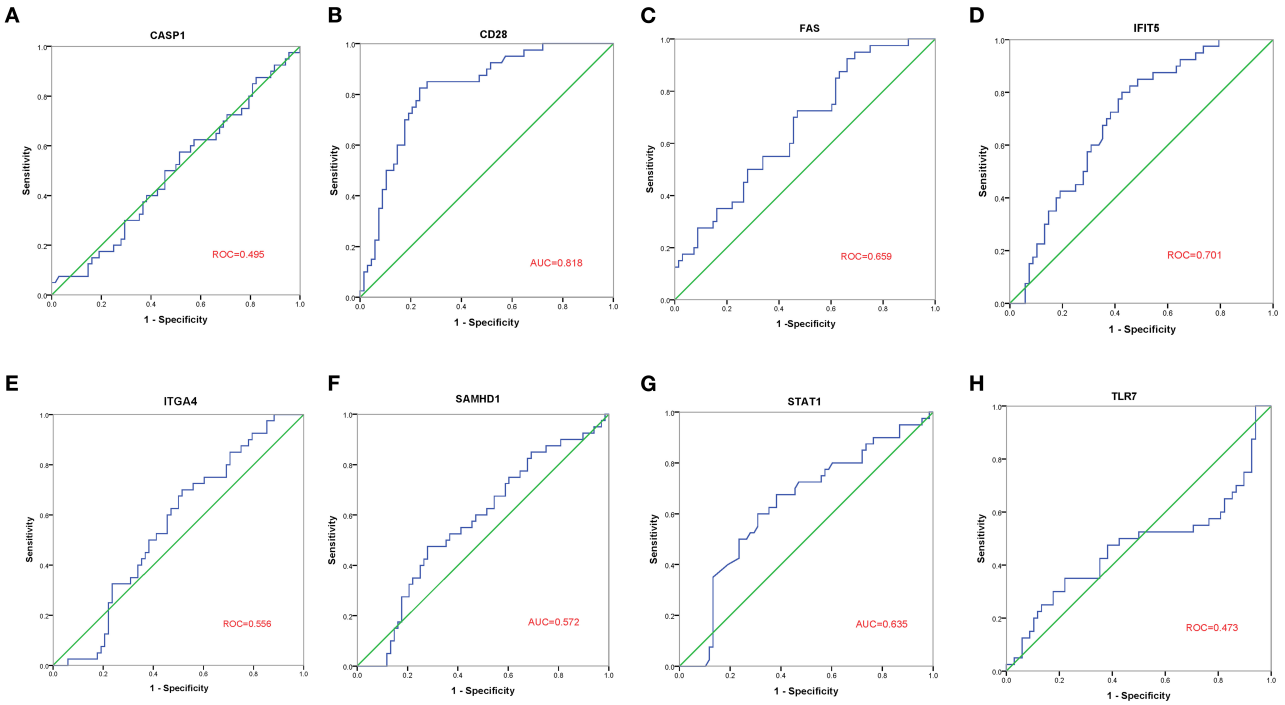

FIGURE 9 | ROC curve analysis of the 8 specifically expressed core genes using the GSE30933 dataset. **(A–H)** ROC curve analysis of the 8 specifically expressed core genes.

been widely developed and applied in various diseases, which reveals the underlying pathogenesis of disease and identifies vital biomarkers related to the diagnosis and prognosis of the disease (26). Nevertheless, the comprehensive research on the relationship between child and adult FRDA based on bioinformatics has not been systematically reported so far.

In this study, we screened out 530 DEGs in child samples, including 177 up-regulated and 353 down-regulated genes, and 857 DEGs in adult samples, including 483 up-regulated and 374 down-regulated genes. Eventually, 88 co-DEGs were identified by intersecting the up-regulated and down-regulated DEGs between the child and adult datasets. The results of GSEA and immune infiltration analysis indicated that these genes in both the child and adult datasets were mainly enriched in the immune response. GO and KEGG pathway enrichment analysis of co-DEGs suggested that the immune response characterized by the activation of immune cells and regulation of innate immune response were significantly stronger in FRDA samples. In addition, Reactome analysis revealed that immune system activation, necrosis, and signal transduction were closely related to the progression of child and adult FRDA.

Dysfunction of the immune system function is vital for the prognosis of diseases, including FRDA. Recently, Nachun et al. demonstrate that there are significant differences in the proportion of natural killer (NK) cells among control, carrier, and FRDA groups through bioinformatics analysis, and they are found significantly decreased in FRDA patients (27). In addition, IL-6, a cytokine produced by macrophages, has been proven to be increased in the blood plasma of FRDA patients, which suggests the activation of macrophages may be implicated in the neuropathology of FRDA (28). The current study, however, found a significant decrease in the number of macrophages and a remarkable increase in the number of activated NK cells in the FRDA_adult group; no statistical significance was found in the number of natural killer cells and macrophages between the FRDA_children and Control_children group. The following facts may have led to this discrepancy: Firstly, different datasets used for analysis create batch effects, thus may result in distinct results. Secondly, subjects from different regions or with different ethnicities may also have a certain impact on the results. Thirdly, in our current study, we have classified FRDA into adult and child groups and explored the association between FRDA and immune cell types in adults and children, which may also be another factor for the inconsistent results. Moreover, in our study, we performed the CIBERSORT algorithm instead of the quadratic programming method for immune infiltration analysis, which suggests that the impact of distinct analysis methods on the results could also not be ignored.

In order to further narrow the scope of research, we constructed the PPI network of co-DEGs, and screened out a total of 10 hub genes by intersecting the results of five algorithms in the CytoHubba plugin. These genes were mainly involved in the immune system process and response to other organisms. Afterward, we selected eight hub genes that interact with target miRNA and verified these hub genes using the GSE30933 dataset. We found the expression of three immune-related genes (CD28,

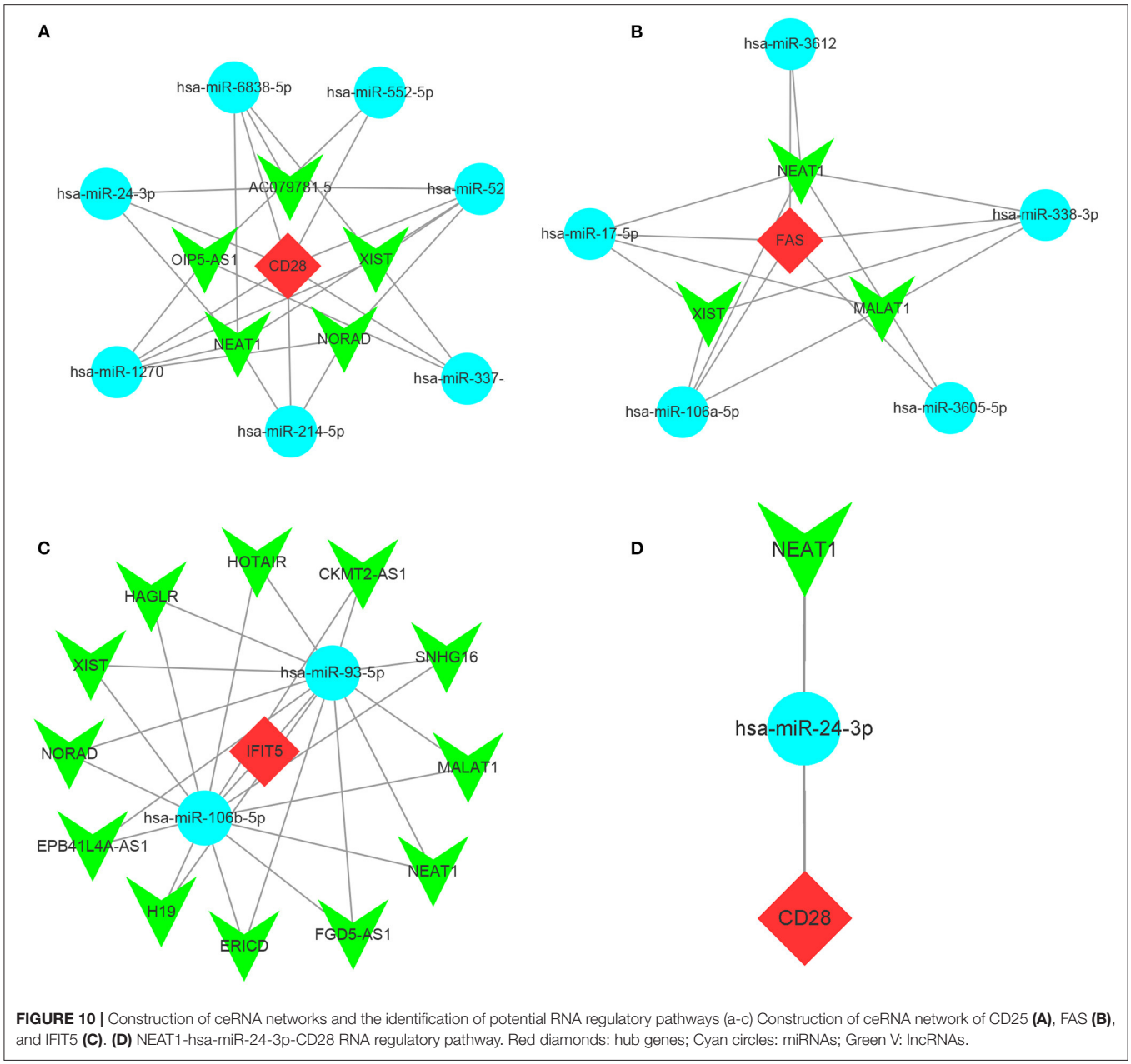

FIGURE 10 | Construction of ceRNA networks and the identification of potential RNA regulatory pathways (a-c) Construction of ceRNA network of CD25 **(A)**, FAS **(B)**, and IFIT5 **(C)**. **(D)** NEAT1-hsa-miR-24-3p-CD28 RNA regulatory pathway. Red diamonds: hub genes; Cyan circles: miRNAs; Green V: lncRNAs.

FAS, and IFIT5) in FRDA samples were significantly lower than that in the Control group. The ROC analysis revealed these genes had greater diagnostic significance for FRDA. Therefore, we hypothesize that the downregulation of CD28, FAS, and IFIT5 may be the potential mechanisms involved in the progression of FRDA.

CD28, a member of cell surface glycoprotein receptor, primarily expressed on CD^{4+} T cells and CD^{8+} T cells, belongs to costimulatory molecules superfamily and plays a vital role in immune system response including T cell proliferation and differentiation, the production of cytokine and chemokines (28, 29). However, CD28 has not been mentioned in FRDA-related studies. In our current study, we found CD28 was remarkably down-regulated in both child and adult FRDA. In addition, the results of GSEA indicated that the T cell receptor signaling pathway was negatively correlated with child FRDA, and the ability of T cell differentiation was markedly inhibited in adult FRDA. These results revealed the insult of FRDA to the T cells-related immune process to some extent. Therefore, we conclude the downregulation of CD28 might play a critical role in the progression of children and adult FRDA.

FAS (also known as CD95 and TNFRSF6), a death receptor, belongs to the tumor necrosis factor (TNF) receptor superfamily and is mainly involved in the regulation of caspase-8-dependent apoptosis by interacting with its ligand FasL (30). Several studies have found the expression of FAS in plasma, gray matter, and

white matter is significantly enhanced in Alzheimer's disease (AD) patients (31–33). This indicates that FAS might be markedly related to the progression of AD. However, recent studies also demonstrate that FAS engagement evokes non-apoptotic signals including cell migration and differentiation and cytokine processing (34, 35). Consistently, our study found plasma FAS decreased in both child and adult FRDA samples. Combined with the result of ROC analysis, we considered FAS as a potent protective factor for FRDA, and the downregulation of FAS may be closely related to the progression of FRDA in children and adults.

IFIT5, a member of the IFIT1 family, can be activated under stress conditions including virus infection, the production of type I interferon, and lipopolysaccharides stimulation (36, 37). IFIT5 has been proven to be implicated in the regulation of a wide variety of functions, such as viral restriction, translation initiation, cell migration and proliferation, and double-stranded RNA signaling (38, 39). Currently, IFIT5 has not been reported in FRDA. In our study, we identified that IFIT5 has a low level of expression in both child and adult FRDA, which indicated that the decrease of IFIT5 may be a key factor leading to pathological changes in FRDA.

Moreover, in order to clarify the potential regulatory mechanisms related to FRDA progression at the transcriptome level, we predicted the target lncRNAs of the miRNAs interacting with CD28, FAS, and IFIT5 genes and constructed a ceRNA network using Cytoscape software. Afterward, we applied the literature search and only found miR-24-3p was linked with ataxia (40). Therefore, we propose that NEAT1-hsa-miR-24-3p-CD28 may be the potential RNA regulatory pathway involved in the progression of child and adult FRDA.

Several limitations need to be highlighted in this study. Firstly, the sample size for analysis and validation is relatively insufficient. A greater number of samples are needed to verify these results. Secondly, our study preliminarily identified the potential RNA regulatory pathways during the progression of child and adult FRDA, which needs to be further clarified *in vitro*, *in vivo*, and clinical trials studies.

CONCLUSION

In summary, our study found the downregulation of three immune-specific hub genes, CD28, FAS, and IFIT5, may be associated with the progression of child and adult FRDA. Furthermore, NEAT1-hsa-miR-24-3p-CD28 may be a potential RNA regulatory pathway related to the pathogenesis of child and adult FRDA. These findings provide a novel perspective for exploring the pathophysiological mechanism of FRDA progression at the transcriptome level.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO), https://www.ncbi.nlm.nih.gov/geo/, GSE11204 and GSE30933.

AUTHOR CONTRIBUTIONS

LL and YL designed the study. ZZ and QF collected and analyzed the data and searched the literature. YL and ZZ interpreted the results. LL wrote and prepared the original manuscript. YL revised the manuscript. All authors have read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.816393/full#supplementary-material

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Cross-Sectional Investigation of Brain Volume in Dyslexia

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The goal of the study was to determine whether dyslexia is associated with differences in local brain volume, and whether these local brain volume differences show cross-sectional age-effects. We investigated the local volume of gray and white brain matter with voxel-based morphometry (VBM) as well as reading performance in three age groups of dyslexic and neurotypical normal reading subjects (children, teenagers and adults). Performance data demonstrate a steady improvement of reading skills in both neurotypical as well as dyslexic readers. However, the pattern of gray matter volumes tell a different story: the children are the only group with significant differences between neurotypical and dyslexic readers in local gray matter brain volume. These differences are localized in brain areas associated with the reading network (angular, middle temporal and inferior temporal gyrus as well as the cerebellum). Yet the comparison of neurotypical and normal readers over the age groups shows that the steady increase in performance in neurotypical readers is accompanied by a steady decrease of gray matter volume, whereas the brain volumes of dyslexic readers do not show this linear correlation between brain volume and performance. This is further evidence that dyslexia is a disorder with a neuroanatomical basis in the form of a lower volume of gray matter in parts of the reading network in early dyslexic readers. The present data point out that network shaping processes in gray matter volume in the reading network does take place over age in dyslexia. Yet this neural foundation does not seem to be sufficient to allow normal reading performances even in adults with dyslexia. Thus dyslexia is a disorder with lifelong consequences, which is why consistent support for affected individuals in their educational and professional careers is of great importance. Longitudinal studies are needed to verify whether this holds as a valid pattern or whether there is evidence of greater interindividual variance in the neuroanatomy of dyslexia.

Keywords: reading, developmental dyslexia, local brain volume, gray matter, VBM, structural MRI

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INTRODUCTION

Developmental dyslexia affects about 5 percent of the population depending on diagnostic criteria (1). Even nowadays affected individuals still struggle to receive adequate support, thus dyslexia has far-reaching consequences for the suffering individual on scholastic, psychological and socio-economic levels (2–4). Individuals affected by dyslexia have problems with fluent and/or accurate reading, spelling and the proper acquisition of grapheme-phoneme correspondence. Dyslexia is not caused by a general cognitive impairment or a lack of an opportunity to learn (5). Several cognitive, sensoric and neurobiological deficits are suggested to cause dyslexia (6) which in turn are supposed to impact the acquisition and automation of the reading and spelling process (7–10). Reading processes take place in a large neural reading network comprised of broad areas in the dorsal superior temporal, ventral inferior temporal, and inferior frontal brain [e.g., (11–13)]. The extent of the involvement of this particular reading network depends on cognitive demands of the reading task (14). Phonological processing strongly involves the dorsal reading system, whereas the ventral system is more involved with visual word form processing as well as the transfer of letter shape to phonological content (transfer of visual input to linguistic output units) (15). The extent to which either the dorsal or ventral system is most involved depends on the skill level involved in the reading process: beginning readers rely more on the phonological dorsal system and skilled readers rely more on the well-trained ventral visual reading system (16–18). In those suffering from dyslexia, the dorsal and inferior frontal components of the neural reading network consistently display functional differences compared to the neural reading network of neurotypical readers (19–21).

Voxel-based morphometry (VBM) is used in neuroimaging studies to determine whether dyslexia is associated with differences in local gray and white brain matter volumes in the reading network. VBM, without using a region of interest approach, is a fully automated method to identify regions of local volume differences in the whole brain.

Studies investigating the differences in local brain matter volume between neurotypical reading controls and readers with dyslexia identify various areas within the oral and written language networks [for an overview see (22–28)]. Regions which repeatedly show differences in local brain volume between neurotypical reading and dyslexic subjects are in inferior parietal (29), temporo-parietal (29, 30) and superior temporal regions (31), the inferior frontal gyrus, the left and right fusiform gyrus (32) and the cerebellum (29, 32).

Recent studies using VBM demonstrate variations in brain matter volume in prereading children at risk for dyslexia (33–35), across different language systems (36, 37) as well as differences in dyslexic children after a reading intervention (38). VBM studies thus indicate that dyslexia-specific morphometric differences can already be observed before the acquisition of written language and that these differences can be referred to as early neuroanatomical signatures for the subsequent reading problems. Finding no differences in dyslexia-specific VBM profiles across different language systems, Silani et al. (36) assume that there could be a common neuroanatomical basis irrespective of the language system. VBM studies also highlight the flexibility of the human brain since these studies demonstrate that these differences can be influenced by reading experience or training.

Several studies on the association between functional and volumetric differences in dyslexia detect that functional and volumetric differences between neurotypical and dyslexic readers are not only coexisting results, but can also serve as indicators of associated disorder characteristics. As these studies demonstrate (30, 39) there is evidence that areas which show dyslexia-specific hypo-activations in functional neuroimaging studies are related to differences in brain matter volumes between neurotypical and dyslexic readers.

Methodological inconsistencies between existing VBM studies on dyslexia make it hard to compare results since they are strongly influenced by the language system (shallow vs. deep orthographic system), experimental designs (subject samples, diagnostic criteria for dyslexia) as well as methods for data acquisition and analysis, [i.e., modulation for absolute GMV, registration algorithms using either a group-specific or a priori template, kernel size used for smoothing as well as differences on the level of statistical analysis (i.e., level of statistical correction for multiple tests)]. These inconsistencies can lead to differences in the findings of the studies (22, 37). The investigation of age dependent differences in brain volume of dyslexic readers by comparing different study-results is thus difficult.

To our knowledge, no study has yet addressed characteristics in brain matter volume with VBM in dyslexic readers over a large age range.

The aim of the present study is therefore to investigate age-dependent differences in local brain matter volume in developmental dyslexia. We hypothesize that dyslexic and neurotypical readers show different local brain volumes in areas related to the neuronal reading network and that these local brain volume differences show cross-sectional age-effects when comparing three groups (children, teenagers and adults with dyslexia compared to neurotypical normal reading, age-matched controls). Since we apply the same experimental design as well as the same parameters for data acquisition and analysis to all groups, we should be able to overcome the methodological pitfalls mentioned above.

MATERIALS AND METHODS

Subjects

The study comprises three groups of subjects with dyslexia (children, teenagers and adults) as well as three groups of neurotypical normal reading subjects matched a posteriori according to age and nonverbal IQ (children, teenagers and adults).

A total of 21 neurotypical children, 24 neurotypical teenagers, 27 neurotypical adults, 22 dyslexic children, 18 dyslexic teenagers as well as 22 dyslexic adults took part in the diagnostic session and MRI data acquisition. Due to inferior MRI Data quality of some participants, these individuals were excluded from the analysis. Thus, the analyses in the present study are based on a final

sample of 20 neurotypical children, 21 neurotypical teenagers, 26 neurotypical adults, 21 dyslexic children, 17 dyslexic teenagers as well as 20 dyslexic adults.

The study was approved by the local Ethics Committee at the Jena University Hospital. Subjects and legal guardians were informed verbally and with written materials about the experimental procedure. All individual participants included in the study and their legal guardians gave written informed consent for their participation. They were informed that all published data are fully anonymized. All procedures performed in this study are in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Assessment of Sample Criteria

During a diagnostic session several standardized tests were applied. These data were used to assess the sample criteria. The IQ of all subjects had to be $\geq$85. Sample criteria for dyslexia was a double discrepancy: $\geq$1.5 standard deviations between nonverbal IQ and reading and spelling performance as well as reading and spelling performance $<$ percentile rank 15. Sample criteria for neurotypical controls was discrepancy between nonverbal IQ and reading as well as spelling performance $<$ 1 standard deviation.

Subjects with uncorrected impairments of sight or hearing, bilingual education, neurological or psychiatric disorders (especially ADHD) were excluded based on the information obtained in a detailed clinical screening interview as well as performance in an attention test. All subjects were right handed according to verbal assessment. For an overview of the results regarding study criteria please refer to **Table 1**.

As a measure of nonverbal intelligence, Raven's Standard Progressive Matrices was administered (40). The test measures the subjects' reasoning ability, the educative ("meaning-making") component of Spearman's g. It is comprised of multiple-choice questions. For each test item, the subject is asked to identify the missing element that completes a pattern. Reading fluency and accuracy both in single word as well as in text reading was assessed by means of a standardized reading test [Zürcher Lesetest, (41)]. In this test subjects read different lists of single words as well as different texts respective to class level. Since the ZLT can be applied to a wide age range, the use of various test procedures was not necessary. The test acquires scores for the time needed as well as the errors made while reading the target words and texts. Spelling performance was assessed by dictation of a gap text by means of standardized spelling tests appropriate for the respective class or age level (42–44). The tests acquire the number of misspelled words.

Attention was assessed via the so-called "cross-out-test." In this test, the subject is confronted with visually similar items and is asked to cross out certain target items. Attention as well as the tendency toward impulsive behavior is assessed via the speed and accuracy with which differentiation between the visually similar items is achieved by the subject. This test was administered in order to ensure that deficits in attention span do not confound the results [d2, (45)].

Reading Experiment: Assessment of Reading and Phonological Skills as Dependent Variables

Since phonological processing plays a crucial role in the reading process, especially for beginning readers, we acquired additional neuropsychological data on reading skill and phonological decoding skills using single word reading (e.g., Baum Bein), reading of pseudowords (e.g., Bilza Bilaz) as well as the rhyming of pseudowords (e.g., Jurde Surde).

We used these tasks in order to trigger phonological reading processes at different levels of difficulty, requiring different levels of phonological skill: Reading of frequently used regular single words should require lowermost phonological skills. These words should engage highly automatized whole word reading strategies. Pseudoword reading should exercise an increased demand for phonological processing, since the pseudowords do not exist in the common vocabulary and have no entry in the mental lexicon. The unknown word material must be read by using phonological grapheme-phoneme correspondence skills. Rhyming of pseudowords is thought to require the most phonological processing skill as, in addition to the grapheme-phoneme correspondence skill, phonological short time storage is needed to keep up the phonological code of the pseudoword in order to make the rhyme judgment (10).

For single word reading, frequently used German words were taken from third grade vocabulary (46, 47). Pseudowords were created on the basis of real words in which first the vowels were exchanged followed by stepwise exchange of consonants, until there was no longer an association for an existing German word. Subjects decided whether two items that were visually presented side by side on the computer screen (i.e., Baum Bein) were identical or not. Each decision required a key press, so that responses were registered through a key press of either index (stimuli are the same) or middle finger (stimuli are not the same) of the right hand. Reaction time and error rate were acquired via ERTS (48). The presentation rate of the stimuli was not fixed but subject-controlled with a maximal stimulus-presentation time of 5 s. If the subject pressed a key within these 5 s, the next pair of stimuli was presented after an inter-trial interval of 500 ms. The computer automatically switched to the next trial if the reaction time of 5 s was exceeded.

We hypothesized that improvement in reading and phonological skills (lower reaction times and error rates) in neurotypical and dyslexic readers should be observed from children to teenagers to adults and a potential phonological processing deficit should be reflected by increased reaction times and error rates over the three tasks in dyslexics compared to neurotypical readers. Performance in word reading, pseudoword reading and pseudoword rhyming is depicted in **Figure 1**.

MRI Data Acquisition

MRI data were acquired using a Siemens Magnetom Vision 1.5 Tesla MRI Scanner (Erlangen, Germany). The head was fixated inside the head coil with extra padding in order to avoid movement artifacts. A high-resolution anatomical dataset of the whole brain (192 slices, T1-weighted, TR = 15 ms, TE = 5 ms, flip

TABLE 1 | Sample criteria.

	NT-child $n = 20$	D-child $n = 21$	NT-teen $n = 21$	D-teen $n = 17$	NT-adult $n = 26$	D-adult $n = 20$	
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	**ANOVA statistics**
Age (year, month)	10.60 (0.94)	10.38 (0.92)	13.31 (1.37)	13.92 (1.50)	26.25 (5.29)	26.20 (9.54)	ME read-lev: F (1.125) = 0.02; n.s.; ME age: F (2.125) = 140.97; $p < 0.001$; inter F (2.125) = 0.09, n.s.
IQ	109.15 (15.52)	108.14 (15.13)	102.97 (12.81)	100.76 (14.61)	106.89 (16.17)	100.90 (12.15)	ME read-lev: F (1.125) = 1.37; n.s.; ME age: F (2.125) = 2.28; n.s.; inter F (2.125) = 0.35, n.s.
Spelling (IQ-scale)	105.34 (11.96)	69.84 (8.45)	102.05 (10.64)	66.18 (13.65)	104.85 (13.67)	63.64 (11.24)	ME read-lev: F (1.125) = 313.07; $p < 0.001$; ME age: F (2.125) = 1.15; n.s.; inter F (2.125) = 0.79, n.s.
Reading (IQ-scale)	101.68 (7.22)	74.20 (8.15)	106.19 (5.68)	83.50 (13.06)	110.77 (3.02)	99.64 (8.72)	ME read-lev: F (1.125) = 207.52; $p < 0.001$; ME age: F (2.125) = 52.71; $p < 0.001$; inter F (2.125) = 12.36, $p < 0.001$

NT-child, neurotypical reading children; D-child, children with dyslexia; NT-teen, neurotypical reading teenagers; D-teen, teenagers with dyslexia; NT-adult, neurotypical reading adults; D-adult, adults with dyslexia; n, sample-size of subgroup; sd, standard deviation; IQ, intelligence quotient; ME, main effect; age, children, teenagers, adults; read-lev, neurotypical readers and readers with dyslexia; inter, interaction; %, percent; age, year, month; spelling and reading scores, transformed to IQ-scale for better comparison.

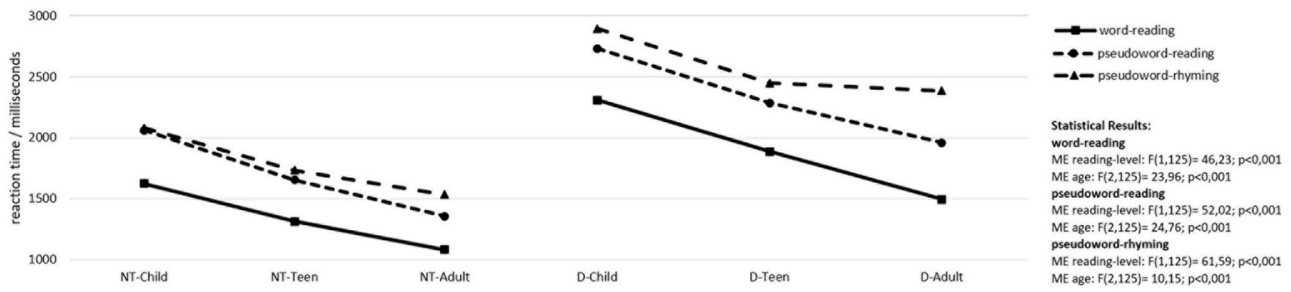

FIGURE 1 | Word reading and phonological processing skills. NT-child, neurotypical reading children; D-child, children with dyslexia; NT-teen, neurotypical reading teenagers; D-teen, teenagers with dyslexia; NT-adult, neurotypical reading adults; D-adult, adults with dyslexia; ME, main effect; age, children, teenagers, adults; reading-level, neurotypical readers, readers with dyslexia. Only significant results are reported.

angle 30°, 1 mm slice thickness, magnetization prepared rapid acquisition gradient echo sequence) was acquired.

Data Analysis / Diagnostic Data

Diagnostic data as well as the performance data on word reading and phonological processing were analyzed using 3 x 2 ANOVA with the factor "age group" (children, teenagers and adults) and the factor "reading-level" (neurotypical readers vs. readers with dyslexia).

Data Analysis / Voxel-Based Morphometry

Data Pre-processing and analysis were performed using Statistical Parametric Mapping software (Institute of Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/software/spm8, SPM, RRID:SCR_007037, version 8). For morphometric analysis of the data, we used voxel-based morphometry (VBM). This method involves the following steps: (1) spatial normalization of all images to a standardized anatomical space by removing differences in overall size, position, and global shape; (2) extracting gray and white matter from the normalized images; and (3) analyzing differences in local gray and white matter values across the whole brain (49). We applied an optimized method of VBM (50) using the VBM8 Toolbox (http://dbm.neuro.uni-jena.de/vbm; VBM toolbox, RRID:SCR_014196, version 8) for both gray and white matter.

The segmentation procedure is further refined by accounting for partial volume effects (51), by applying adaptive maximum a posteriori estimations (52), and by applying a hidden Markov random field model (53). Because spatial normalization expands and contracts for some brain regions we scaled the segmented images by the amount of contraction, so that the total amount of gray or white matter in the images remained the same as it would be in the original images.

Due to the large differences in gray matter brain volume between the groups of children, teenagers, and adults we created a sample-specific template for spatial normalization. An iterative high-dimensional normalization approach provided by the DARTEL toolbox (54) was applied to the segmented tissue maps in order to normalize all images to a template. The DARTEL algorithm started with low-dimensional spatial normalization and calculated the average of all normalized segmentations. This averaged image was used in the next iteration and spatial resolution of the normalization was enhanced. This iteration scheme was repeated while the dimensionality of

spatial normalization was increased. The result was a high-dimensionally warped brain with minimal bias, because a sample-specific template was used.

The resulting gray and white matter images were finally smoothed with a Gaussian kernel of 8 mm FWHM. We restricted the statistical analysis to areas with a minimum probability value of 0.1 to avoid possible edge-effects around tissue borders. Differences in local gray and white matter volume across the whole brain are analyzed with voxel-by-voxel *t*-test using the general linear model. We use a 3 × 2 ANOVA with factors age group (children, teenager and adults) and reading-level (neurotypical readers and readers with dyslexia) to test for differences between each group with dyslexia and their neurotypical control group. Since we use a modulation for Non-linear effects only that considers overall brain size, there is no need to correct for total intracranial volume (TIV) in the statistical model.

Furthermore, we tested for an interaction of the factors age group and reading-level. Results were considered significant for $p < 0.05$, corrected for multiple comparisons using FWE based on threshold-free cluster enhancement (TFCE), which avoids using an arbitrary threshold for the initial cluster-formation (55). Corresponding coordinates for each significant region are reported in Montreal Neurological Institute (MNI) space.

Additionally we performed correlation analyses between reading performance and gray matter volumes as well as age and gray matter volumes of those gray matter clusters which showed significant main effects for reading level in the comparison of neurotypical and dyslexic readers. In clusters with two cluster maxima, we chose the cluster with the larger TFCE values for the correlations (Cluster 1: MNI coordinates −51 −63 25; Cluster 3: MNI coordinates −42 −67 −29; Cluster 5: 47 −60 −32).

RESULTS

Sample Criteria

For a summary of the descriptive statistics as well as the statistical results of the 3 x 2 ANOVAs of the sample criteria data please refer to **Table 1**. Reading and spelling performance of the neurotypical readers stays within the normal range for all three age groups, whereas reading performance shows better performance with rising age for the dyslexic readers, reaching normal reading performance levels in adulthood. However spelling performance remains below average for all three age groups of dyslexic readers.

Reading and Phonological Skills

For performances in word and pseudoword reading and pseudoword rhyming there are highly significant main effects for factors age-group and reading-level. For reading of words and pseudowords there is a steady increase in performance from children to teenagers to adults in the neurotypical as well as in the dyslexic readers even though the latter are consistently slower over all three age groups compared to the neurotypical readers. For rhyming of pseudowords (the task with the highest demands on phonological processing skills), there is also an improved performance from children to teenagers in the neurotypical readers and the readers with dyslexia. However, adults with dyslexia do not show any further increase in performance. Their performance remains at the level of the teenagers with dyslexia.

Voxel-Based Morphometry

Gray Matter

Only children show significant main effects for the factor reading-level regarding local gray matter volume differences between neurotypical readers vs. readers with dyslexia (on a level corrected for multiple statistical comparisons, TFCE-statistic, FWE corrected, $p = 0.05$). Neurotypical children show significantly larger local volumes of gray matter than children with dyslexia in large left-sided temporo-parietal and frontal clusters [encompassing the inferior temporal gyrus, fusiform gyrus, middle temporal gyrus, V5, middle occipital gyrus, superior temporal gyrus, inferior parietal gyrus, angular gyrus, supramarginal gyrus, left and right superior temporal gyrus (encompassing the insulae), left and right cerebellum as well as left and right prefrontal clusters (encompassing the superior, middle and medial frontal gyrus)]. See **Figure 2** for the differences in gray matter volume of the children and **Table 2** for the coordinates of the local cluster maxima.

Three clusters, which are part of the left-sided reading network (angular gyrus and middle temporal gyrus), the left and right cerebellum, and the right fusiform gyrus show significant interaction effects of the factors age-group and reading-level regarding local gray matter brain volume.

The percent signal change in the cluster maxima as depicted in **Figure 3** demonstrates, that the groups of readers with dyslexia (see **Figure 3**) mainly drive this interaction effect. Neurotypical readers present a steady decrease of percent signal change in all clusters from children to teenagers to adults. Whereas the comparison of the percent signal change in the readers with dyslexia displays an inverted U-shape: comparing the groups with dyslexia, children show smaller volumes than teenagers, and for teenagers larger volumes can be detected than for adults. Adults on the other hand show the smallest local volumes of gray matter compared to children and teenagers (see **Figure 3**).

Correlation Analysis

As depicted in **Tables 3** and **4** the correlation analysis between reading performance and gray matter volume shows two significant correlations for neurotypical readers in Cluster 1 and 5, there are no significant correlations between performance and gray matter volume for dyslexic readers. Looking at correlations between age and gray matter volume, there are significant negative correlations for neurotypical readers in Cluster 1 and 5 and significant negative correlations for dyslexic readers in Cluster 3 and 5. Scatterplots for these correlations are depicted in **Figures 4**, **5**.

White Matter

There are no significant differences in local white matter volume between the children, teenagers, and adults.

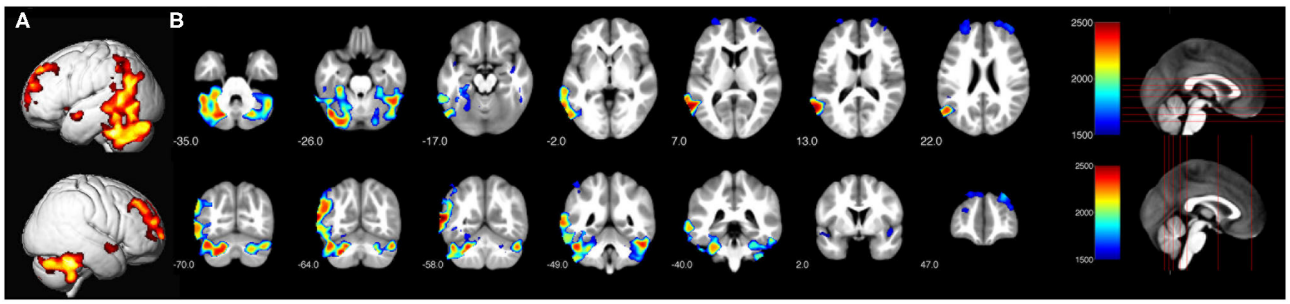

FIGURE 2 | Significant differences in local gray matter (TFCE-statistic, FWE corrected for multiple comparisons, $p \leq 0.05$) for neurotypical reading children > children with dyslexia. **(A)** Render view of all clusters. **(B)** Depiction of axial and coronal slices in order to illustrate the displayed slices.

TABLE 2 | Coordinates and anatomical regions of main effect neurotypical reading children > children with dyslexia.

Cluster size	*p* (FWE-cor)	TFCE	*x*	*y*	*z*	Hemisphere	AAL region
22,069	**0.012**	**2521.43**	**−63**	**−60**	**11**	**Left**	**Middle temporal, angular**
	0.017	2382.85	−23	−68	−38	Left	Cerebellum
	0.018	2342.32	−23	−60	−32	Left	Cerebellum
8,679	**0.017**	**2353.06**	**50**	**−50**	**−29**	**Left**	**Inferior temporal, cerebellum, fusiform**
	0.019	2273.18	47	−62	−32	Right	Cerebellum, inferior temporal
	0.020	2204.81	41	−72	−32	Right	Cerebellum
3,610	**0.029**	**1830.28**	**30**	**45**	**44**	**Right**	**Superior and middle frontal**
	0.029	1813.50	24	39	45	Right	Superior and middle frontal
	0.035	1709.09	42	53	26	Right	Inferior frontal, middle and superior frontal
3,446	**0.036**	**1678.57**	**−27**	**42**	**47**	**Left**	**Superior and middle frontal**
	0.036	1656.90	−5	53	45	Left	Superior frontal
	0.036	1656.17	−17	51	45	Left	Superior frontal
570	**0.039**	**1595.57**	**45**	**3**	**−12**	**Right**	**Superior temporal, insula**
	0.040	1580.81	41	−9	−18	Right	Hippocampus, superior temporal, fusiform
446	**0.042**	**1540.42**	**−51**	**0**	**−12**	**Left**	**Superior and middle temporal**
	0.043	1528.52	−42	5	−15	Left	Superior temporal, insula
209	**0.044**	**1509.94**	**−50**	**−45**	**20**	**Left**	**Superior and middle temporal, supramarginal**
	0.046	1486.35	−42	−36	33	Left	Supramarginal, inferior parietal, postcentral
	0.047	1477.07	−44	−47	29	Left	Supramarginal, angular, superior temporal
152	**0.048**	**1468.62**	**9**	**−54**	**29**	**Right**	**Precuneus, posterior and middle cingulum**
	0.048	1459.51	11	−47	26	Right	Posterior and middle cingulum, precuneus,
93	**0.048**	**1468.55**	**−29**	**15**	**−3**	**Left**	**Insula, putamen, inferior frontal**
23	**0.048**	**1457.90**	**−9**	**−81**	**−8**	**Left**	**Lingual, calcerinus, cerebellum**
16	**0.048**	**1457.70**	**−33**	**53**	**3**	**Left**	**Middle and superior frontal**

p, level of significance, only significant results corrected for multiple comparisons (FWE) are reported; TFCE, Threshold Free Cluster Enhancement; AAL, Automated Anatomical Labeling. Values in bold denote the respective local cluster maxima.

DISCUSSION

Reading and spelling performance of the dyslexic readers shows a typical clinical pattern across the different age groups: as proven by significant main effects for age-group, reading-level and interaction effects the reading performance in the dyslexic readers improves significantly over age, reaching average performance in adulthood, whereas spelling performance in dyslexic readers stays below average in all three age groups. The fact that the three groups of dyslexic readers show slight differences in spelling performance can probably be explained by the fact that different tests appropriate to their respective ages had to be used.

There are three main study results for the VBM data: (1) Only children display a main effect for the factor "reading-level" regarding differences in local gray matter volume. (2) The significant interaction effect for the factors "age-group" and "reading-level" can be explained by a significant correlation of

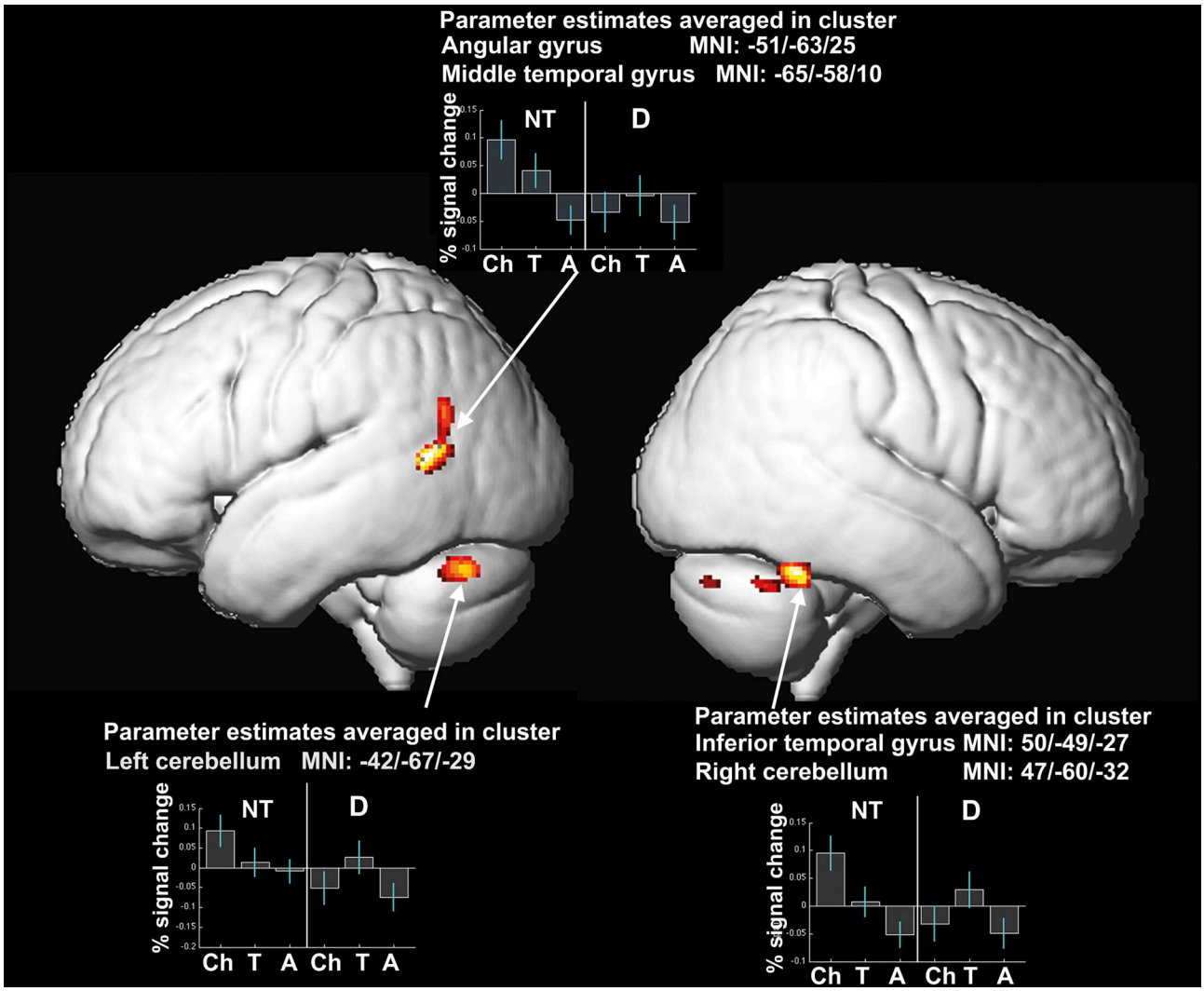

FIGURE 3 | Significant differences in local gray matter (uncorrected, $p \leq 0.001$, expected cluster level according to expected numbers of voxel per cluster: 75 voxel) as well as % signal changes in the cluster centers for interaction effect of factors age-group and reading-level. Coordinates of the clusters and percent signal change of the averaged clusters are depicted. NT, neurotypical readers; D, readers with dyslexia; Ch, children; T, teenagers; A, adults.

performance and gray matter volume in neurotypical readers, showing that performance increase is accompanied by gray matter volume decrease over age. Dyslexic readers do not show these correlations between performance and gray matter volume. (3) There are no significant differences in white matter.

Main Effect for Factor "Reading-Level" in the Children

Children show differences in local gray matter brain volume in temporo-parietal areas [fusiform gyrus, angular gyrus, supramarginal gyrus and superior temporal gyrus (insulae)]. These clusters correspond to findings from other VBM studies in which readers with dyslexia display anatomical differences in these particular brain areas (29–32). These areas are critical parts of the reading network since they are related to phonological processing [superior temporal and temporo-parietal brain areas, (56)], skilled and automated reading, visual-auditive integration and memory for word images [inferior temporal brain areas (15, 18)].

Could This Main Effect Be Due to a Lack of Reading Experience?

Krafnick and colleagues (57) argue that differences in brain volume between neurotypical reading children and children with dyslexia are most likely due to a lack of reading experience, since the comparison to reading-level matched neurotypical children does not display a significant difference in volume. It is likely that the reading experience plays an important role in the shaping

TABLE 3 | Correlational analysis reading performance and gray matter volume.

	Cluster 1		Cluster 3		Cluster 5	
	r	*p*	*r*	*p*	*r*	*p*
Neurotypical readers	0.319	0.008	0.154	0.212	0.315	0.010
Dyslexic readers	−0.015	0.912	0.052	0.699	0.213	0.108

r, correlation coefficient; p, level of significance; Cluster 1, MNI coordinates −51 −63 25; Cluster 3, MNI coordinates −42 −67 −29; Cluster 5, 47 −60 −32.

TABLE 4 | Correlational analysis age and gray matter volume.

	Cluster 1		Cluster 3		Cluster 5	
	r	*p*	*r*	*p*	*r*	*p*
Neurotypical readers	−0.557	0.000	−0.215	0.080	−0.439	0.000
Dyslexic readers	−0.194	0.145	−0.317	0.015	−0.280	0.033

r, correlation coefficient; p, level of significance; Cluster 1, MNI coordinates −51 −63 25; Cluster 3, MNI coordinates −42 −67 −29; Cluster 5, 47 −60 −32.

of the reading network, however differences in brain volume in prereading children with or without risk for dyslexia can also be observed (33, 35). Thus, the effects of the present study do not seem to be only due to a lack of reading experience.

In addition to the differences in gray matter volume observed in the temporo-parietal cluster, the present study also demonstrates differences in bilateral prefrontal regions. Black and colleagues (34) report reduced gray matter brain volume in the prefrontal brain areas of children at risk for dyslexia. They associate these reduced volumes with differences in functions such as naming, verbal fluency (58, 59), executive processing (2) and working memory. The working memory is an important factor in the development of efficient reading skills as this is the means by which speech sounds are sustained during reading. These anatomical differences in brain areas important for the development of automated reading skills, efficient phonological processing skills and verbal working memory could be the neuroanatomical correlate for a markedly poorer baseline in the development of reading and spelling skills.

Association Between Performance and Brain Volume in Neurotypical Readers

The regular pattern of brain matter volume development over the whole life span is that of an inverted U-shape (60). Normally there is an increase of brain volume with a peak of brain volume around the age of 6–8 years, followed by a steady decrease of brain volume as an ongoing lifelong process.

The mechanism behind this phenomenon is called synaptic pruning (13, 61–63). In general it is assumed, that the key to better cognitive performance lies in neural processes like pruning and cortical thinning in order to create a well-functioning cortical network. We know from the neurotypical developing brain, that overproduction of neurons and connections are the starting point in brain development. The initial network is unorganized with many superfluous connections making communication disorganized and inefficient. It is suggested that the subsequent decrease in gray matter brain volume over age in the neurotypical brain reflects the sculpting process for a well-functioning mature brain with efficient neuronal networks (60).

Recent research confirms that experience-dependent changes in brain structures extends throughout the lifespan (13, 26, 64), and that reading experience in the neurotypical reading brain successively leads to a reduction of brain volume. There are also studies on reading development which demonstrate that lower brain volumes correspond to better reading skills (13, 64).

Performance data of the present study indicate that there is a steady increase in reading skill for all reading tasks in neurotypical readers from children, to the teenage years, to adulthood. This improvement in performance is accompanied by a steady decrease of gray matter volume. Thus, findings from our neurotypical reading control group agree with the literature insofar that better reading and spelling skills are related to lower volumes of gray matter brain volume in the reading network.

Dyslexia Specific Differences in Brain Matter Volume

Compared to the brain volumes of the neurotypical readers in our sample, the dyslexic readers do not show these linear effects when looking at correlations of performance and brain volumes. The behavioral data indicate that neurotypical readers as well as dyslexic subjects show progress in reading and spelling performance with age. However, as correlational analyses results demonstrate, this steady increase in performance is not paralleled by a linear decrease of brain volume in dyslexic readers. Children with dyslexia demonstrate smaller gray matter brain volume and lower reading performance compared to the teenagers with dyslexia as can be seen in **Figure 3**. The improvement in reading skills in the teenagers with dyslexia is combined with larger brain volume in this group. This is not in line with the amount of brain volumes of the neurotypical reading control group in which the progress in performance of the teenagers is accompanied by lower brain volume.

Yet that progress in performance can lead to volume increase is in line with various studies, demonstrating that acquisition of a skill is accompanied by increase in gray brain matter (26, 65). Krafnick and colleagues (38) described that behavioral training effects in children with dyslexia are accompanied by rising brain volumes in the left anterior fusiform gyrus/hippocampus, left precuneus, right hippocampus and right anterior cerebellum. After this volume increase, a developmental effect similar to the neurotypical readers is occurring in the form of a decrease of local brain volume accompanied by an increase in the performance level. As these developments can be observed in regions associated to the reading network, this strongly suggests that preexisting structural differences in children are varied by experience-dependent structural change involving dendritic and synaptic pruning (13). The interaction effect can therefore be described as neuronal developmental delay, leading to partial compensation by a subsequent volume increase and then decrease with age and rising performance skills.

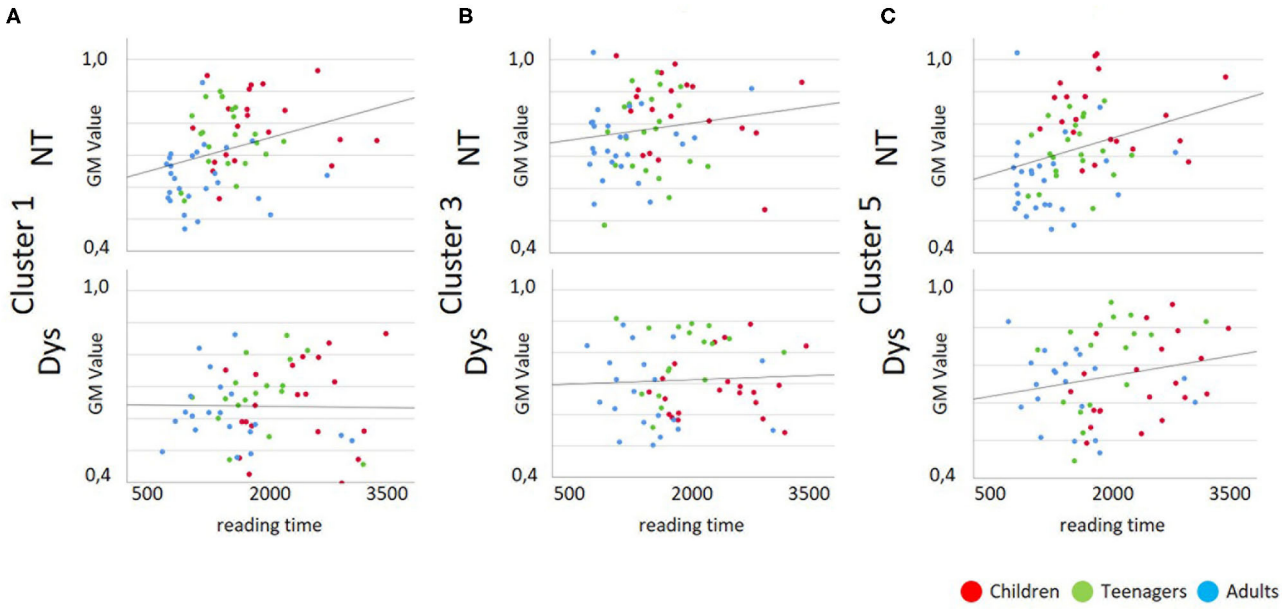

FIGURE 4 | Scatterplots for Gray Matter Volume vs. Reading Performance; Dys, Dyslexic Readers; NT, Neurotypical Readers; GM, Gray Matter Volume; reading time in milliseconds. **(A)** Cluster 1/MNI-coordinates: −51/−63/25, **(B)** Cluster 3/MNI-coordinates: −42/−67/−29, **(C)** Cluster 5/MNI-coordinates: 47/−60/−32.

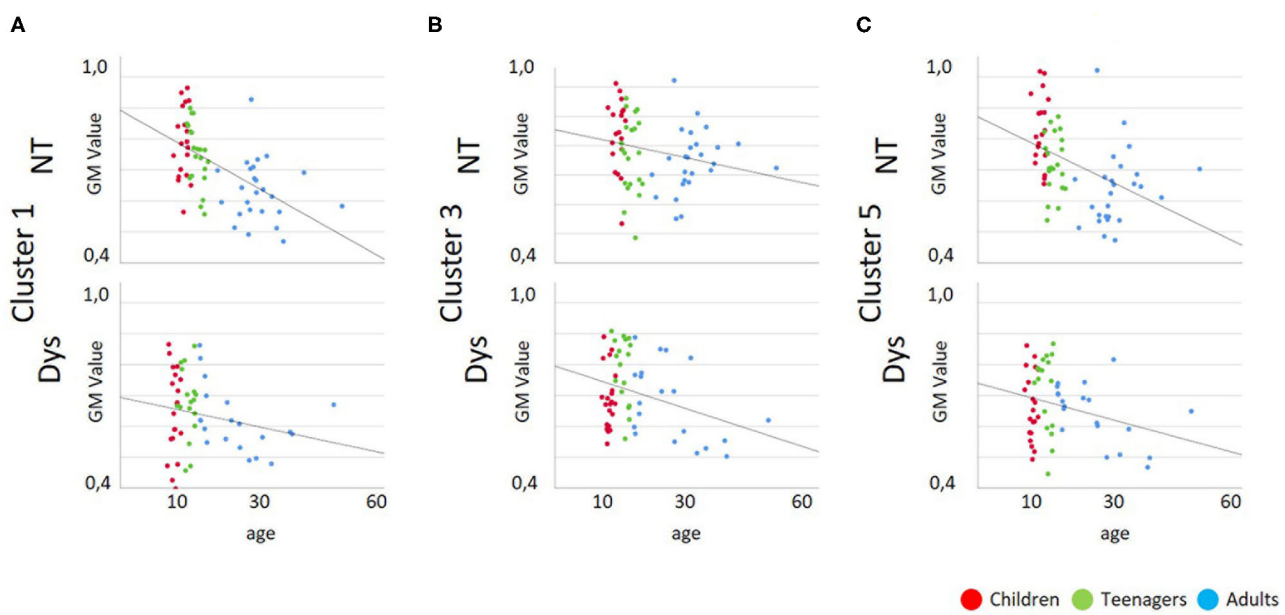

FIGURE 5 | Scatterplots for Gray Matter Volume vs. Age; Dys, Dyslexic Readers; NT, Neurotypical Readers; GM, Gray Matter Volume; age in years. **(A)** Cluster 1/MNI-coordinates: −51/−63/25, **(B)** Cluster 3/MNI-coordinates: −42/−67/−29, **(C)** Cluster 5/MNI-coordinates: 47/−60/−32.

That volume changes do not proceed in a straightforward single direction (only lower brain volumes accompany better performance) is demonstrated by Linkersdörfer and colleagues (13): they describe that both, volume in- as well as decrease is associated with progress in performance. The results of the present study are thus further evidence for the flexibility of the human brain.

In sum, these morphometric and behavioral data combined appear to indicate that the differences in brain matter volume in the children are an early neuroanatomical signature of the dyslexia-specific reading problems. Additionally, even though the patients with dyslexia show progress in performance, it appears that the neuronal processes leading to this increase in performance are different from those in the neurotypical readers.

Whereas neurotypical readers display a steady fine-tuning of the neural reading network and a decrease of gray matter, in readers with dyslexia progress in performance does not show these linear correlations.

Methodological Issues

VBM is primarily a tool to investigate effects in gray matter, whereas its sensitivity in detecting effects in white matter is inferior because in *T1*-weighted MR images, this tissue type is characterized by the presence of large homogeneous regions with only small changes in signal. Changes in white matter fiber tracts can be better detected using Diffusion Tensor Imaging (DTI). However, this does not mean that white matter analysis is less reliable, but it is more difficult to detect effects compared to gray matter. Sophisticated methods such as DTI are simply more powerful at detecting effects in white matter, but when effects are found with VBM, they are no less reliable.

Because our findings are restricted to effects in the children only, one might ask whether our results are exaggerated due to normalizing them to a template for an older age group. Therefore, we additionally preprocessed and reanalyzed our children's data by creating a children-specific template. By using this alternative approach, we observe an almost identical pattern in the differences between the neurotypical reading children and children with dyslexia. Thus, it is very unlikely that the effects in children are driven by the use of a template, which is created from the whole sample of children, teenagers, and adults.

With respect to the bilateral findings across the brain, one might speculate that these findings could be caused by the fact that dyslexic children have smaller brains. However, we account for individual differences in brain size in our approach. Although segmented images are scaled by the extent of contraction or expansion due to spatial normalization, we do not scale by the linear effects of spatial normalization. Thus, overall brain size differences are corrected for, while local differences in the brains are preserved.

Limitations of the Study

The drawback of our design is that it cannot uncover whether differences in brain volume are preexisting before reading acquisition and thus cause differences in reading skill development, or whether these anatomical differences result from the failure of learning to read. Additionally the cross-sectional design does not allow answering developmental questions in a straightforward manner. However as Casey and colleagues point out (60), longitudinal MRI studies investigating the structural brain development in children and teenagers (66, 67) observe similar patterns as cross-sectional studies (68–70). Thus, also cross-sectional investigations provide valuable information.

CONCLUSION

Brain development is a lifelong process with regressive as well as progressive learning and experience based neuronal changes (26, 60). Longitudinal studies provide reliable evidence for a causal relationship between the learning experience and subsequent changes in brain volume. Looking at the current state of the literature, a straightforward conclusion for neuronal differences in brain volume between neurotypical normal and dyslexic readers is compounded due to the heterogeneity of VBM study results. This study attempts to shed light on these questions by maintaining the constancy of certain crucial experimental settings such as the diagnostic criteria, the methods of data measurement and data analysis while comparing different age groups of neurotypical and dyslexic readers. The VBM and behavioral data point out that reading deficits in individuals with dyslexia are associated with gray matter volume differences in the reading network compared to neurotypical readers and that in those two groups behavioral improvement in reading skills is reflected in different neuroanatomical patterns. Even though there is some compensation by an increase in the brain volume and subsequent network shaping, this neural baseline is not sufficient to allow for the development of neurotypical reading skills even in dyslexic adults.

Thus, this study, in its investigation of neuroanatomical and behavioral data over a wide age range is further evidence that dyslexia is a disorder with lifelong consequences, which is why consistent support for affected individuals in their educational and professional careers is of great importance. Longitudinal studies, which include investigations of the individually developing dyslexic brain as well as the question whether longitudinally (until well into adulthood) remediation after reading intervention leads to anatomical profiles similar to those of neurotypical readers are needed in order to come to understand whether these findings hold as a valid pattern or whether there is evidence of greater interindividual variance in the neuroanatomy of dyslexia.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Local Ethics Committee at the Jena University Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CL and ML contributed to patients' recruitment and data collection. All authors contributed to data processing, participated in drawing up the manuscript, involved in the intellectual workup for the article, read, and approved the final manuscript.

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Long-Term Health Outcomes of Infantile Spasms Following Prednisolone vs. Adrenocorticotropic Hormone Treatment Characterized Using Phenome-Wide Association Study

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Objective: To determine differences in long-term health and neurological outcomes following infantile spasms (IS) in patients treated with adrenocorticotropic hormone (ACTH) vs. prednisolone/prednisone (PRED).

Methods: A retrospective, case-control study of patients with an International Classification of Diseases, Ninth Revision, Clinical Modifications (ICD-9) diagnosis of IS, identified over a 10-year period from a national administrative database, was conducted. IS patients treated with ACTH or PRED were determined and cohorts established by propensity score matching. Outcomes, defined by hospital discharge ICD codes, were followed for each patient for 5 years. Related ICD codes were analyzed jointly as phenotype codes (phecodes). Analysis of phecodes between cohorts was performed including phenome-wide association analysis.

Results: A total of 5,955 IS patients were identified, and analyses were subsequently performed for 493 propensity score matched patients, each in the ACTH and PRED cohorts. Following Bonferroni correction, no phecode was more common in either cohort ($p < 0.001$). However, assuming an *a priori* difference, one phecode, abnormal findings on study of brain or nervous system (a category of abnormal neurodiagnostic tests), was more common in the PRED cohort ($p < 0.05$), and was robust to sensitivity analysis. Variability in outcomes was noted between hospitals.

Significance: We found that long-term outcomes for IS patients following ACTH or PRED treatment were very similar, including for both neurological and non-neurological outcomes. In the PRED-treated cohort there was a higher incidence of abnormal

neurodiagnostic tests, assuming an *a priori* statistical model. Future studies can evaluate whether variability in outcomes between hospitals may be affected by post-treatment differences in care models.

Keywords: pediatric, epilepsy, infantile spasms (IS), ACTH, outcomes-health care

INTRODUCTION

Infantile spasms (IS) is a severe pediatric epilepsy disorder typically presenting in the first year of life (1). Hallmarks of IS include spasm-like seizures that occur in clusters with progressive worsening, and a distinctive electroencephalogram (EEG) pattern termed hypsarrhythmia (1, 2). Patients are at risk for adverse long-term outcomes, including increased mortality, risk for intractable epilepsy, and neurodevelopmental impairment (3). Determination of treatment guidelines for IS has evolved over the past several decades (2, 4). First-line treatment for IS consists of steroid or steroid-inducing treatment, but the choice of prednisolone/prednisone (PRED) or adrenocorticotropic hormone (ACTH) has conflicting or equivocal data on efficacy, including time to remission, resolution of hypsarrhythmia, and outcomes (2, 5). Further, ACTH is significantly more expensive without evidence supporting its cost-effectiveness (6). Our goal was to characterize the long-term neurological and other health outcomes for IS, comparing patients who received either ACTH or PRED, using information from a nationwide pediatric clinical administrative database.

METHODS

Study Design and Participants

We performed a retrospective analysis of data from the Pediatric Hospital Information System (PHIS). PHIS is a nationwide database containing pediatric patient data from 52 children's hospitals (7), including inpatient visit data, as well as some observation, emergency department, ambulatory surgery, and clinic visits data. From PHIS, we identified all patients with an International Classification of Diseases, Ninth Revision, Clinical Modifications (ICD-9) code indicative of infantile spasms (ICD-9-CM: 345.6, 345.60, 345.61), from January 1, 2004 to September 30, 2015. Prior work has indicated the effectiveness of using ICD codes for identifying patients with IS (8). Outcomes for each patient were measured for up to five years after initial IS diagnosis.

Standard Protocol Approvals, Registrations, and Patient Consents

This project used de-identified data and was not considered human subjects research, and was exempted by the Institutional Review Boards at the University of Utah and Intermountain Healthcare.

Data Preparation

Data for IS patients was accomplished by identifying patients with an ICD-9-CM code of IS, either 345.60 (Infantile Spasms, without mention of intractable epilepsy) or 345.61 (Infantile spasms, with intractable epilepsy). For inclusion, patients had to receive ACTH or PRED within the 21 days following IS diagnosis. The diagnosis of IS and the administration of ACTH or PRED were determined from PHIS. Patients on both ACTH and PRED (96 patients) or on neither (3,497 patients) were removed. For the remaining 2,362 patients, their ICD-9 codes were converted to phecodes. Phenome-wide association study (PheWAS) is a methodology for evaluating patients by grouped diagnostic codes (9). PheWAS categorizes each ICD-9 code into one of 1866 "phecodes", which are groups of similar diseases or traits. A phecode was assigned to a patient if they had a matching phecode-associated ICD-9 diagnosis. Phecodes were rounded to the lowest whole integer to reduce granularity in the data for analysis. For instance, the phecode 008.xx indicates an intestinal infection, with the numbers after the decimal point indicating more granular details concerning the phecode (for example, 008.52 indicates an intestinal infection due to *C. difficile*).

The data was first filtered based on the medication given. Patients on ACTH or PRED were retained, patients on neither or both were discarded. For patients with multiple IS diagnoses, the earliest visit was used as the index date. To more accurately ensure that ACTH or PRED was given for an IS diagnosis and not a different diagnosis, additional requirements were as follows: 1. The patient's age at the time of IS diagnosis had to be < 1 year ($<$365 days). 2. The patient's age (in days) at the time of IS diagnosis has to be less than or the same as the age when the patient received the first dose of either ACTH or PRED. 3. The administration of medication needed to occur at no more than 21 days after the initial IS diagnosis.

All diagnoses/ICD codes/phecodes between 6 months and 5 years after initial IS presentation were included for analysis, resulting in 1,916 patients and 66,257 phecodes prior to propensity score matching. Duplicates for phecodes and medical record number were removed. Prior to this removal, data was analyzed for Wilcoxon-Mann-Whitney tests to determine changes in phecode frequencies on the individual level. Chronic condition complex (CCC) data was recorded using two categories: (10) non-neurological CCC codes (termed "Non-Neuro CCC") and neurological CCC (termed "Neuro CCC") codes.

CCC determination was used to establish similar patients for matching. For Neuro CCC and Non-Neuro CCC codes, a binary flag (1 yes, 0 no) was used to indicate whether a patient was diagnosed with a chronic condition prior to the initial IS diagnosis. To prevent all patients from receiving a Neuro CCC flag because of their IS diagnosis, and instead only identify those with addition neurological CCCs, the IS CCC flag was removed unless the ICD-9-CM code also indicated a concurrent

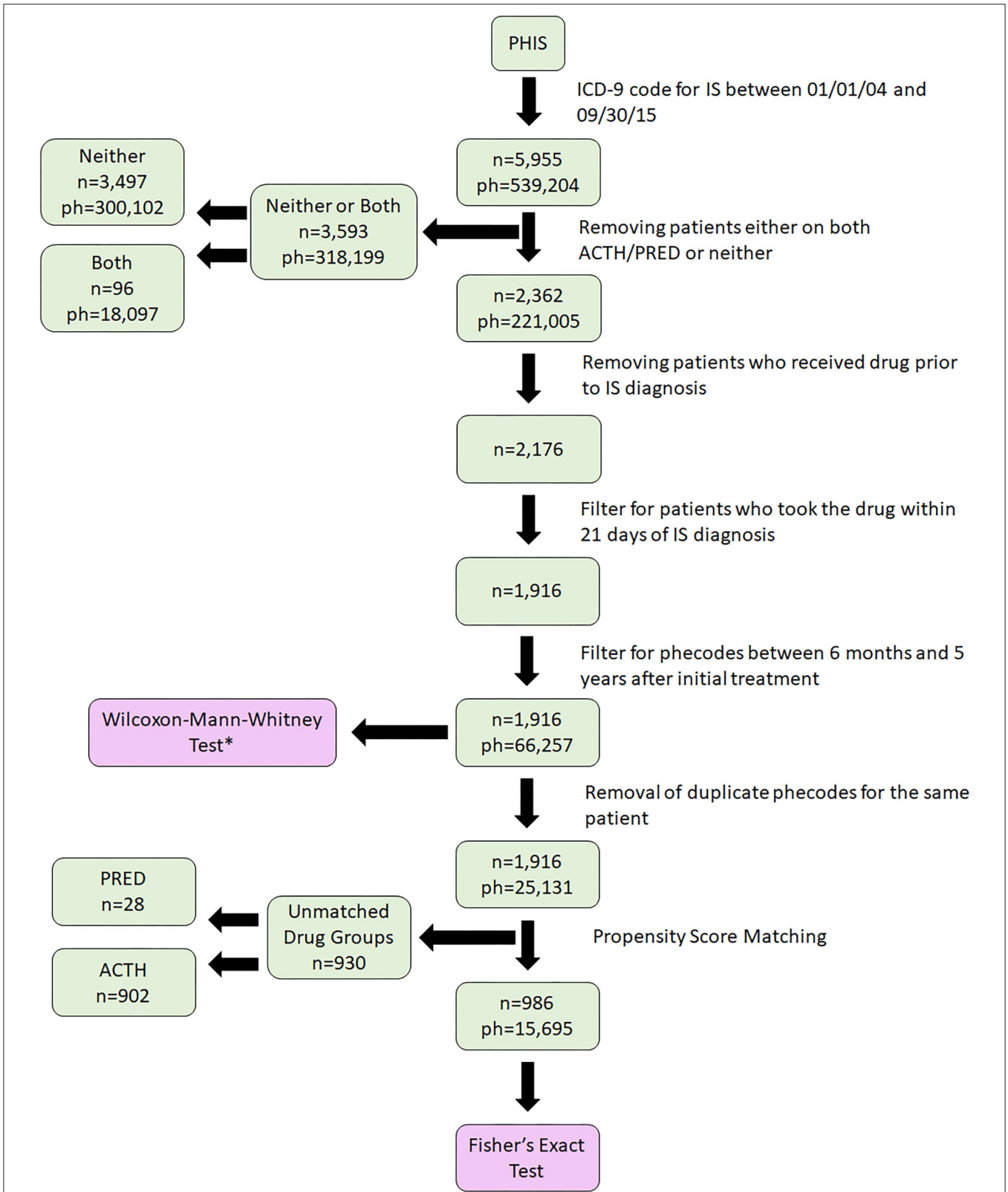

FIGURE 1 | Cohort identification. A total of 5,955 patients with infantile spasms (IS) were identified in Pediatric Hospital Information System (PHIS) using ICD-9 codes. We then filtered for medications and timeframe, performed propensity score matching, and finally, 986 patients were selected for analyses (493 in each drug cohort). IS, Infantile Spasms; ph, phecode; n, number of patients. *The dataset indicated for Wilcoxon-Mann-Whitney test is queried using match results.

diagnosis of intractable epilepsy (345.61: Infantile spasms, with intractable epilepsy).

Discharge year was determined based on the first visit for IS to account for changes in IS treatment over time. Sex, race/ethnicity, urban flag, and payer, were all based on a patient's first visit for any diagnosis. Non-Neuro CCC flag and Neuro CCC flag were determined by filtering for phecodes prior to a patient's initial IS diagnosis.

Propensity Score Matching

Propensity score matching was performed including for sex, payer (government, private, unknown, or other), urban flag (based on Rural-Urban Commuting Area (RUCA) codes), race/ethnicity, year of discharge, Neuro CCC, and Non-Neuro CCC. Propensity score matching was performed in R (version 3.6.1) using the MatchIt package. A 1:1 matching ratio of ACTH to PRED patients was used. Among 1,916 total ACTH or PRED patients, 986 patients matched (493 in each drug cohort). We used k-nearest neighbors for matching and a caliper of 0.20 standard deviations.

To optimize the matching process, the variables discharge year and race were further categorized. Year of discharge was simplified into 3 subgroups: group 1, discharge years 2008–2011; group 2, 2012–2015; and group 3, 2016–2019. Due to the small proportion of persons with Native American, Black Hispanic, and Pacific Island race/ethnicity in our study, these individuals were placed in a single group.

Statistical Analysis

Frequency calculations of the cohort (ACTH or PRED) phecodes were performed using Python. Differences between the groups were evaluated by either a two-sided Fisher's exact test; or

TABLE 1 | Demographics of the infantile spasms (IS) cohort before and after matching.

	Prior to matching		After matching	
	ACTH *n* **(%)**	**PRED** *n* **(%)**	**ACTH** *n* **(%)**	**PRED** *n* **(%)**
Sex				
Female	624 (45%)	233 (45%)	219 (44%)	221 (45%)
Male	771 (55%)	288 (55%)	274 (56%)	272 (55%)
Race				
White Non-Hispanic	774 (55%)	253 (49%)	248 (50%)	247 (50%)
White Hispanic	164 (12%)	86 (17%)	66 (13%)	69 (14%)
Black Non-Hispanic	168 (12%)	64 (12%)	63 (13%)	63 (13%)
Other	150 (11%)	50 (10%)	51 (10%)	47 (10%)
Multiple	53 (4%)	38 (7%)	34 (7%)	37 (8%)
Unknown	35 (3%)	14 (3%)	15 (3%)	14 (3%)
Asian	38 (3%)	10 (2%)	11 (2%)	10 (2%)
Native, Black Hispanic, or Pacific Islander	13 (1%)	6 (1%)	5 (1%)	6 (1%)
Insurance				
Government	575 (41%)	278 (53%)	256 (52%)	260 (53%)
Private	607 (44%)	203 (39%)	207 (42%)	196 (40%)
Other	201 (14%)	35 (7%)	26 (5%)	32 (6%)
Unknown	12 (1%)	5 (1%)	4 (1%)	5 (1%)
Urban flag				
Missing value/other country (−1.0)	31 (2%)	29 (6%)	16 (3%)	21 (4%)
Rural (0.0)	213 (15%)	75 (14%)	68 (14%)	74 (15%)
Urban (1.0)	1,151 (83%)	417 (80%)	409 (83%)	398 (81%)
Discharge year				
Group 1 (2008–2011)	333 (24%)	176 (34%)	184 (37%)	174 (35%)
Group 2 (2012–2015)	331 (24%)	316 (61%)	280 (57%)	290 (59%)
Group 3 (2016–2019)	731 (52%)	29 (6%)	29 (6%)	29 (6%)
Chronic neurological conditions				
At least one	427 (31%)	234 (45%)	195 (40%)	213 (43%)
None	968 (69%)	287 (55%)	298 (60%)	280 (57%)
Non-neurological chronic conditions				
At least one	454 (33%)	237 (45%)	204 (41%)	212 (43%)
None	941 (67%)	284 (55%)	289 (59%)	281 (57%)

distributional, with the number of distinct occurrences for a given phecode between the two cohorts assessed in rank distribution by the Wilcoxon-Mann-Whitney (WMW) test (SciPy v1.6.1). Although each patient in the ACTH cohort was matched with a patient in the PRED cohort, this similarity was not considered causal enough to justify using paired method tests (11). Phecodes were then sorted by lowest (most significant) *p*-value for each cohort. Percentage differences between the phecodes of the two cohorts were also calculated. Adjusted *p*-values were calculated using a Bonferroni correction. To determine whether there were differences in phecode frequencies at the individual level, as well as between the two drug groups, a Wilcoxon-Mann-Whitney Test was performed on the data prior to duplicate phecode removal.

For sensitivity analysis, to determine whether the hospitals with the most IS patients had a disproportionate effect on

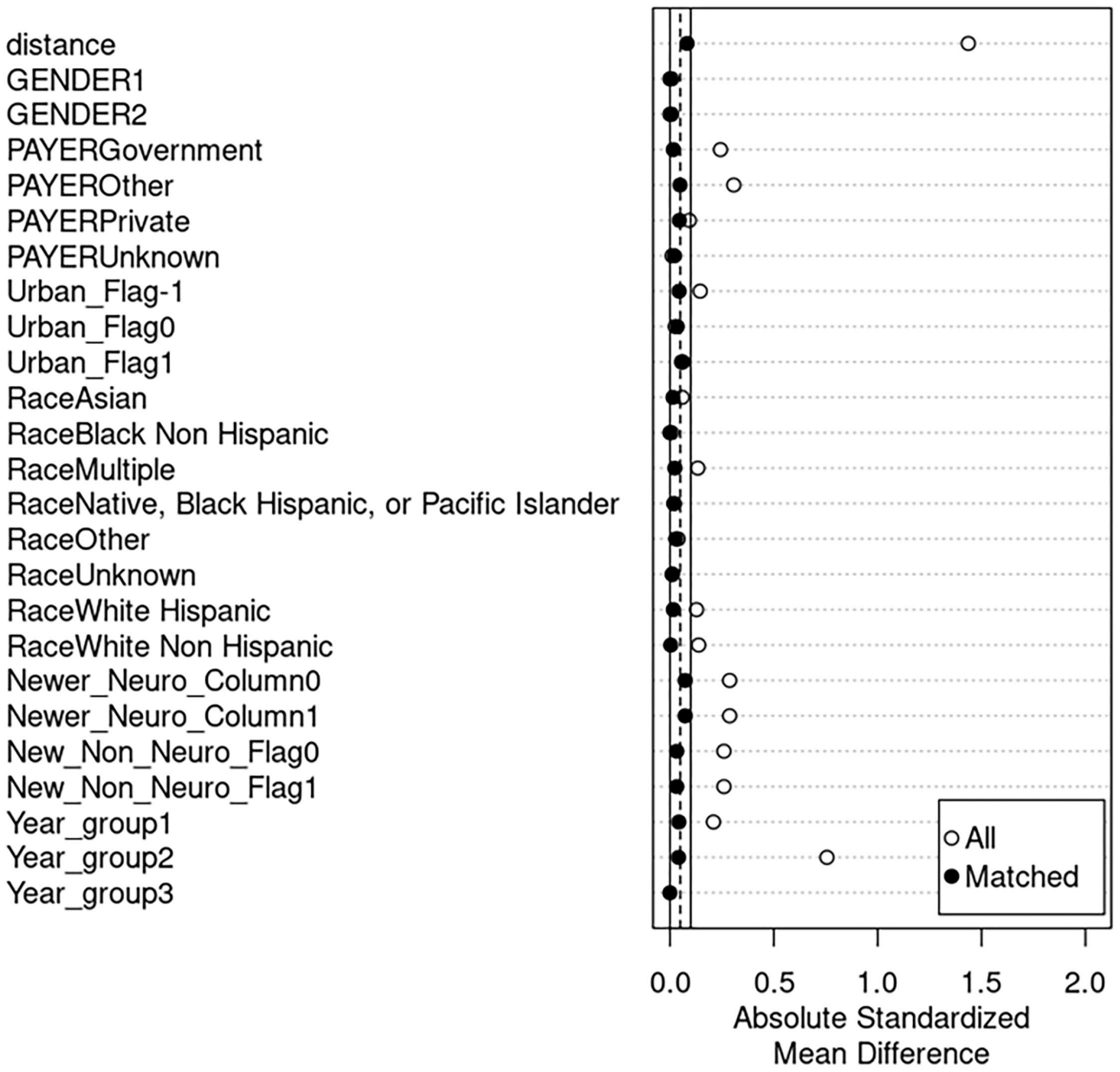

FIGURE 2 | Propensity score matching. Differences in means (y-axis) are shown between the adrenocorticotropic hormone (ACTH) and prednisolone/prednisone (PRED) drug cohorts for covariates before and after matching. Before matching (indicated by white dots), the absolute standardized difference in means has higher values, indicating a higher difference between the cohorts for certain covariates. Following matching (indicated by black dots), the values for the absolute standardized mean difference decrease, indicating a higher degree of similarity between the cohorts when comparing variable frequencies. "Distance" indicates the absolute difference between the propensity scores of matching patients.

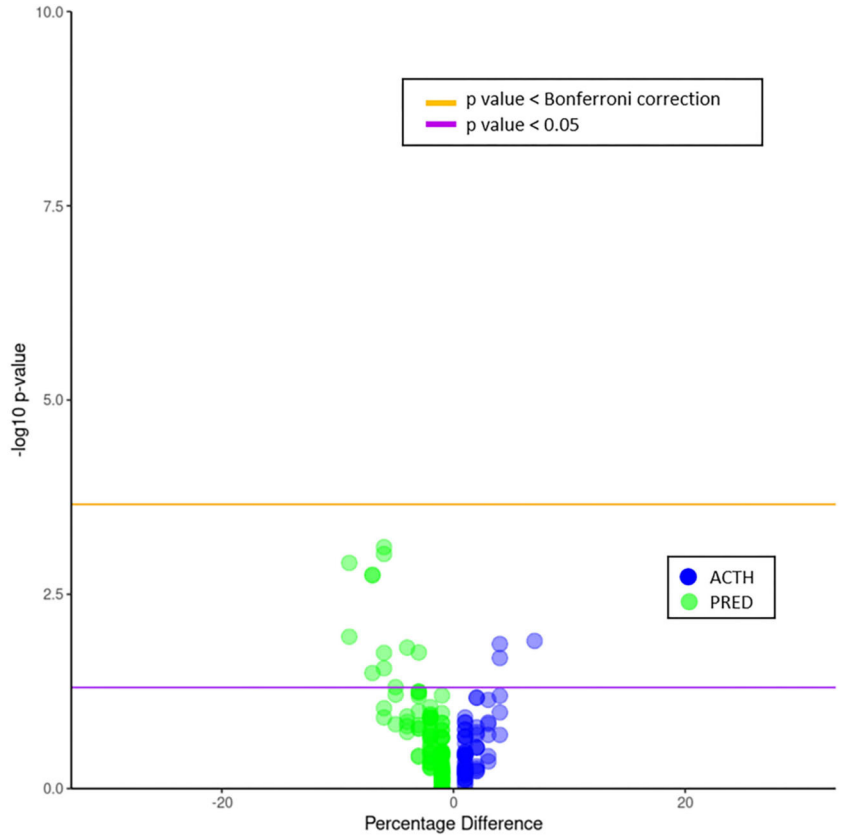

FIGURE 3 | Volcano plot of phecode differences between ACTH and PRED patients. Frequencies are plotted as negative $\log_{10}$ of the *p*-value over the percentage difference. The purple line indicates a *p*-value of 0.05; the orange line indicates an adjusted *p*-value using the Bonferroni correction. Neither drug cohort had phecode frequencies above the adjusted *p*-value. However, the PRED cohort had two neurological phecode frequencies above the $p < 0.05$ line, abnormal findings on study of brain/nervous system, and infantile cerebral palsy (percentage differences of 7% for both phecodes). The ACTH cohort also had a neurological phecode frequency above $p < 0.05$ line, hemiplegia (percentage difference of 4%).

the results vs. an overall trend in the data, the holdout (leave one out) method was performed on the five hospitals with the most patients. The top hospital in terms of patient contribution to data was removed prior to matching, and the data analysis was rerun. This was done sequentially for the top five contributing hospitals.

Data Availability Statement

All data are available on request to the authors. Data preparation was performed in Python (version 3.7.7). Code and software used by the authors are freely available, and if not otherwise indicated, are available at GitHub (https://github.com/Monika-Baker) or upon request.

RESULTS

From an initial 5,955 IS patients identified, following sorting and exclusions, we identified 1,916 patients who had taken either ACTH or PRED (**Figure 1**). Selected demographic data are provided in **Table 1**. After propensity score matching (**Figure 2**), final cohorts of 493 patients each for ACTH and PRED were established. All ICD-9 codes related to each patient were then collected for 5 years after initial date of IS diagnosis and grouped into phecodes.

Phecode frequencies and percentage differences were compared for ACTH and PRED cohorts (**Figure 3**). Neither of the treatment groups had phecode frequencies with significant *p*-values following Bonferroni correction. However, the PRED-treated group had two neurological conditions: abnormal findings on study of brain/nervous system, and infantile cerebral palsy with significant $p < 0.05$ values assuming an *a priori* hypothesis of a difference in neurological outcomes (**Table 2**; percentage rate difference for both was 7%). The ACTH-treated group also had a neurological condition with a frequency greater than $p < 0.05$, hemiplegia (percentage rate difference 4%).

Following sensitivity analysis (leave one out/holdout), abnormal findings on study of brain/nervous system retained significance and did not dramatically fluctuate, but hemiplegia and infantile cerebral palsy did fluctuate with *p*-values rising above 0.05 (**Figure 4**). Analysis with Wilcoxon-Mann-Whitney test indicated that these neurological phecodes were also more prevalent at the individual level (abnormal findings on the

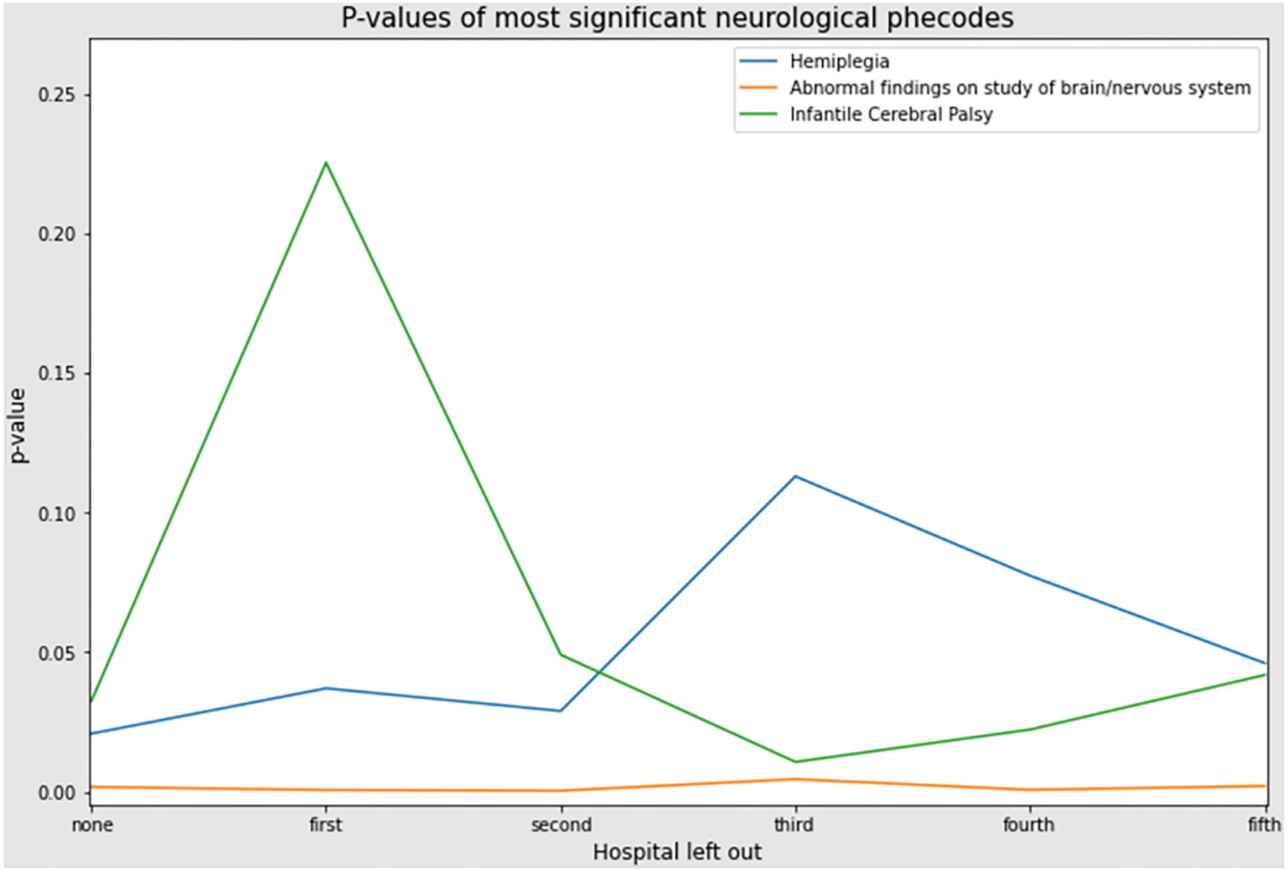

FIGURE 4 | Sensitivity analysis. Graph of leave one out analysis; *p*-value on y-axis and rank order contributing hospitals on x-axis. The top patient contributing hospital was left out prior to propensity score matching and data analysis rerun including Fisher's exact test. This was performed sequentially for each of the top hospitals. Results of *p*-value for the phecode, abnormal findings on study of brain/nervous system, remained similar. However, the *p*-values for infantile cerebral palsy and hemiplegia fluctuated substantially when the first and third hospitals were left out, respectively. This indicates that these hospitals were skewing the frequencies for these two phecodes, and that it is not an overall trend in the data.

study of brain/nervous system and infantile cerebral palsy for PRED patients, hemiplegia for ACTH patients) as well as at the cohort level. However, only abnormal findings on the study of brain/nervous system was robust to the sensitivity analysis.

DISCUSSION

In this large, national-level, long-term analysis of outcomes for IS patients treated with ACTH or PRED, we found no differences in neurological or non-neurological conditions. However, assuming an *a priori* hypothesis of differences in neurological outcomes and thus not performing Bonferroni multiple comparisons correction, PRED-treated IS patients were more likely, and at higher rates, to have two neurological phecodes, abnormal findings on the study of brain/nervous system and infantile cerebral palsy. ACTH-treated IS patients were more likely, and at higher rates, to have the neurological phecode of hemiplegia, assuming the same *a priori* hypothesis.

Only one neurological finding, abnormal findings on the study of brain/nervous, was robust to sensitivity (holdout) analysis. However, the significance for hemiplegia and infantile cerebral palsy did fluctuate, indicating that the differences in frequencies for these phecodes were driven by data from a single (or few hospitals) hospital. Interestingly, two of the phecodes were more common in the PRED-treated group, following analysis with the Wilcoxon-Mann-Whitney test ("Abnormal findings on study of brain/nervous system" and "Hemiplegia, infantile cerebral palsy"). This indicates that the increases in the frequencies of these two phecodes were driven at the individual level, i.e. multiple instances of the same diagnosis (phecode) in the same patient, from different hospital admissions.

Limitations of the study are its retrospective nature, inherent limitations of matching, and that most of the data from PHIS are from in-patient hospitalization. As such, the in-patient source of the majority of data limits quantification of certain disorders, such as developmental delay. However, although we were unable to quantify the absolute number of IS patients with a diagnosis such as developmental delay, our analysis is

TABLE 2 | Unadjusted *p*-values of neurological phecodes, in prednisolone/prednisone (PRED) and adrenocorticotropic hormone (ACTH) cohorts.

Phecode description	**PRED**
Abnormal findings on study of brain and/or nervous system	0.*001812*
Infantile cerebral palsy	0.*032601*
Other conditions of brain	0.148819
Sleep disorders	0.537421
Developmental delays and disorders	0.820108
Epilepsy, recurrent seizures, convulsions	0.917949
Phecode description	**ACTH**
Hemiplegia	0.*02090*
Delirium, dementia, and amnestic and other cognitive disorders	0.0677
Strabismus and other disorders of binocular eye movements	0.104934
Hearing loss	0.382294
Neurological disorders	0.556552
Disorders of optic nerve and visual pathways	0.595292

still able to evaluate for the ratios or proportions, and thus relative differences, between the PRED and ACTH cohorts. Due to the additional complexities in analysis, for this study, we did not evaluate patients who had taken both PRED and ACTH or other medications (e.g., vigabatrin) (12) An additional limitation of the dataset was that a large number of IS patients were listed with neither ACTH or PRED, suggesting that IS patients treated solely with outpatient prescription management, at least within our inclusion time frame, were not included in our analysis. It is important that our data showed no differences in epilepsy outcomes between the PRED and ACTH groups, which has been a concern regarding IS treatment (2, 4).

It is unclear why there is an observed increase in the PRED group of abnormal findings on the study of brain/nervous system. This phecode encompasses multiple nonspecific neurological findings in various neurodiagnostic tests, including cerebrospinal fluid, radiological tests, and EEGs. Further studies should be performed to identify which of these non-specific findings should be focused on, and whether they are due to differences in side effects or disease management.

In conclusion, we have found that PRED and ACTH treatment for IS have similar long-term outcomes for most health conditions, including for most neurological conditions. Further, our study is one of the few for IS, which considers long-term outcomes including that of non-neurological conditions, while most studies are evaluating immediate treatment response (for example, Grinspan et al.) (13) or evaluating long-term outcomes for only cognitive or epilepsy-related aspects (14, 15). As some outcomes appeared to be correlated with specific hospitals, future studies can evaluate whether variability in outcomes may be affected by post-treatment differences in care models.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Utah IRB. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The study was conceived by JB. MB and JW collected data. All authors analyzed data, prepared data for publication, edited drafts of the manuscript, and approved the final manuscript for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.878294/full#supplementary-material

Supplementary File 1 | Excel file of Fisher's exact test results for phecodes with higher frequencies in the PRED-treated cohort.

Supplementary File 2 | Excel file of Fisher's exact test results for phecodes with higher frequencies in the ACTH-treated cohort.

Supplementary Figure 1 | Wilcoxon-Mann-Whitney test. To determine if there were differences in frequencies for the same phecode at the patient level, a Wilcoxon-Mann-Whitney test was performed. The phecode file before duplicate removal was used to determine phecode frequencies, and was queried with a patient list after propensity score matching. The results were similar to the results from the Fisher's exact test with abnormal findings on study of brain/nervous system, infantile cerebral palsy, and hemiplegia with $p < 0.05$. However, following Bonferroni correction, there were no significant non-neurological phecodes.

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Predictors of Functional School Outcome in Children With Pediatric Acquired Brain Injury

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Objective: Among the variety of domains that may be impacted after pediatric acquired brain injury (pABI) are functional school outcomes. The purpose of this study was to identify demographic, medical, and psychological factors associated with impairments in functional school outcomes, defined as school absence, need of educational and psychological services, quality of life (QoL) in the school setting, and academic performance in children with pABI, with a specific emphasis on the significance of fatigue.

Materials and Method: We used baseline data from a randomized controlled trial. The sample consisted of seventy-six children aged 10 to 17 ($M = 13$ yrs) with pABI in the chronic phase (>1 year). All completed assessments of school-related QoL, academic performance, global functioning, fatigue, IQ, behavioral problems, and executive function.

Results: Fatigue, IQ, global functioning, behavioral problems, and sex emerged as potential predictors for functional school outcomes. Of note, overall fatigue emerged as the strongest potential predictor for parent-reported QoL in school ($\beta = 0.548$; $p < 0.001$) and self-reported QoL in school ($\beta = 0.532$; $p < 0.001$).

Conclusions: Following pABI, specific psychological, medical, and demographic factors are associated with functional school outcomes. Neither of the injury-related variables age at insult and time since insult were associated with functional school outcomes. Overall, our findings may suggest that a reintroduction to school with personalized accommodations tailored to the child's specific function and symptoms, such as fatigue, is recommended.

Keywords: fatigue, school, cognition, pediatric acquired brain injury, disability

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INTRODUCTION

Pediatric acquired brain injury (pABI), such as traumatic brain injury (TBI) or non-traumatic injuries (e.g., brain tumor, stroke, hypoxia, or infections /inflammation to the brain), is one of the leading causes of lifelong disabilities in school age children (1, 2). Consequences may include persistent impairments in cognition, emotional health, adaptive behavior, and school

functioning (3–11). Functional school outcome is of particular concern, here broadly defined as school absence, aid from Educational and Psychological Service (EPS), quality of life (QoL) in the school setting, and academic performance in children (12, 13). School is the principal location for the development of not only academic skills such as mastering the school curriculum, but also cognitive, social, and community-related skills during childhood. For many, return to school life after pABI represent an indicator of a return to normality (14). However, despite significant improvements in medical treatment after pABI, functional school impairments often emerge over time, for example when returning to school after cancer treatment, and are characterized by poor school performance, high rates of grade retention, and need of external educational services (10, 11, 15–17).

For many children, pABI can be viewed as a chronic disease process that initiates ongoing and possibly lifelong changes that influence several organ systems, physical and sensory limitations, in addition to neurocognitive impairment, emotional distress, and fatigue, that may have a cumulative negative effect on functional school outcome. Indeed, functional school outcomes may be influenced and explained by multiple variables and factors which display the complex and interdependent relationship between demographic [e.g., age and sex; (18, 19)], medical [e.g., injury-related variables such as age at injury, time since injury and functional outcome; (20)], and psychological variables [e.g., IQ, executive function (EF), behavioral problems, fatigue; (9, 21–24)].

Typically, functional school outcome is negatively affected during the initial 6 months after pABI, when compared to healthy children or children with orthopedic injuries (25, 26). While some aspects may improve in the first 6 to 12 months after injury, longitudinal studies indicate significant long-term impairments several years after pABI [e.g., (11)]. Age at injury is also a known predictor of outcome (27). Early injury [i.e., 7 years or younger; (28)], has been associated with poorer neurocognitive functions including IQ, attention, memory and EF, in addition to persisting disability (22, 23, 29, 30).

Importantly, neurocognitive and behavioral impairments may have adverse effects on school outcomes (9, 24). Post-pABI cognitive sequelae include deficits in IQ, attention, EF, memory and learning, and language skills (31–33). In particular, concurrent and longitudinal associations between EF and functional school outcomes have been demonstrated, with EF also being longitudinally predictive of academic difficulties and school dropout (34). Executive functions can be described as distinct, but related, higher-order neurocognitive processes responsible for purposeful, goal-directed behavior (35, 36). Relatedly, children with pABI are at risk for persistent symptoms of behavior problems, with potential debilitating effects on children's long-term functioning [e.g., (9, 26, 33, 37, 38)].

Demographic, medical, and psychological factors are central factors influencing functional school outcomes for children with pABI. However, fatigue has been largely overlooked as a potential predictor of functional school outcomes, despite being described as one of the most universal and debilitating symptoms following pABI (39–41), that may have adverse effects on academic outcomes. The etiology of fatigue after pABI is complex, primarily relating to changes in the central and peripheral nervous system and endocrine disturbances (42, 43). In addition, fatigue is related to exacerbating factors such as emotional disorders and cognitive impairment, and in particular executive dysfunction (42, 44). Previous research suggest that fatigue is associated with decreased QoL, interfering with everyday activities (e.g., social and physical activities) and school function (41, 45–48). There is preliminary evidence to suggest an association between fatigue and unfavorable functional school outcomes (i.e., schoolwork being negatively affected and worse academic performance) (46, 47, 49). Beyond these studies, the associations between fatigue and functional school outcome are scarcely examined in the pABI population. However, studies of other conditions provide support to the relationship; for example, there is evidence showing that fatigue is associated with worse cognitive and academic outcomes in pediatric multiple sclerosis (50), as well as with unfavorable functional outcomes of young adult cancer survivors and stroke patients (51, 52). Similarly, disadvantageous social outcomes relating to employment and substantial government benefits in long-term survivors of pediatric brain tumors have been found to be strongly associated with fatigue and executive dysfunction (31). Furthermore, Berrin et al. (53) observed that fatigue is an important determinant in understanding how diagnostic subtypes of cerebral palsy translates into problems with school functioning.

There are, however, several methodological shortcomings in existing studies on functional school outcome in the field of pABI, such as small sample size, inferior measures of fatigue (e.g., yes/no questions) and too narrow definition and assessment of functional school outcome (e.g., no inclusion of objective functional school data), limiting the generalizability and validity of findings. Importantly, most studies evaluating functional school outcome in the context of post-pABI fatigue have been limited by the use of relatively narrow definitions of functional school outcome, such as describing it merely in terms of school performance/work (46, 47, 49). Beyond performance/work, domains of functioning including QoL in the school setting and objective functional school data, such as information regarding school absence and aid from external educational services, have not been formally investigated in children with pABI. To our knowledge, functional school outcome in relation to fatigue after pABI has only been assessed with questionnaires (46, 47) and interviews addressing school performance/work (54). Notably, no studies have examined the association between fatigue and different categories of specific functional school outcomes, or examined these associations in children aged 10–17 years, including both TBI and non-traumatic brain injuries.

In sum, there is an urgent need to employ a broader, more holistic approach to assessing functional school outcome and identify potential predictors of functional school outcome in children with pABI that may assist in developing evidence based personalized interventions to advance school functioning. Thus, the purpose of this study was to investigate how demographic, medical, and psychological factors, with a specific emphasis on fatigue, are associated with impairments in functional school

outcomes in children with pABI. Given the lack in previous studies, the present study will have a more exploratory approach and attempt to answer the following questions:

1. Do Demographic, Medical, and Psychological Factors Predict Impairments in Functional School Outcomes, Indicated by School Absence, aid From the EPS, Self- and Parent Reported QoL in School Setting, and Academic Performance?
2. Do Parent-Reported Fatigue Emerge as a Significant Predictor for any of the Functional School Outcomes?

MATERIALS AND METHODS

This cross-sectional study presents baseline data derived from a dual site, evaluator-blinded, parallel group randomized controlled trial (RCT) on the efficacy of cognitive rehabilitation for children and adolescents with pABI (55, 56). The original RCT was preregistered at clinical.trials.gov (NCT03215342), approved by the Regional Committee for Medical and Health Research Ethics (2017/772), Norway, and conducted in accordance with principles of Good Clinical Practice, the Helsinki Declaration and the standards for Ethical Research Involving Children (ChildWatch International and UNICEF).

Participants and Design

Seventy-six participants (mean age 13.4 years, *SD* = 2.3, 57% girls), with pABI resulting from non-traumatic (brain tumor, stroke, hypoxia/anoxia, and brain infections/inflammations; *n* = 58) and traumatic (TBI; *n* = 18) injuries, and who were between the age of 10 to 17 years at time of invitation, were recruited from trauma referral centers from the north, mid- and south-east regions of Norway (see **Table 1** for demographic and medical characteristics). Information related to the inclusion and exclusion criteria was collected by a semi-structured interview [for more details about the semi-structured interview see (56)]. Inclusion required executive complaints in daily life as determined by a free description of the child's function in daily-life and specific EF questions (e.g., "*Does the child handle doing more than one thing at the same time? Does the child manage to plan activities? Is the child easily distracted?*) in the semi-structured interview, in addition to minimum 12 months since injury/illness or completion of cancer therapy. The exclusion criteria consisted of: (i) pABI before 2 years of age; (ii) cognitive, sensory, physical or language impairment affecting the capacity to attend regular school (i.e., follow educational goals of peers and regular teaching) and/or complete the intervention; (iii) preinjury neurological disease, severe psychiatric disorder and/or stimulant medication; (iv) recently detected brain tumor relapse [see also (56)].

Potential participants were identified based on discharge diagnosis and received a written invitation (*n* = 223). In the case of a positive response, written informed consent was obtained from participants (>16 years) or primary caregivers (participants < 16 years). Following this, a semi-structured screening interview was conducted to determine eligibility for study inclusion. Parents reported for participants <16 years of age, and participants older than 16 years of age could attend the interview [for more details about the semi-structured interview see (56)]. Ninety-nine individuals were eligible for screening. Out of these, ten participants did not meet inclusion criteria (i.e., 9 with insufficient EF complaints, and 1 was excluded based on information indicating obvious violation of eligibility not previously communicated) and were as such excluded, while two participants declined to participate. Following randomization (*n* = 87), 76 participants completed a baseline assessment. Pre-inclusion attrition comprised 11 participants (e.g., due to worsening of illness) (see also 57). In the present study, the children were assessed pre-intervention (baseline) at the hospital during one workday by experienced test-technicians, a study nurse, and psychology students (master level) under the supervision of clinical neuropsychologists. To compensate for the potential variation associated with several assessors and to ensure consistent results for the study, a Standard Operating Procedure (SOP) described the protocol and procedures for the assessments, and the test administrators received training from experienced clinical neuropsychologists in addition to being blinded to the type of intervention to be received.

TABLE 1 | Demographic and medical characteristics in study sample.

	(*n* = 76)
Age years (*M*/*SD*)	13.4 (2.3)
Sex (Girls/boys)	43/33
Primary injury, *n* (%)	
Brain tumor	29 (38)
Traumatic brain injury	18 (24)
Cerebrovascular accidents	17 (22)
Inflammation	7 (9)
Anoxia	5 (7)
Cerebral imaging, *n* (%)[a]	76 (100)
Confirmatory pathological findings, *n* (%)[b]	67 (88)
Neurological impairments, *n* (%)	33 (43)
Admission to intensive care unit, *n* (%)	49 (64)
Brain surgery in the brain tumor group	25 (86)
Chemotherapy	11 (14)
Radiation therapy	8 (11)

[a] *All had conducted magnetic resonance imaging (n = 65) or computed tomography (CT; n = 11) at some point.*

[b] *Out of the 9 individuals without confirmatory imaging (normal), six had traumatic brain injury, One anoxia, and two with brain infection/inflammation. Three out of these nine had conducted a CT only and all had traumatic brain injury.*

Measures

Demographic and Medical Characteristics

Demographic information, in addition to school absence and aid from EPS, was collected in a structured interview. Medical and injury characteristics were extracted from medical records. This data included brain imaging findings [computerized tomography (CT), magnetic resonance imaging (MRI)] from the first year post injury or disease onset, data regarding admission to intensive care unit, and treatment (e.g., chemotherapy, radiation therapy, brain surgery in brain tumor group). Neurological status (cranial nerve,

motor function, balance, sensibility), was obtained in a medical examination by a physician.

Functional School Outcomes

School Absence

School absence was measured by asking parents: "To what degree has your child been absent from school during the last 6 months?" The degree of absence was indicated using the following response set: <10, 10–50 or >50%. Due to a very low number of responses indicating >50%, we ended up using categories <10 and >10% for the present study.

Aid From the Educational Psychological Service

Typically, EPS provides assessments and advice regarding special educational needs in the school setting. In the present study, parents were asked the following question in order to obtain data on EPS aid: "Has your child received any support from EPS?" The following two categories were employed: "no aid" and "current or previous aid."

Quality of Life in the School Setting

Quality of life in the school setting was investigated by employing the 5-item School Functioning subscale from the Pediatric Quality of Life Inventory (PedsQL parent report) (57). The format, instructions, Likert response scale, and scoring method are identical to the PedsQL MFS, with higher scores indicating better QoL in the school setting. Items on the PedsQL school scale assess problems regarding "keeping up with schoolwork," "paying attention in class," and "forgetting things." Furthermore, the scale score has good reliability ($\alpha = 0.72$) and validity in several health conditions, including pABI (58–60). Finally, good internal consistency ($\alpha = 0.93$) was demonstrated for the PedsQL (parent) in the present study.

Academic Performance

The participants' teachers were administered the Teacher's Report Form ages 6-18 (TRF/6-18) from the Achenbach System of Empirically Based Assessment (ASEBA; 62) to assess the children's academic performance. The TRF/6-18 provide scores for the child's current performance in academic subjects. Here, teachers rate the child's school performance using a 5-point scale ranging from 1 (far below grade level) to 5 (far above grade level) for each academic subject. For adaptive characteristics in school, teachers rate the child on 7-point scales ("Much less," "Somewhat less," "Slightly less," "About average," "Slightly more," "Somewhat more," "Much more") in four areas: dedication to schoolwork, appropriateness of behavior in school, ability to learn, and happiness. Acceptable internal consistency was found for academic performance ($\alpha = 0.72$).

Potential Predictors

Fatigue

Symptoms of fatigue were measured using the 18-item Pediatric Quality of Life Inventory-Multidimensional Fatigue Scale (PedsQL MFS, parent report) (60). It is comprised of three dimensions, General, Sleep/rest and Cognitive fatigue, as well as a total score. The total score was used as outcome in the present study. Items are rated on a 5-point scale ("Never," "Almost Never," "Sometimes," "Often," and "Almost Always"). Respondents are asked how much of a problem each item have been during the past month (e.g., "feel too tired to spend time with friends"). Items are reverse scored and linearly transformed to a 0–100 scale, so that higher scores indicate better QoL (i.e., less fatigue symptoms). The scale scores of this measure have demonstrated strong evidence of reliability and validity across various pediatric health conditions, including pABI (61–63). In the present study good internal consistency was found for the parent-report ($\alpha = 0.93$).

Behavioral Problems

Behavioral problems was assessed with the Child Behavior CheckList for ages 6–18 from ASEBA (CBCL/6-18; 62). It consists of 113 questions, scored on a three-point Likert scale (0 = absent, 1= occurs sometimes, 2 = occurs often). Here, a parent is instructed to report on the child's problems. The form yields various subscales and three composite scores, of which Total problems ($M = 50$, $SD = 10$), the sum of scores of all the problem items, i.e., behavioral problems, was employed in the present study. The questionnaire has robust psychometric properties with adequate internal consistency ($\alpha = 0.82$) (64).

Executive Function

The Behavioral Assessment of the Dysexecutive Syndrome for Children (BADS-C; 66) consists of six subtests; the Playing Cards Test, the Water Test, the Key Search Test, Zoo Map Tests 1 and 2, and the Six Part Test. It was developed to reflect different EFs (e.g., shifting, planning and goal-directed behavior, estimation abilities and inhibition) in everyday life and is used as a global measure of EF in the present study. A total age-scaled score is converted to an overall scaled score, ranging from 49 to 146. The scores can be classified functionally like this: impaired performance (overall scaled score range 49–68); borderline performance (overall scaled score range 70–78); low average performance (overall scaled score range 80–88); average performance (overall scaled score range 90–109); high average performance (overall scaled score range 111–119), and superior performance (overall scaled score range 121–146) (65).

Intellectual Ability

Full scale IQ was estimated by using six subscales (i.e., Vocabulary, Similarities, Digit Span, Coding, Block Design and Matrix reasoning) from the Wechsler Intelligence Scale for Children- Fifth Edition (WISC-V) (66). The WISC-V is an individually administered test battery ($M = 100$, $SD = 15$, subscales $M = 10$, $SD = 3$).

Global Functioning

The Glasgow Outcome Scale Extended, pediatric version (GOS-E) is designed to provide a functional outcome, assessing global disability and recovery after brain injury, i.e., inside and outside the home, capacity for work/school, participation in social and leisure activities, and family and peer interactions (67). It consists of a scale with 19 items and eight levels: Level 1 = dead, Level 2 = vegetative state, Level 3 = low severe disability, Level 4 = upper severe disability, Level 5 = low moderate disability, Level 6 = upper moderate disability, Level 7 = low good recovery, and

Level 8 = upper good recovery. The GOS-E has demonstrated good psychometric properties (67, 68), and has been found to be strongly associated with functional independence (69).

Statistical Analyses

Frequency distributions, means, medians, standard deviations (*SD*), and range were calculated for demographic characteristics, potential predictor variables and functional school outcomes (dependent variables). School absence, aid from EPS, QoL in school setting (parent- and self-report), and academic performance were the dependent variables in separate multiple linear regression equations with demographic, medical, and psychological factors as potential predictor variables. Bivariate correlations between potential predictor variables (independent variables) and functional school outcome variables (dependent variables) were computed using Spearman's rho. Variables showing significant correlations ($p < 0.05$) with the functional school outcome variables were then entered into regression equations. Multiple linear regression models were employed for the continuous dependent variables and logistic regression was used for the dichotomized categorical dependent variables. Preliminary analyses were conducted to ensure no violation of the assumptions of normality, linearity, and homoscedasticity, including Mahalanobis distances to find multivariate outliers (removed if present in each equation) (70). To avoid multicollinearity, independent variables demonstrating correlations of >0.70 with other independent variables were removed from the equation (71). In addition, variance inflation factor and tolerance statistics were checked in relation to collinearity (70). In deciding the strength of the relationships, Cohen's (72) guidelines were employed: r = 0.10 to 0.29 (small), r = 0.30 to 0.49 (moderate), and r > 0.50 (large). All statistical testing used an alpha value of 0.05 (two-tailed). Data analyses were conducted using IBM-SPSS version 26.

RESULTS

Sample Characteristics

Seventy-six participants were included in the present study. Demographic and medical characteristics are summarized in **Table 1**. Brain tumor was the dominant cause of injury (n = 29), followed by TBI (n = 18), and other etiologies accounted for 29 (38%). Almost all had confirmatory cerebral imaging findings (88%), 43% had clinical neurological findings and 64% had been admitted to an intensive care unit. For the brain tumor group, almost all had conducted brain surgery, 38% had received chemotherapy, and 28% had received radiation therapy.

Scores on potential predictor variables and functional school outcomes are presented in **Table 2**. The mean age when injured was 8 years (SD = 3.6), ranging from 1 to 15 years of age. Mean time since insult was almost 5 years (SD = 2.7). Regarding global functioning, the GOS-E yielded a mean score of 5.7 (SD = 1.4) placing the majority of the sample in the category "upper moderate disability." Concerning the PedsQL MFS, the group mean fell below the clinically important cutoff score (<70) (60). The sample displayed general intellectual ability, executive test performance and perceived behavioral functioning within the normal range, i.e., the group mean was within 1.5 *SD* from the normative scores on the IQ measure (i.e., WISC-V), the executive test (i.e., BADS-C), and the questionnaire CBCL, respectively.

TABLE 2 | Scores on potential predictor variables and functional school outcomes.

Measure	*n* (%)	Mean (*SD*)	Median (range)
Potential predictor variables			
Age yrs. (n = 76)		13.4 (2.3)	13 (10, 17)
Sex (girls) (n = 76)	43 (57)		
Age at insult yrs. (n = 76)		8 (3.6)	8 (1, 15)
Time since insult yrs. (n = 76)		4.8 (2.7)	5 (1, 12)
GOS-E (n = 73)		5.7 (1.4)	6 (3, 8)
PedsQL MFS scaled (n = 76)		55.3 (19.2)	56.3 (10, 97)
Est. full scale IQ (n = 72)		92.5 (13.3)	92 (67, 122)
BADS-C overall scaled score (n = 75)		83.9 (20.1)	84 (34, 125)
CBCL (n =71; total problems T-score)		57.6 (9.9)	58 (38, 79)
Functional school outcomes			
School absence < 10% (n = 76)	48 (63)		
Aid from EPS (n = 75)	46 (60.5)		
QoL in school setting Parent-report scaled (n = 76)		56.1 (22)	55 (15, 100)
QoL in school setting Self-report scaled (n = 75)		58 (20.1)	60 (15, 100)
Academic performance T-score (n = 69)		44 (6.7)	44 (35, 64)

GOS-E, the Glasgow Outcome Scale Extended; PedsQL MFS, The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale, parent report, total score; BADS-C, Behavioral Assessment of the Dysexecutive Syndrome for Children; CBCL, The Child Behavior Checklist Total problems (M = 50, SD = 10); EPS, Educational Psychological Service; Estimated full scale IQ from Wechsler Intelligence Scale for Children–Fifth edition (M = 100, SD = 15). IQ scores are scaled scores. Higher score represents more daily life difficulties in CBCL. For PedsQL MFS and QoL in school setting higher scores indicate less problems (e.g., fatigue). QoL in school setting, Quality of life in school setting from the 5-item School Functioning subscale from the Pediatric Quality of Life Inventory (parent report). Academic performance scores are from the Teacher's Report Form ages 6-18 (TRF/6-18) from the Achenbach System of Empirically Based Assessment (M = 50, SD = 10). Clinical cutoffs, CBCL and academic performance T ≥ 65; PedsQL MFS and QoL in school setting T < 70.

As seen in **Table 2**, a majority (n = 48) had <10% school absence during the last 6 months, and a majority had received aid from EPS (n = 46). The group means for both self- and parent-reports of QoL in school setting were below normal (<70) (60). Finally, academic performance was rated by the participant's teachers to be normal, with a group mean T-score of 44 (SD = 6.7).

Bivariate Analyses

Correlations between potential predictors (independent variables) and functional school outcome variables were first examined and are presented in **Table 3**. Statistically significant

TABLE 3 | Correlations between functional school outcome variables and potential predictor variables.

	School absence	Aid from EPS	QoL school (parent)	QoL school (self)	Academic performance
Potential predictor variables					
Age	0.182	−0.033	−0.116	−23*	0.151
Sex	0.119	−0.187	0.012	−0.226	−0.393**
Age at insult	0.204	−0.108	−0.052	−0.185	0.180
Time since insult	−0.138	0.154	−0.069	0.018	−0.103
GOS-E	−0.429**	−0.41**	0.503**	0.352**	0.369**
PedsQL MFS	−0.454**	−0.278*	0.742**	0.707**	0.104
IQ	−0.232*	−0.505**	0.403**	0.177	0.721**
BADS-C	−0.114	−0.36**	0.273*	0.085	0.419**
CBCL	0.32**	−0.045	−0.463**	−0.364**	−0.022

*QoL school, Quality of life in school setting from the 5-item School Functioning subscale from the Pediatric Quality of Life Inventory (parent report); PedsQL MFS, The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale parent report, total score; EPS, Educational Psychological Service; GOS-E, the Glasgow Outcome Scale Extended; BDS-C, Behavioral Assessment of the Dysexecutive Syndrome for Children; CBCL, The Child Behavior Checklist Total problems; Estimated full scale IQ from Wechsler Intelligence Scale for Children–Fifth edition. Academic performance scores are from the Teacher's Report Form ages 6–18 (TRF/6-18) from the Achenbach System of Empirically Based Assessment. *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).*

correlations are described below, including the strength of the relationships:

School Absence

PedsQL MFS, GOS-E, and IQ were negatively correlated with school absence, indicating that more overall fatigue, lower global functioning, and poorer IQ is associated with less school attendance ($p < 0.05$). CBCL (total problems) was correlated with school absence, indicating that fewer behavioral problems are associated with higher school attendance ($p < 0.01$). All correlations between school absence and potential predictors were moderate except IQ (small).

Aid From EPS

GOS-E, PedsQL MFS, IQ, and BADS-C were negatively correlated with aid from EPS, indicating that that lower global functioning, more fatigue, poorer IQ, and worse EF performance is associated with receiving more support from EPS ($p < 0.05$). Moreover, all correlations between aid from EPS and potential predictors were moderate except PedsQL MFS (small).

QoL School (Parent)

Higher QoL school (parent) scores were significantly associated with higher GOS-E, PedsQL MFS, IQ, and BADS-C scores ($p < 0.05$), indicating that better parent-reported QoL in the school setting is associated with higher global functioning, lower levels of overall fatigue, higher IQ and better EF performance. Additionally, QoL school scores were negatively associated with CBCL scores, indicating that worse parent-reported QoL in the school setting is associated with more behavioral problems ($p < 0.01$). All correlations between QoL school (parent) and potential predictors were moderate to large except BADS-C (small).

QoL School (Self)

Higher QoL school (self) scores were significantly associated with higher GOS-E and PedsQL MFS ($p < 0.01$), indicating that better self-reported QoL in the school setting is associated with higher global functioning and lower levels of overall fatigue. Furthermore, QoL school scores were negatively associated with age and CBCL scores, indicating that poorer self-reported QoL in the school setting is associated with lower age and more behavioral problems ($p < 0.05$). All correlations between QoL school (self) and potential predictors were moderate to large except age (small).

Academic Performance

Academic performance was negatively associated with sex, indicating that better academic performance is associated with being female ($p < 0.01$). Moreover, GOS-E, IQ, and BADS-C scores were positively correlated with academic performance, indicating that better academic performance is associated with higher global functioning, higher IQ, and better EF performance ($p < 0.05$). Of note, all correlations between academic performance and potential predictors were moderate to large.

Post-hoc Analysis

A *post-hoc* correlation analysis was conducted to further explore the relationship between the PedsQL MFS subscales (General fatigue: $M = 58$, $SD = 22.6$; Sleep/rest fatigue: $M = 59.2$, $SD = 23.7$; and Cognitive fatigue: $M = 49.1$. $SD = 21.6$) and functional school outcomes.

All PedsQL MFS subscales were significantly associated with QoL in school (self and parent) and school absence ($p < 0.05$), indicating that lower levels of overall fatigue are associated with better parent- and self-reported QoL in the school setting and lower rates of school absence. General and Cognitive fatigue scores were additionally associated with aid from EPS, indicating that less symptoms of General and Cognitive fatigue are associated with less aid from EPS ($p < 0.05$). All correlations between the PedsQL MFS subscales and potential predictors were moderate to large except between Cognitive fatigue and school absence and Cognitive fatigue and aid from EPS (small).

TABLE 4 | Logistic regression predicting likelihood of school absence.

				95% CI for OR	
	B	*p*-value	OR	Lower	Upper
GOS-E	−0.474	0.077	0.623	0.368	1.053
PedsQL MFS	−0.032	0.087	0.968	0.934	1.005
IQ	−0.003	0.901	0.997	0.944	1.052
CBCL (total)	0.06	0.111	1.062	0.986	1.144

*GOS-E, the Glasgow Outcome Scale Extended; PedsQL MFS, The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale parent report; IQ, Estimated full scale IQ from Wechsler Intelligence Scale for Children–Fifth edition; CBCL, The Child Behavior Checklist Total problems. *$p < 0.05$.*

TABLE 5 | Logistic regression predicting likelihood of aid from EPS.

				95% CI for OR	
	B	*p*-value	OR	Lower	Upper
GOS-E	−0.399	0.15	0.671	0.390	1.155
PedsQL MFS	−0.014	0.396	0.986	0.956	1.018
IQ	−0.063	0.035*	0.939	0.886	0.996
BADS-C	−0.011	0.557	0.989	0.953	1.026

*GOS-E, the Glasgow Outcome Scale Extended; PedsQL MFS, The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale parent report; IQ, Estimated full scale IQ from Wechsler Intelligence Scale for Children–Fifth edition; BADS-C, Behavioral Assessment of the Dysexecutive Syndrome for Children; CBCL, The Child Behavior Checklist Total problems. *$p < 0.05$.*

Multivariate Analysis

Potential predictor variables showing significant correlations ($p < 0.05$) with the functional school outcome variables were entered as independent variables into regression equations, with the functional school outcome variables as the dependent variables (**Tables 4–6**). When running the model's residual plots as well as normality plots were produced and visually inspected. No violation of the assumptions of normality, linearity, multicollinearity, nor homoscedasticity was detected.

Potential Predictors of Functional School Outcomes

School Absence

The full model containing all predictors was statistically significant, X^2 (4, $N = 64$) = 17.92, $p = 0.001$. The model explained between 24.4 % (Cox and Snell R square) and 33.7% (Nagelkerke R square) of the variance in school absence and correctly classified 73.4 % of cases. None of the independent variables made a unique statistically significant contribution to the model (**Table 4**).

Aid From EPS

The full model containing all predictors was statistically significant, X^2 (4, $N = 68$) = 21.11, $p < 0.001$. The model explained between 26.7 % (Cox and Snell R square) and 35.8 % (Nagelkerke R square) of the variance in school absence and correctly classified 75 % of cases. Only IQ made a unique statistically significant contribution to the model [OR.939, 95% CI (0.886, 0.996); **Table 5**].

QoL School (Parent)

A total of 63% of the variance in QoL school (parent) was explained, $F_{(5,60)} = 23.1$; $p < 0.001$, with PedsQL MFS [$\beta = 0.548$; $p < 0.00$; *B* 95% CI (2.131, 4.165)], GOS-E [$\beta = 0.206$; $p < 0.05$; *B* 95% CI (1.669, 29.796)], and CBCL total problems [$\beta = -0.205$; $p < 0.05$; *B* 95% CI (-1.03, 1.224)] as significant predictors (**Table 6**).

QoL School (Self)

For QoL school (self), 48% of the variance was explained, $F_{(4,63)} = 16.18$; $p < 0.001$, with PedsQL MFS [$\beta = 0.532$; $p < 0.001$; *B* 95% CI (1.717, 3.983)] as a significant predictor (**Table 6**).

Academic Performance

A total of 68% of the total variance in academic performance was explained, $F_{(4,63)} = 34.83$; $p < 0.001$, with IQ [$\beta = 0.663$; $p < 0.001$; *B* 95% CI (0.239, 0.427)] and sex [$\beta = -0.481$; $p < 0.001$; *B* 95% CI (-8.377,−4.506)] as significant predictors (**Table 6**).

DISCUSSION

The aim of this study was to extend our knowledge about the ability of demographic, medical, and psychological factors, and in particular fatigue, to predict different categories of specific functional school outcomes in a pABI sample. The main finding of this study was that these factors were associated with all functional school variables examined, except school absence. Secondly, fatigue made the strongest unique contribution in explaining self- and parent reported QoL in the school setting.

Return to school after pABI may pose several challenges for families and school personnel. The complex constellation of neurocognitive, emotional and physical symptoms can hamper learning and cause increased absence rates. In the present study, fatigue and IQ emerged as the strongest contributors in explaining less favorable functional school outcomes, followed by global functioning, behavior problems and sex. Indeed, fatigue was the strongest contributor for overall QoL in the school setting (including both parent- and self-reports) when the variance explained by all other variables in the models was controlled for. Specifically, greater fatigue severity was associated with poorer school-related QoL. This is a central aspect of school functioning, and to our knowledge, no previous research has demonstrated these specific associations in a pABI sample with different etiologies. Although more conjectural, the *post-hoc* analysis showing that all dimensions of fatigue (i.e., General, Sleep/rest and Cognitive) were significantly associated with overall QoL in school, suggest that this relationship is driven by several aspects of fatigue. However, a high level of caution is needed in making inferences from the exploratory analyses. In relation to these findings, it is also important to consider the potential overlap in item content between fatigue and QoL measures. The association between fatigue and QoL measures is potentially driving the relationship (i.e., correlations of > 0.7 reported in **Table 3**). Nevertheless, these findings are in accordance with

TABLE 6 | Multiple linear regression models with functional school outcome variables as dependent variables and demographic, medical, and psychological factors as independent variables: β, *B*, CIs, *p*-values and adjusted R^2.

Potential predictor variables	QoL school (parent)					QoL school (self)					Academic performance				
	β	*B*	95% CI for *B* Lower	95% CI for *B* Upper	*P*-value	β	*B*	95% CI for *B* Lower	95% CI for *B* Upper	*p*	β	*B*	95% CI for *B* Lower	95% CI for *B* Upper	*p*-value
Age	-				-	–0.148	–6.55	–14.636	1.526	0.11	-				-
Sex	-				-	-				-	–0.481	–6.442	–8.377	–4.506	<0.001*
GOS-E	0.206	15.733	1.669	29.796	0.029*	0.151	10.699	–2.471	23.87	0.109	0.060	0.278	–0.506	1.061	0.481
PedsQL MFS	0.548	3.148	2.131	4.165	<0.001*	0.532	2.85	1.717	3.983	<0.001*	-				-
IQ	0.136	1.124	–0.447	2.696	0.158	-				-	0.663	0.333	0.239	0.427	<0.001*
BADS-C	0.015	0.097	–1.03	1.224	0.172	-				-	0.087	0.029	–0.03	0.088	0.33
CBCL (total)	–0.205	- 2.273	- 4.158	–0.388	0.019*	–0.152	–1.569	–3.644	0.505	0.136	-				-
Adjusted R^2			0.63					0.48					0.68		

QoL school, Quality of life in school setting from the 5-item School Functioning subscale from the Pediatric Quality of Life Inventory (parent report); PedsQL MFS, The Pediatric Quality of Life Inventory Multidimensional Fatigue Scale parent report; EPS, Educational Psychological Service; GOS-E, the Glasgow Outcome Scale Extended; BADS-C, Behavioral Assessment of the Dysexecutive Syndrome for Children; CBCL, The Child Behavior Checklist Total problems; Estimated full scale IQ from Wechsler Intelligence Scale for Children–Fifth edition. Academic performance scores are from the Teacher's Report Form ages 6–18 (TRF/6-18) from the Achenbach System of Empirically Based Assessment.

*$p < 0.05$.

findings from previous research. Fatigue has been associated with functional school outcomes (i.e., schoolwork being negatively affected and worse school performance) in a variety of pABI types [e.g., (46, 47, 49)]. In addition, post-ABI fatigue has also been associated with health-related QoL, with increased fatigue being associated with greater perceived negative impact of health issues on a range of daily activities in adult ABI populations [e.g., (73)]. In a study by Macartney et al. (49), pediatric brain tumor survivors described how they felt too tired to attend school following pABI, in addition to experiencing cognitive problems (e.g., memory and concentration problems) that contributed to learning difficulties. We were able to extend previous findings by providing preliminary evidence of associations of post-pABI fatigue also being associated with other functional school outcomes (i.e., QoL in the school and school absence), beyond questionnaires and interviews only addressing performance/work employed in previous research [e.g., (46, 47, 49)]. Although none of the independent variables were able to predict school absence, there was a certain trend toward significance for fatigue ($p = 0.087$), and the *post-hoc* analysis showed that all dimensions of fatigue (i.e., General, Sleep/rest and Cognitive) were significantly associated with school absence. In a similar vein, the association between fatigue (PedsQL MFS total score) and aid from EPS may primarily be driven by Cognitive and General fatigue. Although speculative, given the exploratory nature of the analyses, these findings might indicate that fatigue also may be associated with school absence. However, our study included only children with reported EF problems in daily life, which may have contributed to a sample with higher levels of fatigue compared to the general pABI population. Moreover, despite fatigue being one of the most common symptoms across ABI conditions, there is no consensus framework, and importantly no single, valid and reliable instrument for the assessment of fatigue due to its subjective and multidimensional nature (41, 74).

IQ emerged as a potential predictor for aid from EPS and academic performance, suggesting that higher IQ is associated with receiving less aid from EPS and better academic performance. This finding was not unexpected when considering that IQ is vital for independent participation in activities such as education, self-care, and later in life, employment and living independently [e.g., (75)]. Notably, there is broad agreement that there is a moderate to strong association between cognitive or general intellectual ability and educational achievement overall (21, 76, 77). The role of EPS is to ensure that expert assessments are prepared when this is necessary, in order to improve the adaptation of the education for pupils with special needs. Special educational needs typically refer to children with learning problems or disabilities that make it more difficult for them to learn compared to most children at the same age (78). The need for this kind of service is less likely when having a higher IQ (21). A high IQ may indicate a good cognitive reserve (i.e., cognitive enrichment) and may as such may be advantageous after a pABI, with less negative behavioral manifestations (79). Of note, many studies have reported that lower cognitive reserve is associated with worse outcomes after ABI [e.g., (80)]. Our findings are also in accordance with a study by Prasad et al. (20) who found that

children with TBI have higher rates of school support services than children with orthopedic injuries and healthy comparison children. In a similar vein, Lahteenmaki et al. (81) reported that children who had been treated for cancer had a greater need for help from extra lessons. It is also important to keep in mind that academic performance may be affected by the adverse effect on the learning opportunities caused by neurological changes due to chemotherapy and radiotherapy, and absence from school due to hospitalization.

Global functioning (GOS-E) emerged as a potential predictor for parent-reported QoL in the school setting. Specifically, worse global function after brain injury was associated with poorer school-related QoL. This finding is perhaps not surprising when considering that the GOS-E was designed to provide a global outcome with developmental specificity in the pediatric population, assessing functional status, independence, and participation in relevant societal roles (67). For example, the physical and/or psychological changes caused by cancer treatment can delay a child's return to school, reduce the desire to attend school, and cause more absence from school (82). It is, however, important to keep in mind that the broad categories in GOS-E may inadequately account for the multidimensional nature of pABI outcomes with limited sensitivity to change within specific functional domains (83). The lack of available normative rates of school absence makes it difficult to interpret our findings and understand the extent and implications. Since school is a fundamental context for development, reduced QoL in this context, with the risk of increased absence, has the potential to produce deviations in normal development (84), adding to the existing risk caused by the injury in a developing brain. Acquired brain injuries can disrupt subsequent brain development, causing significant short- and long-term alterations in several functional abilities (4, 33). Interestingly, only parent-reported, not self-reported, QoL in the school setting was associated with global functioning. This can be a result of parents overestimating the impact of global functioning to QoL in the school setting, or that the children underestimate the impact of global functioning, or a combination of the two. It is, however, important to keep in mind that there are several factors that may contribute to the discrepancy between self- and parent reports (85), such as response bias due to parental anxiety or expectations (86), and reduced awareness (87). The nature of self- and parent reporting is complex (88), emphasizing the need of including healthy controls in addition to self- and parent report in future studies.

Children with pABI, and in particular TBI, are at higher risk than non-injured children of behavioral problems, with potential detrimental effects on children's long-term functioning and QoL [e.g., (9)]. There are many studies that provide data for the CBCL as a valid tool to assess co-occurring emotional and behavioral problems in children (89). In the present study, the overall extent of both emotional and behavioral problems (CBCL total behavioral problems) emerged as a potential predictor for parent-reported QoL in school, suggesting that behavioral problems have adverse effects on QoL in school. Of note, behavioral impairments after pABI is common (37), and may negatively impact school performance and educational progress, by hindering both the continued development of current skills and acquisition of new skills (9, 24, 26, 33, 38, 90). For example, behavioral impairments may put children at risk for ineffective interactions with the environment, leading to poor functional school outcome. In fact, emotional ill health in children has been associated with a range of adverse functional school outcomes such as educational failure and higher rates of school absence (91–93). Although not specifically assessed in the present study, it is important to mention that difficulties in emotional regulation are among the most common consequences of ABI, with potential detrimental effects in all life domains [e.g., (5, 94–96)]. Emotional regulation can be described as an important aspect of EF (97). In our study, EF did not emerge as a significant predictor for any of the functional school outcomes. This is somewhat surprising, as EF include cognitive processes such as shifting, inhibition, and updating of working memory (35, 98), processes that are essential to learning, academic achievement, and behavioral competence [e.g., (34, 99, 100)]. However, there is a lack of consensus on the definition of EF, and the assessment of the construct is a known challenge (e.g., "task impurity") (101, 102). In particular, the highly structured and examiner-guided setting in which the examination takes place (e.g., BADS-C), makes less demand on the child's goal setting, structuring and decision-making abilities than the real-life setting (101, 102).

Finally, sex emerged as a potential predictor for academic performance (academic subjects and adaptive characteristics in school), suggesting that better academic performance is associated with being female. Previous research has shown that boys perform less well in school assessments when compared to girls, despite similar cognitive test scores (103, 104). There are, however, a couple of issues that needs to be considered when discussing these findings. Although the teachers rate the child's school performance for each academic subject it is possible that there are more complex academic skills not captured by the CBCL (e.g., comprehension, written expression). Moreover, for adaptive characteristics in school, teachers rate the child regarding commitment to schoolwork, appropriateness of behavior in school, ability to learn, and happiness. These areas may be difficult to rate, especially when considering the context of many of these children, being survivors of life-threatening insults, in addition to the consequences following pABI that may include changes in emotional, behavioral, and cognitive functions. Hence, further research into this area is warranted.

Surprisingly, neither of the injury-related variables age at insult and time since insult showed significant correlations with functional school outcome variables. This is not in commensurate with previous research whereby age at insult and time since insult has been associated with functional school outcomes (e.g., 25, 26–28). Most of the cited previous research, however, involved TBI samples, while the present sample consisted of different pABI etiologies with brain tumor as the dominant cause of injury. Additionally, since we included children ranging from 1 to 12 years post-injury, they were at different stages in their recovery processes. Relatedly, the impact of developmental factors has not been studied in the present

study. A young brain has the capacity for more efficient neural restitution, by neural regrowth and anatomical reorganization (105, 106), in addition to being more vulnerable to more severe, diffuse and persistent impairments after pABI compared to the adult brain (107, 108). Furthermore, previous research has demonstrated that insult severity (e.g., Glasgow Coma Scale) is as a predictor of outcome after TBI [e.g., (7)]. However, a generally accepted categorization of severity in atraumatic insults is lacking. Since there is no widely recognized measure of injury severity across different etiologies, we were unable to explore the potential impact of insult severity in the present study. Although severe psychiatric disorder was an exclusion criterium and the sample as a whole displayed normal behavioral functioning, some of the participants may have concurrent mental disorders such as anxiety, depression, or posttraumatic stress disorder. Hence, it is recommended to examine factors such as developmental factors, insult severity, and concurrent mental disorders in more detail in future studies.

Research evidence on how to optimize the return to school process after pABI is lacking. Our findings may suggest a reintroduction to school with academic accommodations tailored to the child's specific symptoms, such as fatigue and behavioral problems, and global functioning and intellectual ability (e.g., GOS-E and IQ). Importantly, taking a broader approach to assessing functional school outcomes may be necessary to get a more nuanced understanding of the impact of pABI and to help inform targeted strategies that can support a child's successful return to school and improve educational outcomes following pABI.

Strengths and Limitations

Strengths of the present study include the large sample size relative to other pABI studies, robust and standardized assessment methods with both child and proxy report and objective functional school data, and the inclusion of both traumatic and non-traumatic brain injuries, increasing the generalizability and validity our findings. However, future research might also want to consider additional fatigue outcomes, such as objective fatigue measures [e.g., (109)]. In addition to the potential overlap in item content between fatigue and QoL measures, factors such as awareness, social desirability bias, response set bias (tendency to respond similarly to all/many items), and over/under reporting of symptoms may influence the accuracy of questionnaire responding. Also, for future research inter-rater reliability should be measured to ensure consistent results. Importantly, our results are based on correlational analyses and self/parent reports, so it cannot be definitively determined whether described predictors had a direct causal impact on the functional school outcomes. In the present study we focused on a restricted number of outcomes. However, there are other variables of interest that may be considered for future research that could impact school outcomes and/or performance on cognitive assessments, such as sleep, pain, parental health and education, other psychosocial and medical variables (e.g., depression, health-related quality of life, social function). Importantly, the present study did not explore the potential impact of injury severity. This should be investigated in future studies. Data regarding level of TBI, type of tumor, cause of cerebrovascular accidents or anoxia should also be collected in future research to better describe the sample. Another important limitation in the present study is that only participants with reported executive difficulties were included, with all being motivated for a cognitive rehabilitation intervention addressing cognitive difficulties. Perceived executive dysfunction may have contributed to a higher fatigue load in the sample. Awareness questionnaires should be considered for future studies. Moreover, a larger sample size may allow for analyses exploring potential between- and within-injury differences. Given the novelty of the study, with very few studies that have examined functional school outcomes and fatigue in a pABI context, a more exploratory approach was employed to the statistical analyses; we did not correct for multiple comparisons. Accordingly, our selection of predictors exceed the recommended predictor/sample size ratio (71). Hence, cautious interpretation of findings is necessitated by the exploratory nature of the analyses and design of the study. Relatedly, regression analyses in a cross-sectional design study with a limited sample also necessitates caution when interpreting findings. Regarding the generalizability of our findings outside of Norway; the participants were recruited from trauma referral centers from the north, mid- and south-east regions of Norway (nationwide), which allows us to generalize the results to other countries with a healthcare and school system comparable to the Norwegian, e.g., the Nordic countries. A potential advantage to our study is that factors such as race, ethnicity, socioeconomic status, health insurance status, care access (rural/urban), and school access/quality inequities do not play a large role in health outcomes in Norway (110). Finally, the cross-sectional design of our study prevents investigation of the trajectory of functional school outcome over time in addition to factors that might influence change in school outcome over time. To expound these matters, future research could include a larger sample and longitudinal methods to examine the course of predictors and school outcomes over time.

Conclusions

Following pABI, fatigue, IQ, global functioning, behavioral problems, and sex is associated with functional school outcomes. This may suggest that a reintroduction to school with personalized adaptations to the child's specific symptoms, such as fatigue and behavioral problems, and global outcome and intellectual ability is recommended. Importantly, fatigue represents a potentially modifiable treatment target for children with pABI and may, in turn, improve functional school outcome. Finally, out findings support a recommendation to employ a broad approach with information from different sources when assessing functional school outcome to obtain a more comprehensive understanding of pABI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Committees for Medical and Health Research Ethics, Norway. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JS, RH, AB, TF, ES, SA, KR, and TR have developed study protocol for this study. JS wrote the article with input from all authors. All authors contributed to the final manuscript, including final approval of the version published. Finally, all authors have agreed to be accountable for all aspects of the work.

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Analysis of the Group of Pediatric Patients With Relapsing-Remitting Multiple Sclerosis: Data From the Czech National Registry

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Importance: Multiple sclerosis can also affect children. Approximately 3–10% of patients develop multiple sclerosis before the age of 16.

Objective: The aim of this analysis is to describe the characteristics of pediatric patients with multiple sclerosis who started their treatment with disease-modifying drugs in 2013–2020, with data obtained from the Czech National Registry of patients with multiple sclerosis.

Design and Setting: A method of retrospective analysis conducted with 134 pediatric patients with multiple sclerosis was used.

Results: The findings reveal that the mean age at the date of the introduction of the first disease-modifying drugs treatment is 15.89 years, and gender does not play any role. In addition, moderate (51.6%) and mild (45.2%) relapses are predominant in these young patients. Seventy five percent of patients will not experience a confirmed progression of the expanded disability status scale within 54.7 months from starting the treatment. Furthermore, the results confirm that the first-choice treatment is interferon beta-a and glatiramer acetate, which is common for adult patients. However, some factors, such as a low efficacy or a lack of tolerance may impact on treatment discontinuation in children.

Conclusion: More research should be performed on novel disease-modifying drugs for this target group.

Keywords: pediatric multiple sclerosis, relapsing-remitting form, disease-modifying drugs, interferon beta-a, glatiramer acetate

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease associated with pathological processes in the central nervous system affecting mostly younger adults (20–40 years of age) (1, 2). However, multiple sclerosis can also affect children. Approximately 3–10% of patients develop MS before the age of 16, and in 1% it is even before the age of 10. Research shows an incidence of pediatric MS between 0.13 and 0.66 per 100,000 children per year (3, 4). The relapsing-remitting form of MS occurs in 98% of patients (1). Compared to adults, the pediatric form has more frequent relapses, more rapid lesion expansion early in the disease with more pronounced aspects of inflammation, worse cognitive decline, and worse physical disability over a longer time frame (5).

An important characteristics of childhood MS, compared to adults, is the longer time between disease onset and disability accumulation. This suggests that children have a greater ability to compensate for inflammatory brain damage despite high rates of relapse. The ARR (annualized relapse rate) is used to characterize the average number of relapses per patient per year. In contrast, the transition time from mild to severe disability, which is approximately 10 years, is similar in children and adults and is thought to be mainly due to neurodegeneration (5, 6). A scoring system called the expanded disability status scale (EDSS) is used to quantify disability in MS patients.

Early and correct diagnosis connected with prompt initiation of appropriate treatment is of paramount importance for improved long-term prognosis of the patient, including lower rates of relapse and worsening disability, as evidenced by recent publications on pediatric-onset multiple sclerosis (7, 8) and also pediatric guidelines for MS therapy recommend starting treatment as early as possible to prevent disease (7, 8). As in the treatment of adults, the drugs of choice for pediatric patients are disease-modifying drugs (DMDs), which target the peripheral immune system to reduce the risk of MS relapses. Currently, only two molecules from the DMD family are approved (FDA/EMA) for pediatric use and have been studied in Phase III clinical trials. These are fingolimod and teriflunomide. However, it is quite common that interferon-β and glatiramer acetate are also used in clinical practice. Clinical practice and recent publications show that an increasing number of pediatric patients are being treated with dimethyl fumarate or natalizumab. However, it should be noted that a number of phase II and III trials are currently underway to evaluate the efficacy and safety of unapproved molecules in pediatric MS patients (1, 9–11). The treatment of pediatric patients is strictly indicated in specialized centers and is mostly based on recommended treatment protocols for adult patients (12).

The aim of this analysis is to describe the characteristics of pediatric patients with multiple sclerosis who started their treatment with disease-modifying drugs in 2013–2020, with data obtained from the Czech National Registry of MS patients (ReMuS).

MATERIALS AND METHODS

Design

This was a retrospective analysis of the Czech National Registry of MS patients ReMuS. The details about the ReMuS were described in another research study, please consult Pavelek et al. (2).

Analyzed Population

For the purpose of this analysis, a group of patients with multiple sclerosis who started their first treatment with DMD drugs between 2013 and 2020 and were <18 years old on the date of starting this therapy was selected from the ReMuS registry. Three time points were defined throughout the follow-up period: the BL (first line; treatment start of a 2-yr follow-up); M12 (follow-up at 12 months after the introduction of treatment); and M24 (follow-up at 24 months after the introduction of treatment). Patients were divided into three groups: group A are patients who started their first DMD therapy between 2013 and 2018 and were younger than 16 years (i.e., had at least 2 years of follow-up before the age of 18). Group B are patients who received their first DMD therapy between 2013 and 2018 and were both older than 16 years and younger than 18 years (i.e., had reached adulthood during the 2-year follow-up period). Group C are patients who started treatment in 2019 or 2020 and therefore do not have the full two-year follow-up period covered.

Endpoints

The aim of this analysis is to describe the characteristics of pediatric patients with multiple sclerosis who started their treatment with disease-modifying drugs between 2013 and 2020. The molecules monitored are as follows: (a) state-approved molecules (i.e., in the Czech Republic) for pediatric use: fingolimod and teriflunomide; (b) drugs approved for use in adults with MS: interferon beta-1-a, peginterferon beta-1-a, interferon beta-1-b, glatiramer acetate; dimethyl fumarate; natalizumab; (c) an off-label molecule: rituximab. In addition to the basic demographic data, the following data were analyzed. In terms of relapses that were observed in group A and B, these were ARR and severity of relapses and time to the first relapse in the treatment setting.

In this analysis the EDSS obtained may not have been captured accurately at the three time points (BL, M12, M24) and therefore had to be extrapolated in the follow-up period. Disability was monitored only for group A, and from several perspectives: changes in EDSS, including absolute changes according to their magnitude, were monitored; time to the confirmed EDSS progression was analyzed; and the relationship between disability and severity of the first relapse was determined. For the purpose of calculating the time to the confirmed progression, the EDSS at the date of the introduction of the first DMD treatment was rounded to the nearest valid EDSS value. For example, the EDSS on the day of treatment onset was calculated as follows: the nearest measured EDSS before the date of the onset of the first DMD treatment and the nearest measured EDSS after the date of the onset of the first DMD treatment were connected using a straight line. Subsequently, the EDSS at the treatment start date is estimated using this straight line. In addition, a check was

also made to ensure that the EDSS measurement was not too far from the observation date (e.g., from the start of the first DMD treatment). If there was more than a year between EDSS measurements, then the EDSS that was within 90 days (inclusive) of the observation date (for example, from the start of the 1st DMD treatment) was used. If no such value was available, the EDSS was considered missing and the value was therefore not further processed. The confirmed progression is defined as a change in EDSS of 1.5 points or more for patients with a first line EDSS of 0 and 1 point for patients with a first line EDSS of 1 or more if the change persists for at least 6 months. The analysis was performed only for group A. Time to the confirmed progression was calculated using the Kaplan-Meier estimator. As the confirmed progression did not occur in a sufficient number of patients to estimate the median time to confirmed progression, the upper quartile was estimated.

Regarding the therapy, the initial DMD therapy was analyzed in detail according to the active molecules. It was also observed how many patients discontinued the first DMD therapy and after how long. Adverse effects and reported reasons for changing/terminating the first DMD therapy were monitored simultaneously. Subsequently, changes in lines of therapy in patients who discontinued their first DMD therapy were also monitored.

Statistical Analysis

The aim of this analysis is to describe the characteristics of pediatric patients with multiple sclerosis who started their treatment with disease-modifying drugs or other off-label drugs (fingolimod, teriflunomide, interferon beta-1-a, peginterferon beta-1-a, interferon beta-1-b, glatiramer acetate, dimethyl fumarate, natalizumab, rituximab, alemtuzumab, cladribine or ocrelizumab.) between 2013 and 2020. In addition to the basic demographic data, the following data were analyzed. In terms of relapses that were observed in group A and B, these were ARR and severity of relapses and time to the first relapse in the treatment setting. Disability was monitored only for group A, and from several perspectives: changes in EDSS, including absolute changes according to their magnitude, were monitored; time to the confirmed EDSS progression was analyzed; and the relationship between disability and severity of the first relapse was determined. Regarding the therapy, the initial DMD therapy was analyzed in detail according to the active molecules. It was also observed how many patients discontinued the first DMD therapy and after how long. Adverse effects and reported reasons for changing/terminating the first DMD therapy were monitored simultaneously. Subsequently, changes in lines of therapy in patients who discontinued their first DMD therapy were also monitored.

RESULTS

Demography of the Analyzed Groups

The analysis included 134 pediatric patients who were divided into 3 groups (see Methods–Study population; **Table 1**). There were 44 patients in group A (yellow), 57 patients in group B (green), and 33 patients in group C (blue). Of the 134 patients studied, 36 (26.9%) were boys and 98 (73.1%) were girls. A more detailed analysis by group revealed that 29.5% of boys and 70.5% of girls were in group A, with a mean age of 14.6 and 14.1 years at the time of the introduction of the first DMD treatment. In group B, there were 24.6% of boys and 75.4% of girls with a mean age of 17 and 17.2 years at the introduction of the first DMD treatment. Group C included 27.3% of boys and 72.7% of girls who were 15.7 and 16.1 years old, respectively, at the time of the introduction of the first DMD treatment.

Relapses

The average annual relapse rate was analyzed only for groups A and B, as the entire 2-year period was monitored and the relapse rates can therefore be compared (**Table 2**). During the follow-up period, 27 (61.4%) patients in group A did not experience any relapse, while in group B, 31 (54.4%) patients did not have any relapse. Time to the first relapse was calculated as the difference between the date of the first relapse after the introduction of the follow-up and the date of the introduction of the follow-up (i.e., the introduction of the first DMD treatment) and calculated using the Kaplan-Meier estimator. A significant difference ($p = 0.035$) was found in the data obtained, with half of the boys relapsing within 42.6 months, whereas half of the girls relapsed within 26.7 months from the introduction of the first DMD treatment.

Disability

The disability was analyzed only in group A. The patients for whom the EDSS value could not be determined at the time of the start of the follow-up were excluded from the disability analysis ($N = 2$). The mean EDSS was 1.47 at BL, 1.5 at M12 and 1.48 at M24.

It can be assumed that 75% of patients will not experience the confirmed EDSS progression within 54.7 months from the introduction of treatment. In addition, the relationship between the first relapse and disability was also examined in group A (**Table 3**). Of the 44 patients studied, 42 patients were further considered for the analysis, as 2 patients had not relapsed before the recorded introduction of treatment.

Therapy

For all groups, the first DMD treatment was analyzed by looking at the number of patients in each group relative to the active molecule and the mean time to the treatment completion (**Table 4**). The change in the strength of the drug was also included in the DMD drug discontinuation and change. If we do not consider this change in the strength of the drug as a discontinuation of the therapy, then 77% of patients in group A, 68% of patients in group B and 24% of patients in group C would have discontinued the therapy. The mean time to the completion of the first DMD treatment would be 27.2 months in group A, 20.2 months in group B and 7.4 months in group C. The uncompleted therapies were not included in the mean time to the completion.

For the patients who were not given an identical drug of different intensity after the discontinuation of the first line DMD, the reasons for stopping the treatment were monitored. There

TABLE 1 | Demographic characteristics of the pediatric population at the time of the introduction of DMD treatment (by age and by year).

Age	Introduction of the first DMD treatment								Total
	2013	2014	2015	2016	2017	2018	2019	2020	
8	0	1	1	0	0	0	0	0	2
9	0	0	1	0	0	0	0	0	1
10	0	0	1	0	1	0	0	0	2
11	0	0	0	0	0	0	0	1	1
12	0	0	1	0	1	1	1	0	4
13	0	0	2	1	0	1	0	2	6
14	1	1	2	2	4	0	1	1	12
15	6	5	3	2	5	1	4	4	30
16	6	2	3	3	4	6	7	4	35
17	6	6	2	7	5	7	4	4	41
Total	19	15	16	15	20	16	17	16	134

Green colour - patients on injectables; Blue colour - patients on newer DMT; Yellow colour - patient without DMT.

TABLE 2 | Average annual relapse rate (AAR) and the characteristics of relapses during the follow-up period.

Group	Number of patients	ARR	Relapses in the monitored period (24 months)			
			Total	Mild	Moderate	Severe
A	44	0.352	31	45.2%	51.6%	3.2%
B	57	0.404	46	56.5%	43.5%	0.0%

were a total of 28 such patients in group A, of whom 21 discontinued due to efficacy, 5 due to poor tolerability of the drug, 1 due to adherence/comfort, and one due to an unknown reason. The mean time to discontinuation was 22.4 months for discontinuation due to efficacy and 18.1 months for tolerability. In group B, the most common reasons for discontinuation of the first DMD treatment were efficacy (21 patients) and tolerability (7 patients). The mean time to the treatment discontinuation was 19.8 months for efficacy and 18.3 months for tolerability. Only one adverse event (immediate post-injection reaction) was recorded, and this was for group B and glatiramer acetate 20 MG. The treatment was discontinued after less than three (specifically 2.76) months.

Changes in the treatment lines in the patients who completed their first DMD treatment are analyzed only in group A, given that 89% of patients in this group discontinued their first DMD treatment (**Table 5**). The first DMD drug was a first line drug for 38 patients. Of these, 22 patients switched to another first line drug after completing this treatment, 15 patients switched to a second line drug after completing the first DMD treatment, and 1 patient did not start any additional DMD drug.

DISCUSSION

Research studies reveal that ∼3–5% of all individuals diagnosed with MS will experience their first attack before the age of 16 (13–15). Our analysis expands these findings, showing that of the 134 pediatric patients studied, 44 were aged 0–15 years and 57 were aged 16–17 years. Even the two youngest patients started their first DMD at the age of eight in 2014 and 2015. One nine-year-old and one ten-year-old patient also started their first DMD treatment in 2015. However, the mean age at the date of the introduction of the first DMD treatment is 15.89 years, and gender does not play a role (girls/boys −15.90/15.80).

Childhood MS is considered to be a highly active form with more frequent relapses (2–3 times more frequent relapses than adults with early-onset MS), lesions early in the disease, and worse cognitive and physical impairment earlier in life (16, 17). Research shows that the increased frequency of relapses persists for about the first 6 years of the disease (18). Interestingly, children recover from relapses faster than adults, on average 4 weeks compared to 6–8 weeks for adults (19). Our analysis shows that in group A, 31 relapses were recorded in the two-year follow-up period, i.e., the ARR is 0.352 relapses/year. This means one relapse per year for approximately one in three patients. Moderate (51.6%) and mild (45.2%) relapses were predominant in the study period. In group B, 46 relapses were recorded in the two-year follow-up period, i.e., ARR is 0.404 relapses/year and mild (56.5%) and moderate (43.5%) relapses were prevalent. Thus, it can be said that the frequency of relapses is higher in group B than in group A, but in this latter group they are more severe in nature.

In pediatric MS patients, there is a trend that the disability occurs at a younger age (6, 20). At the time of the start of the follow-up period, the mean EDSS for Group A was 1.47 and appeared not to have changed significantly over the follow-up period. On the basis of the available data, we assume that 75%

TABLE 3 | Relationship between the first relapse and disability (Group A).

Disability of the first relapse before the introduction of DMD treatment	Number of patients	EDSS at the time of the introduction of the first DMD treatment				EDSS in M12				EDSS in M24			
		Average	SD	Median	NA	Average	SD	Median	NA	Average	SD	Median	NA
Mild	12	1.46	0.891	1.5	0	1.65	0.906	1.8	0	1.52	0.842	1.5	0
Moderate	30	1.47	0.675	1.5	0	1.43	0.807	1.5	1	1.46	0.608	1.5	0

TABLE 4 | Overview of the first DMD treatment for all groups by active molecules.

Group	The first DMD treatment	Number of patients	Completed		
			Number	Percentage	Mean time to the completion of treatment (months)
A	Glatiramer-acetate 20 MG	20	19	95%	24.83
	Interferon beta-1a 44 MCG	9	7	78%	15.58
	Glatiramer-acetate 40 MG	7	5	71%	19.49
	Interferon beta-1a 30 MCG	4	4	100%	38.85
	Interferon beta-1a 22 MCG	2	2	100%	24.25
	Peginterferon beta-1a	1	1	100%	13.34
	Natalizumab	1	1	100%	36.17
B	Glatiramer-acetate 40 MG	17	8	47%	20.33
	Interferon beta-1a 44 MCG	14	11	79%	23.09
	Glatiramer-acetate 20 MG	13	13	100%	11.42
	Interferon beta-1a	6	4	67%	28.62
	Peginterferon beta-1a	4	4	100%	13.4
	Interferon beta-1b	1	1	100%	16.43
	Fingolimod	1	1	100%	27.63
	Natalizumab	1	-	0%	-
C	Interferon beta-1a 44 MCG	10	2	20%	233%
	Glatiramer-acetate 40 MG	9	2	22%	830%
	Peginterferon beta-1a	7	2	29%	5.59
	Interferon beta-1a	3	-	0%	-
	Glatiramer-acetate 20 MG	2	2	100%	15.82
	Teriflunomid	1	-	0%	-
	Interferon beta-1a 22 MCG	1	1	100%	6.41
A		44	39	89%	23.89
B		57	42	74%	18.5
C		33	9	27%	7.83

of patients will not experience a confirmed progression of EDSS within 54.7 months of starting the treatment.

It is quite common clinical practice that DMDs that are registered for adult patients are prescribed for pediatric patients. The first-choice treatment is interferon beta-a and glatiramer acetate. This was confirmed in our analysis of the ReMuS registry data. For example, in group A, glatiramer acetate (45.5% of patients) and interferon beta-a (20.5% of pediatric patients) were the most frequent first DMD drugs. The safety and efficacy of these drugs have been demonstrated in small retrospective studies, case studies and unblinded controlled trials (21, 22). However, these molecules still need to have an official approval by state authorities. A lack of tolerance or continued disease progression despite these therapies may require the use of other therapies.

In our analysis, 77% of patients in group A, 68% of patients in group B, and 24% of patients in group C discontinued the first DMD treatment. The mean time to the discontinuation of the first DMD treatment would be 27.2 months in group A, 20.2 months in group B and 7.4 months in group C. In group A, 21 patients discontinued treatment due to low efficacy, and the mean time to discontinuation for this reason was 22.4 months. In group B, low efficacy was also the most common reason for discontinuation (21 patients) and the mean time to discontinuation for the same reason was 19.8 months. However, as mentioned above, none of the pediatric patients should be

TABLE 5 | Summary of changes in the treatment lines in patients who have completed their first DMD treatment.

1. DMD	2. DMD	Number	Percentage	Average delay (months)
First line	First line	11	28.21%	0.06
First line	Only the change in in the strength of the drug	11	28.21%	0.03
First line	Escalation line	15	38.46%	0.55
First line	Unprescribed	1	2.56%	NA
Escalation line	Escalation line	1	2.56%	2
Total		39	100.00%	

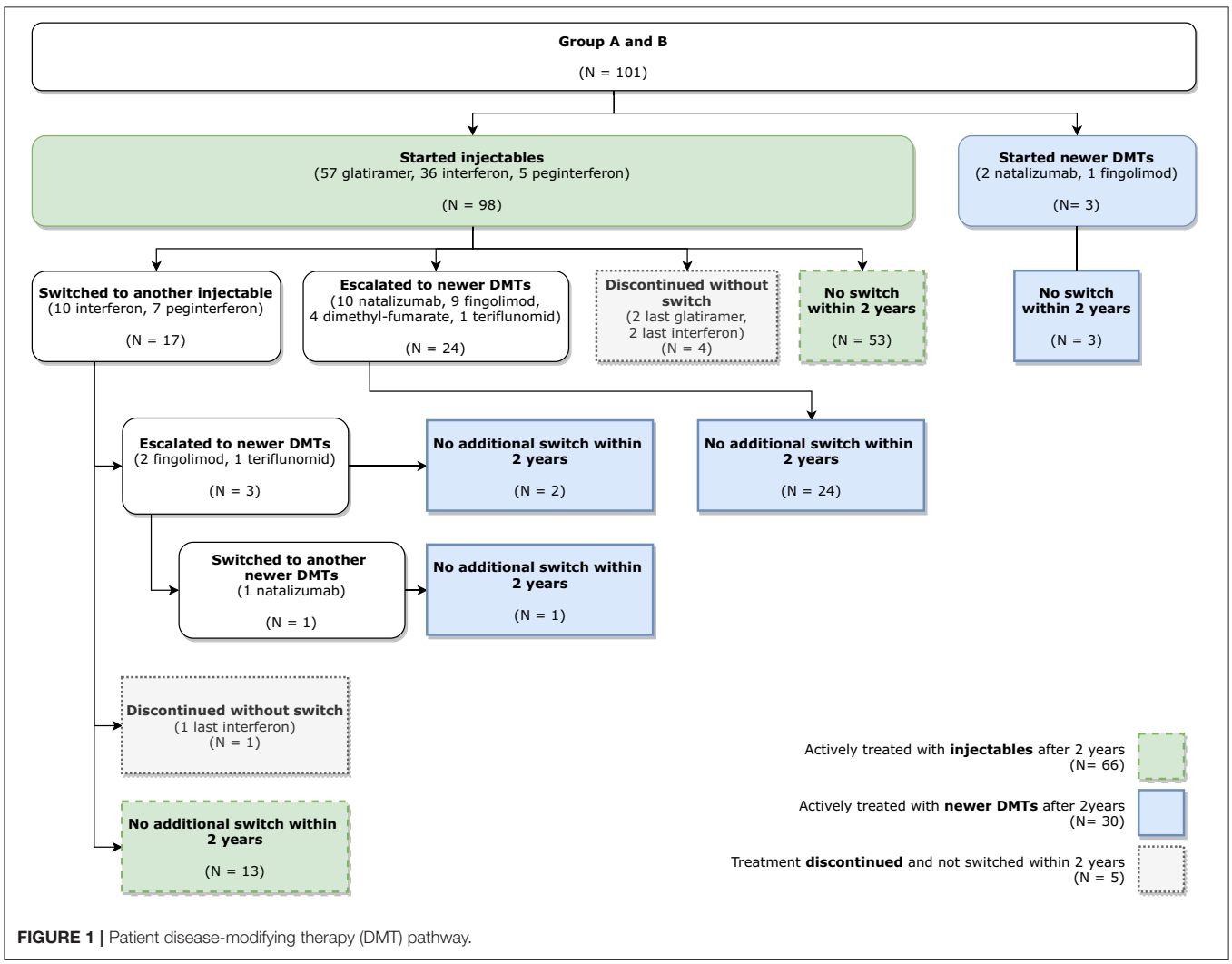

FIGURE 1 | Patient disease-modifying therapy (DMT) pathway.

left untreated; 28.21% (11 patients) were switched to another first-choice medication, 28.21% (11 patients) had a change in strength of the drug, and 38.46% (15 patients) were put on an escalation line.

As **Figure 1** below shows, out of 101 patients, 56 patients (53 with first line drug treatment and 3 with second line drug treatment) receive the same treatment after 2 years = 55.45%. In addition, out of 98 patients who started on first line drug treatment, 27 (27.55%) patients switched to the second line drug treatment within 24 months. Similar findings were confirmed by a UK study (12).

In conclusion, it should be stated that this certainly does not exhaust all treatment options and other therapies for MS, including dimethyl fumarate or rituximab that are currently under investigation in clinical trials for the treatment of pediatric MS (5, 23). For example, in a cohort study comparing the first-line treatment with novel DMDs in children with MS, the newer

DMDs provided better disease control, but further studies are still needed, particularly on safety (24). For completeness, it can be added that due to the highly active nature of pediatric MS, some authors recommend starting the treatment with novel DMTs with expected higher efficacy (17).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors have equally contributed to drafting, writing, and revising this article. They all have approved its final version.

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Ketogenic Diet Therapy for Drug-Resistant Epilepsy and Cognitive Impairment in Children With Tuberous Sclerosis Complex

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Objective: Tuberous sclerosis complex (TSC) is a rare disease with a high risk of epilepsy and cognitive impairment in children. Ketogenic diet (KD) therapy has been consistently reported to be beneficial to TSC patients. In this study, we aimed to investigate the efficacy and safety of KD in the treatment of drug-resistant epilepsy and cognitive impairment in children with TSC.

Methods: In this multicenter study, 53 children (33 males and 20 females) with drug-resistant epilepsy or cognitive impairment caused by TSC were retrospectively recruited from 10 hospitals from January 1, 2010, to December 31, 2020. Intention-to-treat analysis was used to evaluate seizure reduction and cognition improvement as outcomes after KD therapy.

Results: Of the 53 TSC patients included, 51 failed to be seizure-free with an average of 5.0 (range, 4–6) different anti-seizure medications (ASMs), before KD therapy. Although the other two patients achieved seizure freedom before KD, they still showed psychomotor development delay and electroencephalogram (EEG) abnormalities. At 1, 3, 6, and 12 months after the KD therapy, 51 (100%), 46 (90.2%), 35 (68.6%), and 16 patients (31.4%) remained on the diet therapy, respectively. At these time points, there were 26 (51.0%), 24 (47.1%), 22 (43.1%) and 13 patients (25.5%) having ≥50% reductions in seizure, including 11 (21.6%), 12 (23.5%), 9 (17.6%) and 3 patients (5.9%) achieving seizure freedom. In addition, of 51 patients with psychomotor retardation, 36 (36 of 51, 70.6%) showed cognitive and behavioral improvements. During the KD therapy, no serious side effects occurred in any patient. The most common side effects were gastrointestinal disturbance (20 of 53, 37.7%) and hyperlipidemia (6 of 53, 11.3%).

The side effects were gradually relieved after adjustment of the ketogenic ratio and symptomatic treatment.

Conclusion: KD is an effective and safe treatment for TSC-related drug-resistant epilepsy and cognitive impairment in children. KD can reduce seizure frequency and may potentially improve cognition and behavior.

Keywords: tuberous sclerosis complex, comorbidity, drug-resistant epilepsy, cognitive impairment, ketogenic diet, multi-center clinical trial, children

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal-dominant multi-system neurocutaneous syndrome characterized by hamartomas involving the skin, central nervous system, heart, lungs, kidneys, and other organs. The estimated incidence of TSC is between 1:6000 and 1:10,000 in live births. TSC is mainly caused by mutations in *TSC1* at 9q34 or *TSC2* at 16p13 (1). The nervous system manifestations of TSC mainly include epilepsy, cognitive impairment, developmental delay, and other neurological defects, among which epilepsy is the most common (2, 3). In TSC patients, the incidence of epilepsy is 80–90%, with drug-resistant epilepsy accounting for 55–62% (4, 5). Mutations in the *TSC1* or *TSC2* gene can lead to the over-activation of the mammalian target of rapamycin (mTOR) signaling pathway, resulting in subcortical tubers, which are the leading cause of seizures in TSC (6). Moreover, multidrug-resistant proteins and related genes, such as multidrug resistance type 1 gene and multidrug resistance-associated protein-1 transporters, are widely expressed in subcortical tubers (7, 8).

Tuberous-sclerosis-associated neuropsychiatric disorders (TAND) is an umbrella term encompassing the full range of neurodevelopmental, behavioral, psychiatric, and psychosocial manifestations associated with TSC (9). Almost all patients with TSC would have some of these neuropsychiatric manifestations in their lifetime (10). The common neurodevelopmental disorders associated with TSC are autism spectrum disorders (ASDs) (40–50%) and attention deficit hyperactivity disorder (30–50%) (11–13). Tubers have traditionally been considered as the critical pathological substrate: the tubers can directly cause seizures and the tuber load correlates with intellectual disability and autism (14). Recently, studies in knockout mice with *TSC* gene inactivation specifically in glial cells have shown cell-autonomous effects within glia. Different types of glial cells have emerged as major contributors to TAND and other neurological phenotypes of the genetic disorder TSC (15).

The ketogenic diet (KD) is a high-fat, low-carbohydrate, adequate-protein diet, with additional adequate, balanced nutrients (16). KD was first used as a therapeutic method for epileptic seizures in 1921 and was introduced into China in 2004 (17). Current studies suggest that the KD can play an anti-epileptic role by inhibiting the over-activated mTOR signaling pathway and through other multi-target mechanisms involving neurotransmitters, brain energy metabolism, oxidative stress, and ion channels (18–20). Ketone bodies (KBs) have recently been reported to act as neuroprotective agents by increasing ATP levels and reducing the production of reactive oxygen species in neurological tissues, together with increased mitochondrial biogenesis, which may enhance the regulation of synaptic function (21). Moreover, increased brain ketone uptake is positively related to episodic memory, language, executive function, and processing speed (22). Although successful treatment of TSC-related epilepsy and cognitive improvement by KD has been reported in recent years, there is still a lack of sufficient data on seizure reduction and cognitive improvement, particularly multi-center data in children. In this study, we conducted a multi-center retrospective study to analyze the efficacy and safety of KD in the treatment of TSC-related drug-resistant epilepsy and cognitive impairment in children with TSC, to improve the prognosis in children with TSC.

Abbreviations: TSC, Tuberous sclerosis complex; KD, ketogenic diet; KBs, Ketone bodies; mTOR, mammalian target of rapamycin; ASM, anti-seizure medication; EEG, electroencephalogram; ASD, autism spectrum disorder; IQR, interquartile range (25–75 percentiles); IQ: intelligence quotient; DQ, developmental quotient.

MATERIALS AND METHODS

Patients

Fifty-three children with cognitive impairment or drug-resistant epilepsy caused by TSC were retrospectively enrolled. They received KD therapy at Shenzhen Children's Hospital, Shanghai Neuromedical Center, Second Affiliated Hospital of Xi'an Jiaotong University, Guangdong 999 Brain Hospital, Children's Hospital of Fudan University, Shandong University Cheeloo College of Medicine, Peking University First Hospital, People's Hospital of Ningxia Hui Autonomous Region, Jiangxi Provincial Children's Hospital, or Children's Hospital Affiliated to Zhengzhou University, from January 1, 2010, to December 31, 2020. The patients were recruited according to the following inclusion criteria. First, the patients should meet the diagnostic criteria of TSC following the International TSC Consortium in 2012 and 2018 (23, 24); that is, patients having a pathogenic variant in *TSC1* or *TSC2*. For patients not having genetic test reports, clinical criteria (the presence of two major criteria or one major and two minor criteria) of TSC should be met. Second, the patients should be diagnosed with drug-resistant epilepsy, i.e., failure to achieve sustained seizure freedom after adequate trials of two tolerated, appropriately chosen anti-seizure medications (ASMs) schedules (whether as monotherapies or in combination) (25). Patients diagnosed with drug-resistant epilepsy may have failed to respond to epilepsy surgery or did not receive surgery evaluation before KD. Besides, TSC patients with West syndrome

or infantile spasms who failed to respond to adrenocorticotropic hormone and another ASM were also recruited. Third, two patients still having an abnormal electroencephalogram (EEG) discharge or global development delay after administration of two or more kinds of ASM schedules were also included. Abnormal EEG refers to EEG with the presence of epileptic discharges. Global development delay was defined as motor and mental development lagging behind normal children of the same age. The intelligence quotient (IQ) and developmental quotient (DQ) tests were not commonly conducted, only 14 patients had the tests before KD, and four had the tests after KD. Development delay was diagnosed mainly based on a thorough clinical history and a detailed physical examination by a trained specialist. The 51 patients were diagnosed with psychomotor development delay at KD initiation, and three of them were diagnosed with ASD. At last, the patients were 0.4–14 years of age and had a KD treatment and follow-up time of $\geq$1 month. To avoid the influence of possible late response, we used the efficacy at 3 months after KD to the analysis of the KD's effect on reducing seizures and improving cognition and behavior. The exclusion criteria for patients were: (1) patients having severe diseases of vital organs, such as severe hepatic, renal or cardiac insufficiency, and immune deficiency; and (2) children lacking major medical records after KD and whose parents disagreed to participate in this study.

Study Design

Implementation of KD

The patients initiated KD as inpatients. 50 patients initiated the classic KD, of whom 30 patients initiated KD with a ketogenic ratio (the ratio of fat to carbohydrate and protein) of 2:1, 15 with 4:1, 4 with 3:1, and 1 with 2.5:1. While the other 3 patients were treated with a modified Atkins diet (MAD), of which the lipid-to-nonlipid ratio was not strict, ranging from 1:1 to 1.5:1, but carbohydrates were kept at 10–20 g/day. During the maintenance period of KD therapy, 43 patients continued the classical KD, in which 29 with 1.5–4 :1, 9 with 4:1, 4 with 2:1, and 1 with 3:1 ketogenic ratio, while 10 patients received the MAD. Before KD initiation, the dietitians designed the KD meal plans based on the patients' food habits and body weights. During the KD treatment, other diets were stopped, and the original ASMs did not change within 3 months after KD initiation. Meanwhile, potassium citrate, multi-vitamins, essential minerals, and calcic agent without sucrose and lactose were supplemented in the daily diet. For the first week of KD, all patients were inpatients and were closely monitored for any possible adverse effect, and their parents or caregivers were trained on how to calculate the dietary ratio and make ketogenic foods at home. After discharge, the patients were asked to make daily records of seizures, calories, KD foods, and side effects. At 1, 2, 3, and 6 months after KD, the patients revisited the hospitals. After 6 months of KD, the revisit interval was extended to half a year if the KD was effective. In addition, the dietitians followed up with the patients by telephone or WeChat monthly. In any emergency, patients could go to the nearest hospital or contact doctors and dietitians in the KD group.

Data Collection

A unified information collection form was developed to collect data on clinical manifestations, cognitive and behavioral status, ASMs, and seizure frequency before and after KD. Information on the age of KD initiation, dietary ketogenic ratio, duration, efficacy, and adverse effects of KD therapy was also collected *via* this form. The seizure types were classified following the 2017 International League against Epilepsy classification criteria (26). At KD initiation, the parents or caregivers received specialized training from neurologists on identifying the seizure type and frequency and assessing psychomotor developmental issues during the hospital stay. Correlations between TSC genotype and KD efficacy were assessed based on the results of whole-exome sequencing. Information of cortical tubers, subependymal nodules, and subependymal giant cell astrocytoma confirmed by magnetic resonance imaging (MRI) was also collected.

KD Efficacy Analysis

KD efficacy was evaluated in terms of seizure reduction and psychomotor development. For assessment of seizure reduction, baseline (Pre-KD) seizure frequency was recorded by parents or guardians 1 month before initiation of KD, defined as the total seizure frequency during the 1 month before the initiation of KD. Patients with $\geq$50% reduction in total seizures during a particular month after KD were defined as responders to KD. Seizure freedom after KD was defined as the absence of seizures for at least one month. As the developmental test is relatively scarce, only 14 patients underwent the test before KD, while 4 had the test after KD. The psychomotor improvement was assessed based on pediatric neurologists' examination and caregiver' evaluation at 3 months after KD. For development evaluation by family members, the evaluation was made based on language expression, instruction execution, and learning ability. There were three levels of cognitive and behavioral improvement: Grade I for obvious improvement, Grade II for no obvious change, and Grade III for regression in cognition and behavior. Patients with obvious improvement (Grade I) were defined as responders to KD.

KD Safety Analysis

Blood and urine routine examination, liver and kidney function, blood electrolytes, blood lipids, gallbladder, and urinary system color ultrasound examinations were conducted at baseline, and at 1, 3, 6, and 12 months after KD. In addition, any adverse clinical events presumed to be related to KD were recorded.

Data Analysis

We used intention-to-treat analysis to assess the outcomes in terms of seizure reduction, development, and cognition improvement. The Chi-square test was used to analyze the effects of sex and cortical tubers on the efficacy of KD. Fisher's exact probability method was used to analyze the effects of seizure type, genotype, and dietary ketogenic ratio on the efficacy of KD. The nonparametric test was used to analyze the effects of the age at epilepsy onset, the age at KD initiation, or the duration of epilepsy before KD. Paired *t*-test was used to analyze the change of ASMs pre and after KD. Comparisons were considered

statistically significant when p-values were below 0.05 in a univariate test or below corrected p-value (corrected p-value = 0.05/n, n is the times of comparisons) by Bonferroni in multiple univariate tests. Data analyses were performed using the IBM SPSS statistics 25.0 software.

Ethics Approval, Clinical Registrations, and Patient Consents

This study was approved by the Ethics Committee of Clinical Research of Shenzhen Children's Hospital (permission number, 2021010). The study was registered with the Chinese Clinical Trial Registry, and the registration number is ChiCTR2100047909. All guardians of patients consent for this retrospective analysis and publication of information relating to them.

RESULTS

Patient Characteristics

Demographics and epileptic characteristics of the included patients are presented in **Table 1**. There were 33 males and 20 females. Thirty-four patients had genetic testing. *TSC1* gene mutation was found in 6 patients and *TSC2* in 24 patients, 4 with negative results. Fifty-one patients were diagnosed with psychomotor retardation. The mean age of epilepsy onset was 6.0 (4–16) months, and the mean duration of epilepsy before KD was 22.7 (12–41) months. Each patient suffered 2.0 (1–3) types of seizures on average. In addition, 22 patients (41.5%) were diagnosed with West syndrome, one (1.9%) with Lennox-Gastaut syndrome. There were 30 patients (56.6%) diagnosed with non-syndromic epilepsies, in whom 17 patients with focal onset, six patients with tonic seizures, five patients with epileptic spasms, one patient with the tonic-clonic seizure, and another patient with myoclonic seizure. An average of 5.0 (4–6) ASMs were used among the 53 patients before KD, two 2 of them experienced seizure freedom before KD initiation but had psychomotor development retardation and EEG abnormalities. A total of 29 patients (54.7%) were treated with mTOR inhibitors, three patients (5.6%) with Everolimus, and 26 patients (49.1%) with Sirolimus. The mean age at KD initiation was 40.0 (20–57) months, and the mean duration of KD was 34.7 (17.4–56.46) weeks, by December 31, 2020.

TABLE 1 | Patient characteristics.

Characteristics	Total ($n = 53$)
Gender, *n* (%)	
Male	33 (62.3%)
Female	20 (37.7%)
TSC gene, *n* (%)	
TSC1 gene mutation	6 (11.3%)
TSC2 gene mutation	24 (45.3%)
TSC genetic test with negative results	4 (7.5%)
Not tested	19 (35.8%)
Characteristics of head MRI, *n* (%)	
Cortical tubers	35 (66.0%)
Subependymal nodules	39 (73.6%)
Subependymal giant cell astrocytoma	1 (1.9%)
Age at onset of epilepsy, median (IQR[a]), months	6.0 (4–16)
Psychomotor development at KD initiation, *n* (%)	
Normal	2 (3.8%)
Delayed	51 (96.2%)
Seizure type at KD initiation, *n* (%)	
Focal onset	20 (37.7%)
Generalized onset	
Epileptic spasms	19 (35.8%)
Tonic	7 (13.2%)
Tonic-clonic	2 (3.8%)
Clonic	1 (1.9%)
Myoclonic	1 (1.9)
Atonic	1 (1.9%)
Seizure-free	2 (3.8%)
Epilepsy syndrome prior to KD, *n* (%)	
West syndrome	22 (41.5%)
Lennox-Gastaut syndrome	1 (1.9%)
Non-syndromic epilepsy	30 (56.6%)
Number of ASMs used prior to KD, median (IQR)	5.0 (4–6)
Epilepsy duration prior to KD, median (IQR), months	22.7 (12–41)
Age at KD initiation, median (IQR), months	40.0 (20–57)
Duration of KD, median (IQR), weeks	34.7 (17.4–56.46)

[a] *IQR: interquartile range (25–75 percentiles).*

Response to KD

The Response Rate to KD in Terms of Seizure Reduction, Overall and at Each Time Point

For the 51 patients who did not achieve seizure freedom after use of ASMs, 51 (100%), 50 (98.0%), 46 (90.2%), 35 (68.6%), and 16 (31.4%) patients of them remained on the diet at 1, 2, 3, 6 and 12 months after KD, respectively, and 26 (51.0%), 23 (45.1%), 24 (47.1%), 22 (43.1%), and 13 (25.5%) of them experienced ≥50% reduction of seizures at the corresponding time points. In addition, 11 (21.6%), 13 (25.5%), 12 (23.5%), 9 (17.6%), and 3 (5.9%) patients achieved seizure freedom at 1, 2, 3, 6, and 12 months after KD, respectively (**Table 2**).

Efficacy in Improving Cognition and Behavior

Before the initiation of KD, 51 of the 53 patients were diagnosed with psychomotor retardation, and two of the 51 patients had achieved seizure freedom before KD. At 3 months after KD, 36 patients (36/51, 70.6%) had obvious improvement in cognition and behavior assessed by neurologists' physical examination and parents' comprehensive judgment based on language expression, instruction execution, and learning ability (**Table 3**). In particular, at 3 months after KD, 3 patients (3/51, 5.9%), who were diagnosed with ASD before KD, were reported with an obvious improvement in behavior assessed by caregivers and doctors based on concentration and learning abilities, and social behavior and interactions of children.

Of the 49 patients diagnosed with psychomotor retardation and had seizure onset at the initiation of KD, 34 (34/49, 69.4%)

TABLE 2 | Response rate in seizure reduction at 1, 2, 3, 6, and 12 months after KD (*n*, %).

Reduction in seizures	1 month after KD	2 months after KD	3 months after KD	6 months after KD	12 months after KD
Seizure-free	**11 (21.6)**	**13 (25.5)**	**12 (23.5)**	**9 (17.6)**	**3 (5.9)**
Reduced by 90∼99%	1 (2.0)	0	2 (3.9)	3 (5.9)	4 (7.8)
Reduced by 50∼90%	14 (27.4)	10 (19.6)	10 (19.6)	10 (19.6)	6 (11.8)
Reduced by<50%	13 (25.5)	15 (29.4)	12 (23.5)	6 (11.8)	0
No reduction	12 (23.5)	12 (23.5)	10 (19.6)	7 (13.7)	3 (5.9)
Total	51 (100)	50 (98.0)	46 (90.2)	35 (68.6)	16 (31.4)

TABLE 3 | Psychomotor improvement at 3, 6, and 12 months after KD (*n*, %).

Items	3 months after KD	6 months after KD	12 months after KD
Number of patients remaining on KD	48 (94.1)	36 (70.6)	20 (39.2)
Psychomotor improved	**36 (70.6)**	29 (56.9)	17 (33.3)

had cognition and behavioral improvement at 3 months after KD. In addition, 26 patients (26/34, 76.5%) experienced ≥50% reduction in seizures, including 10 patients (10/34, 29.4%) with seizure freedom. As for ASMs, the 29 patients with psychomotor improvement at 6 months after KD, using 3.17±1.07 different ASMs, compared to 3.38±1.21 at the initiation of KD (P=0.161). The 17 patients with psychomotor improvement at 12 months after KD, using 3.06 ± 1.34 different ASMs, compared to 3.24 ± 1.39 at the initiation of KD (P = 0.455). Ten patients (10/36, 27.8%) continued to use mTOR inhibitors from KD initiation to 6 months after KD.

Comparison of Seizure Reduction Between the Responders and the Non-responders

For the 51 patients with seizure onset at the initiation of KD, at 3 months of KD, 46 patients remained on KD, including 24 responders and 22 non-responders. There was no statistical significant difference in age of epilepsy onset (P = 0.349), age of KD initiation (P = 0.531), epilepsy duration before KD (P=0.138), cortical tubers (P = 0.686), or sex (P = 0.958) between the two groups.

We also assessed the correlation between TSC genotype and KD efficacy. At 3 months after KD, there were two patients (2/6, 33.3%) with *TSC1* gene mutation and nine patients (9/22, 40.9%) with *TSC2* gene mutation experienced ≥50% reduction in seizures, respectively. There was no statistical significant difference in KD efficacy among groups with *TSC1* and *TSC2* gene mutations (P = 0.622).

KD Efficacy in Groups of Different Seizure Types at KD Initiation

In the first month of KD, none of the patients withdrew from KD. The proportions of KD responders in children with seizure types of focal onset, epileptic spasms, tonic, and clonic seizures were 60.0, 52.6, 42.9, and 100.0%, respectively. At the same time, four patients (20.0%) with the focal onset and seven patients (36.8%) with epileptic spasms experienced seizure freedom. However, two patients with generalized tonic-clonic seizures, one patient with myoclonic seizures, and one patient with tonic seizures did not significantly benefit. None of the patients with tonic or clonic seizures could achieve seizure free. There was no significant difference in KD efficacy among patients with different seizure types at KD initiation (P = 0.490).

KD Efficacy in Patients With Different Ketogenic Ratios

At the first month of KD, the proportions of responders with ketogenic ratios of 2:1, 4:1, 3:1, 1:1, and 2.5:1 were 60.0, 46.2, 0.0, 33.3, and 100.0%, respectively. There were 6 (20.0%) and 5 patients (38.5%) with seizure freedom for at least 1 month in the 2:1 and 4:1 groups, respectively, while none achieved seizure freedom in the 3:1, 1:1, and 2.5:1 groups (**Table 4**). There was no significant difference in the efficacy of KD among groups of different ketogenic ratios at initiation (P = 0.133).

At the third month of KD, the proportions of responders in children with maintenance ketogenic ratios of 1.5–4:1, 4:1, 2:1, 3:1 and 1–1.5:1 were 46.4%, 25.0%, 75.0%, 0.0%, and 60.0%, respectively. There were 5 (17.9%), 2 (25.0%), 1 (25.0%), and 4 patients (40.0%) with seizure freedom in the groups with ketogenic ratios of 1.5–4:1, 4:1, 2:1, and 1–1.5:1, respectively (**Table 5**). There was no significant difference in KD efficacy among the groups of different ratios during the maintenance period (P = 0.493).

Safety of KD Therapy

During the KD treatment, none of the 53 patients reported severe side effects. The main side effect was gastrointestinal disturbance (20/53, 37.7%), including abdominal pain, vomiting, diarrhea, and constipation. The second most common side effect was hyperlipidemia (6/53, 11.3%), leading to total cholesterol level ≥5.18 mmol/L (200 mg/dl) and/or triglyceride level ≥1.70 mmol/L (150 mg/dl) in plasma. The third most common side effect was infection (3/53, 5.7%), including one patient with pneumonia caused by severe coughing and two patients with upper respiratory tract infection. Other side effects included kidney stones (2/53, 3.8%), slow growth (2/53, 3.8%), hypoproteinemia (1/53, 1.9%), and hyperuricemia (1/53, 1.9%) (**Table 6**). All of the above side effects were relieved after adjustment of the ketogenic ratio and symptomatic treatment.

TABLE 4 | KD efficacy in groups of different ketogenic ratios at initiation.

Ketogenic ratio at KD initiation	Enrolled *n* (%)	Responded *n* (%)	No effect *n* (%)	Seizure freedom *n* (%)
2:1	30 (100.0)	**18 (60.0)**	12 (40.0)	**6 (20.0)**
4:1	13 (100.0)	**6 (46.2)**	7 (53.8)	**5 (38.5)**
3:1	4 (100.0)	0	4 (100.0)	0
1:1	3 (100.0)	**1 (33.3)**	2 (66.7)	0
2.5:1	1 (100.0)	**1 (100.0)**	0	0

TABLE 5 | KD efficacy in groups of different ketogenic ratios in the maintenance period.

Ketogenic ratio in the maintenance period	Enrolled *n* (%)	Remained *n* (%)	Responded *n* (%)	No effect *n* (%)	Seizure freedom *n* (%)
1.5∼4:1	28 (100.0)	26 (92.9)	**13 (46.4)**	13 (46.4)	**5 (17.9)**
4:1	8 (100.0)	6 (75.0)	**2 (25.0)**	4 (50.0)	**2 (25.0)**
2:1	4 (100.0)	4 (100.0)	**3 (75.0)**	1 (25.0)	**1 (25.0)**
3:1	1 (100.0)	1 (100.0)	0	1 (100.0)	0
1–1.5:1(MAD)	10 (100.0)	9 (90.0)	**6 (60.0)**	3 (30.0)	**4 (40.0)**

TABLE 6 | Adverse effects reported during the KD treatment.

Adverse effect	*n* (%)
No adverse effect	30 (56.6)
Gastrointestinal disturbance	**20 (37.7)**
Hyperlipidemia	**6 (11.3)**
Infectious disease	3 (5.7)
Kidney stones	2 (3.8)
Slow growth	2 (3.8)
Hypoproteinemia	1 (1.9)
Hyperuricemia	1 (1.9)

DISCUSSION

In our study, we evaluated the efficacy and safety of KD in the treatment of drug-resistant epilepsy and cognitive impairment related to TSC in children. The overall response rates in terms of seizure reduction at 1, 2, 3, 6, and 12 months after KD were 51.0, 45.1, 47.1, 43.1, and 25.5%, respectively. In addition, 36 of the 51 patients (70.6%) with psychomotor retardation exhibited obvious improvement of cognitive function after KD therapy.

The efficacy of KD for TSC-associated epilepsy has previously been evaluated in single-center or smaller sample-size studies. For example, Kossoff et al., (5) first reported that at 6 months after starting KD, 11 of 12 children with TSC (92%) experienced >50% reduction of seizures. Park et al. (27) analyzed 12 children with drug-resistant epilepsy related to TSC who received KD therapy and found that at 3 months of KD treatment, 10 patients (83.3%) had a >50% reduction of seizures. Youn et al. (28) also studied the long-term efficacy of KD for drug-resistant epilepsy in patients with TSC and found that at 3 months of KD, 21 of 31 patients (67.7%) had >50% reduction of seizures. All these studies suggest that KD may be effective in the treatment of drug-resistant epilepsy associated with TSC; however, more studies on the application of KD in this rare disease group are needed.

The findings of cognitive improvement in this multicenter study are similar to the results of a single-center study by Park et al. (27), which showed that in 12 patients with TSC-related epilepsy, four patients (33.3%) were "much improved" and five patients (41.7%) were "somewhat improved" after 3 months of KD. In particular, there are three patients (3/51, 5.9%) diagnosed with ASD before KD, were reported with an obvious improvement in behavior, after receiving KD therapy for a mean duration of 20.2 (17.37 to 26) weeks. Therefore, KD might be an effective therapy for cognitive impairment and ASD in TSC.

During KD therapy, KBs replace glucose to be the main brain fuel. This process benefits the potassium channel, which is sensitive to adenosine triphosphate, adenosine, and γ-aminobutyric acid (GABA) energy activity; increases the expression of brain-derived neurotrophic factor; expands energy reserves; and improves mitochondrial function, ensuring stabilization of neuron action potential (21, 29). Ketone substrates also improve the structural and functional synaptic plasticity and lead to the activation of the signaling pathway that reinforces neural bioenergy and resistance to oxidative stress (30, 31). The benefits of cognition can be translated into improvements in verbal and recognition memory, verbal fluency, executive function, and global cognition (21, 32, 33). Nevertheless, further research is needed to clarify the mechanisms by which KD improves cognition and behavior function of patients with developmental delay and ASD.

Although patients with psychomotor improvement did not reduce ASMs at 6 and 12 months after KD ($P = 0.161$ and $P = 0.455$, respectively), some patients with seizure freedom

did have a reduction in the burden of ASMs. In our study, 18 patients (18/51, 35.3%) experienced seizure freedom, and four of the 18 patients (4/51, 7.8%) tried to reduce the use of ASMs; among them, three patients (3/51, 5.9%) still maintained seizure freedom, but one patient (1/51, 2.0%) experienced seizure recurrence. In addition, one patient treated with KD who experienced >90% reduction of seizures maintained the state of >90% reduction of seizures after reducing ASMs. Therefore, KD is beneficial in some children with drug-resistant epilepsy related to TSC, when the use of ASMs is reduced, which is similar to the conclusion of Youn et al. (28). When KD treatment fails, surgery may be an alternative option, and one patient in our study chose surgery after KD failure.

Consistent with the report by Youn et al. (28), our study showed that there was no direct correlation between KD efficacy and seizure type at KD initiation, *TSC* gene mutation, or the interval from seizure onset to KD initiation. Meanwhile, the efficacy of KD in the treatment of drug-resistant epilepsy associated with TSC was not affected by the age of epilepsy onset, age at KD initiation, sex, cortical tubers, or the ketogenic ratio. However, Youn et al. (28) found that the ages at seizure onset and KD initiation were significantly earlier in patients who experienced a recurrence of seizures after reaching seizure freedom than in patients with sustained seizure freedom ($P = 0.005$ and $P = 0.005$, respectively). Furthermore, patients who experienced a recurrence of seizures after seizure freedom were treated with significantly more ASMs than patients with sustained seizure freedom ($P = 0.009$).

Another issue is the relationship between the ketogenic ratio and KD efficacy. In our study, there was no significant difference in KD efficacy either among groups of different ketogenic ratios at KD initiation ($P = 0.133$) or among groups of different ketogenic ratios during the maintenance period ($P= 0.493$). However, a previous report by Seo et al. (34) showed that the 4:1 KD had greater antiepileptic efficacy than the 3:1 KD ($P < 0.05$) at 3 months after initiating the diet. Bough et al. (35) also found that the seizure threshold was significantly elevated with increased ketogenic ratios in rats. The discrepancies between our study and the above studies may be due to the following reasons. First, the sample size in each ketogenic ratio group was too small. Second, the lack of association of ketogenic ratio at KD initiation with the efficacy in the first month after KD may be because the time was too short for patients to show response. Third, the lack of effect of ketogenic ratio in the maintenance period on the KD efficacy in the third month after KD maybe because most (26/37, 70.3%) patients using the classical KD had adjusted the ketogenic ratio (1.5–4:1) during the maintenance period, while a few (11/37, 29.7%) patients used a fixed ratio (4:1 or 3:1 or 2:1) during the maintenance period.

The mTOR signaling pathway plays a crucial role in brain development, and the TSC-related neuropsychological abnormalities are related to the over-activation of the mTOR signaling pathway (10, 36). McDaniel et al. have confirmed that KD could inhibit the over-activation of the mTOR signaling pathway in animal models (37). In addition, Warren et al. (20) confirmed that decanoic acid, a vital component of the medium-chain triglyceride KD, could decrease the mTORC1 activity in rat hippocampus *ex vivo* and TSC patient-derived astrocytes. These results provide a biological mechanism of KD efficacy in TSC. However, in the case series of five TSC patients, KD did not induce tumor regression or suppress the growth of TSC-related tumors (38). KD did not appear to be able to provide the same level of mTOR inhibition required to cause tumor regression. The exact mechanism of KD-mediated improvement of seizures and cognitive behavior in TSC patients is still not fully understood and needs further study.

There are several limitations to our study. First, this was a retrospective study, so the data was not complete enough. In our study, for the lack of IQ and DQ tests, the evaluation of psychomotor improvement was mainly based on subjective assessment by neurologists and parents. Future studies are needed to compare psychomotor states before and after KD by some objective assessment such as formal IQ and DQ tests, and formal questionnaires of assessment of life quality or even childhood autism rating scale in TSC with ASD. Second, since the follow-up time of this study was 12 months, we mainly analyzed the short-term efficacy of KD in the treatment of TSC. More studies are needed to explore the long-term outcomes of KD in the future. Third, we did not analyze the relationship between KBs and KD efficacy, although KBs were regularly monitored in children on KD. Fourth, due to the limited medical resources, we did not perform enough EEG recording during the KD. The cycle of EEG reexamination in children with epilepsy is usually 6 to 12 months, so it is difficult to perform follow-up EEG every 1 to 2 months after KD initiation in clinical practice. Therefore, we should continue collecting the relevant data to further explore the long-term outcomes in the future.

CONCLUSIONS

KD is an effective and safe treatment for children with TSC-related drug-resistant epilepsy and cognitive impairment. KD can reduce seizure frequency and may potentially improve cognition and behavior.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Clinical Research of Shenzhen Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YF, JL, LY, and HL designed the study. YF drafted the article. All authors analyzed and interpreted the clinical and diagnostic data, critically reviewed the manuscript, and read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.863826/full#supplementary-material

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Interictal Discharge Pattern in Preschool-Aged Children With Tuberous Sclerosis Complex Before and After Resective Epilepsy Surgery

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Objective: To analyze the interictal discharge (IID) patterns on pre-operative scalp electroencephalogram (EEG) and compare the changes in IID patterns after removal of epileptogenic tubers in preschool children with tuberous sclerosis complex (TSC)-related epilepsy.

Methods: Thirty-five preschool children who underwent resective surgery for TSC-related epilepsy were enrolled retrospectively, and their EEG data collected before surgery to 3 years after surgery were analyzed.

Results: Twenty-three (65.7%) patients were seizure-free post-operatively at 1-year follow-up, and 37–40% of post-operative patients rendered non-IID on scalp EEGs, and patients with focal IIDs or generalized IID patterns on pre-operative EEG presented a high percentage of normal post-operative scalp EEGs. IID patterns on pre-operative scalp EEGs did not influence the outcomes of post-operative seizure controls, while patients with non-IID and focal IID on post-operative EEGs were likely to achieve post-operative seizure freedom. Patients with new focal IIDs presented a significantly lower percentage of seizure freedom than those without new focal IIDs on post-operative EEGs at 3-year follow-up.

Conclusion: Over 1/3 children with TSC presented normal scalp EEGs after resective epileptsy surgery. Patients with post-operative seizure freedom were more likely to have non-IIDs on post-operative EEGs. New focal IIDs were negative factors for seizure freedom at the 3-year follow-up.

Keywords: epilepsy, epileptogenic tuber, interictal discharge (IID), scalp electroencephalographs (EEGs), tuberous sclerosis complex (TSC)

HIGHLIGHTS

- The pre-operative interictal discharge patterns on scalp EEGs had no obvious effect on post-operative seizure control in TSC-related epilepsy.
- The interictal discharge patterns on post-operative scalp EEGs were consistent in TSC patients who underwent resective epileptic surgery.
- In 37–40% of post-operative patients, interictal discharge on scalp EEGs were not present.
- Patients with post-operative seizure freedom were more likely to have absent interictal discharge on post-operative scalp EEG.

INTRODUCTION

Approximately 90% of patients with tuberous sclerosis complex (TSC) suffer from epilepsy, which are often medically resistant and can present with multiple seizure types (1–3). Epilepsy surgery, especially resective surgery, is the most efficient approach for patients with TSC-related intractable epilepsy, and 68–75% of patients with TSC present post-operative seizure freedom and a worthwhile reduction (>90%) in seizure frequency after resective surgery (4–7).

Due to an autosomal dominant epilepsy with multiple tubers in most patients with TSC-related epilepsy, the biggest questions to the resective surgery for TSC are whether the numbers and volumes of cortical tubers, and the number of epileptogenic tubers will increase with age, and whether new epileptogenic tubers will appear after removal of epileptogenic tubers, besides the difficulty in localizing the epileptogenic tuber(s). It has been reported that the number and relative volume of cortical tubers will not change after 1 year of age in TSC patients (8), and consistent location of interictal epileptiform activity on scalp electroencephalographs (EEGs) indicated the relative stability of epileptogenic tubers in patients with TSC-related epilepsy (9).

As previously reported, total resection of the actual seizure-onset zone does not always lead to seizure freedom in focal

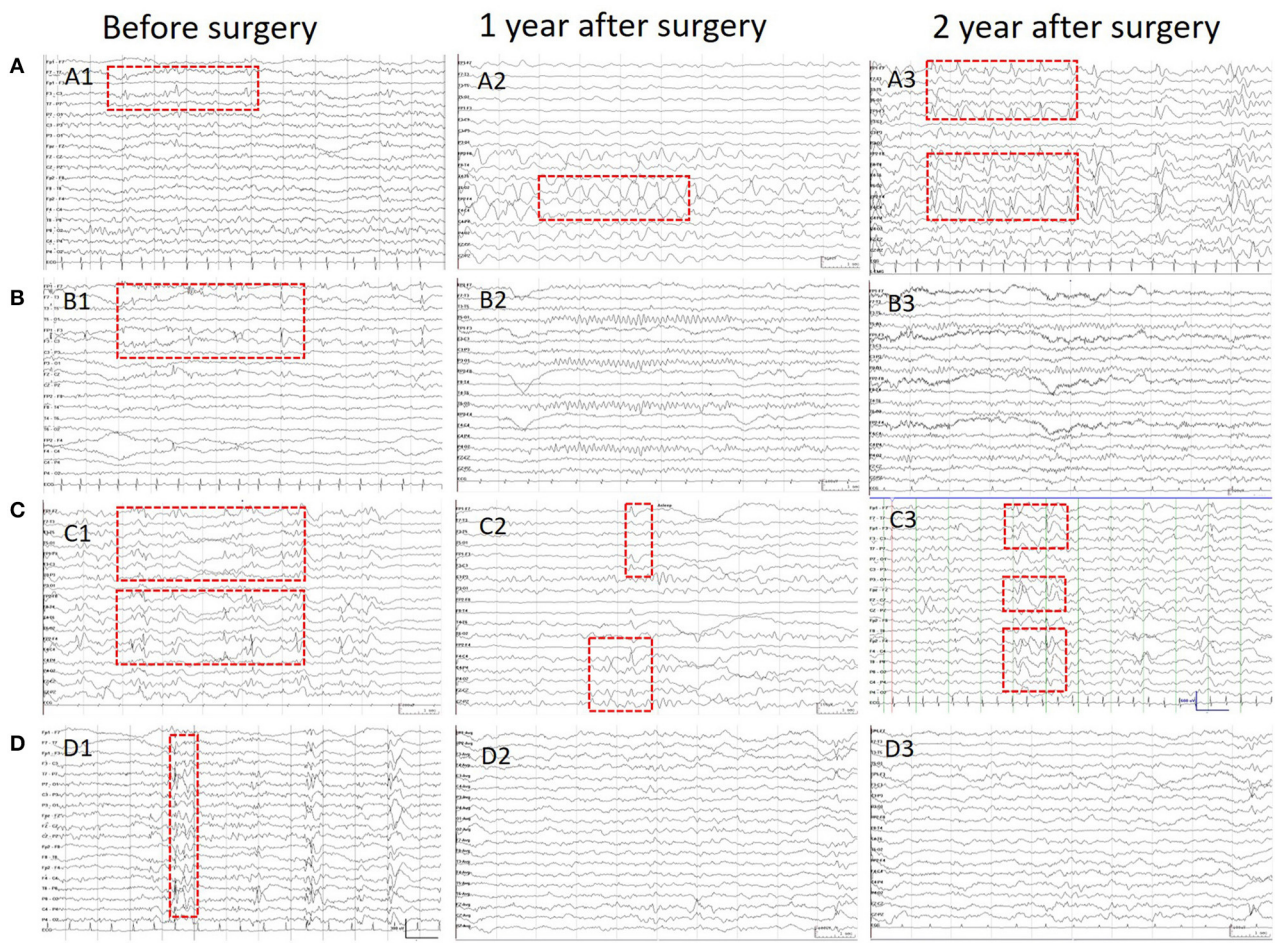

FIGURE 1 | Patients' IIDs patterns on EEG before and after surgeries. This figure shows the different IID patterns of four cases on pre-operative and post-operative scalp EEGs. **(A)** Shows the focal IIDs on the left frontal areas on pre-operative EEG (A1), focal IIDs and slow waves on the right frontal areas on EEG in 1 year after surgery (A2), and multifocal IIDs on the EEG in 2 years after surgery. **(B)** Shows the focal IIDs on pre-operative EEGs (B1), and non-IIDs on EEGs in 1- (B2) and 2-year after surgery (B3). **(C)** Shows the multifocal IIDs on pre-operative EEGs (C1), and multifocal IIDs on EEGs in 1- (C2) and 2-year (C3) after surgery. **(D)** Shows the generalized IIDs on pre-operative EEGs (D1), and non-IIDs on EEGs in 1- (D2) and 2- year (D3) after surgery.

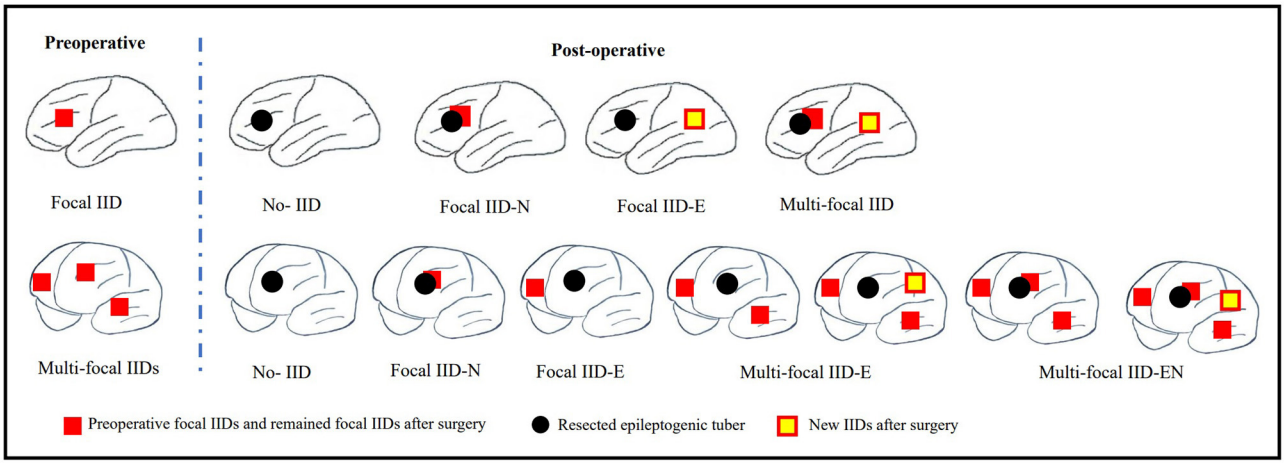

FIGURE 2 | Schematic diagram of pre-operative focal IID and multi-focal IID patterns and their post-operative IID patterns. The top row of this figure shows the pre-operative focal IID pattern and its post-operative patterns, including non-IID, focal IID-N (continuous IID located near the resected region), focal IID-E (new IID located elsewhere to the resected region), and multifocal IID-EN (new IID located elsewhere to the resected region and continuous IID located near the resected region). The bottom row of this figure shows the pre-operative multiple focal IID pattern and its post-operative patterns, including non-IID, focal IID-N, focal IID-E (continuous IID located elsewhere to resected region), multifocal IID-E (continuous IID or continuous IID and new IID located elsewhere to regions with pre-operative IIDs), multi-focal IID-EN (continuous IID or new IID located elsewhere to regions with pre-operative IID, and continuous IID located near the resected region).

seizures. In some cases, additional post-surgical recordings suggest that areas adjacent to the resection trigger epileptic seizures. These observations led to the concept of potential seizure-onset zones (10). Therefore, surgical resections did not result in seizure freedom either because of incomplete resection of the actual seizure-onset zone or incomplete resection of the potential seizure-onset zone (10). Weiner et al. reported a three-stage operation in patients with TSC-related epilepsy (11). They conserved subdural intracranial electrodes for several days after the first resective surgery and found that the margins of the resection and other tubers could induce seizures. However, there is no long-term study on whether new epileptogenic tubers will present and provoke seizures after resective surgery. Hence, the long-term IID patterns on post-operative EEG were analyzed to study whether there were new epileptogenic tubers after the removal of epileptogenic tubers.

METHODS

Patients

Patients were enrolled retrospectively according to the following criteria: patients who were no more than 6-year-old; subjects who underwent resective surgery from January 2015 to June 2020 in the comprehensive epilepsy centers of our hospitals; subjects who had been diagnosed with TSC according to the revised diagnostic criteria of Northrup et al. (12); and patients who had pre-operative scalp EEG recordings and post-operative scalp EEG recording at 2-year follow-up. The study was approved by the ethics committee of the Fourth Medical Center of the PLA General Hospital (Permit No. 2019KY004-HS001).

Pre-operative Evaluation

Non-invasive pre-operative evaluations included neurological physical examinations, high-resolution magnetic resonance imaging (MRI), long-term video EEG recordings, interictal positron emission tomography (PET), and Gesell development quotient tests. MRI scans included 3.0T routine axial T1- and T2-weighted, diffusion-weighted, and sagittal T1-weighted imaging and 2-mm thickness/zero interval axial and coronal T2-flair imaging. Each scalp EEG recording had to include more than three habitual seizures. MRI-PET co-registration was performed for each patient. Stereo-EEGs were recorded to detect epileptogenic cortex tubers when the predominant cortex tuber on MRI or the areas with focal ictal symptoms were discrepancies with the region with the focal scalp EEG (7, 13). The epileptogenic tuber was defined as the first tuber with initial rhythmic discharge on scalp EEG or stereo-EEG before a clinical seizure attack.

Pre-operative and Post-operative Scalp EEG Recording and Analysis

Scalp EEGs were made with 21-channel recordings with electrode positions according to the 10–20 system. Digital recordings of EEG traces were available for assessment. All scalp EEGs were recorded for no $<$24 h. Two observers (L.Y and S.C) reviewed all the EEGs. Both observers were blinded to the previous reports and recording time of EEG, information about patients' name and seizure semiology or frequency, and MRI findings. A final consensus reading was performed to identify the location of the epileptiform abnormalities. Consistency was defined as the presence of interictal epileptiform activity at the same location in all EEGs reviewed. If the assessment did not correspond to the original assessment, a third observer (S.L)

Pts No.	Before surgery	Postoperative		
		1 year	2 years	3 years
1		SF	SF	SF
2		SF	SF	SF
3		SF	SF	SF
4		SF	SF	SF
5		SF	Non-SF	Non-SF
6		Non-SF	Non-SF	Non-SF
7		SF	SF	SF
8		SF	SF	SF
9		SF	SF	SF
10		SF	SF	SF
11		SF	SF	SF
12		SF	SF	SF
13		Non-SF	Non-SF	Non-SF
14		SF	SF	
15		SF	SF	
16		SF	Non-SF	
17		Non-SF	Non-SF	
18		SF	SF	SF
19		SF	SF	SF
20		SF	SF	SF
21		SF	SF	SF
22		SF	SF	SF
23		SF	SF	Non-SF
24		SF	SF	SF
25		SF	Non-SF	Non-SF
26		Non-SF	Non-SF	Non-SF
27		Non-SF	Non-SF	Non-SF
28		Non-SF	Non-SF	Non-SF
29		Non-SF	Non-SF	Non-SF
30		Non-SF	Non-SF	Non-SF
31		SF	SF	SF
32		SF	SF	SF
33		SF	SF	
34		Non-SF	Non-SF	Non-SF
35		Non-SF	Non-SF	Non-SF

FIGURE 3 | IID patterns on scalp EEGs in different times for each patient. This figure shows the interictal epileptiform discharge (IID) patterns from 3 years before the surgery to 3 years after surgery in every patient. Blue box for EEG without IID; green box for focal IIDs; orange box for multiple focal IIDs, and golden box for generalized IIDs. pts, patients; pre-op, pre-operative; post-operative, post-operative; SF, seizure freedom.

reviewed the recordings. The results of interictal EEG were divided into four groups: non-interictal discharge (IID) (without interictal epileptiform discharges, IIDs) (**Figure 1B**,B3), focal IID (unique unilateral focal IID with or without generalized IID) (**Figures 1A**,A1,A2, **2**), multifocal IID (two or more independent focal IID or unilateral IID with or without generalized IID) (**Figures 1C**,C1,C2, **2**), and generalized IID (any bilaterally synchronous and symmetric pattern IID without focal IIDs, but it can be in a restricted field) (14) (**Figure 1D**,D1,D2). For the post-operative interictal EEG patterns, focal/multifocal IID was subdivided into focal/multi-focal IID-E (new IID located elsewhere to region with pre-operative IIDs) a focal/multi-focal IID-N (continuous IID located near the resected region), multi-focal IID-EN (new IID located elsewhere to region with pre-operative IIDs and continuous IID located near the resected region), multi-focal/focal IID (focal/multi-focal IID located regions with pre-operative multiple focal IID but the resected region), and non-IID (**Figure 2**).

Surgical Methods and Post-operative Medicine Treatment

The patient underwent lobectomy and/or tuberectomy. Tuberectomy was used for the epileptogenic tuber within or near the eloquent area. Lobectomy was performed for large epileptogenic tubers or multiple epileptogenic tubers in the anterior temporal lobe, frontal pole, or occipital pole. Multiple tuberectomies or lobectomy combined with tuberectomy were considered when multiple epileptogenic tubers or epileptogenic and propagating tubers could not be removed by a single lobectomy. Pre-operative and post-operative medicine treatment was provided to all patients with 2–4 types of optimal anti-seizure medications.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 26.0; SPSS, Inc., Chicago, IL, USA). The outcomes were described as percentages, means, and standard deviations. McNemar-Bowker's test was used to analyze influence of the IIDs patterns on pre-operative EEG on the outcome of IIDs patterns on post-operative EEG. Generalized estimating equation (GEE) was used to analyze the influences factors on the post-operative seizure control of the three time points of follow-up. Chi-square and Fisher's exact tests were performed for univariate analyses of categorical variables. When the two-tailed error probability "p" was <0.05, the outcome was considered significant.

RESULTS

Patients

Fifty-three preschool children (0–6 years old) with TSC-related intractable epilepsy underwent epilepsy surgery in our hospital from January 2015 to December 2019, and 35 of them, who had pre-operative and post-operative EEG recordings and surgical outcomes, were enrolled in this retrospective study. There were 11 (31.4%) girls and 24 (68.6%) boys. The average age at surgery was 3.51 [SD = 1.69, range (0.7–6.0), medium: 3.0, interquartile range (2.0–5.0)] years, the average age at first unprovoked seizure was 0.90 [SD = 1.13, range (0.0–5.0), medium 0.6, interquartile range (0.3–1.0)] years, and the pre-operative history of seizure ranged from 0.7 to 5.7 [mean = 2.55, SD = 1.50, medium 2.2, interquartile range (1.2–3.5)] years. Stereo-EEGs were performed on 11 (31.4%) of the 35 children.

TABLE 1 | Relationships between IIDs patterns on pre- and post-operative EEGs.

Post-operative IID in EEG	Pre-operative IID in EEG			Chi-square	*P*-value
	Focal IIDs	Multiple IIDs	Generalized IIDs		
1 year follow-up					
No IID	4 (67%)	7 (29%)	3 (60%)	21.36	0.0016
Focal IID	1 (17%)	10 (42%)	0 (0%)		
Multiple IIDs	1 (17%)	7 (29%)	0 (0%)		
Generalized IIDs	0 (0%)	0 (0%)	2 (40%)		
2 years follow-up					
No IID	4 (67%)	6 (25%)	3 (60%)	19.23	0.0038
Focal IID	0 (0%)	11 (46%)	0 (00%)		
Multiple IIDs	2 (33%)	7 (29%)	0 (0%)		
Generalized IIDs	0 (0%)	0 (0%)	2 (40%)		
3 years follow-up					
No IID	4 (67%)	5 (25%)	2 (50%)	13.78	0.0322
Focal IID	0 (0%)	7 (35%)	0 (0%)		
Multiple IIDs	2 (33%)	8 (40%)	0 (0%)		
Generalized IIDs	0 (0%)	0 (0%)	2 (50%)		

IIDs, interictal epileptiform discharges.

Surgery Approach and Post-operative Seizure Freedom

The surgical approach consisted of 15 tuberectomies, 9 lobectomies, and 11 multiple tuberectomies or lobectomies combined with tuberectomies. All 35 patients completed 1- and 2-year follow-up, and 25 (71.4%) and 23 (65.7%) of them had post-operative seizure freedom. Eighteen (60%) out of the 30 patients who completed the 3-year follow-up had post-operative seizure freedom.

Pre-operative and Post-operative IIDs

All pre-operative scalp EEGs indicated IIDs. All patients had scalp EEGs 1-year (40% without IID) and 2 years (37.1% without IID) after surgery, respectively, and 30 (36.7% without IID) children had post-operative scalp EEGs at the 3-year follow-up. No significant difference was found in the percentage of non-IID on scalp EEGs in the different post-operative periods ($p = 0.8929$). Eleven patients did not present with IIDs on post-operative scalp EEGs at all three follow-ups, and one case (3%) with focal IID on scalp EEG at 1-year follow-up reached normal EEG at 2-year follow-up, and two patients with normal EEGs at 1-year follow-up presented with focal or multi-focal IIDs at 2- and 3-year follow-up (**Figure 3**). There were significant differences in the outcomes of IID patterns on post-operative scalp EEGs at 1- ($p = 0.0016$), 2- ($p = 0.0038$) or 3-year ($p = 0.0322$) follow-up among patients with different IID patterns on pre-operative scalp EEG (**Table 1**; **Figure 3**).

Patients' Characteristics and Post-operative Seizure Freedom

The influence of age at surgery, age at first seizure, seizure type at onset, pre-operative history, and number of resected tubers on the post-operative seizure control was not found. However, patients with pre-operative seizure history <2 years presented statistically significant higher percentage of post-operative of seizure free than those with seizure history longer than 2 years (OR = 6.387, 95%CI: 1.174–34.746, $p = 0.032$) (**Table 2**).

IIDs Patterns and Post-operative Seizure Freedom

IID patterns on scalp EEGs before surgery did not affect the outcomes of post-operative seizure control ($p > 0.05$, **Table 2**). Significant differences were found in patients' seizure controls at 1-, 2-, and 3-year follow-up among patients with different IID patterns on scalp EEGs at the same time after surgery ($p < 0.001$, **Table 3**). Non-IIDs and focal IIDs on post-operative EEGs indicated the highest percentage of seizure freedom (**Table 3**).

IIDs in Areas of Resected Tubers and New Finding of Focal IIDs After Surgery

There were 21 patients who underwent a single area resection (six lobectomies and 15 tuberectomies), and 14 patients underwent multiple area resections (three lobectomies combined tuberectomies, and 11 multiple tuberectomies). In the resected areas, 25 (71.4%), 22 (62.8%), and 19 (63.3%) patients' scalp EEGs did not have IIDs in the resected areas at 1-, 2-, and 3-year follow-up, respectively (**Table 3**), and no significant difference was found among the percentages of patients without IIDs in the resected area among the three follow-ups (**Table 3**). No significant difference was found in post-operative seizure control between patients with IIDs in the resected area and those without IIDs in the resected area (**Table 3**).

Compared to all pre-operative scalp EEG results before surgery in each patient, there were two (5.7%), four (11.4%), and four (13.3%) patients with new focal IIDs on scalp EEGs at 1–3 years of follow-ups, respectively, and there was no significant

TABLE 2 | Relationship of pre-operative influence factors and number of resected tuber and post-operative seizure freedom.

Factors	Number (%) of seizure freedom			OR	95%CI	*P*-value
	1-year FU	2-year FU	3-year FU			
Age at surgery						
<3 years	12 (71%)	11 (65%)	9 (64%)	1.737	0.709–4.254	0.227
≧3 years	13 (72%)	11 (61%)	9 (56%)	-	-	-
Age at first seizure						
<1 year	18 (72%)	15 (60%)	12 (55%)	2.500	0.635–9.841	0.190
≧1 years	7 (70%)	7 (70%)	6 (75%)	-	-	-
Seizure type at onset						
Focal seizure	12 (80%)	10 (67%)	8 (62%)	1.154	0.158–8.415	0.888
Generalized spasm	9 (64%)	8 (57%)	6 (55%)	0.719	0.100–5.187	0.743
Other seizures	4 (67%)	4 (67%)	4 (67%)	-	-	-
History of pre-operative seizure*						
<2 years	13 (87%)	13 (87%)	11 (85%)	6.387	1.174–34.746	0.032
≧2 years	12 (60%)	9 (45%)	7 (41%)	-	-	-
Number of resected tubers						
1	14 (70%)	12 (60%)	11 (65%)	1.850	0.101–33.942	0.679
2	10 (77%)	9 (69%)	6 (55%)	2.083	0.106–40.780	0.629
3	1 (50%)	1 (50.00)	1 (50%)	-	-	-
Pre-operative EEG pattern						
Focal IIDs	5 (83%)	4 (67%)	4 (67%)	1.950	0.174–21.871	0.588
Multiple IIDs	17 (74%)	16 (67%)	12 (60%)	1.375	0.192–9.842	0.751
Generalized IIDs	3 (60%)	3 (60%)	2 (50%)	-	-	-

IIDs, interictal epileptiform discharges; FU, follow-up.
$^{*}P < 0.05$ with GEE analysis.

difference in the percentage of new focal IIDs among the three follow-ups ($p = 0.5588$). However, significant difference was found in patients' seizure controls at 3-year follow-up between patients with or without new focal IID on post-operative EEGs ($p = 0.018$, **Table 3**).

DISCUSSION

To the best of our knowledge, this is the first study to examine post-operative scalp EEGs in patients with TSC-related epilepsy after resective surgery. Although ictal scalp EEG and intracranial EEG have been used to localize the onset zone of seizures, interictal EEG is usually used to define the irritation area (2, 6, 7, 15, 16). However, some studies have confirmed that scalp EEG can also be a biomarker significantly associated with an underlying cortical pathology and aid in localizing suspicious brain regions (2, 17–20). Furthermore, interictal EEG patterns have been reported to be associated with post-operative seizure control in patients with TSC-related epilepsy (4, 21). Because over 70% of patients reach post-operative seizure freedom at the 1-year follow-up and 2–4 h of scalp video-EEG is routinely used for post-operative EEG examination, ictal EEG cannot be recorded in most patients at follow-up. Moreover, intracranial EEG is not suitable for post-operative follow-up. Therefore, interictal epileptiform discharge on scalp EEG is one of the best tools to observe long-term EEG changes after removal of the epileptogenic tubers in TSC patients.

The outcomes in this cohort show that patients with multifocal or generalized IIDs, besides focal IIDs, on pre-operative scalp EEG can also present non-IIDs on post-operative EEGs at 1–3 years of follow-up, which proves that focal epileptogenic tubers could lead to multifocal or generalized IIDs. Furthermore, patients with pre-operative multifocal or generalized IIDs do not have a low percentage of post-operative seizure freedom compared to those with pre-operative focal IID, which indicates that pre-operative IID patterns have no obvious effect on post-operative seizure control in carefully selected patients and comprehensive pre-operative evaluations. Due to the lower myelinization of the brain and less organized brain networks (22), young children with focal onset zone can presence diffuse interictal activity pre-operatively.

The maximal brain growth rate occurs around birth, and the brain is ∼95% of the size of the adult brain by 6 years of age. The bulk of this early growth comes from a variety of sources, including increases in synapses and dendrites, as well as myelination (23). Therefore, it may be the most obvious period of EEG discharge pattern changes. Nevertheless, 6–12% of patients presented new focal IIDs in 1–3 years of follow-up after removal of epileptogenic tubers compared to pre-operative IID patterns on scalp EEG, and 37–40% patients did not have obvious IIDs on post-operative scalp EEG,

TABLE 3 | Influence of post-operative EEG patterns on post-operative seizure freedom.

IIDs in EEG	1-year follow-up		2-year follow-up		3-year follow-up	
	SF	*P*-value	SF	*P*-value	SF	*P*-value
EEG pattern at follow up*						
Non-IID	13 (93%)	<0.001	13 (100%)	<0.001	11 (100%)	<0.001
Focal IIDs	10 (91%)		8 (73%)		6 (86%)	
Multiple IIDs	2 (25%)*		1 (11%)*#		1 (10%)*#	
Generalized IIDs	0 (0%)*#		0 (0%)*#		0 (0%)*#	
Post-operative continuous IID located near the resected region						
Yes	5 (45%)	0.421	6 (46%)	0.157	5 (45%)	0.266
No	20 (83%)		17 (77%)		13 (68%)	
New IID located elsewhere to the resected region on post-operative EEG compared to pre-operative EEG						
Yes	0 (0%)	0.076	1 (25%)	0.134	0 (0%)	0.018$
No	25 (76%)		21 (68%)		18 (69%)	

IIDs, interictal epileptiform discharges; SF, seizure freedom.

$^{*}P < 0.05$, the SF percentage in the group compared to the data in non-IID group at same follow-up.

$^{\#}P < 0.05$, the SF percentage in the group compared to the data in focal-IID group at same follow-up.

$^{\$}P < 0.05$, the SF percentage in the group compared to the data in other group at same follow-up.

including 11 patients without IID for 3 years. Furthermore, obvious correlations in IIDs patterns in post-operative EEG were found among 1-, 2- and 3-year follow-up, which indicated stability of IIDs patterns in post-operative EEG. Concurrently, 60% of post-operative patients achieved seizure freedom for 3 years. Therefore, we first showed that the epileptogenic tubers in patients with TSC-related epilepsy were relatively stable, and reconfirmed that not all tubers were independent epileptogenic tubers.

Continuous IIDs near the resected tubers can also be found in 29–37% of scalp EEGs in post-operative patients. The reasons for the continuous IID may include incomplete removal of tubers, propagation from IID in other tubers, and hyperexcitability of the cortex near the epileptogenic tubers. However, the presence of continuous IID did not affect the post-operative seizure freedom, which indicated that the incomplete removal of tubers should not be the main reason.

This study has some limitations. First, not all patients had all the 3-year EEG results before and after surgery due to the limitations of a retrospective study. Second, the sample of enrolled subjects was not large because of the low incidence of TSC-related epilepsy and the limited number of patients who underwent resective surgery.

In conclusion, pre-operative IID patterns had no obvious effect on post-operative seizure control after comprehensive pre-operative evaluations, while patients with focal IIDs or generalized IID patterns on pre-operative EEG presented a high percentage of normal post-operative scalp EEGs. In 37–40% of post-operative patients, non-IID on scalp EEGs was shown, and patients with post-operative seizure freedom were more likely to have non-IID on post-operative EEGs. New focal IIDs were negative factors for seizure freedom at the 3-year follow-up. The IID patterns on post-operative scalp EEGs were consistent, and epileptogenic tubers in patients with TSC-related epilepsy were relatively stable.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Fourth Medical Center of the PLA General Hospital. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SL: conceptualization, methodology, writing-original draft preparation, writing-reviewing and editing, funding acquisition, and investigation. LY and YW: methodology, writing-original draft preparation, and investigation. SZ and JZ: subjects collections and investigation. TL: investigation and data analysis. SC and GZ: supervision and writing-reviewing and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.868633/full#supplementary-material

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Computed Tomography and Magnetic Resonance Imaging in the Diagnosis of Cerebral Paragonimiasis in Children

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Objective: To investigate the computed tomography (CT) and magnetic resonance image (MRI) manifestations of cerebral paragonimiasis (CP) in children and to improve the understanding of the disease.

Methods: The cranial CT and MRI data of 12 children with positive intradermal tests for *Paragonimus*-specific antigens were retrospectively analyzed. Additionally, the lesion locations, morphology, and imaging characteristics were analyzed.

Results: The lesions were located in the cerebral parenchyma in 12 cases, with 10 in the supratentorial area and two in the subtentorial area, among which three cases included involvement of the meninges. The morphology of the lesions was mainly nodular and striated, with clear or indistinct borders and varying degrees of surrounding edema. The lesions showed isodense or slightly hyperdense opacities on the CT scans, heterogeneous equal or slightly decreased signal intensities on the T1-weighted images (T_1WI), heterogeneous equal or slightly increased signal intensities on the T2-weighted images (T_2WI), and equal or slightly increased signal intensities on the diffusion-weighted images (DWI) in MRIs. In four cases, the cyst wall showed equal T1 and short T2 signals, and in six cases, the characteristic "tunnel sign" and "worm-eaten sign" were visible. The contrast-enhanced MRI showed strip-shaped enhancement in five cases, nodular or ring-shaped enhancement in three cases, linear enhancement in two cases, and uneven enhancement in two cases. The meninges adjacent to the lesions were thickened with significant enhancement in four cases.

Conclusion: CP was mostly located in the cerebral parenchyma with involvement of the adjacent meninges. CT and MRI scans had certain imaging characteristics, and the MRI may particularly be of great value for the diagnosis of CP.

Keywords: cerebral, paragonimiasis, children, computed tomography, magnetic resonance imaging

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INTRODUCTION

Cerebral paragonimiasis (CP) is a condition in which larvae of *Paragonimus* in the abdominal or thoracic cavity migrate upward from the mediastinum, travel up through the soft tissues surrounding the carotid artery, and enter the brain along bony holes such as the carotid canal and rupture foramen, forming an intracerebral abscess or granuloma. Clinically, the patient has

a history of eating raw crayfish or stone crabs; they may have fever, convulsions, epilepsy, vomiting, and other symptoms, as well as exhibit subcutaneous migratory masses and test positive for *Paragonimus* (1, 2). Early diagnosis of CP is the key to achieving a good prognosis, thus proper imaging is particularly important to provide a reference for early diagnosis and to help clinicians develop the correct therapeutic plan (3, 4). Computed tomography (CT) and magnetic resonance imaging (MRI) facilitate the localization and qualitative diagnosis of lesions and are of great value for the diagnosis of CP (5). In the present study, the CT and MRI manifestations of 12 cases in our hospital with a clinically confirmed diagnosis of CP were retrospectively analyzed to improve the understanding of the disease. The diagnosis of CP was based on the application of an enzyme-linked immunosorbent assay (ELISA) with confirmation of serum antibodies to *Paragonimus* (4).

MATERIALS AND METHODS

Clinical Data

The clinical data, along with CT and MRI imaging, were collected from 12 pediatric patients with CP, including 8 males and 4 females, aged 6–14 years, with a median age of 9 years old. All cases originated from Guizhou Province, China. Of the 12 patients, 9 had dizziness and headache, 6 had nausea and vomiting, 5 had limb movement or sensory disturbances, and 4 had seizures. Nine patients were treated with the insect repellents Praziquantel. Two patients were treated with oral insect repellents Praziquantel after lesion resection + decompressive craniectomy. One patient was discharged after intracranial decompression and went to another hospital for surgical treatment with unknown information concerning the drug administration and surgical therapy. The patients were followed up 1 year after discharge. There were no significant abnormal symptoms in eight patients, significant improvement of the symptoms in two patients, and the existence of limb weakness in one patient. While one patient was lost during the follow-up.

Scanning Technique

The patients were scanned using a Siemens SOMATOM Sensation 16-row spiral CT scanner and a GE Signa 1.5T superconducting head-specific MRI scanner. Both plain CT and contrast-enhanced scanning were conducted with a thickness layer of 5 mm. The contrast enhancement was conducted with conventional scanning after an injection of 80 ml of non-ionic iodine contrast agent through the elbow vein. The parameters in MRI scanning were as follows: T1-weighted images (T_1WI), T2-weighted images (T_2WI), T2 fluid-attenuated inversion recovery (T_2/FLAIR), and diffusion-weighted images (DWI) were scanned. The T_1WI axial, coronal, and sagittal scans were conducted with an injection of gadopentetate glucosamine (Gd-DTPA) through the elbow vein at 0.1 mmol/kg of body weight at the time of enhancement.

TABLE 1 | The location distribution of cerebral paragonimiasis.

Location of the lesion	Cases (%)
The supratentorial area	10 (83)
The subtentorial area	2 (17)

RESULTS

The Clinical Characteristics

The clinical manifestations in the 12 pediatric patients with paragonimiasis were mainly dizziness, headache, vomiting, seizure, and hemiparesis, with varying degrees of respiratory symptoms. With inquiry into the relevant clinical history, it was found that all 12 children had a history of eating raw stream crabs and drinking raw stream water, thus the clinical diagnosis should be highly suspicious of CP. The insect repellents Praziquantel is the preferred choice for the treatment of cerebral paragonimiasis. Praziquantel was administered and achieved good therapeutic effects in the remaining 11 patients except for one case who went to another hospital for treatment in the present group.

The CT and MRI Manifestations

Cranial CT and MRI scans were conducted in the 12 pediatric patients with CP, and the lesions in all 12 cases were located in the cerebral parenchyma, with 10 in the supratentorial area, 2 in the subtentorial area, and involvement of the meninges in three cases (as shown in **Table 1**). The morphology of the lesions in the CT and MRI was mainly nodular and striated, with clear or indistinct borders and varying degrees of surrounding edema. The lesions showed isodense or slightly hyperdense opacities on the CT scan. The MRI manifested as ring-shaped cystic lesions, with heterogeneous equal or slightly decreased signal intensities on the T_1WI scans, heterogeneous equal or slightly increased signal intensities on the T_2WI scans, and equal or slightly increased signal intensities on the DWI scans. In four cases, the cyst wall showed equal T1 and short T2 signals, and in six cases, the characteristic "tunnel sign" and "worm-eaten sign" were visible. The contrast-enhanced MRI showed strip-shaped enhancement in five cases, nodular or ring-shaped enhancement in three cases, linear enhancement in two cases, and uneven enhancement in two cases. The meninges adjacent to the lesions were thickened with significant enhancement in four cases (as demonstrated in **Figure 1**, **Table 2**).

DISCUSSION

Pathogenesis of Cerebral Paragonimiasis

Paragonimiasis is a Zoonotic Parasitic disease mostly caused by eating raw or semi-raw crabs and crickets or drinking raw stream water in endemic areas (6). *Paragonimus* is pathogenic to humans and includes the *Paragonimus westermani* and *Pagumogonimus skrjabini Chen* species. The main pathological features of Paragonimiasis are the formation of sinus and multilocular cysts (7). The pathogenic mechanism is mainly that the metacercariae enter the intestine through the mouth

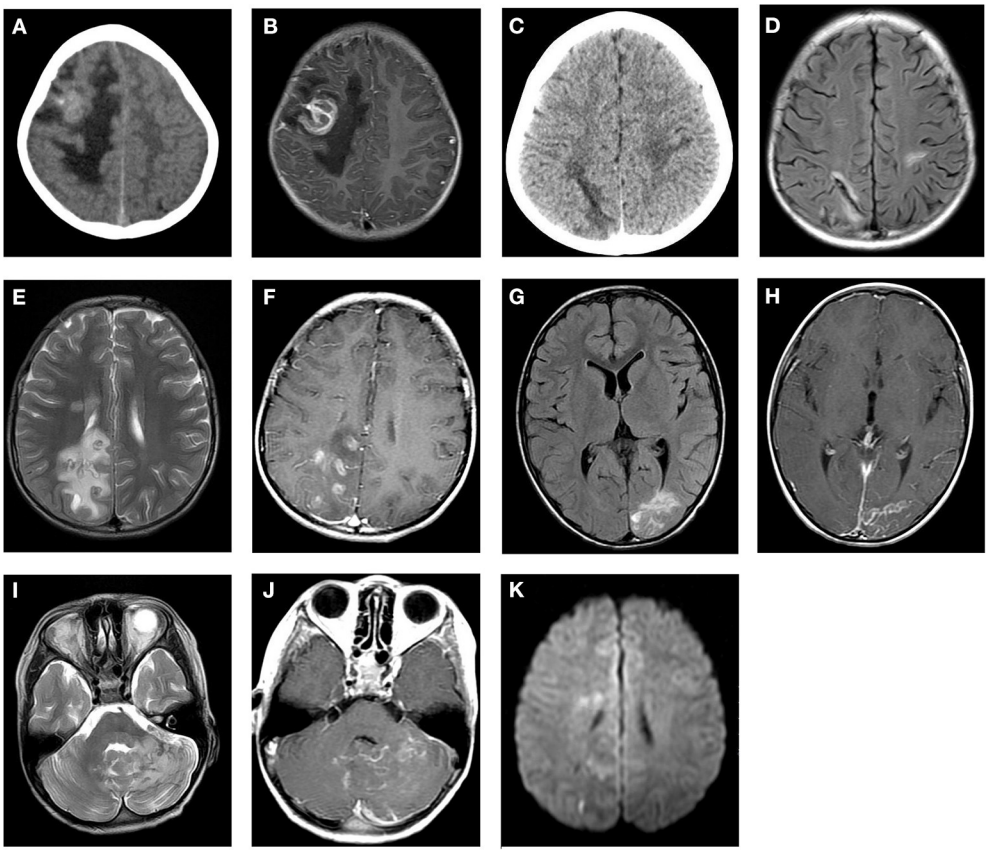

FIGURE 1 | Cranial CT and MRI features of the patients with cerebral paragonimiasis are shown. CT images showed the lesion in the right frontotemporal lobe, which is a mass of equal or slightly high density, surrounded by patchy low-density edema **(A)**. The T1WI enhancement scans showed that the lesion was a ring-like enhancement, surrounded by edema **(B)**. CT images showed a strip-like low-density lesion in the right parietal lobe surrounded by mild edema **(C)**. On the T2/FLAIR images, the lesion was a strip-like low signal surrounded by a high signal, which is the classic "tunnel sign" **(D)**. On the T2WI scans, the lesion in the right parietal occipital lobe was nodular and a circular low or slightly high signal, accompanied by peripheral patchy edema **(E)**. The lesions showed obvious nodular and circular enhancement on the T1WI enhancement scans **(F)**. On the T2/FLAIR images, the left occipital lobe lesions were strip-like and sheet with slightly high signals, and the peripheral edema was not obvious **(G)**. On the T1WI enhancement scans, the lesion showed strip enhancement **(H)**. On the T2WI scans, the left cerebellar hemisphere showed an uneven increased signal, which had nodular and striped mixed signals with unclear boundaries **(I)**. On the T1WI scans, the lesions showed ring-like and strip-like enhancements, and the adjacent meninges were thickened and significantly enhanced **(J)**. On the DWI scans, an equal or slightly high signal is shown **(K)**.

and decapsulate in the small intestine, becoming juvenile. The juveniles enter the abdominal cavity through the diaphragm and enter the lungs where they develop into adult worms. Both adults and larvae of *Paragonimus* have the habit of invasion and migration (1, 8). The mechanism of *Paragonimus* migration to the brain is not very clear. One hypothesis is that immature larvae migrate through the loose connective tissue surrounding the jugular vein or carotid artery, penetrate the meninges, and invade the cerebral tissue. This is consistent with the finding that the distribution of lesions tends to be in the middle cerebral artery and posterior artery. The lesions are often located in the temporal lobe, parietal lobe, and occipital lobe (1). Migration and catabolism of the *Paragonimus* in the cerebral tissue cause an inflammatory response, leading to eosinophil infiltration and granuloma formation with edema of the surrounding cerebral tissue (9, 10). As time progresses, the worm destroys the cerebral tissue, causing necrosis and the formation of partially calcified fragments, which are encapsulated by a cyst wall of granulation and fibrous tissue (8). Meanwhile, eosinophilia can extensively erode the cerebrovascular system and eventually cause rupture of the eroded blood vessels, resulting in various degrees of hemorrhage (10).

TABLE 2 | The MR manifestations of cerebral paragonimiasis.

The MR manifestations of the lesion	Cases
The cyst wall showed equal T1, short T2	4
The tunnel sign and the worm-eaten sign	6
The linear enhancement	2
The strip enhancement	5
The nodular, ring-shaped enhancement	3
The uneven enhancement	2
The thickening and enhancement of adjacent meninges	4

The Clinical Manifestations and Laboratory Examinations

CP is most common in children and adolescents, with a significantly higher incidence in children than in adults, and more common in males than in females (1). Most patients with CP have a slow onset and progressive exacerbation. In a few patients, especially the pediatric patients, the onset may be acute, with the neurological clinical manifestations being mostly headache, dizziness, vomiting, seizure, partial sensory disorders, and other localized brain symptoms; these are often accompanied by respiratory symptoms (11–13). According to the literature, most patients with CP have had respiratory symptoms before the appearance of neurological symptoms, with cough, shortness of breath, and chest pain as the main manifestations (8). In addition, symptoms and manifestations caused by lesions at other sites may develop, and even result in sudden death (6). Due to the lack of specificity, the clinical manifestations of CP are complex and varied, thus it is highly susceptible to being misdiagnosed.

The laboratory examinations for CP include eosinophil counting, intradermal testing for the *Paragonimus* antigen, and ELISA (4, 14). Eosinophil counting is the most simple and easy examination to perform. A large number of eosinophil aggregates can be visible in the cerebral tissue in patients with CP, which can also be accompanied by a large number of plasma cells, neutrophils, lymphocytes, etc. It is an important screening method commonly used in clinical practice. The confirmed diagnosis of CP relies on detection by ELISA of *Paragonimus* antibodies in the serum of the patient (4).

The Imaging Manifestations and Pathological Analysis

The lesions of CP are often located in the cortical and medullary junction areas of the supratentorial area of the cerebral hemispheres, mostly in the temporal, parietal, and occipital lobes, but can also occur in the subtentorial area (1, 15). The morphology of the lesions in CT and MRI scans is mainly nodular and striated, with varying degrees of surrounding edema. The image appearance varies at different stages of the lesion. For most patients, the most common CT presentation is an isodense or hyperdense lesion, and MRI presents as a ring-shaped cystic lesion (16). With a review of the previous literature, the authors suggested that the "tunnel sign" might be the characteristic imaging manifestation in CP, which appeared on the CT as a strip lesion with decreased intensity and opacity and varying degrees of surrounding edema. On the MRI, the tunnel sign appeared as a decreased intensity signal on the T1WI scans and an increased intensity signal on the T2WI scans, showing a tubular structure with an aperture of 1–3 mm. On the T2/FLAIR images, it appeared as a strip of decreased intensity signal surrounded by increased intensity signal, and it might also appear as a strip or a piece of increased intensity signal. On the T1WI enhanced scans, the lesion appeared as a significantly enhanced tunnel opacity or a significantly enhanced tunnel opacity between two adjacent lesions, which was the migration trajectory of the *Paragonimus* in the brain. The "tunnel sign" reflected the pathological changes that occurred after the penetration of the *Paragonimus* into the cerebral tissue (3, 8, 16). In the present study, the tunnel sign was observed in four cases, and the imaging findings were consistent with those reported in the literature. In addition, the manifestations of ring-shaped, nodular, and linear enhancement on the T1WI enhanced scans were also due to different pathological changes (17). The ring-shaped enhancement was mostly caused by cavities formed by stagnant worms destroying the cerebral tissue, with necrotic and liquefied brain tissue inside, surrounded by a cyst wall composed of granulation and fibrous tissue (7), while the granulomatous hyperplasia and vasculitis in the infiltrative stage might manifest as a nodular enhancement. If the lesion involved the meninges, the adjacent meninges might be thickened with significant enhancement in the T1WI enhanced scan, which might be caused by the invasion of *Paragonimus* migration into the meninges. In the present study, the ring-shaped and nodular enhancements observed in three patients, as well as the meningeal involvement,

TABLE 3 | The similarities and differences in the CT and MRI manifestations between other similar lesions and cerebral paragonimiasis.

	The similarities and differences in images	
	The difference	**The similarity**
Cerebral parenchymal tuberculosis	The tuberculosis is generally small with rare bleeding and mild peripheral edema. If multiple aggregates fuse to form a tuberculous granuloma, there is bead-like or plum-like enhancement, often accompanied by hydrocephalus	With the occurrence of calcification, nodular, ring-like enhancement
Brain abscess	The wall is thin, smooth and regular, with restriction in the pus cavity in DWI	With ring-like enhancement, and obvious peripheral edema
Glioma	Mostly located in the deep white matter of the brain, generally with patchy or wreath-like enhancement, and obvious space-occupying effect	Generally with heterogeneous density/signal, and necrosis and hemorrhage may occur within the lesion, with significant peritumoral edema
Brain metastasis	Mostly with disseminated distribution, and calcification is less common	Prevalent in the corticomedullary junction area, with nodular, circumferential enhancement and significant peri-lesion edema

thickening, and significant enhancement in four patients, were consistent with previous reports in the literature. Two cases manifested as liner enhancement. The different features of enhancement in these cases might be correlated with the different stages of the disease. Intracranial hemorrhage can occur in children with CP, and the hemorrhage signal has a variety of manifestations on MRIs, mainly showing a high T1 signal and an equal, slightly high, or low T2 signal (11, 18). Susceptibility-weighted imaging (SWI) is a sensitive examination for the micro-hemorrhagic foci (19). However, no significant hemorrhage was observed in the cases enrolled in the present study, and no additional SWI sequence scanning was conducted. Therefore, a larger sample size is needed for further investigation.

Differential Diagnosis

In the diagnosis of cerebral paragonimiasis, attention should be paid to differentiating from cerebral tuberculosis, metastases, brain abscess, glioma, etc: ① Patient with brain tuberculoma mostly has poisoning symptoms or history of tuberculosis, with small tuberculous granuloma in the single brain parenchyma, with the rare occurrence of bleeding and mild peripheral edema, often accompanied by hydrocephalus. ② Patients with brain abscess have local or systemic signs and symptoms of infection, with elevated leukocytes, and one or more thin and luminous annular enhancements on the enhancement scans, with restriction spreading of pus cavity on DWI having the diagnostic value. ③ Brain metastases are common in middle-aged and elderly patients, usually with a history of the primary tumor, and present as multiple scattered nodular or ring-shaped enhancing lesions in the corticomedullary junction area, with unsmooth walls, rare calcifications, and significant perifocal edema disproportionate to the size of the lesion. ④ Gliomas are mostly located in the deep white matter of the brain. There may exist hemorrhage and necrosis within the tumor, which manifest as plaque-like or wreath-like enhancement with obvious space-occupying effect (as shown in **Table 3**).

CONCLUSION

CT and MRI scanning can be of great value in the clinical diagnosis of CP. The lesions often manifest as a collection of multiple ring-shaped lesions of different sizes, and the "tunnel sign" with peripheral edema might be the most typical imaging feature in CP. In addition, bilateral cerebral hemisphere involvement, multiple foci in the brain, extensive invasion of adjacent meninges and ventricular walls, and migration of lesions were other noteworthy imaging features. The analysis of imaging features of CP could provide a reference for early diagnosis, but the definitive diagnosis of CP should also be combined with epidemiological history, clinical manifestations, and laboratory examinations. Early diagnosis and timely treatment could reduce the need for surgery and prevent further injury to brain function, which could be critical for patients to achieve a good prognosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki (as was revised in 2013). The study was approved by Ethics Committee of the Affiliated Hospital of Guizhou Medical University. Written informed consent was obtained from all participants.

AUTHOR CONTRIBUTIONS

JD and XL: conception and design of the research. JD and LL: acquisition of data and writing of the manuscript. HF and YY: analysis and interpretation of the data. YL and HY: statistical analysis. XL: obtaining financing. XL and HY: critical revision of the manuscript for intellectual content. All authors read and approved the final draft.

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The Clinical Impact of Methotrexate-Induced Stroke-Like Neurotoxicity in Paediatric Departments: An Italian Multi-Centre Case-Series

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Introduction: Stroke-like syndrome (SLS) is a rare subacute neurological complication of intrathecal or high-dose (≥500 mg) Methotrexate (MTX) administration. Its clinical features, evoking acute cerebral ischaemia with fluctuating course symptoms and a possible spontaneous resolution, have elicited interest among the scientific community. However, many issues are still open on the underlying pathogenesis, clinical, and therapeutic management and long-term outcome.

Materials and Methods: We retrospectively analyzed clinical, radiological and laboratory records of all patients diagnosed with SLS between 2011 and 2021 at 4 National referral centers for Pediatric Onco-Hematology. Patients with a latency period that was longer than 3 weeks between the last MTX administration of MTX and SLS onset were excluded from the analysis, as were those with unclear etiologies. We assessed symptom severity using a dedicated arbitrary scoring system. Eleven patients were included in the study.

Results: The underlying disease was acute lymphoblastic leukemia type B in 10/11 patients, while fibroblastic osteosarcoma was present in a single subject. The median age at diagnosis was 11 years (range 4–34), and 64% of the patients were women. Symptoms occurred after a mean of 9.45 days (± 0.75) since the last MTX administration and lasted between 1 and 96 h. Clinical features included hemiplegia and/or cranial

nerves palsy, paraesthesia, movement or speech disorders, and seizure. All patients underwent neuroimaging studies (CT and/or MRI) and EEG. The scoring system revealed an average of 4.9 points (± 2.3), with a median of 5 points (maximum 20 points). We detected a linear correlation between the severity of the disease and age in male patients.

Conclusions: SLS is a rare, well-characterized complication of MTX administration. Despite the small sample, we have been able to confirm some of the previous findings in literature. We also identified a linear correlation between age and severity of the disease, which could improve the future clinical management.

Keywords: stroke-like syndrome, methotrexate, pseudo-stroke, neurotoxicity, subacute toxicity

INTRODUCTION

Methotrexate (MTX) is an antimetabolite agent acting as a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR), hence blocking the synthesis of folate and tetrahydrofolate and inhibiting DNA synthesis during the S-phase of the cell cycle. Folate antagonists were among the first developed antineoplastic agents, and methotrexate is still a mainstay of treatment for leukemia, lymphomas, gastric, breast, and bladder cancer. Neurological complications of anticancer therapy may either result from direct neurotoxicity or from indirect drug-induced metabolic derangements, cerebrovascular disorders or, in the case of checkpoint inhibitors, autoimmune disorders. MTX yields poor drug penetration across the blood–brain barrier due to its ionization and hydrophobicity (1); neurological toxicity mostly results from intrathecal (IT) administration or from high-dose intravenous (IV) treatments leading to intrathecal inflow.MTX neurotoxicity syndrome may cause acute, subacute, or chronic symptoms (2, 3). The overall incidence of MTX neurotoxicity ranges from 3 to 10% and varies according to dose, route, and frequency of administration (4, 5). Factors are high-dose therapy, intrathecal route, young age, and cranial irradiation (6).

While delayed/chronic neurotoxicity may take from months to years to manifest as leukoencephalopathy, acute to subacute neurotoxicity usually occurs within hours to weeks after MTX administration (7). In particular, the weekly or biweekly administration of high-dose MTX (HD-MTX), a prolonged low-dose oral treatment, and IT administration may produce a subacute MTX neurotoxicity called stroke-like syndrome (SLS), possibly characterized hemiplegia, hemisensory deficits, aphasia, dysarthria, dysphagia, diplopia, and occasionally seizures (2). Symptoms develop approximately 2–14 days after drug administration, they last from 15 min to 72 h, and then resolve spontaneously without sequelae. Watanabe et al. observed that the neurological events did not occur immediately after the first IT-MTX administration but started about 1–2 weeks after IT-MTX administration and often fluctuated until they resolved completely (8). Neuroimaging studies are usually normal, although changes have been described on MRI, such as areas of restricted diffusion on diffusion-weighted imaging and non-enhancing T2 hyper-intense lesions in the white matter. Additional investigations such as CSF analysis, and haematologic exams (e.g., thrombophilic profile) are usually normal, whereas electroencephalography (EEG) might show diffuse slowing of the background activity (9, 10). Treatment may consist of observation and supportive care alone. Dextromethorphan, dexamethasone, aminophylline and folic acid have also been successfully employed in MTX neurotoxicity (11–15), such as SLS. However, as most of these cases resolve spontaneously, the value of these medications is not clear. MTX has been eliminated from the therapeutic regimen in most of the cases of subacute neurotoxicity, but cases have also been reported in which further doses of MTX were given without any complication (15).

The incidence of subacute neurotoxicity is still unclear; in 369 children with diagnosed acute lymphoblastic leukemia (B-ALL) treated with both IV-MTX and/or IT-MTX, subacute encephalopathy occurred in 14 patients (3.8%) (16). In other studies, the incidence was higher, ranging from 0.8 to 3.8% (16–18), whereas in a recent paper the incidence of SLS was much lower (0.2%) (19).

MATERIALS AND METHODS

We retrospectively recruited 11 patients aged 4–34 years (median 11.02) from the Pediatric Onco-Hematology Centers of Pisa, Bologna, Pavia, and Cagliari who had been diagnosed with leukemia or osteosarcoma and had presented a stroke-like event, defined as the acute or subacute presentation of 1 or more of the following symptoms after MTX administration: hemiplegia, altered consciousness, seizures, hemianopsia, cranial nerve palsy, unilateral sensory disorders or speech disorders. We attributed the neurological event to MTX-induced stroke-like neurotoxicity if the first neurological symptom occurred within 3 weeks after IT or HD MTX administration once other possible causes had been excluded. Patients with neurological symptoms or patients who had presented with evident signs of symptoms on their first visit were excluded, as were those who had clear extracranial problems (i.e. septic shock). Each patient underwent brain imaging using MRI and/or computed tomography (CT). Electronic medical records were reviewed, and data regarding the mode of MTX administration, the temporal relationship to MTX administration, the type, duration and severity of stroke-like symptoms, and the neurological outcome were recorded.

To assess the severity of the disease, we developed an arbitrary ranking scale based on the clinical judgment assigning a score to a

TABLE 1 | Severity score for each symptom observed.

Symptom	Score
Hyposthenia	1
Hemiplegia	2
Paresthesia	1
Tongue deviation/Protrusion of oral opening	2
Speech disordes (aphasia and/or dysarthria)	2
Impaired consciousness	2
Deafness	1
Corea	2
Tremor	1
Sialorrhea (or drooling)	1
Isolated VII cranial nerve deficit	1
Seizure	2
Hypertension	2

total of 13 observed symptoms (**Table 1**). Each symptom received a score of 0 (no signs/symptoms); 1 (mild symptoms, with a low impact on daily life) or 2 (major symptom, or potentially life-threatening), with a possible maximum sum score of 20.

Statistical Analysis

For the statistical analysis of quantitative variables, we evaluated mean, median, and standard deviations, which were compared through *t*-tests. To study the relationship between these variables, the Pearson correlation coefficient was calculated. We described the most relevant correlations with a scatter plot and regression line, calculating the regression coefficient, and the coefficient of determination. The Fisher exact test was employed for the analysis of categorical variables which were expressed as percentages,. Finally, we described the overall survival rates through Kaplan –Meier curves (**Figure 1**). We used IBM SPSS, ver. 26. for the statistical analysis.

RESULTS

Total eleven patients with MTX-induced stroke-like neurotoxicity were included in the study. The main characteristics of our cohort are reported in **Table 2**. Of 11 patients, 64% were women. Of these, ten subjects had acute lymphoblastic leukemia type B (B-ALL) and 1 had fibroblastic osteosarcoma of the right femur. The median age at the time of diagnosis was 11 years (range, 4 – 34 years). None of the patients with SLS showed evidence of CNS leukemia or CNS metastasis at the original presentation. As regards the treatment of patients with B-ALL, 5 (55%) were treated according to the AIEOP-BFM 2009 protocol, and the others (45%) according to the AIEOP-BFM 2017 protocol. The patient with osteosarcoma was treated according to the ISG/OS-2 PGOP NEG protocol. About 6 patients (55%) received IT administration of 12 mg of MTX, 3 (27%) also underwent HD intravenous therapy with 5 g/m (2), only 1 patient received intravenous therapy exclusively, while 1 was treated with oral and IT MTX.

The mean interval between the most recent MTX exposure and SLS was 9.45 days (± 0.75), with a median of 9 days (range 2–13). **Figure 1** shows the survival rate of the analyzed population.

SLS episodes occurred in 1 patient during the induction phase, in 4 during re-induction and in 3 patients during the maintenance phase.

Symptoms lasted 24 h in 5 patients, 72 h in 3 patients, and 96 h in 3 patients, as seen in **Table 3**. The clinical presentation typically included mild paresis and paraesthesias, disturbances of speech and eventually motor impairment

In detail, 7 patients (63.6%) showed limb hyposthenia, 3 (27.3%) had hemiplegia, and 3 (27.3%) developed paraesthesias. In 4 patients (36.4%) tongue deviation and/or protrusion of the buccal opening was observed. About 8 patients (72.7%) had speech disorders, mainly aphasia and/or dysarthria, whereas an altered mental status occurred in 2 cases (18.2%).

Other observed symptoms were deafness, choreic movements, tremors, drooling, or facial nerve palsy. Of these 1 patient experienced seizures and increased blood pressure.

The analysis of these symptoms revealed a different gender distribution: limb hyposthenia occurred in 5 (71.4%) women and in 2 (50%) men; no men presented paraesthesia while buccal opening and/or tongue deviation manifested in 28% of females and 50% of males. Impaired consciousness was not observed in men, whereas it occurred in 28% of women (**Figure 2**).

Our analysis displayed a severity score ranging from 1 to 10, with a mean value of 4.9 (± 2.3), and a median of 5.

We did not observe any correlation between the severity of the clinical picture and the age of the female patients, or with the administration modality. On the other hand, a significant correlation was detected in the male subgroup, in which age showed a linear correlation with the severity of the clinical picture (r: 0.98; *p-value*: 0.017), as shown in **Figure 3.** A trend toward a linear correlation was also observed between severity and the time elapsed since the last administration of MTX (**Figure 4**), although no statistical significance has been detected (r: 0.56; *p-value*: 0.71).

Total 3 (27%) MRIs were reported as normal, while 8/11 (73%) showed focal or diffuse hyperintensity of the periventricular or subcortical white matter on T2-weighted images; these lesions showed restricted diffusion on ADC map, suggesting cytotoxic oedema (**Figure 5**). As expected, CT was much less sensitive for white matter changes and was reported as normal in 9/11 cases; only 1/11 patients (Patient 4) presented with mild white matter hypodensity and slight dilation of the liquor spaces which was perhaps due to therapy outcomes. EEG was performed on all patients, and resulted abnormal for mild interhemispheric asymmetry in 2 subjects (Patients 7 and 9). A cerebro-spinal fluid analysis was not performed.

Treatment decisions were made in each case by the local oncology group in joint sessions. As shown in **Table 3,** 5/11 patients received no therapy, whereas 6/11 patients received pharmacological treatments: 4/11 antiseizure medications (midazolam, clonazepam, levetiracetam), 2/11 (Patients 2 and 6) anti-hypertensive agents (amlodipine, nebivolol), 2/11 (Patients

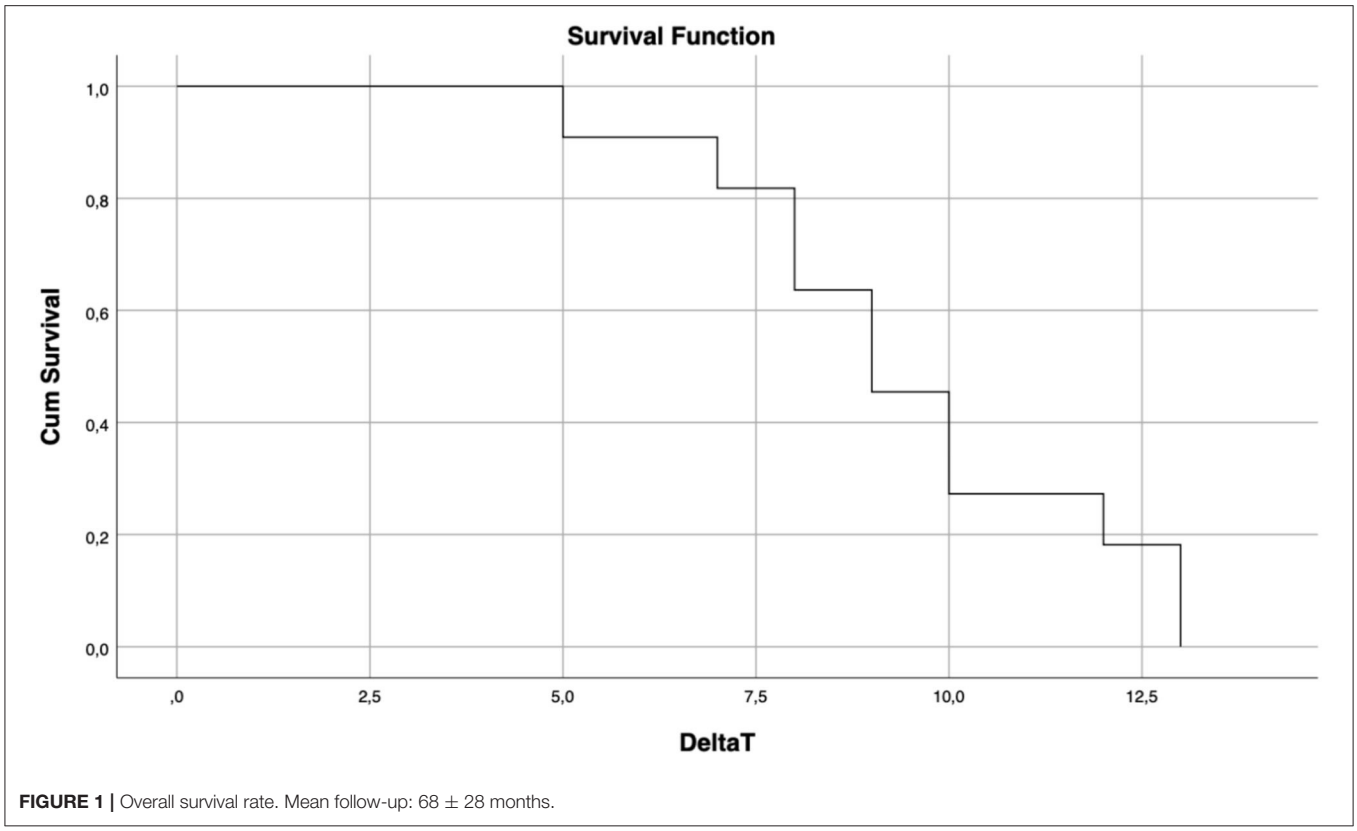

FIGURE 1 | Overall survival rate. Mean follow-up: 68 ± 28 months.

TABLE 2 | Clinical data of our study cohort.

Characteristics	Number (%)	
Sex	Male	4 (36%)
	Female	7 (64%)
Age	≤ 10 years old	5 (45%)
	>10 years old	6 (55%)
Diagnosis	LAL-B common	10 (90%)
	Fibroblastic Osteosarcoma	1 (10%)
Involvement of CNS at diagnosis	Yes	0 (0%)
	No	11 (100%)
Trial	AIEOP-BFM 2009	5 (45%)
	AIEOP-BFM 2017	4 (36%)
	ISG/OS-2 PGOP NEG	1 (10%)
	Unknown	1 (10%)

1 and 8) supplemental folinic acid, $n = 1/11$ (Patient 1), empiric anticoagulant therapy with low-molecular weight heparin, dexamethasone, and gabapentin.

In 5 patients IT-MTX was re-established following the initial SLS. Patients 5 and 6, who experienced clinical symptoms during Protocol M, did not receive the fourth dose of MTX. Patients 4 and 8 discontinued reinduction and phase IB with a subsequent therapy modification. Only 1 patient presented a MTX-induced SLS relapse upon re-exposure during the re-induction therapy (20%), with right paraesthesia, right hemiparesis and dysarthria. The brain MRI showed a T2-weighted hyperintense lesion in the left subcortical white matter. The patient was treated with midazolam with a subsequent remission of symptoms. During maintenance, after 12 doses of oral MTX, the patient re-presented with right hemiparaesthesia and re-flaring of the above mentioned hyperintense lesion. The oncologist decided to stop 6 MP and MTX and to recommence therapy by reducing the dose.

DISCUSSION

Methotrexate-induced stroke-like syndrome is a rare complication of intrathecal or high dose administration of MTX [≥500 mg/m (2)]. Its peculiar and worrisome clinical features, together with its predisposition to a spontaneous resolution, have elicited the interest of the scientific community since its first descriptions (1). A review of the literature showed 11 reports of neurological serious adverse events associated with MTX administration, determined to be consistent with MTX-induced stroke-like neurotoxicity, characterized by focal neurological dysfunction that could occur with disturbances in speech, vision, or altered mental status, sensorial or motor deficits.

Herein, we examined the main features of such toxicity in a pediatric population through the analysis of a multicentric cohort of 11 patients, so as to highlight the main characteristics of this manifestation and eventually achieve a better understanding of its pathogenesis.

Different hypotheses have been proposed on the mechanisms underlying SLS. Direct damage induced by MTX might result

TABLE 3 | Main clinical features of our study cohort.

Patient	Sex	Tumor	Age at onset of SLS	Duration (hours)	MRI latency (days)	MRI findings	Therapy adopted	Relapse
1	M	B-ALL	5	72	1	Normal	Heparin, dexamethasone, folinic acid, gabapentin, cefixima	No
2	F	Fibroblastic osteosarcoma	34	6	3	Normal	Amlodipine, nebivolol	No
3	F	B-ALL	6	72	1	Normal	None	No
4	M	B-ALL	7	72	1	White matter hyperintensity in rolandic area	None	No
5	F	B-ALL	13	96	1	Signal alterations in T2 and DWI images in periventricular white matter	None	No
6	M	B-ALL	16	96	3	Hyperintensity in T2-weighted images of deep white matter, DWI showing cytotoxic edema	Midazolam, Trazodone, amlodipine	No
7	F	B-ALL	15	24	2	Hyperintensity in T2-weighted images of deep white matter, DWI showing cytotoxic edema	Midazolam	Yes, after 10 months
8	F	B-ALL	11	96	0	Cytotoxic edema in white matter	Midazolam, folinic acid, Levetiracetam	No
9	F	B-ALL	4	24	0	Hyperintense lesion of the No left corona radiata and bilateral hypeintensity in the frontal white matter in T2-weighted images, without any restriction to diffusion.	Clonazepam	No
10	M	B-ALL	13	24	1	Areas of restricted diffusion with slight hyperintensity in the left centrum semi-ovale and bilaterally in the frontal white matter.	None	No
11	F	B-ALL	9	24	0	Hyperintensity in T2-weighted images of deep white matter, DWI showing cytotoxic edema	None	No

in astrocytosis, axonal loss, and demyelination (20, 21). Several MTX-related biochemical changes could also indirectly affect the central nervous system, such as higher levels of homocysteine and a lower production of methionine resulting from the inhibition of DHFR. Decreased levels of S-adenosylmethionine have been associated with demyelination (22), whereas homocysteine appears to have a direct toxic effect on vascular endothelium (23), elicit oxidative stress (24) and alter coagulation (25), although abnormalities in hemostasis have never been observed in patients with MTX-related neurotoxicity (8, 15).

Moreover, homocysteine-derived metabolites, namely sulfur-containing amino acids, are known to be excitatory agonists of the N-methyl-D-aspartate (NMDA) receptor (26), whose intensive stimulation could lead to seizures and excitotoxicity, eventually leading to neuronal damage and degeneration (27). Furthermore, MTX can decrease the levels of adenosine, biopterins, homovanillic acid, and 5-hydroxyndoleacetic acid (22, 28), which also seem to be involved in the development of neurological alterations (7).

According to the literature, the main risk factors for MTX-induced neurotoxicity include high-dose or intrathecal therapy, association with cranial radiation and age >10 years old (9). Some authors proposed a high MTX/leucovorin ratio as an additional SLS trigger (29).

Our findings support these parameters as risk factors; all of our patients were on IT or HD MTX, and the median age was 11 years (mean: 12 years ± 8.4). However, it is not infrequent to observe SLS in younger patients (8, 30), as we observed in Patient 9. Interestingly, we diagnosed SLS also in a 34-year old patient (Patient 2). The onset of SLS in this patient could be partially explained by the higher doses of MTX used in ISG/OS-2 PGOP NEG protocol and the tendency toward a reduced clearance of MTX in older patients (8). Consistently with this hypothesis, we detected a linear correlation between the age and the severity of the clinical picture in our male patients, which had not been described in previous works. However, no studies have linked SLS with pharmacokinetic parameters of methotrexate.

In our series, the onset of SLS could be observed in induction, re-induction and maintenance treatments.

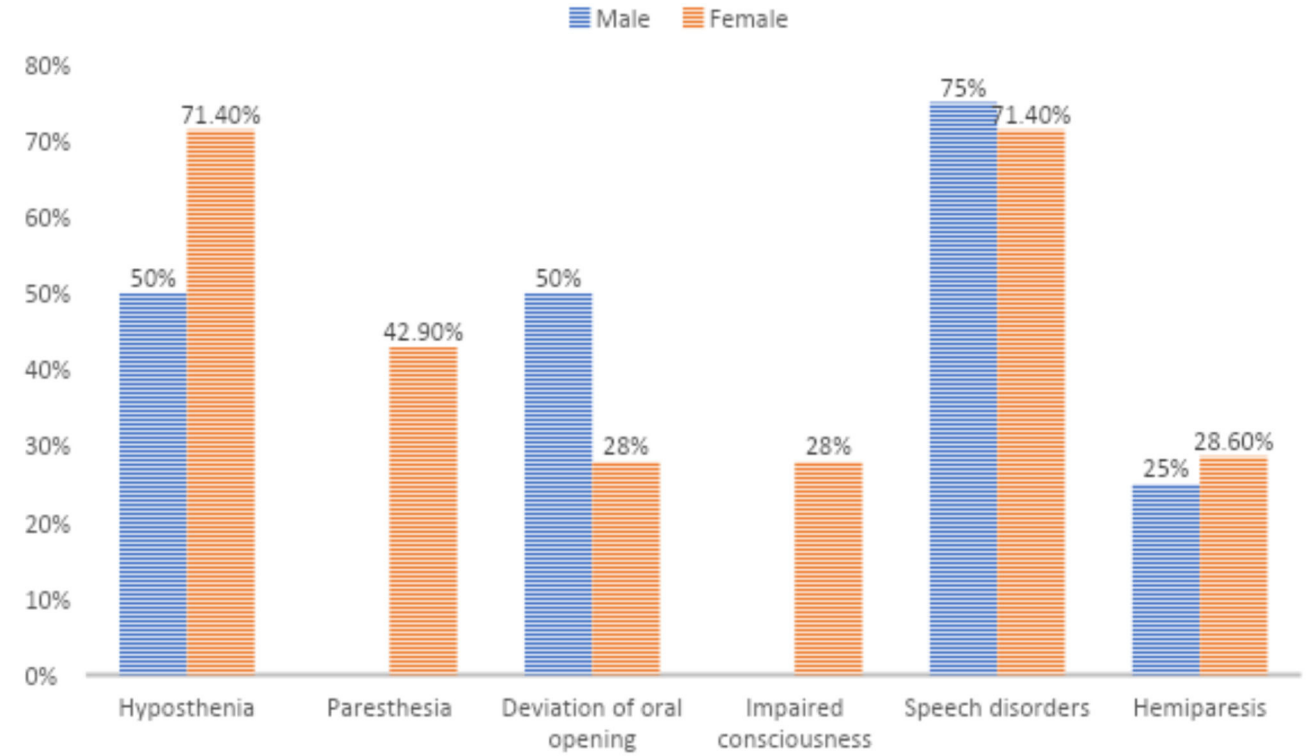

FIGURE 2 | Gender differences in occurrence of symptoms.

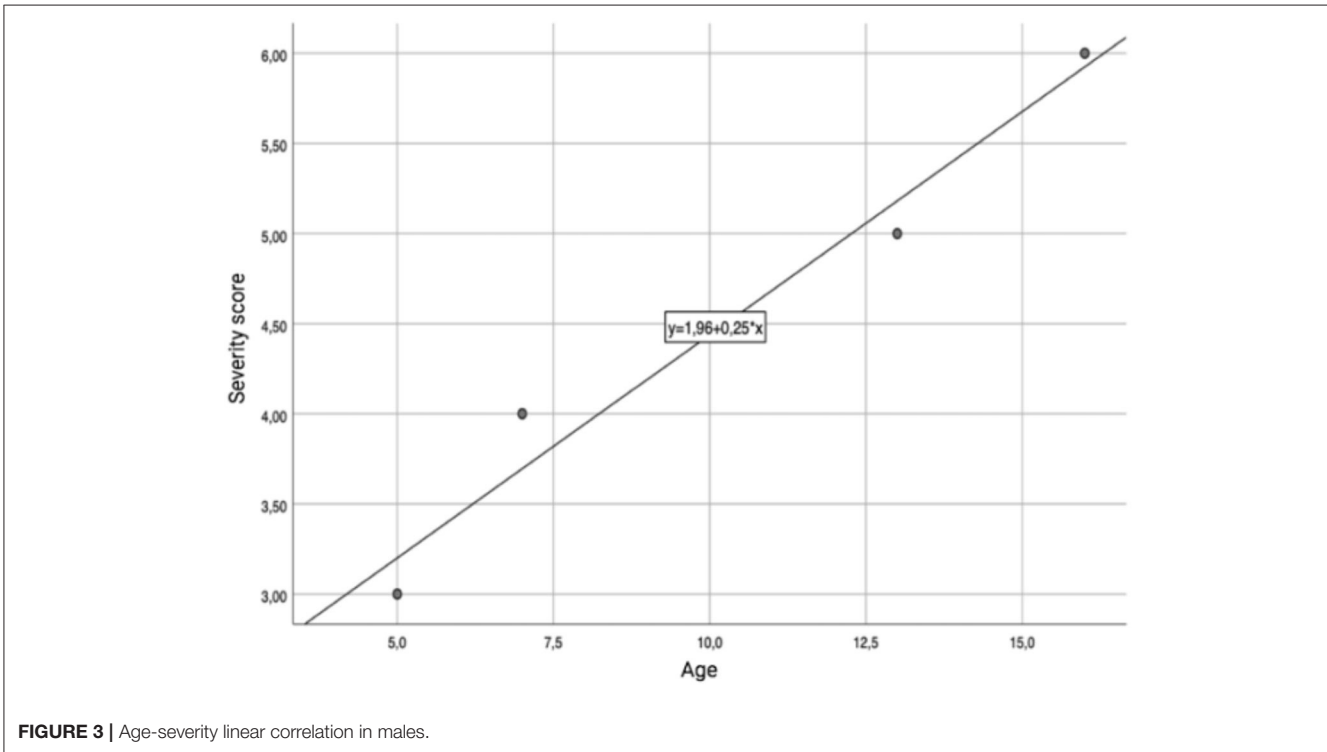

FIGURE 3 | Age-severity linear correlation in males.

All these phases included the co-administration of Ara-C and Cyclophosphamide. These drugs may promote neurological complications in high-dose regimens, and different authors have hypothesized their role in facilitating

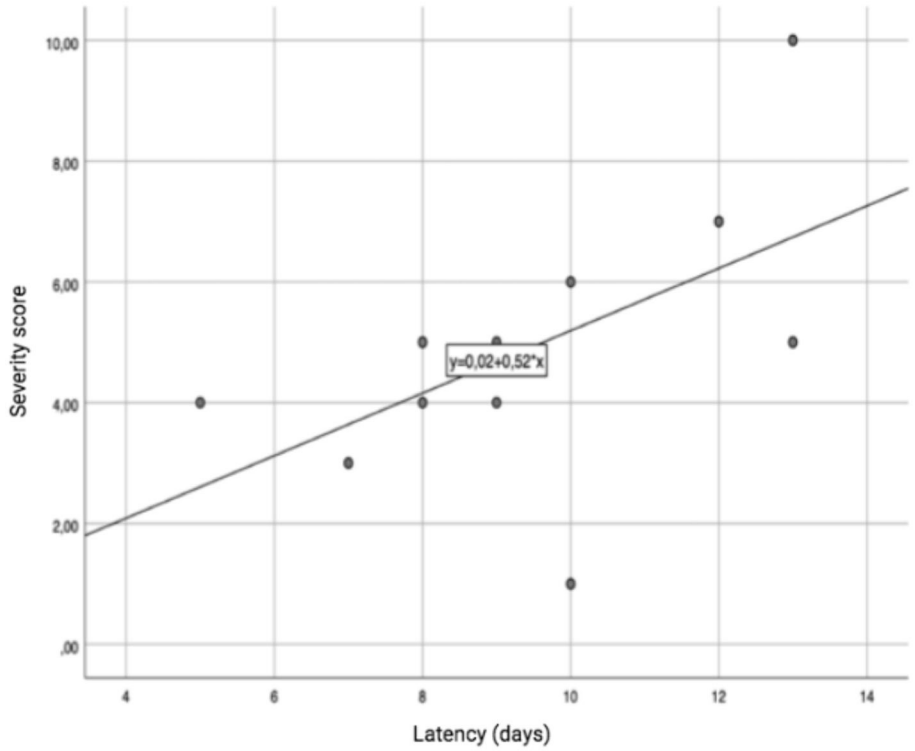

FIGURE 4 | Correlation between the latency time since the last administration of MTX and the severity of the clinical picture.

MTX-induced neurotoxicity. In particular, we could presume that cyclophosphamide plays a contributing role, since it is not employed in other IT /HD MTX-based schedules which are not characterized by SLS (e.g. trials for acute myeloid leukemia).

Furthermore, SLS was not observed upon the first administration of IT or HD-MTX. This could lead us to presume that there is a possible sensitization or progressive accumulation of this drug as a predisposing factor for its neurotoxicity.

In all our cases, the onset of SLS occurred within 1 or 2 weeks after the last administration of MTX (median 9 days), all patients presented fluctuating symptoms which eventually resolved spontaneously and involved mostly 1 haemisoma. These results were in agreement with those in previous literature. Moreover, 2 patients presented transitory increased blood pressure, 1 diffuse tremor and another patient presented seizures.

The severity of the clinical picture was assessed through an arbitrary scale with a total score ideally ranging from 0 to 20. All patients presented a total score between 1 and 10. According to our observations, the main symptoms presented by our patients determine a significant impairment in their quality of life, such as speech disorders, limb hyposthenia, deviation of the oral opening and hemiplegia.

Interestingly, Patient 6 showed choreic movements. Choreoathetosis had been previously reported in SLS patients and could lead us to presume a possible involvement of basal ganglia.

We failed to identify a correlation between the severity of the clinical picture and the time which had elapsed since the last administration of MTX, although this could be due to the small group of patients.

All patients underwent a CT scan, which was normal in 9/11 cases. Brain MRI, performed between 1 and 3 days after the onset of symptoms, showed a hyperintensity in *centrum semiovale* with restricted diffusion in the same area on T2-weighted sequence. Such findings have been observed in other studies also (8, 31–33), and are similar to those seen in the early phases of cerebral infarction (8). This might suggest that the pathogenesis of SLS could be an ischemic lesion of deep white matter.

Interestingly, 3 of our patients showed a normal MRI. Such findings could be related to inter-subject variability, or to the waxing/waning phase of the disease in which the exam was performed.

MTX administration is associated with higher concentrations of different molecules, even in CSF, such as adenosine and homocysteine, which could lead to damage of the vascular endothelium. It has been therefore hypothesized that the deep white matter, which is less vascularised, may be more prone to developing ischaemic lesions (7).

Moreover, other authors have suggested that the peculiar fluctuating manifestations of SLS might be the sign of a progressive depolarization of neuronal and axonal membranes (1, 9), similarly to migraine-associated cortical spreading depression (CSD). In this case, a pivotal role could be played by the MTX-associated astrocytosis (20, 25). It has been observed that astrocytes may show intracellular calcium waves that spread

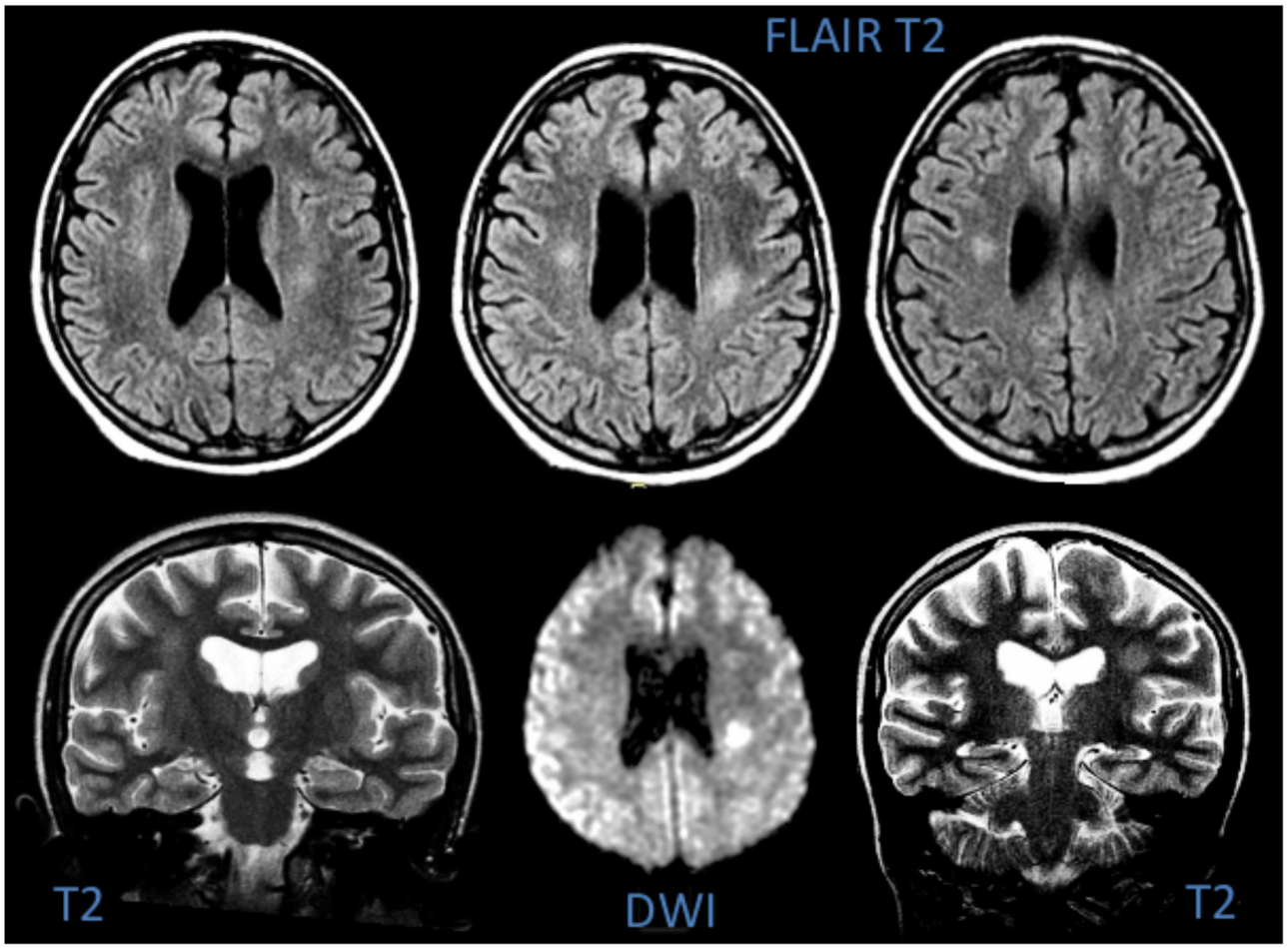

FIGURE 5 | MRI of Patient 6, showing hyperintensity of the deep white matter on T2-weighted images and signs of cytotoxic oedema in DWI.

over long distances (34) and can modulate neuronal and vascular activity.

As regards the treatment of SLS, different drugs could reverse the biochemical effects of MTX (7). Two of our patients received additional folic acid, which may have an effect on the above-mentioned ratio with MTX concentrations, preventing relapses or fluctuations and possibly improving recovery. In both cases, the treatment was followed by remission of the symptoms. Notably, rescue with leucovorin has been successfully employed by some authors in patients rechallenged with HD or intrathecal MTX to prevent relapses of SLS (16). Aminophylline, due to its action as a competitive antagonist of adenosine, has also been widely administered as secondary prophylaxis in patients with MTX-induced stroke-like syndrome (9, 14, 16, 30–32). Moreover, other authors (31, 35), have reported the use of dextromethorphan in some patients. The efficacy of this treatment could be related to its antagonizing action on the NMDA receptor, which might inhibit the excitatory effects of homocysteine metabolites. Total 2 patients required anti-hypertensive therapy.Of these, 1 patient, (Patient. 6), who also presented choreic movements, required the administration of midazolam, the latter being also effectively employed in our patients with tremor and seizures. To our knowledge, no specific treatment has been proposed for SLS besides supporting therapy. Given the predisposition to a spontaneous resolution of symptoms, a gold standard of management is still to be defined.

CONCLUSIONS

Although SLS is a well-known complication of MTX administration, its clinical features and correct treatment are still largely debated. The worrisome clinical picture, which occurs in other complications of chemotherapy, such as Posterior Reversible Encephalopathy Syndrome (36), represents a major concern for the clinician. Better comprehension of the syndrome is therefore needed. Through the analysis of 1 of the largest cohorts of patients in literature, we have been able to achieve a better understanding of the clinical features and severity of MTX-induced SLS. Our results, despite the limited sample, have confirmed some of the main assessments

observed in literature thus far. Nonetheless, we have also detected a linear correlation between age and severity of the disease, which could lead to changes in the management of SLS.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AS, AO, GN, IT, and VF contributed to conception and design of the study. AS, GN, IT, and VF organized the database. GM performed the statistical analysis. AS wrote the first draft of the manuscript. AS, GN, IT, EB, AO, and RB wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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International Consensus Recommendations for the Assessment and Management of Individuals With CDKL5 Deficiency Disorder

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CDKL5 Deficiency Disorder (CDD) is a rare, X-linked dominant condition that causes a developmental and epileptic encephalopathy (DEE). The incidence is between ~ 1:40,000 and 1:60,000 live births. Pathogenic variants in *CDKL5* lead to seizures from infancy and severe neurodevelopmental delay. During infancy and childhood, individuals with CDD suffer impairments affecting cognitive, motor, visual, sleep, gastrointestinal and other functions. Here we present the recommendations of international healthcare professionals, experienced in CDD management, to address the multisystem and holistic needs of these individuals. Using a Delphi method, an anonymous survey was administered electronically to an international and multidisciplinary panel of expert clinicians and researchers. To provide summary recommendations, consensus was set, *a priori,* as >70% agreement for responses. In the absence of large, population-based studies to provide definitive evidence for treatment, we propose recommendations for clinical management, influenced by this proposed threshold for consensus. We believe these recommendations will help standardize, guide and improve the medical care received by individuals with CDD.

Keywords: CDKL5 deficiency disorder, cyclin-dependent kinase-like 5, developmental and epileptic encephalopathy, care guideline, consensus methods, Delphi methods

INTRODUCTION

CDKL5 deficiency disorder (CDD) is a rare and X-linked dominant condition (1, 2), with many aliases, including Developmental Epileptic Encephalopathy 2 (DEE2) (3, 4). It is caused by loss-of-function variants in the *CDKL5* gene (5) which maps to Xp22.13, a gene with 20 coding exons (6, 7). The gene codes for Cyclin-Dependent Kinase-like 5 (CDKL5) protein, previously known as Serine-Threonine Kinase 9 (STK9) (8). *CDKL5* was first mapped by Montini et al. (9) before subsequently seeing an update to its described genomic structure in 2003 (6) by Kalscheuer and colleagues. It was at this time that *CDKL5* was reported as the second cause of X-linked infantile spasms (ISSX), for the first time highlighting genetic heterogeneity in this clinical syndrome. Further genetic reports followed, describing *CDKL5* variants as disease causing while also being genetically and clinically distinct from Rett syndrome (10–12).

As an X-linked dominant condition, CDD is more frequently found in females, with a varying report of female-to-male ratio of between 4:1 (2) up to 12:1 (13). Males are described as displaying a more severe phenotype. The incidence is estimated at between ∼ 1:40,000 and 1:60,000 live births, approximating to one-third of the frequency of Dravet syndrome (1:20,000–1:50,000) (14, 15) or one-quarter of the frequency of Rett syndrome (1:10,000) (16). It is detected in 10–20% of females with early-onset DEEs presenting within the first 6 months of life and should be considered as part of a differential diagnosis for children, females and males, presenting with severe, early-onset epilepsy (17).

CDD presents with a broad phenotype that includes intellectual disability, and impairments in speech, gross and fine motor abilities (18), sleep, gastrointestinal function (19) and vision. Approximately 75% of individuals have cortical visual impairment (20, 21). Seizures typically present in early infancy, with a wide spectrum of semiologies, and are often refractory to treatment (22, 23). Criteria for recognition and diagnosis have been proposed to guide clinicians (2). Evidence is emerging of genotype phenotype correlations for CDD gene variants (24). Evidence-based guidelines have recently been suggested for another DEE, Rett syndrome (25) but there is currently a paucity of evidence and no published consensus to guide clinical management in CDD. Given the broad phenotype, unique features and rarity of CDD, an initial document describing comprehensive care is needed to assist specialist and primary care practitioners caring for individuals diagnosed with CDD. Accordingly, we reviewed the literature and used consensus methods to establish recommendations for clinical management in CDD.

Abbreviations: CBD, Cannabidiol; CDD, CDKL5 Deficiency Disorder; CDKL5, Cyclin Dependent Kinase-like 5; DEE, Developmental Epileptic Encephalopathy; DEXA, Dual-Energy X-ray Absorptiometry; DTI, Diffusion Tension Imaging; ECG, Electrocardiogram; EEG, Electroencephalogram; MRI, Magnetic Resonance Imaging; SUDEP, Sudden Unexpected Death in Epilepsy.

METHODS

Study design: Delphi method.

Literature review and initial guideline development: We performed a literature search and considered mortality, morbidities, diagnosis, treatment, and surveillance of CDD. We used Medline/Pubmed and the Cochrane Library to perform the search.

The main search terms were: "CDKL5" and "Cyclin-dependent Kinase-Like 5." Other associated search terms, as relevant to the topics of interest, were also searched for in combination with the main search terms.

Searching of the Cochrane Library (26) yielded no articles featuring either of the two main search terms. Searching Medline (Pubmed) (27) for the presence of either of the two main search terms, within title or abstract, yielded 452 articles. The search term, "CDD" was not used, to avoid unrelated terms or conditions with the shared abbreviation e.g., "cervical degenerative disease" (CDD). To inform the questions of the survey, the evidence on each topic was reviewed by filtering the main search term results ($n = 452$) to identify topics of interest as included in the title or abstract (**Figure 1**).

Based on these findings, we identified 84 questions for consideration in the Delphi process. The items queried all aspects of CDD including initial assessments, diagnosis, treatment options, follow-up, and surveillance.

The questions were formed by a core committee and reviewed by a subcommittee. Families and caregivers also contributed by review of the design. The Delphi results were analyzed by committees, which consisted of experts in different aspects of CDD from the US, Europe, and the UK. Patient advocacy groups were also part of this process.

Delphi consensus method: The Delphi process provides consensus guidance for the delivery of best clinical care. It is important that the participants are selected carefully. One potential pitfall in a consensus process is that when questions address issues without an evidence base, some respondents may provide answers despite a lack of specific knowledge. It is crucial, therefore, that the respondents are experts in the field. It is generally believed that 15–20 subjects could be sufficient to take part in a Delphi process but the higher the number of the subjects and homogeneity of response, the better the outcome. Some papers have cited that consensus thresholds can be accepted even low as 51%, "in keeping with most other Delphi studies" (28) with others recommending that consensus thresholds should be higher or require unanimous agreement, depending on the gravity of the decisions being made (29). Reviews of Delphi methodology describe the varying nature of the consensus thresholds but all note the importance of having a pre-defined threshold level for consensus, to avoid author bias upon review of responses (30). For this project, *a priori* consensus was defined as 70% agreement for all areas.

General pediatricians, pediatric neurologists, ophthalmologists, developmental pediatricians, geneticists, orthopedic surgeons, adult neurologists, rehabilitation clinicians, allied health professionals, gastroenterologists and nutritionists were invited to take part. All the people surveyed were based in

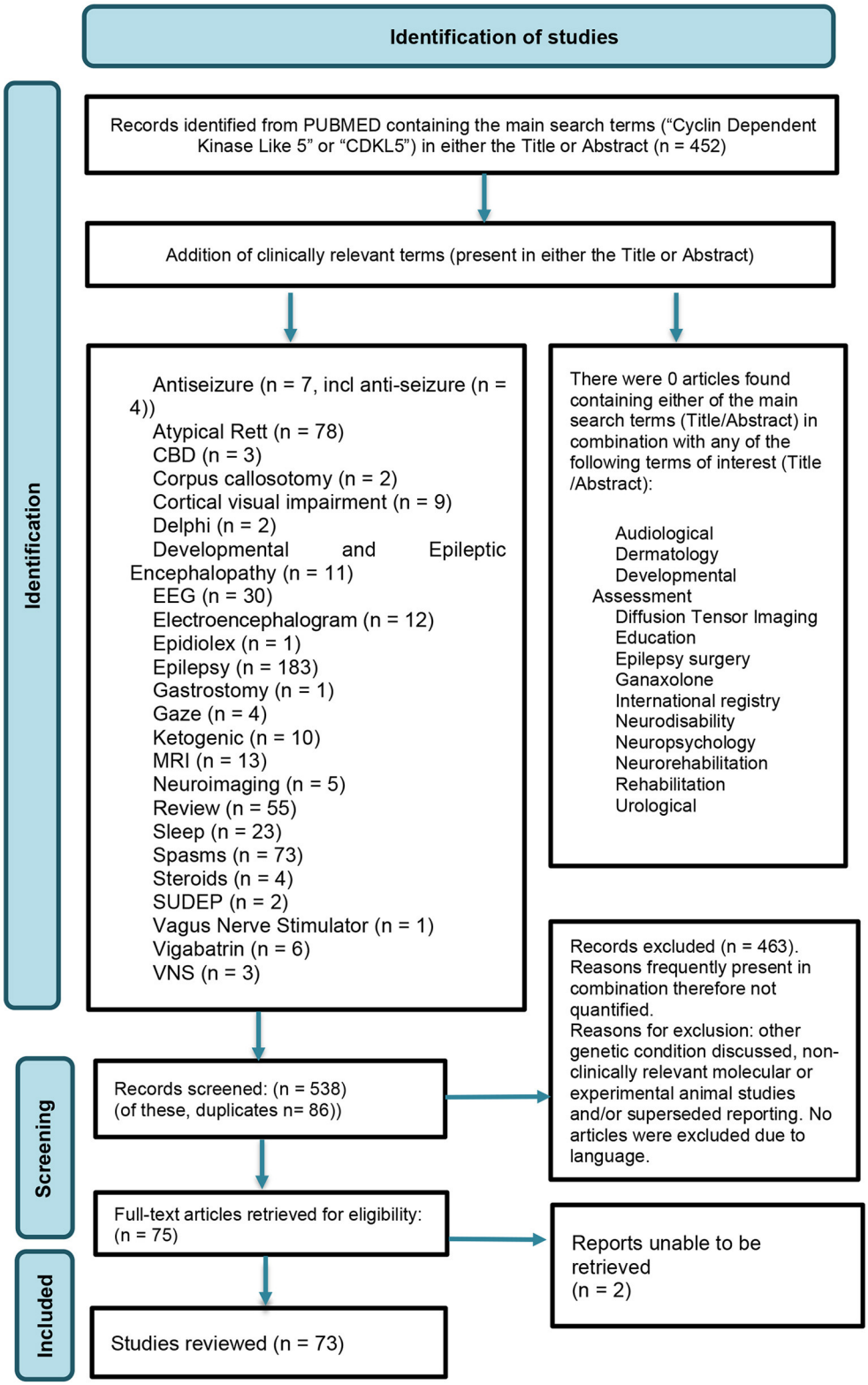

FIGURE 1 | Identification of studies.

the US, Europe, and the UK. Clinicians were identified through CDD clinics and Centers of Excellence, and researchers were identified through publications. The surveys were conducted over 6 months from August 2020 to January 2021. A weekly electronic reminder was sent to the responders. Forty-seven experts responded to the survey. The responders were pediatric neurologists ($n = 30$), epileptologists ($n = 10$), geneticists ($n = 2$) as well as a general pediatrician, a development/community pediatrician and an allied health professional. Two of the 47 respondents did not describe their specialty. The number of years of experience within their current specialty favored highly experienced professionals, with 58% ($n= 27$) having >15 years, followed by 34% ($n = 16$) with >5 years of experience. Professionals had a mixed range of experience in managing CDD; under half had managed <5 individuals ($n = 22$, 47.8%) followed by nearly a fifth that had managed 6–10 individuals ($n = 9$, 19.6%) with nearly a third (32%) having managed >10 individuals with CDD ($n = 15$). While CDD exposure had been mixed, most of the surveyed professionals ($n = 46$, 97.9%) had significant (>20 individuals) experience in managing DEEs. Many members of the core and subcommittee had a wealth of expertise in managing patients with CDD, leading their national centers of excellence in their practicing countries.

RESULTS

The survey contained questions relating to current practice in CDD and was sent to the respondents. Answers where respondents did not feel they had relevant experience to be able to answer, indicated by selection of "I am not qualified to answer" or "I do not know," were excluded, for the purposes of assessing the degree of consensus of opinion among experienced responders for each particular topic. Questions referring to "at baseline" were in reference to where the diagnosis of CDD had already been made, with the exception of genetic testing (CDD is considered a genetic diagnosis).

Genetic Screening and Counseling

ACMG (American College of Medical Genetics and Genomics) guidelines state that genetic counseling should be "offered at all stages of genetic testing".

Survey

There was consensus in favor (45 responses, 97.8%) of offering a genetic test, before diagnosis was established, to all individuals with DEE. There was no consensus when asked when they would offer genetic counseling; with responses nearly equally divided into "Prior to genetic testing" (17 responses, 44.8%) and "After genetic testing" (21 responses, 55.2%). There was no agreement between the geneticists who responded to this question.

Neurological Assessment

Clinical Management

CDD is a disorder associated with DEE. Seizures often take the form of spasms, with or without hypsarrhythmia demonstrated on electroencephalogram (2, 11, 20, 31, 32), tonic seizures, and hypermotor (mixed) seizures (20). In addition, individuals may present with hypotonia (33). Male children with *CDKL5* mutations are believed to be more severely affected and have a higher frequency of epileptic (infantile) spasms and brain atrophy (34).

Survey

Regarding formal assessments by a pediatric neurologist, 97.6% (40 responses) of respondents felt individuals should be seen at baseline, and thereafter regularly. Asked whether individuals should be seen by a pediatric epilepsy specialist at baseline and regularly, the response was the same with 95.2% (40 responses) in agreement.

While Sudden Unexpected Death in Epilepsy (SUDEP) is reported to occur in individuals with CDD, data from large cohort studies suggests the frequency of SUDEP within the CDD population is lower than for Dravet syndrome or SCN8A-DEE, given the frequencies of these disorders (35–37). The annual risk of SUDEP among individuals with CDD remains uncertain due to the limited data.

Survey

Respondents were asked whether families should be informed about SUDEP at baseline. The responses were mainly in favor (86.5%, 32 responses), meeting the threshold for consensus.

Survey

The respondents were asked which laboratory tests should be carried out at baseline. Mixed responses included: "blood count" (18 responses, 64.3%), "vitamin D" (18 responses, 64.3%) and "urea and creatinine" (16 responses, 57.1%). Twenty-five percent (7 responses) felt no blood tests should be routinely performed. Similarly, when asked which should be carried out annually, leading responses were "blood count," "vitamin D" (each having 16 responses, 59.3% each) and "metabolic profile with urea and creatinine" (14 responses, 51.9%). The predominance of these basic profiles and a vitamin D level suggest that the purpose of such tests is not for diagnostic benefit but to reduce the risk from associated comorbidities e.g., from gastrointestinal dysfunction or reduced mobility with associated fracture risk, as in other DEEs. There were 7 respondents who believed no annual laboratory requests should be performed (25.9%).

Neuroimaging

In terms of neuroimaging, there are limited, non-quantitative reports on the findings associated with CDD. One study (38) reported "cortical atrophy" in 13 of 20 girls, associated with areas of increased T2 signal in the white matter, especially in the temporal lobes in some.

Survey

Respondents were asked whether all individuals should have a brain MRI scan at baseline for those who have not been investigated with an MRI previously. The responses did reach a consensus with 70.3% (25 responses) responding "Yes." As a follow-on, those who had responded "Yes" were asked whether all individuals should have a DTI brain MRI scan at baseline. Currently DTI is an area of research interest with no reports

published in relation to CDD. The majority did not feel this was required; "No" (76.7%, 23 responses).

Electroclinical Findings and Use of Electroencephalogram

Individuals with CDD typically present with epileptic spasms within the first 4 months of life and subsequently manifest epileptic encephalopathy (32, 38, 39). Electroclinical findings in the first year of life include a peculiar seizure pattern with "prolonged" generalized tonic-clonic events, lasting 2–4 min, consisting of a tonic-vibratory contraction, followed by a clonic phase with a series of spasms, gradually transitioning into repetitive distal myoclonic jerks (40). The EEG during these seizures shows a bilateral, synchronous initial flattening, followed by repetitive sharp waves and spikes. Atypical hypsarrhythmia is often seen in infancy, developing into multifocal abnormalities in older individuals (38). Typical EEG findings develop over time and are not manifest in young infants. This likely reflects limited functional cortical organization in young infants, necessary to propagate and sustain an electrical discharge, and limited interhemispheric transmission from commissural immaturity (41, 42). Early EEG findings can vary from normal background to moderate slowing, with superimposed focal or multifocal interictal discharges and rarely, a burst-suppression pattern (40). In a follow-up of children older than 3 years, about half experienced seizure remission while continuing anti-seizure drugs, with the other half continuing to have intractable spasms, often associated with multifocal and myoclonic seizures (38, 39).

Survey

Most (86.0%, 37) respondents supported an EEG at baseline, regardless of clinical seizures. Most (76.9%, 30) respondents favored EEG performed to capture epileptic spasms before treatment. For less typical seizure-like events, respondents were asked whether an EEG should be repeated to capture and classify spells of unclear clinical significance. Responses were in favor, with 97.6% recommending this (40 responses). There was no consensus when asked what duration of an EEG to request. The leading response was "Routine (under 2 h)" (18 responses, 51.4%). The variation of responses may reflect the availability of prolonged EEG.

Seizure Management–Use of Antiseizure Drugs and Ketogenic Diet

Seizures associated with CDD typically present in early infancy, with a wide spectrum of semiologies, and are often refractory to treatment (22, 23). The most common seizure types in CDD are epileptic spasms (often without hypsarrhythmia) and tonic seizures that may cluster (20). It is uncertain what proportion of epileptic spasms are attributable to CDD, however one study identified 3 patients with pathological variants in *CDKL5* among 73 patients with epileptic spasms (43). Other seizure types have been described including atonic, atypical absence, focal with motor components, myoclonic, typical absence and tonic-clonic (44). To have pathological variants in *CDKL5* without associated seizures is extremely rare but has been reported (22) although this is unlikely to affect CDD being considered a DEE.

The treatment of epileptic spasms encompasses aspects of seizure control, side-effects and longer-term neurodevelopmental outcomes. O'Callaghan et al. performed a multicentre, open-label randomized controlled trial to investigate the effect of treatment options, either oral prednisolone (10 mg four times a day) or intramuscular tetracosactide (0.5 mg (40 IU) on alternate days), with or without oral vigabatrin (100 mg/kg per day) (45). The primary outcomes at 18 months, independently assessed, were neurodevelopmental outcomes and the frequency of seizures. While this study was not focussed on the epileptic spasms associated with CDD, it identified that earlier seizure control was a predictor of better developmental and epilepsy outcomes at 18 months. While earlier seizure control was obtained in the combination therapy group, it was surprising that there was no statistically significant difference in developmental or epilepsy outcomes at 18 months between the two groups (combination therapy or hormonal therapies alone). The authors explained this incongruity with the suggestion that those who had not responded to hormonal therapy alone would have rapidly received additional vigabatrin and therefore received combination therapy. Furthermore, any improvement in development associated with earlier cessation of seizures with combination treatment, may be undermined by the potential negative side-effects of vigabatrin such as drowsiness and visual field defects, as listed among others in the British National Formulary for Children. Studies assessing neurodevelopmental and seizure outcomes would be welcome for individuals with epileptic spasms associated with CDD, in light of reports of worse seizure outcomes with hormonal therapy for individuals with CDD. One study (22) assessed seizure variables in relation to CDD genotype and found that with a median age of questionnaire completion at 5 years, those who had previously been treated with corticosteroids had more frequent seizures than those who had never been treated, irrespective of a history of epileptic spasms.

Studies looking at the efficacy of anti-seizure drugs in the treatment of CDD-related epilepsy have frequently shown only temporary and frequently paradoxical (exacerbation) responses to various anti-seizure drugs, despite the use of medications with different mechanisms of action (23). In one study looking at the effect of anti-seizure drugs in 39 individuals with CDD (23), the highest, but still very low, responder rate after 12 months was reported with sodium valproate (9%, 3 individuals) whereas there was a very low number of individuals that responded to phenytoin, felbamate, carbamazepine and clonazepam. Drug response was defined as a more than 50% reduction in the preceding 4 weeks, compared to 4 weeks in the baseline period before starting the new anti-seizure drug. In this study, steroids/ACTH had a 19% (5) responder rate at 3 months but 0% response rate at 12 months. Similarly, vigabatrin had a 32% (8) responder rate at 3 months but just a 4% (1) responder rate at 12 months (23). For patients with earlier onset epilepsy with focal epileptiform activity, there is evidence supporting the use of sodium channel blockers, such as oxcarbazepine, carbamazepine and lacosamide (46).

Initial apparent benefit with subsequent loss of anti-seizure drug efficacy over time in the management of epilepsy associated

with CDD has been described as the "honeymoon effect" (2, 22). This was first described following analysis of caregiver reports on the effects of anti-seizure medication on seizures from caregivers of 163 individuals with CDD with epilepsy registered in the CDKL5 Disorder Database (22). It was found that fewer than half (43%, 71/163) of caregivers reported ever having had more than 2 months of seizure freedom. Typically the honeymoon period had a median onset of 2 years (for 74%, 52/70) and a median duration of 6 months (for 84%, 59/70).

Survey

Respondents were asked to rank their first, second, third and fourth-line therapies for epileptic spasms associated with CDD. There was no consensus for any of the first, second, third or fourth line suggested therapies, although the standard treatments of vigabatrin, steroids and the combination of these featured most strongly. For first line therapy, 37.5% (15 responses) favored combination therapy (steroids and vigabatrin), 35% (14 responses) favored steroids alone and 27.5% (11 responses) favored vigabatrin alone. No responder suggested use of ketogenic diet as a first line therapeutic option. Similarly, there was no consensus among second line therapy options, however among a choice of steroids, vigabatrin, combination of these or the ketogenic diet, the ketogenic diet was selected by nearly a quarter (23.1%, 9 responses) as a second line therapeutic option. The ketogenic diet similarly made up an increasing preference (17 (54.8%) and 10 (41.7%) responses) for third and fourth line therapy preferences. The ketogenic diet was considered by respondents as early in the management of seizures as a second or third line therapy option, with few other epilepsies, e.g., *SLC2A1* mutation (47), prompting such early consideration.

Lim et al. (48) studied the use of the ketogenic diet to manage refractory epilepsy associated with CDD. They found that of the approximately half of individuals with CDD who have tried the ketogenic diet, some 59% of individuals experienced improvement in seizure frequency, duration, or intensity. However, none of the individuals on the ketogenic diet became seizure-free. This lack of complete resolution of seizures, along with side-effects of the diet, led to poor long-term adherence (median duration 17 months). In a study on quality of life domains for individuals with CDD, 20% (5 of the 25 surveyed) were currently on a ketogenic diet (49).

Survey

The respondents were asked whether individuals should be treated with a ketogenic diet as soon as they fail their first line treatment for epileptic spasms. The responses were mixed with most in favor (23 responses, 53.5%). This response may be interpreted as encouragement for starting a ketogenic diet at the soonest moment that a first line therapy has proven inadequate for controlling epileptic spasms and that the diet may be in addition to a second line medication option (differentiating this nuance from the preceding survey responses).

While several studies looking at the use of CBD for the treatment of drug-resistant epilepsy have shown promising results, few have provided specific results for the performance of CBD in the CDD subpopulation (50). Devinsky et al. (51) undertook an open-label study exploring the use of CBD in individuals with severe, treatment-resistant, childhood-onset epilepsy including CDD, among other disorders. In individuals with CDD, the median monthly convulsive seizure frequency decreased from baseline (66.4 [$n = 17$], IQR: 25.9-212.0 to week 12 (35.8 [$n = 11$], IQR: 8.9-141.6) which was found to be statistically significant ($p = 0.032$). Further placebo-controlled randomized trials in a larger population sample are necessary to formally assess the safety and efficacy of cannabis-based products in CDD.

Survey

There was consensus on whether CBD (Epidiolex) should be offered for epilepsy in CDD. The responses provided strong support for this option with 92.6% (25 responses) in favor with 7.4% against (2 responses). This reflects an increasingly positive view of CBD for medicinal uses, including in the pursuit of reducing seizure burden among populations of children with mixed etiologies of drug-resistant epilepsy (52, 53).

Ganaxalone is a synthetic methyl derivative of allopregnanolone, a neurosteroid, which acts as a high-affinity allosteric modulator of $GABA_A$ receptors. Ganaxalone has been trialed for epilepsies including epileptic spasms, status epilepticus and protocadherin 19 related epilepsy (2). The Marigold Study (NCT03572933) is the first Phase 3, randomized, placebo-controlled trial that evaluated adjunctive ganaxolone in patients with refractory epilepsy associated with CDD. Patients on ganaxolone experienced a median of 30.7% reduction in major motor seizure frequency compared to a 6.9% reduction in the placebo group during the treatment period relative to baseline ($p = 0.0036$, Wilcoxon Rank-Sum Test). Ganaxolone demonstrated improving trends but did not achieve statistical significance in the key secondary endpoints. Adverse events occurred in 86% of ganaxolone patients and 88% of placebo patients. Ganaxolone was generally well-tolerated with a <5% discontinuation rate in the treatment arm, with somnolence being the most frequent adverse event (36% of patients) (54).

Survey

Respondents were asked whether Ganaxolone should be offered, if available (dependent on regulatory approval). The unanimous response was "Yes" (27 responses, 100%), meeting the threshold for consensus. The FDA has just approved ganaxolone (Ztalmy; Marinus Pharmaceuticals) for the treatment of seizures associated with CDD, in patients aged 2 years and older.

Epilepsy Surgery

The effects of vagus nerve stimulation (VNS) for the treatment of refractory epilepsy for CDD has been studied (55). Of 222 patients with *CDKL5* variants where there was adequate information, 38, the equivalent of 1/6 or 17% had previous or current use of VNS. Improvement in seizure control was reported in 69% (25/36) and of them, this related to improvements in frequency in 68% (17/25), duration in 72% (18/25) or intensity in 60% (15/25). No patient with a VNS became seizure-free and termination of VNS occurred in 1 in 10 cases.

Survey
Respondents were asked whether individuals should be considered for VNS insertion if seizures are refractory to medications. The responses were mainly in favor (89.7%, 35 responses).

Patients with non-resectable, drug-resistant seizures with spread between hemispheres, i.e., generalization, may be considered for corpus callosotomy. In a meta-analysis of the effects of corpus callosotomy in epilepsy surgery, analyzing the impact of corpus callosotomy on 1,742 children and adults from 58 studies, it has been shown to be associated with drop attack freedom in 55.3% and complete seizure freedom in 18.8% (56). For those achieving complete seizure freedom, this favored patients whose etiology included infantile spasms (OR 3.86, 95%. CI 1.13-13.23), normal MRI (OR 4.63, 95%. CI 1.75-12.25), and a shorter epilepsy duration of <15 years (OR 2.57, 95%. CI 1.23-5.38). Interestingly, neither the presence of lateralising EEG abnormalities nor the selection of complete vs. partial corpus callosotomy made a significant impact on the outcome, unlike in the analysis of patients with drop attacks where these were associated with improved outcomes.

Survey
Respondents were asked whether individuals should be considered for corpus callosotomy if seizures were refractory to medications. The leading response was 71.0% in favor (22 responses), meeting the threshold for consensus.

Stereotypes and Movement Disorders
Hand stereotypies are reported in 80% of individuals and can negatively affect functional hand movements in 59% of females and 12.5% of males with CDD (1). Olson and colleagues (unpublished) describe self-stimulatory hand movement syndrome and repetitive leg crossing in CDD patients. Unquantified episodes of persistent, occasionally severe, choreoathetosis, akathisia, dystonia and parkinsonian features have been reported, potentially having been unmasked during temporary periods of improved seizure control or potentially secondary to polytherapy with antiseizure drugs (2).

Survey
Respondents were asked whether individuals should be screened for movement disorders at baseline. The responses were: "Yes" (39 responses, 100%), achieving consensus. The respondents were also asked whether individuals should be screened for movement disorders at regular clinical appointments, annually, with 100% in favor (38 responses). Respondents were 100% in favor with regard to movement disorders being treated if causing problems. Asked what would be the most suitable option, the leading responses were: "Gabapentin" (15 responses, 62.5%), "Clonidine" (13 responses, 54.2%) and "Benzodiazepines" (10 responses, 41.7%).

International Registry
With increased attention on therapies for CDD, prospective, randomized, and double-blind clinical trials are considered essential to establish statistical significance and thus will necessitate international collaboration (57).

Survey
When asked whether individuals should be offered to be enrolled in an international registry or other research studies, 100% were in favor (46 responses).

Neuropsychological Assessment
Survey
When asked whether individuals should have a neuropsychology assessment at baseline (where the diagnosis has already been made), there were mixed responses with 59.4% in favor (19 responses). Similarly, when asked whether individuals should have a neuropsychology assessment regularly, responses were: "Yes" (26 responses, 68.4%). This did not meet the threshold for a consensus of opinion.

Somnology
Sleep-related difficulties are reported in over 85% of individuals with CDD, sometimes dubbed "all night parties" with problematic night-waking reported in up to 58.5% (1, 2, 19) and males more severely affected (19). Sleep apnoeas have been documented in both individuals and mouse models of CDD (58, 59). The odds of reported sleep difficulties was higher in the 5–10 year age group than the under 5 year group (19).

Survey
Respondents in our survey were asked whether individuals should have their sleep assessed at baseline. The leading response met the threshold for consensus with 92.3% (36 responses) in favor. Similarly, there was consensus when respondents were asked whether individuals should have their sleep assessed annually with 85.7% (30 responses) in favor. When respondents were asked which drug or drugs could be used to help with sleep, the leading response, "Melatonin" (35 responses, 53.8%), did not meet the threshold for consensus of recommended first choice, however, was more popular than the second most selected answer, "Clonidine" (16 responses, 24.6).

Therapy Assessments and Interventions
Neuro-Rehabilitation Assessment
Neuro-rehabilitation services, sometimes referred to neuro-developmental or neuro-disability services, are part of the care of individuals with CDD. Assessing function and response to therapies is important in guiding and interpreting the findings of future research into therapies for CDD (60). A collaborative professional and caregiver-based standardized assessment method was designed using four cycles of a Delphi process, the CDD Clinical Severity Assessment (CCSA). This involved clinicians from the International Foundation for CDKL5 Research Centers of Excellence (COE) consortium and the National Institutes of Health' Rett Syndrome, MECP2 Duplication Disorder, and Rett- Related Disorders Natural History study consortium (U54 HD061222; ClinicalTrials.gov: NCT00299312/ NCT02738281). Initial consensus was provided by clinicians, researchers, industry, patient advisory groups and the parents of a child. The CCSA reviewed 53 items, 27 reported

by parents and 26 reported by clinicians. It has recently been developed (61) and validated to enable its implementation for the assessment of outcome measures, as per FDA requirements (62, 63).

The final CCSA will be 50% clinician assessment of motor, cognition, behavior, vision, speech and autonomic function domains. The other 50% will be parent-led assessment, complimentary to the design and structure of the clinician assessment. The aims of the CCSA are to support design and interpretation of research, evidence-based management choices in CDD and identification of current patient needs. Specific items capture levels of functioning in the gross motor, hand function, communication and behavior domains.

Survey

We asked whether individuals should be offered a referral to a neuro-rehabilitation service at baseline, to assess equipment needs and diagnose or improve problems with mobility and hand function and to prevent contractures. There was strong support for this with 91.9% of respondents (34 responses) in favor. Similarly, when asked whether individuals should be offered a referral to a neurorehabilitation service annually for the same purpose, 92.1% (35 responses) were in favor.

Development Assessments

CDD is associated with global developmental delay including intellectual disability. Most individuals are severely impaired. In one study (18), data for 108 females and 16 males, registered with the International CDKL5 Disorder Database, were collected. Over half of females could sit on the floor and nearly a quarter could walk 10 steps. Most females and few males were able to pick up a large object. Those with a late truncating variant displayed better levels of ability than those with no functional protein. Subsequent research has expanded the correlations of the genotype-phenotype (20).

This work was also performed using an expanded cohort from the same International CDKL5 Disorder Database (24). The study looked at genotype-phenotype findings for 385 individuals with CDD. They then assessed genotype-phenotype relationships for 13 recurrent *CDKL5* variants and compared these with previously analyzed historic variant groups. Developmental scores and severity assessments were performed using the CDKL5 Developmental Score (CDS) and an adapted CDKL5 Clinical Severity Assessment (CCSA). Individuals with the missense variant, p.Arg178Trp, had the highest mean adapted CCSA and lowest mean developmental scores. They also found that p.Arg559* and p.Arg178Gln produced severed phenotypes whereas p.Arg134*, pArg550* and p.Glu55Argfs*20 produced milder phenotypes. This study identified trends between variants and phenotypes and updated historic genotype-phenotype reports.

Regression, if encountered, is often related to worsening of seizure control and the presumed effect of epileptic encephalopathy (1, 18, 32, 33, 64). In girls, walking is attained by 22%, raking grasp by 49% by 5 years and pincer grasp by only 13% at any point (18, 65).

Survey

We asked whether individuals with CDD should have developmental assessments and 100% were in favor (44 responses), with 75% (24 responses) proposing, "Soon after diagnosis," meeting the threshold for consensus. Nearly all (95.3%, 41 responses) of respondents felt developmental status assessment should be repeated. Nearly all (92.3%, 36 responses) felt the assessments ought to occur at key developmental points and periods of transition, proposed as during infancy (0–3 years), preschool age (3–6 years), pre-middle school age (6–9 years), adolescent age (12–16 years, early adulthood (18–25 years) and as needed thereafter.

Ophthalmology

CDD is associated with cortical visual impairment (CVI) with approximately 75% having cortical visual impairment (20).

Survey

The respondents were asked whether individuals should have a detailed vision assessment at baseline. The responses were: "Yes" (38 responses, 100%). Similarly, respondents felt individuals should have an annual vision assessment with all in favor (29 responses, 100%). When asked whether individuals with CDD should be referred to an ophthalmology specialist familiar with cortical visual impairment, for assessment, the responses were strongly (100%, 37 responses) in favor. For management by an ophthalmology specialist familiar with CVI, the responses were also 97.1% (34 responses) in favor.

Speech and Language Assessment and Communication Aids

As part of global developmental delay and associated cortical visual impairment, individuals with CDD experience difficulties with communication (18). In one study (65), it was found that under half of individuals could babble by the age of six (43/97, 44%) and under a quarter could say single words by the age of seven (17/105, 16%). Only 7.5% of females achieve speaking in full sentences (18) with males 80% less likely than females to be able to use advanced communication methods (OR 0.17, 95% CI 0.04–0.71). Upon assessment and categorization of highest communication ability, it was found that 26% were able to use spoken language, sign language and abstract symbols, followed by 39% who were able to use complex gestures, vocalizations and concrete symbols with 33% able to use only simple communication alone (such as body language, early sounds, facial expressions and simple gestures). While speech difficulties can present with other features suggestive of autism, this diagnosis is infrequently made while in the context of severe global developmental delay (2).

There have been few studies published reviewing the use of non-verbal communication aids for individuals with CDD. Unpublished data by Olson et al., reviewed the use of devices such as switches and eye gaze technology-based communication aids. They found that in those unaffected or mildly affected by cortical visual impairment, such devices provided assistance for some with CDD. A recent systematic review has investigated outcomes and uptake barriers for

the pediatric population with complex disabilities using eye gaze assistive technology (66). This analysis reviewed the use of eye gaze technology on the World Health Organisation's International Classification of Functioning, Disability and Health Framework. There were 11 articles suitable for review, of which eight assessed communication and of which six reported enhanced communication outcomes. The review highlighted poor methodological quality and/or low level evidence, limiting the review's findings and reflecting a need for further published and high-quality evidence.

Survey
When asked whether individuals with CDD should be checked and assessed for augmentative and assistive communication aids such as switches, touch pads or eye gaze aids, respondents were unanimously in favor (41 responses, 100%).

Orthopedic, Physiotherapy and Occupational Therapy Assessments

Orthopedic concerns are a potential consequence of hypotonia and can lead to scoliosis, with 68.5% of individuals affected by 10 years (1, 19).

Survey
Asked whether individuals should have a hip and spine X-ray, most responses were: "If there is a clinical concern" (31 responses, 77.5%), reaching the threshold required for consensus. Respondents did not favor individuals with CDD having a routine orthopedic (specialist surgeon) review at baseline, with the leading response being not in favor (22 responses, 73.3%). Equally, when asked whether individuals should have a routine yearly orthopedic review, the responses leaned toward not being in favor (15 responses, 53.6%). Whether reflecting concerns (e.g., pertaining to reduced mobility or a ketogenic diet) when asked whether individuals with CDD should be offered a screening test for osteopenia (such as wrist X-ray or DEXA scan), the leading responses was: "If clinically indicated" (28 responses, 82.4%).

Consensus guidelines for the approach to screening and management of scoliosis and osteopenia are not available for CDD however a consensus of routine management for optimal bone health in Rett syndrome has been developed and is likely relevant to individuals with CDD until higher level evidence becomes available (25, 67–69).

Fu et al. provided observational data for 913 females with classic Rett Syndrome. They identified that severe scoliosis was found in 251 participants (27%), 113 of whom developed severe scoliosis during follow-up assessments with 168 (18%) having surgical correction. The study proposed the implementation of spinal bracing when spinal curvature reaches 25°, in the hope of retarding or minimizing further progression. Beyond 40°, the authors strongly promoted surgical intervention. Each study suggests annual evaluations for both of these issues along with guidelines for management and referrals.

Survey
There was consensus in favor when asked individuals with CDD should be offered Physical Therapy (PT) assessment at baseline (where diagnosis has already been made) with 97.8% of respondents in favor (44 responses). Equally 97.8% (44 responses) felt that individuals with CDD should have access to PT regularly for ongoing issues.

Survey
Asked whether individuals should be offered an occupational therapy (OT) assessment at baseline (where diagnosis has already been made), the responses strongly in favor (38 responses, 92.7%). Similarly, when asked whether individuals with CDD should have access to OT regularly for ongoing issues, the responses were strongly in favor (42 responses, 100%).

Educational

Individuals with CDD face difficulties such as communication difficulties and cortical visual impairment. Interventions, such as visual attention tracker, may assist in informing the wider team whether educational interventions are providing benefit (70).

Survey
Asked whether educational accommodations for visual impairment should be provided, 97.6% (41 responses) were in favor. More broadly, 92.1% (35 responses) were in favor when asked whether educational support provided in formal educational plans should be reviewed at baseline. Similarly, respondents felt a review of these should be performed annually, with 94.9% (37 responses) in favor.

Systemic

Auxology

Five individuals with CDD were reported to have normal head circumferences at birth and over the subsequent 2 years develop postnatal microcephaly (64). Similarly, deceleration of head growth has been described in 11 out of 20 (55%) individuals with CDD (33). Microcephaly has been associated with an increased degree of functional impairment (71).

Survey
When asked whether head circumference, weight, height should be each checked at baseline, respondents were in favor; 100% (46 responses), 97.8% (45 responses) and 97.6% (42 responses), respectively. Similarly, when asked whether height and weight should be checked annually, 100% (43 responses) were in favor.

Gastrointestinal Management Including Assessment and Management of Feeding

Patients with CDD may experience dysphagia and require gastrostomy (2). Evidence suggests that gastrostomy tube feeding for pediatric patients with neurological impairments may reduce the risk of death although associated with an increased the risk of severe pneumonia (72). Guidelines produced by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition, for the evaluation and treatment of gastrointestinal and nutritional complications in children with neurological impairment, recommends the use of enteral tube feeding in cases of unsafe of inefficient oral feeding, preferably before the development of undernutrition, and that a gastrostomy is the preferred way to provide intragastric access for long-term tube

feeding for this population. Aside from nutritional difficulties affecting growth, a gastrostomy tube may improve caregiver quality of life, assist in the administration of fluids and/or a ketogenic diet and, through compliance with medications and/or ketogenic diet, may reduce seizure burden (73, 74). A review of patients from the CDKL5 Disorder Database found that 20.7% of individuals were fed exclusively by gastrostomy or nasogastric tube (19) but this prevalence may be as high as 43% among individuals with CDD, following analysis of patients based in the United States of America (75) (154 individuals identified from data held by Centers of Excellence and 40 identified from the NIH's Natural History of Rett and Related Disorders database). In a smaller study on quality of life domains for those with CDD, as many as 56% (14/25 surveyed from the CDKL5 international registry) had a gastrostomy (49).

Survey

Respondents were asked whether gastrointestinal complications such as constipation, air swallowing and acid reflux should be assessed at each clinic visit annually. The responses were strongly in favor (43 responses, 97.7%). Asked whether individuals should be referred to a Gastrointestinal specialist, responses were in favor (92.0%, 23 responses). When asked whether individuals should be referred to a Nutrition specialist, responses were also in favor (30 responses, 96.8%). When asked when swallowing coordination should be formally assessed (i.e., by Speech and Language Specialists) most felt this should be, "Only if there are concerns" (25 responses, 61.0%). Respondents were more strongly in favor of individuals being offered an informal speech therapy assessment at baseline (where diagnosis has already been made) (38 responses, 92.7%). Similarly, a large majority felt that non-specialist feeding, and swallowing should be assessed at annual clinical reviews (36 responses, 90.0%). Respondents were asked when a gastrostomy should be considered, with responses meeting consensus in the selection of, Either (including, "When weight or BMI inappropriately plateaus or tails" or "When swallowing is considered unsafe") (31 respondents, 72.1%). A third of respondents (14 responses, 32.6%) felt this should be limited to "When swallowing is considered unsafe".

Respiratory Assessment

Breathing abnormalities with CDD have been reported and include hyperventilation in 13.6%, breath holding in 26.4% and aspiration in 22.6% (19). The respondents were asked whether a formal respiratory review should be offered routinely at baseline, including a sleep study, to all individuals. There was no consensus however the lead response was "Only if clinically indicated" (28 responses, 66.7%). Similarly, when asked whether individuals should be referred to a pulmonologist/respiratory clinician, 81.0% reported "Only if clinically indicated" (34 responses). However, when respondents were asked whether a non-specialist assessment for breathing disorders, including hyperventilation, breath-holding and other conditions should be offered at each clinic visit annually, the leading response met the threshold for consensus with 90.5% (38 responses) in favor.

Cardiovascular Assessment

Parents of children with CDD may have concerns about the risk of cardiac arrhythmias and, in one caregiver survey, arrhythmia was reported in 11 out of 29 individuals with CDD who had been investigated with electrocardiogram (ECG) (76). Despite parental reports of arrhythmias, there is a lack of data on the rates of arrhythmia among individuals with CDD [from published reviews based on a cohort of 93 individuals published from the International Foundation for CDKL5's Research Centers of Excellence (2)].

Survey

When asked whether individuals should be routinely screened for cardiac issues at baseline (where the diagnosis has already been made), the most common responses were: "Yes" (26 responses, 78.8%), meeting the threshold for consensus. Similarly, when asked whether individuals should have an ECG at baseline (where the diagnosis has already been made) the most cited response was "Yes" (31 responses, 86.1%) achieving consensus. However, there was a lack of consensus when respondents were asked whether individuals should have a routine annual ECG, the leading responses were "Yes" (19 responses, 63.3%). Equally, when respondents were asked whether the individuals should have an echocardiogram at baseline (where the diagnosis has already been made), the leading responses were: "No" (15 responses, 57.7%) with fewer in favor of this (11 responses, 42.3%). Furthermore, when respondents were asked whether individuals should have a routine annual echocardiogram, leading responses were: "No" (23 responses, 88.5%). Lastly, when asked whether individuals should have a routine annual cardiological review by a cardiology specialist, the leading response was "No" (17 responses, 73.9%).

Dermatology

Survey

The respondents were asked whether individuals should have a routine check for pressure ulcers and skin breakdown at baseline (where the diagnosis has already been made). The lead response was in favor (38 responses, 90.5%). Asked whether individuals should have a regular skin check at their annual clinic review, responses were similarly in favor (38 responses, 95%).

Urinary Tract Care

Survey

When respondents were asked whether bladder-related issues should be checked regularly (e.g., urinary retention and urinary tract infections), it was felt this was appropriate with 94.1% of respondents in favor (32 responses).

Audiological

Survey

All survey respondents were in favor of individuals with CDD having an audiological assessment in the form of Automated Auditory Brainstem Response (AABR) screening (100%, 36 responses).

Dental Care

Survey

All survey respondents were in favor that individuals should have baseline and regular dental checks upon diagnosis of CDD (100%, 40 responses).

Financial

Survey

Respondents were asked whether financial support options should be explored as a baseline assessment upon diagnosis of CDD and annually, during clinic reviews. The responses were 100% with 43 responses and 39 responses respectively, both in favor.

Summary of Areas Not Meeting Threshold for Consensus

While there was no consensus in the current study regarding the timing of genetic counseling, the ACMG has provided recommendations for genetic counseling prior to and following genetic testing (77).

Notably, for a condition predominantly regarded as an epileptic encephalopathy in the domain of epilepsy management, there was no consensus on the first, second or third line choices of anti-seizure drug. This may reflect varying clinician preferences or clinicians individually tailoring management to meet the specific needs and varying seizure types of their patients. Nevertheless, vigabatrin, steroids and the combination of these featured most strongly, favoring combination therapy as first line (37.5%, 15 responses) for the management of epileptic spasms.

Summary of Areas Meeting Threshold for Consensus

The following table (**Table 1**) outlines the responses in the survey which met the pre-defined 70% requirement for consensus status, and their recommended timepoints ("baseline," "annually" or "if clinically indicated").

There were many areas of consensus recommendations identified. The majority of these are for completion at baseline. There is an emphasis upon holistic care, such as the monitoring of systemic functions and educational needs, with certain areas recommended to be reviewed, not only at baseline, but also annually and if clinically indicated. These included the monitoring of growth, the need for a regular review of feeding and swallowing, and non-specialist screening for respiratory difficulties.

A comprehensive neurological assessment is encouraged at baseline. The consensus recommendations are for the individual with CDD to be reviewed by a pediatric neurologist with experience in managing epilepsy, clinician discussion to inform families about the risk of SUDEP, completion of a baseline MRI and EEG, consideration for epilepsy surgery, screening for the presence of a movement disorder, registration with the CDKL5 international registry and a review of the individual's sleep. Despite limited published evidence on the use of novel antiseizure drugs for CDD in the literature, Ganaxolone and Epidiolex are encouraged to be offered for epilepsy associated with CDD, if clinically indicated, dependent on FDA and EMA approvals and legal and regulatory requirements, respectively.

DISCUSSION

CDD is a debilitating condition where there is an urgent need for further development of management options. To achieve these necessary advances will require large scale and international, collaborative efforts to evaluate potentially effective interventions in sufficiently powered clinical trials. Progress will rely heavily on cooperation between international medical and scientific professionals, affected families, industry and funding organizations (57). The extensive experience of the author group includes those with direct experience in CDD management including authors of a clinically relevant CDD severity assessment tool (78). We hope that this survey adds to the current knowledge base concerning clinical aspects of care and provides a useful proposed standard of care elucidated by the agreed areas of consensus. These recommendations can support clinicians with less experience of CDD and act as a catalyst for further research that would aim to increase capacity for evidence-based management in CDD.

LIMITATIONS OF SURVEY

In the survey there were occasions when incomplete responses were obtained, ie. fewer than 47 responses per question. This could represent difficulties in selecting the options available (for example, when no "other" option for selecting preferred first-, second- or third-line antiseizure drug preferences) or technical difficulties with the online survey.

For answers where respondents did not have experience in this area, answering "I am not qualified to answer" or "I do not know," responses were excluded from analysis which led to a reduced number of responses included in the analysis. This was notable for certain technical questions, such as whether an MRI with DTI should be performed at baseline (8 respondents selected "Do not know/Do not feel strongly" and 6 selected "I am not qualified to answer") and also for evolving areas of research interest, such as whether CBD (Epidiolex) should be offered for epilepsy in patients with CDD, where 6 respondents selected "Do not know/Do not feel strongly" and 7 selected "I am not qualified to answer."

Certain answers provided professional discretion and may have been subject to personal interpretation, for example, in the use of screening tests for osteopenia, the leading response was 'If clinically indicated' however the indications (e.g., poor mobility, fracture, poor height velocity, bony malformations) in this and other situations were not directly specified.

We invited respondents to provide additional feedback on areas of CDD management that were not covered in the survey. While the survey was designed and constructed with broad support at the outset, we acknowledge that some detail may have been overlooked and therefore we invited comments and suggestions for any missed areas at the end of the survey. There were few responses (4 out of 47) possibly suggesting

TABLE 1 | Recommendations for the management of individuals with CDD with suggested timepoints for completion.

	Baseline	Annually	If clinically indicated
Genetic testing	Genetic testing should be offered to all individuals with DEE to confirm diagnosis.		
Neurological			
Clinical management	Review by a pediatric neurologist and (if not the same professional) an epilepsy specialist. Families should be informed about Sudden Unexpected Death in Epilepsy.	Review by a pediatric neurologist and (if not the same professional) an epilepsy specialist.	
Neuroimaging	Individuals should be investigated with a brain MRI scan.		
EEG	EEG (regardless of clinical seizure status).		An EEG should be repeated to capture and classify spells of unclear clinical significance.
Anti-seizure drugs			Individuals with seizures should be offered Ganaxolone, if available. Equally, CBD (Epidiolex) should be offered for epilepsy with CDD, provided this met legal and regulatory requirements.
Epilepsy surgery	Individuals should be considered for a VNS insertion if seizures are refractory to medications. Individuals should be considered for corpus callosotomy if seizures are refractory to medication.		
Stereotypes and movement disorders	Individuals should be screened for movement disorders and have these treated if causing problems.	Individuals should be screened for movement disorders and have these treated if causing problems.	
International registry	All individuals with CDD should be offered to be enrolled in an international registry of other research studies		
Somnology	Individuals should have their sleep assessed by their clinician.	Individuals should have their sleep assessed by their clinician.	
Therapy assessments and interventions			
Neurorehabilitation	Referral to a neuro-rehabilitation service to assess equipment needs and diagnose problems causing impairment of mobility or hand function and to prevent contractures.	Referral to a neuro-rehabilitation service to assess equipment needs and diagnose problems causing impairment of mobility or hand function and to prevent contractures.	
Development			Development should be assessed during infancy (0–3 years), preschool age (3–6 years), pre-middle school age (6–9 years), adolescence age (12–16 years, early adulthood (18–25 years) and as needed thereafter.
Ophthalmology	Individuals should have a detailed vision assessment. Individuals should be referred for assessment and management of cortical visual impairment by an ophthalmologist familiar with this condition.		
Communication	Individuals should be offered a speech therapy assessment and assessed for augmentative and assistive communication aids such as switches, touch pads or eye gaze aids.		
Orthopedics			Hip and spine X-ray if there is a clinical concern. Screening test for osteopenia (such as wrist X-ray or DEXA scan) if there is a clinical concern
Physiotherapy (PT)	Individuals should be offered PT assessment.		Access to PT regularly for any ongoing issues.
Occupational therapy (OT)	Individuals should be offered an OT assessment.		Access to OT for any ongoing issues.

(Continued)

TABLE 1 | Continued

	Baseline	Annually	If clinically indicated
Educational	Formal educational plans should be reviewed.	Formal educational plans should be reviewed.	Educational accommodations should be made if visual impairment is present.
Systemic			
Auxology	Assessment of head circumference, weight and height.	Assessment of head circumference, weight and height.	Assessment of head circumference, weight and height.
Gastrointestinal management including assessment and management of feeding	Assessment of gastrointestinal complications such as constipation, air swallowing and acid reflux. Individuals should be referred to a Gastrointestinal specialist as well as a Nutrition specialist. Non-specialist feeding and swallowing should be assessed during clinic reviews.	Assessment of gastrointestinal complications such as constipation, air swallowing and acid reflux. Non-specialist feeding and swallowing should be assessed during clinic reviews.	A gastrostomy should be considered either when weight plateaus or BMI tails inappropriately or when swallowing is considered unsafe.
Respiratory	A non-specialist respiratory assessment to screen for breathing disorders, including hyperventilation, breath-holding or other conditions.	A non-specialist respiratory assessment to screen for breathing disorders, including hyperventilation, breath-holding or other conditions.	Referral to a pulmonologist/respiratory clinician.
Cardiology	Screening for cardiac issues and this should include an ECG.		
Dermatology	Individuals should have a routine skin check for pressure ulcers and skin breakdown.	Individuals should have a routine skin check for pressure ulcers and skin breakdown.	
Urology	Bladder related issues should be checked regularly (e.g., to assess for urinary retention and urinary tract infections)	Bladder related issues should be checked regularly (e.g., to assess for urinary retention and urinary tract infections).	
Audiology	Individuals should have an audiological assessment in the form of auditory brainstem response (AABR) screening.		
Dental care	Individuals should have a dental check	Individuals should have a dental check.	
Financial	Financial support options should be explored.	Financial support options should be explored.	

the survey was felt to be sufficient by the majority. Of the responses, the feedback included a need to explore access to support groups and the contacting of other families. Another responder questioned whether mosaicism should be discussed within genetic counseling. This response may be in reference to reported findings of somatic mosaicism in patients with CDD (79, 80) or germline mosaicism with *CDKL5* which was described in one family with two daughters with CDD found to have the same *CDKL5* variant (c.283-3_290del) with parents that tested negative for *CDKL5* variants in all tissues (81).

Further comments included reference to gynecological needs, not described in the survey. The responder queried whether clinicians should consider screening for precocious puberty or referring to gynecology, in the event of problems with menses. This suggestion addresses the unaddressed gynecological facet of CDD holistic care but may also be in reference to precocious puberty which has been described with CDD (82).

Reflective of increasing literature on CDD, one of the respondents suggested whether individuals should have an "anticipatory care plan" and whether this should be reviewed at least annually. This countered another piece of feedback: a concern that being too prescriptive with a potentially "exhaustive" list of management recommendations could heighten parental anxiety (if they feel they or those looking after their child are not fulfilling it). Clinicians managing CDD may need to decide whether to be "anticipatory" or, conversely, more "problem-driven" and which approach may be more appropriate for the individual and their family.

As with other work aiming to bring consensus to the understanding and management of CDD, our project lacks an objective "gold standard," instead being designed with the topics and subtopic questions selected through limited published data, Delphi consensus and expert opinion. In the absence of a high level of evidence, Delphi consensus is considered the best available guidance. We recognize that despite our collective experiences, we are each limited by these experiences and the field still has much to learn regarding the breadth of patient experiences, potential treatments and outcomes. The concept of an "expert" is quite relative with regards to rare disorders such as CDD.

Given these shortcomings, additional discussion and study is needed regarding several issues. While our panel was equivocal, ACMG guidelines that genetic counseling should be provided at all phases of genetic testing (77) seems most prudent. Similarly, an approach toward scoliosis and osteopenia similar to that proposed for Rett Syndrome (25, 67–69) should be provided. All treatments carry risk of potentially significant morbidity and mortality that should be carefully reviewed with families so that informed treatment decisions should be made. Addressing a complete algorithm for use of anti-seizure medications, including variations with age and seizures types, was beyond the scope of our approach, but should be considered as a completely separate

effort. A standard approach to epilepsy management in CDD including avoidance of polypharmacy should be considered, even though the literature indicates significant medical resistance (22).

Consistent with this, our survey indicates that medication and surgical options that may be offered to other individuals with medically resistant epilepsy, due to other causes, should also be offered to individuals with CDD. There has not been strong evidence until recently to support any specific treatment interventions in this population including steroids, surgery or any other specific anti-seizure medications. However, following the large international placebo controlled trial of ganaxolone, the FDA has just approved ganaxolone (Ztalmy; Marinus Pharmaceuticals) for the treatment of seizures associated with CDD, in patients aged 2 years and older.

Families should be part of the decision-making process and presented with both the clinician's experience and that of the broader community and literature. Our approach has been that management in rare diseases should be a "team sport." This study was prompted by frequent emails to each other to discuss potential approaches to increase our collective pool of experience; the community is encouraged to join us.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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Retained Primitive Reflexes and Potential for Intervention in Autistic Spectrum Disorders

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We provide evidence to support the contention that many aspects of Autistic Spectrum Disorder (ASD) are related to interregional brain functional disconnectivity associated with maturational delays in the development of brain networks. We think a delay in brain maturation in some networks may result in an increase in cortical maturation and development in other networks, leading to a developmental asynchrony and an unevenness of functional skills and symptoms. The paper supports the close relationship between retained primitive reflexes and cognitive and motor function in general and in ASD in particular provided to indicate that the inhibition of RPRs can effect positive change in ASD.

Keywords: top-down processing, bottom-up processing, neuronal synchrony, maturational delay, autism spectrum disorders, retained primitive reflexes

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INTRODUCTION

What Are Retained Primitive Reflexes and What Is the Controversy?

The term "primitive reflex" was first used by Buckley (1). Primitive or infantile reflexes are sensory/motor reflexes that are present at birth. Most of these reflexes are present in the womb (2), and one of their functions is to help the child "birth itself." The primary function of primitive reflexes is to allow the infant to move and react to their environment leading to the maturation of the motor system (3, 4). Children need to move, feed, protect, and orient to engage their senses and muscles and create sensory and motor feedback that will activate genes allowing the brain to be built from the bottom up (3–5). The control of these reflexes arises from multiple brainstem regions. The lower reflexes in the medulla are thought to be active first, followed by reflex control associated with the pons and mesencephalon (6–10).

Nevertheless, if there is a delay or disruption to this bottom-up projection known as "bottom-up interference," then the later, more advanced areas of the brain may be delayed in development. This could then delay or prevent the top-down maturational processes that ultimately inhibit these reflexes (11–13). Babinski also noted that not only the response delay of the downward toes in the plantar reflex but also the asymmetry of this response had clinical significance. Asymmetry of the Babinski sign is significant and may relate to a functional maturational dysfunction of the corticospinal tract (14–16).

Several authors have emphasized that frontal lobe development eventually leads to top-down control and inhibition of primitive reflexes. If there is degeneration or damage to the frontal lobe or corticospinal tract later in life, these reflexes can return. They are considered to be frontal release signs (17–20).

The controversy surrounding RPRs is not whether they exist or not. Although not typically part of the current pediatric examination, primitive reflex testing was previously included as part of a routine pediatric neurology examination. They are, however, a well-accepted part of the evaluation of effective child development (3, 7, 21–23), whose normal temporal trajectory is reported in **Figures 1**, **2**. The controversy surrounds the inhibition of these reflexes. In mainstream pediatrics, it is assumed that primitive reflexes are completely inhibited by the end of the infant's first year postpartum. However, many studies have indicated that in a certain percentage of the population, primitive reflexes are not inhibited in the first year of life and persist into middle childhood and even into adulthood (24, 25).

It has also been documented that in children, adolescents, and adults with RPRs, a neurobehavioral disorder or "learning disability" coexists (4, 26, 27). Individuals with ADHD, (25) autism, (28) Tourette's, (29) dyslexia (30), or other neurobehavioral disorders, frequently demonstrate RPRs that are thought to be related to maturational delay in the nervous system (3, 31–34).

Movement allows us to interact with the environment in more sophisticated ways and can help improve our chances of survivability. Interacting with the environment purposefully and beneficially requires the development of sensory organs that supply the individual with information about our location, potential danger, and satisfaction, allowing one to negotiate the surroundings and develop a sensory-motor map of the world that we can use for prediction and goal-directed adaptive behavior (35, 36).

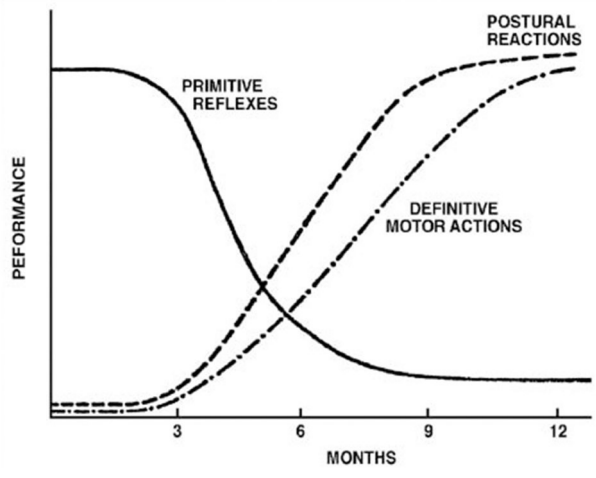


FIGURE 1 | Development of postural reflexes. The diminishing of primitive reflexes and the growing importance of postural reactions indicate the development of requisite conditions for the development of the purposeful movement. The collective time course of primitive reflexes under normal circumstances is compared with the time course of the maintenance of static deep tendon reflexes and postural reactions. Reproduced with permission Pedroso FS. Reflexes. In: Haith MM, Benson JB, editors. *Encyclopedia of Infant and Early Childhood Development.* San Diego, CA: Academic Press (2008). p. 11–23.

Organisms developed brains because they moved, and as they moved, they interacted with their environment in increasingly complex ways, leading to the development of a more complex brain (37, 38). Bipedalism is the most complex of movement strategies that have evolved in any organism, and it is, for the most part, unique to hominids. The relatively large brains of humans support the distinctive erect bodily position and cognitive abilities driven by bipedalism (39). Upright bipedalism permits less flexibility of the human pelvis's anatomy and size and structure in the human pelvis compared with quadrupeds (40). Relatively large-headed infants are born to mothers with relatively small birth canals. The infant's skull and brain cannot be fully developed at birth for the mother and neonate to survive the birth process unscathed (41, 42).

To stimulate the growth and development of the brain, an infant needs to move and interact with its environment (43–45). Movement can be impeded by a brain and nervous system that is not sufficiently mature at birth. What makes some volitional movement possible are the infantile primitive reflexes already intact at birth that allow for reflexogenic movement and interaction with the environment in fundamental ways that help increase the chances of survival. These reflexes appear prenatally and are thought to aid in the birth process. Most of these reflexes are present at birth and then become inhibited within the first few months, with the longest (the plantar reflex) remaining until the end of the first year postnatally. These reflexes allow for basic reflexogenic movement contributing to early motor milestones such as rolling over, creeping, crawling, grasping, sucking, and eventually crawling and walking (43, 44). Postural reflexes that allow for more sophisticated individualized movements are replaced with voluntary movement in most cases. Primitive reflexes allow for basic movements, which allow for simple interaction with the environment and form the basis of the early movement as well as in the stimulation of sensory organs and receptors. This increase in sensory feedback and stimulation is thought to result in the expression of genes related to protein synthesis and the building of functional connections (46). The stimulation of glial cell proliferation increases the size and connectivity of neurons (47). As neurons grow in size, density, and connectivity, they will eventually inhibit, through propriospinal projections, lower or more primitive areas of the brain. They will stimulate the growth and activation of higher, more sophisticated regions of the brainstem and neocortex. While primitive reflexes eventually become inhibited or integrated, they are never entirely eliminated (48). Ultimately, all reflexes seem to come under the control of the frontal lobe (17, 18, 49–51).

In individuals with frontal lobe damage, dysfunction, or degeneration, the reappearance of primitive reflexes known as frontal release signs is oftentimes manifested (17, 18, 34, 49–56).

Upper motor lesions also do not infrequently result in the reappearance of primitive reflexes such as the Babinski reflex or

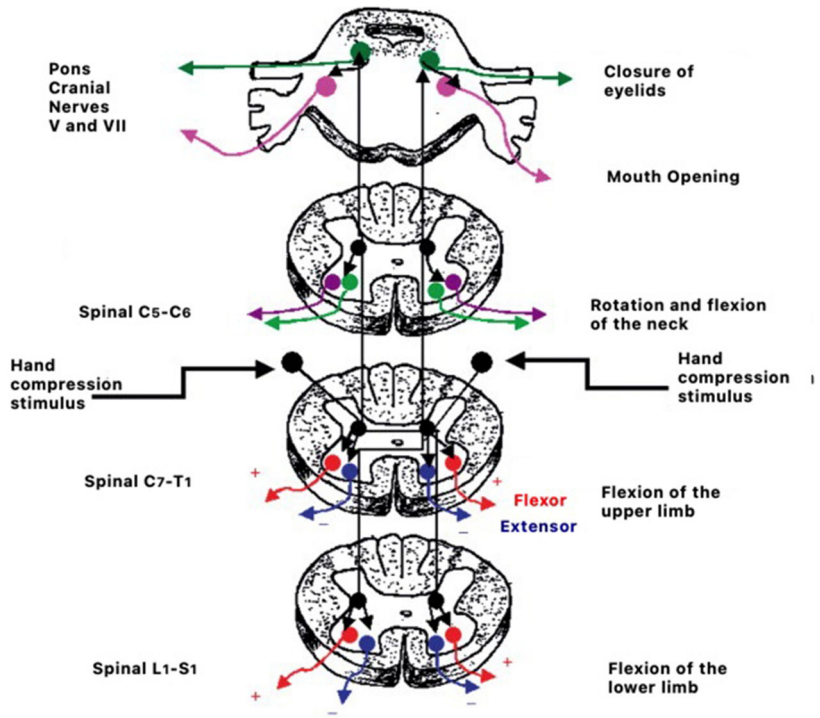


FIGURE 2 | Example of the circuitry of reflex responses to hand compression stimulation.

plantar reflex (57). This is thought to be associated with the loss of the descending inhibitory connections from the cortical spinal tract, which reflects the maturation and growth of the frontal lobe and the sensory-motor cortex.

It has also been noted (58) that the presence of RPRs is a common feature of children with Autism Spectrum Disorder (ASD) (28, 58–60). In most of these disorders, there is no visible damage, injury, lesion, or degeneration as a basis for hypothesizing that the RPRs reflect a maturational delay of brain areas that would typically inhibit these reflexes, especially those in feedback with the frontal lobes (61–63).

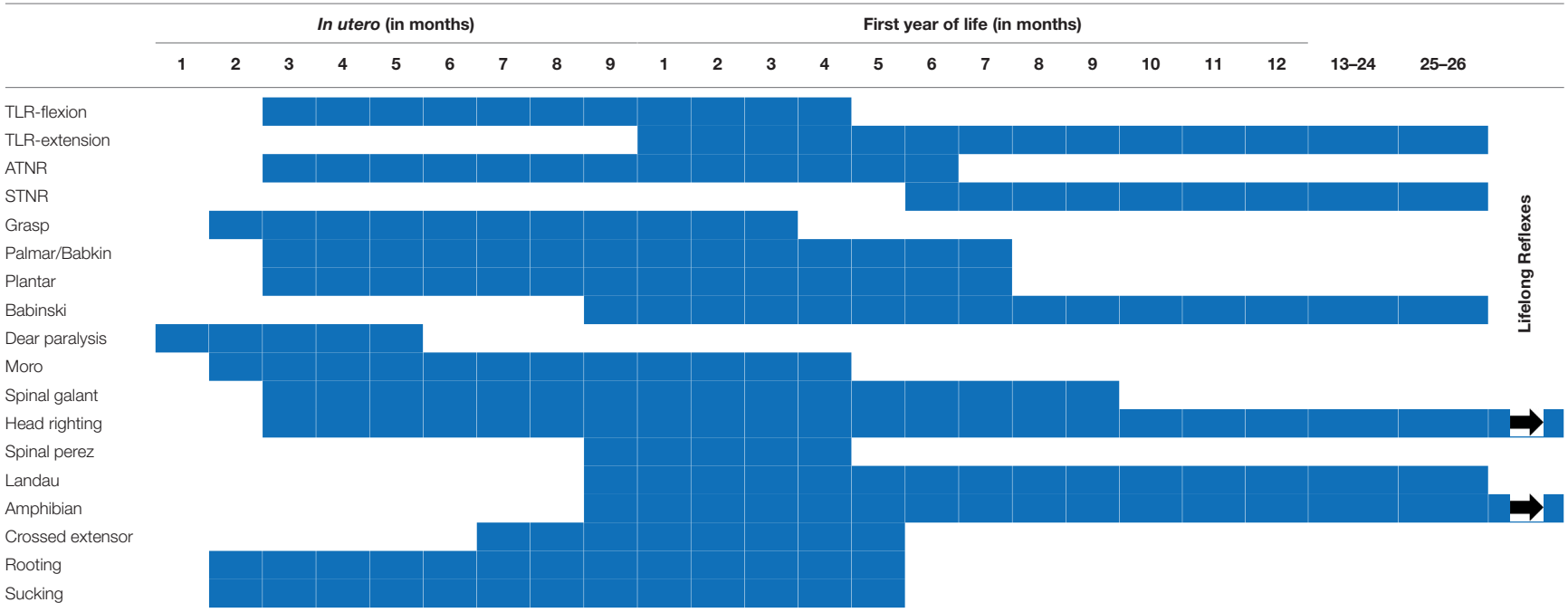
The absence or reduction of environmental influences that would generally promote growth and development, and neuroplasticity within higher brain regions, would typically lead to the inhibition of primitive reflexes and the expression of postural reflexes (3, 64) according to the timeline represented in **Figure 1** and in **Table 1**. The persistence of these primitive reflexes can reflect a maturational lag. The RPRs, especially with asymmetric persistence, will reflect not only a maturational delay of the brain but may also indicate, depending on the timing, abnormal asymmetrical development of the hemispheres (64, 65). In children with cerebral palsy, an injury on one side of the brain can lead to asymmetric retention or lack of development of PRs (28, 66, 67).

According to our current understanding, the prevalence of RPRs is considered variable, and there is disagreement about the pathological significance of these reflexes in both aging and child development. However, evidence from large data sets indicates a significant relationship between RPRs, maturation, and cognitive function (3, 25), and the description of these reflexes is presented in **Table 1**.

Retained Primitive Reflexes vs. Returned Primitive Reflexes

The relationship between cognitive deficits and RPRs has been controversial. Some authors consider these reflexes predictive of diffuse cerebral dysfunction as these signs are significantly correlated with cognitive deficits in a wide age range of individuals (68–73). It is therefore important to differentiate "*retained primitive reflexes*" from "*returned primitive reflex*" (RtPR). Recently, RtPRs have been described in dementia and Parkinson's Disease (74–78). While primitive reflexes are considered adaptive responses that are present in the neonate and disappear or are inhibited as the brain matures, RtPRs can reappear in childhood, adolescence, and adulthood (78) and when they do so, they are reportedly invariably associated with cognitive effects (74, 78). Some authors consider that RtPRs (in particular the Babinski and grasp reflex) are indicative of diffuse cerebral dysfunction as there exists a significant correlation between these signs and cognitive dysfunction in a wide age range of individuals (69–73). Regarding RtPRs, some authors reported that in individuals with Alzheimer's disease, no relation existed between cerebral atrophy based on psychometric testing (e.g., Wechsler Memory Scale) or CT-scan and grasp, snout, or glabellar reflexes.

TABLE 1 | Primitive reflex development and integration timetable.



Retained primitive reflexes indicate cortico-subcortical neuronal network impairment or possibly neuronal developmental delay. Some authors have stated that RPRs are evidenced in neurotypical populations. The palomental reflex, for example, was found in 6–27% of individuals aged between 20 and 50 years, and 28–60% of those above 60 years (64), snouting in 13% of individuals between 40 and 57 years (64); 22–33% of those above 60 years of age (64), and the sucking reflex, which some authors associate with "frontal lobe disease" (79), and Tarawneh and Galvin (80) had noted that in neurotypical individuals between 73 and 93 years of age, the palmomental reflex was evidenced in six percent them.

The Babinski sign (i.e., plantar response) and grasp reflex are two reflexes that are clinically accepted as indicators of central nervous system disease or disorder. Some of the arguments may be accounted for by differences in opinion and interpretation of the reflexes, which can vary significantly from clinician to clinician (4, 26, 81–83).

Another study examined the relationship between cognitive functioning and RPRs in individuals with dementia and without in order to determine the most predictive elements of cognitive testing or the neurological examination for brain dysfunction (84). Using the Cognitive Abilities Screening Instrument-Short Form (CASI-S), Gellis (85) concluded that in those with dementia, individuals with the highest primitive reflex (PR) scores tended to be associated with the lowermost cognitive scores and, in particular, to SPECT scan configurations. Therefore, these researchers concluded that the existence of numerous PRs and cognitive scores could be effective in predicting diffuse cerebral dysfunction. In particular, the presence of the Babinski and grasp responses, or the combination of the snout, suck, paratonia, and palmomental reflexes, are effective indicators of diffuse brain dysfunction, in particular when RtPRs are evidenced and complemented by deficits in cognitive testing scores.

The presence of multiple primitive reflexes is an indicator of diffuse brain dysfunction in elderly populations. Their persistence and presence in children and adolescents may indicate diffuse cortical maturational delay and correlate with cognitive and executive developmental absence or delay. If developmental milestones are not appropriately achieved, we hypothesize that synchronicity, optimization, the efficiency of behavioral-environmental interaction, coordination of movement, and synchronization of the overlapping brain will all be affected (25, 27, 43, 44, 86).

PRIMITIVE REFLEXES, NEURONAL SYNCHRONY IN CORTICAL DEVELOPMENT IN ASD, ADHD, AND OTHER NEUROBEHAVIORAL DISORDERS

Maturational Delays and Lateralization

One of the unique features of the human brain is its degree of lateralization or asymmetry. Humans have the most asymmetrical and lateralized brains of any species. This is thought to be another factor that leads to the significant differences in intelligence between humans and other species. A more lateralized brain allows for the development of a greater variety of centers that can individually process and control numerous functions, combining these individual centers into various networks leading to the unique cognitive abilities shared by humans. This lateralization develops with increasing age, brain, and nervous system development (87–93).

A small child does not have the same degree of lateral asymmetry as an adult. Laterality is a product of the maturity of the brain and especially of the neocortex and the frontal lobes (43, 89). The development of laterality and asymmetric control of functions increases the cognitive potential and requires greater coordination and synchronization of cortical networks (94, 95). For various functions to bind together, all associated brain regions that control complex functions and their networks must be activated simultaneously. This coordination is a byproduct of maturity (4, 21, 43).

As the brain grows and as neurons become interconnected, the speed and coordination of inter- and intra-hemispheric cortical networks increases, allowing for synchronization and integration of a significantly greater number of functions. The two hemispheres of the brain do not develop simultaneously; the right hemisphere is thought to develop more rapidly and earlier than the left, with the most significant development being prenatal and for the first 2–3 years of life (96, 97). Then the left hemisphere is significantly more greatly emphasized in development for the next 2–3 years of life. Once the differences and advantages are established during the first 6 years of life, this forms the basis of hemispheric specialization and lateralization that will increase throughout development (87, 90–93, 97).

Asymmetric Development Can Lead to Underconnectivity, Desynchronization, and Functional Disconnection

Where neural connections (anatomic and functional) are not adequately developed in infancy and early childhood, asynchronicity, the inefficiency of behavioral-environmental interaction, coordination of movement, and synchronization of brain networks may be evidenced (40, 98–104).

A global immaturity of the function of cortical networks in childhood can be associated with a reduction in motor activity (3), spatial exploration (105), experience-dependent plasticity (106), RPRs and delayed postural reflexes (107, 108). A more specific dysfunctionality would be expected if there was asymmetric development of RPRs. If there existed unilateral RPRs and, in particular, unilateral delay of postural reflexes, we would expect an asymmetric maturity and growth of the brain since this would be associated with an asymmetry of tone, in turn altering sensory and muscle feedback, potentially impairing the main driving factors to brain development (109).

Futagi et al. examined the relationship between plantar grasp response asymmetry during infancy and neurological outcome. They, during a follow-up period of between 2.8 and 11.9 years, reviewed the neurologic consequences of 61 children having demonstrated, during infancy, asymmetric plantar grasp responses. All children demonstrated neurological

signs or perinatal risk factors during infancy. Futagi and colleagues reported intellectual disabilities in three, borderline intelligence in nine individuals, cerebral palsy in 38, delayed motor development in six, and neurotypical function in five. The majority exhibited a relationship between the side of the retained plantar grasp response, the side of the motor function deficits, and the side of the abnormal CT results (6). These findings were supported in a systematic review by Hamer and Hadders-Algra (110).

The asymmetry that Futagi et al. (1995) observed in the plantar grasp response strongly suggested brain dysfunction (6, 111, 112). Their studies showed an association between the persistence of motor abnormalities related to the same body side and the asymmetric development of primitive reflexes (113). The plantar response is one of the reflexes most related to brain dysfunction, whether due to injury or functional developmental delays (108, 114). This type of asymmetrical development of the developing brain has been commonly noted in almost all neurobehavioral disorders, especially ASD (115, 116). Along with anatomical asymmetries, there have also been functional asymmetries noted with a characteristic "unevenness" of skills observable in all of these disorders, to varying degrees (25).

A significant feature of those with ASD is the "unevenness" of cognitive function (117). We have proposed (43) that the diverse aberrant behaviors noted in ASD and in other neurobehavioral disorders can be understood better by viewing ASD in the context of functional brain disconnectivity, of the kind that has been noted in minimally conscious states (118, 119) and even in sleep (120), or as reported in people with dyslexia (121, 122). Functional asymmetry within widespread cortical networks could decrease temporal coherence in certain functional networks and enhance temporal coherence in others (123). Recent research has suggested that increased functional ability or intelligence is related to augmented activity in specific networks (124). It is also possible that an increase in the complexity and integration of functional networks may be related to increased temporal coherence that may impart a selective advantage in particular regions of the brain (125, 126). This could explain how certain talents and abilities seem to be inherited and run in families (127).

As optimized brain function implies more efficient neural processing than non-optimized, one might expect optimized execution of motor tasks to be related to greater degrees of activity. However, the converse appears to be the case in the cerebral cortex where increased task effectiveness has been reported that has included: figural, numeric, and spatial reasoning (128) and verbal ability (129) is associated with reduced energy consumption in various cortical regions. This phenomenon has additionally been examined electrophysiologically. When examining resting-state activity [event-related desynchronization (ERD)] during cognitive tasks, there is a reported decrease in background power (7.5–12.5 Hz) decreases which has been reported to be related to the activity recorded in those with higher scores on IQ tests (130, 131), or with significantly greater performance after practice, that in turn is related to a more effective cognitive processing strategy (132, 133). Yet, the issues should include not only the expenditure of energy but also the nature of the functional connectivities between brain regions (94). Smaller regions of activity have been consistently evidenced in brain areas in those with ASD. These diminished areas of activity appear to be developmentally delayed brain regions as opposed to being reflective of pathological processes or damage (94, 95).

Individuals with ASD and other neurobehavioral disorders have also evidenced a reduction of interregional brain connectivity (25, 27, 43, 44, 94, 95, 102, 134–139). The corpus callosum appears to be the brain area associated with the reduced cortical connectivity found in individuals with ASD (140). This implies that the most frequently evidenced functional disconnectivity observed in childhood involves hemispheric interaction. This is a notable reported characteristic difference between ASD and normally developing toddlers (141).

We think that reduced inter-hemisphere coherence is associated with a reduction in the several sensory, motor, and cognitive functions coordinated by the ipsilateral brain hemisphere, and the higher proficiencies are sometimes related to enhanced within-hemisphere coherence (43, 94, 95). We have also described diminished coherence and connectivity in longer inter-hemispheric connections with augmented coherence and connectivity with shorter intra-hemispheric connections (94, 95) that we have hypothesized to be associated with enhanced performance abilities such as those that have been observed in forms of savantism (142).

RETAINED PRIMITIVE REFLEXES, MOTOR FUNCTION, AND NEUROBEHAVIORAL DISORDERS

Retained primitive reflexes have been noted in several neurobehavioral disorders, including ADHD and ASD and are understood to be associated with or absent or delayed developmental milestones in these (25, 27, 28, 44, 143–148). RPRs have been reportedly associated with the presence of clumsiness (25, 27, 43, 149, 150) incoordination (149), awkward posture (151), gait (152–154), and other motor disturbances (25, 155, 156). Most neurobehavioral disorders seem to be associated with motor incoordination and cognitive dysfunction (25, 27, 43, 44, 157, 158).

Teitelbaum et al. (159) theorized that in infants with movement disturbances, reflexes may have "gone astray" and may be early markers of ASD. They observed that some infants demonstrated RPRs that continued far beyond long infancy in the children they examined, whereas other primitive reflexes first appeared in infancy significantly later than though ought. The asymmetric tonic neck reflex, they thought, might be retained in ASD. The verticalization of the head as a consequence of body tilt, was reportedly absent in a subgroup of "autistic-to-be" infants, according to Teitelbaum and colleagues. They suggested that these reflexes might serve as a marker for ASD, and pediatricians could use them to screen for neurological dysfunction (160). In

their earlier work, Teitelbaum et al. (161) showed that infants with a tendency to ASD demonstrated a distinctive constellation of disturbances in patterns of movement as early as 4–6 months of age, measured by Teitelbaum and colleagues in conjunction with laser disc still-frame analysis. Eshkol and Wachman (162) had earlier reported similar findings.

The Galant and Moro reflexes are among the most critical postnatal primitive reflexes that diminish later in development. At the same time, there exists no definitive evidence that these reflexes play a role in ADHD. Konicova and Bob studied school-aged ADHD children between 8–11 years who demonstrated Galant and Moro RPRs compared to an age-matched control group (72, 163). They found that ADHD children demonstrated a significantly greater occurrence of Moro and Galant reflexes than did the control group, indicating that ADHD symptoms may compensate for an immature brain.

Callcott (164) reported that children's learning difficulties relate to reduced movement proficiency, including school-readiness. Calcott investigated the prevalence and severity of the Asymmetrical Tonic Neck Reflex (ATNR) and studied the proficiency of movement in preprimary-aged Western Australian indigenous children. She found that 65% of those tested demonstrated moderate to high ATNR levels that were significantly related to academic achievement (164).

The findings of the Millennium Cohort Study in the United Kingdom (165) supported a relationship between the delayed achievements of motor milestones at 9 months of age and significantly lower cognitive development at age five (165). The Australian Early Development Index reported that nearly a fourth of school-aged are "at risk" in their physical and cognitive development (166). Williams and Holley (167) offered support for these findings linking motor development and cognition by addressing the influence that infant motor experiences in infancy and early childhood may have on higher-level cognitive abilities required for academic achievement in school. As we have already noted, motor function and gesture development typically require the effective inhibition of mouth and hand-related primitive reflexes (167). ASD children not infrequently demonstrate difficulty in executing skilled movements and possess as well as exhibit a poor gesture repertoire (168).

Chinello et al. (144) examined the association between three RPRs, motor behavior, and parental autistic-like traits, in infants aged between 12 and 17 months of age. Independent of age, RPRs were associated with infants' deficient motor skills and were highly correlated with parental autistic-like characteristics.

Numerous authors have reported on an association between clumsiness and incoordination, particularly in gait and posture, and ASD, ADHD, and other neurobehavioral disorders of childhood (163, 169, 170). The kind of gait and motor dysfunction has been mainly thought to possess either basal ganglia or cerebellar origins (25, 27, 40, 43, 171). Developmental Coordination Disorder (DCD), or more simply put, motor incoordination or "clumsiness," is also usually of the same type, primarily involving the muscles that control gait and posture or gross motor activity (172, 173).

Sometimes, we observe that fine motor coordination can also be affected (21, 174, 175). Several authors have noted both differences and similarities between ASD and DCD. DCD individuals demonstrated greater fine and gross motor coordination, theory of mind, and emotional perception than did the ASD individuals, but evidenced comparable difficulties with response inhibition. These authors observed that based on symptom severity, children with ASD who were measured to be "more able" did not diverge on any measured skills from DCD children, in contradistinction to children classified as "less able." The authors wondered whether DCD and ASD vary more in the range of symptom severity than in a singular behavioral domain (174).

Similar comorbidities have been found in children with ADHD (72, 163, 176–180) and those with developmental dyslexia (4, 181–186).

Sumner et al. (187) also found numerous overlapping features in verbal expression, speech, gaze, and face-processing, expression, in ASD and DCD individuals. These findings suggest that children with DCD may also demonstrate difficulties in processing social information. However, when examined with measures of socialization, the DCD individuals scored at an intermediate level in two other socialization measures. The authors concluded that socialization in DCD may not be as manifest as in individuals with ASD (187).

The greatest similarity between ASD and DCD in Sumner and colleagues' review was a paucity of significant effects of cognitive intervention. Concerning treatment, no significant improvement effects were noted in both DCD and ASD groups of individuals (174) or in IQ (188). The ability to train and improve in various domains, especially cognitive, is similar for both conditions. Additionally, no significant disparities were found in a qualitative study examining transitions from primary to secondary school, possibly because the main variables of the study's interest concerned the children's motor behaviors. On the other hand, cognitive intervention has been reported to be effective in reducing symptoms of ADHD (189).

It might seem somewhat confusing initially to observe that fine motor skills seem to be disrupted at almost equal levels as a gross motor. The literature supports the notion that manual dexterity is less effective for high functioning ASD individuals, but only for the non-dominant hand. This suggests a lateralized difference (190, 191). This would show that although fine motor coordinative skill is decreased in those with ASD and fine motor skill is primarily decreased in the left hand, associated with right hemisphere function. This is consistent with a deficit in effective coherence between the right and left hemispheres. Perhaps a parallel situation exists in ADHD individuals and in individuals with other neurobehavioral disorders (192–196).

Variations in the manifestations of ASD and DCD may be associated with differences in the maturation of asymmetries as a consequence of different maturational rates of the left and right hemispheres (197–199). Asymmetric RPRs may also be an early marker of developmental brain immaturity. This aberrant configuration of hemispheric asymmetry may be related to underconnectivity and desynchronization, and eventually to functional disconnectivity between lower brain regions and the neocortex (197–200).

Retained Primitive Reflexes in ASD

Autistic Spectrum Disorder is a neurobehavioral disorder identifiable by dysfunction of communication, behavioral flexibility, eye contact, and social interaction as well as deficits in language, and executive function (201–204). Although there is a consensus about the symptoms that comprise ASD, there exist controversies regarding the precise definitions of ASD and the boundaries between manifestations of related disorders. Researchers have increasingly recognized that motor ability can have a significant effect on other developmental functions, such as language and social cognition (28, 205–209).

Retained primitive reflexes can disturb the natural course of development and create difficulties in social and educational functions in children (21, 210) as well as impact psychomotor development (211). Mature responses in a child's psychomotor behavior can only occur if the central nervous system has reached the appropriate level of maturity (21, 210, 212). The process consists of the transition from brainstem reflex response represented in **Figure 2** to cortically controlled responses (213).

It has been argued that independent of a child's age, RPRs are significantly related to an infant's ability to interact with objects (i.e., agency) (4, 21, 144, 156, 210) as well as with others (i.e., copying gestures) (22), meaning that high scores in the assessment of primitive reflexes, is associated with an increased likelihood of RPRs, which, in turn, are also associated with low scores in motor responsivity, independent of the age of the infant.

As previously indicated, children with ASD demonstrate impediments in the performance of skilled movements and gestures. Numerous investigators have noted that delays in the maturation of motor function during the early years of development foretell the primary dysfunctions characteristic of individuals with ASD (214–216).

This hypothesis has been examined in the infant siblings of ASD children, who purportedly have an increased probability of developing ASD. In longitudinal studies of 3- to 6-month-old infants' motor development of high-risk (HR) infants, over 70% of infants with motor delay later demonstrated communication impairment. Motor development is associated with a normally automatic progression in which infant maturation inhibits more primitive motor responses (217–219).

Assessing RPRs in autism is essential for at least multiple purposes. Firstly, RPRs may be an encouraging early sign of ASD that, along with the early signs of difficulty in eye contact, attentional deficits, as well as other elements, might assist in characterizing the developmental trajectory of the wider ASD phenotype during infancy. Consistent with this thinking, it has been stated that slight disparities in initial periods of the brain's development (e.g., the persistence of primitive reflexes) can produce an adverse progressive effect not just on motor skills that develop later but also on a range of other behaviors (i.e., communicative and social behaviors as well as in object exploration) (144, 220, 221). Secondly, the ability to identify motor abnormalities early in life might also be encouraging for the differential diagnosis of ASD. The proportion of children with ASD and concomitantly with developmental motor and coordination dysfunction varies widely. The variability in these deficits in ASD is likely a result of the heterogeneity of ASD. That heterogeneity, on the other hand, allows us a unique opportunity to classify subtypes of ASD (101, 194, 195).

Retained primitive reflexes have the ability to disturb the normal maturation processes decreasing the ability of the brain to effectively process sensory information. RPRs then that are still present (beyond the average age of 12 months postpartum) can impede the subsequent development and maturation as well as serve as a potential biomarker of neurological dysfunction (144).

Do RPRs Indicate a Dysfunctional Neurological System in ASD?

In supporting the cognitive effects of primitive reflexes and cognitive function, some authors consider the palmomental reflex (PMR) as being related to dementia. The PMR is a polysynaptic reflex that can be evoked by nociceptive stimulation of the thenar eminence, resulting in an ipsilateral involuntary mentalis muscle contraction (19). The extant PMR is found in infants up to ∼12 months of age and then wanes and disappears, largely due to the frontal lobe maturation (19, 222). Consequentially, its recurrence in aged individuals with the pathology of the frontal lobe is thought of as a "cortical release" or "frontal lobe" sign, with a presumption of a lack of frontal inhibition on subcortical motor networks (223–225).

Anatomic (AC) and functional connectivity (FC) studies of linking the PMR with dysfunction of interrelating loops connecting the thalamus and basal ganglia with the motor, premotor, and prefrontal cortices, are consistent with our hypothesis (95, 190, 226–229).

Neuroimaging studies of individuals with ASD have also detected brain areas with atypical lateralization of motor function, with the capacity to detect subtle neuroendocrine phenotypes. Most studies agree that ASD individuals demonstrate an amplified rightward asymmetry that incorporates cerebral cortex volume, corpus callosum, premotor cortex, the sensorimotor resting network, and the inferior parietal lobule (190, 227, 228, 230, 231).

Floris et al. (190), in studying intra-hemispheric connectivity in ASD, demonstrated that high-functioning ASD children aged between 8 and 12 years demonstrated strong rightward lateralization in their motor circuitry's connectivity which was found to be necessary for effective motor responsivity. Notably, motor connectivity's rightward lateralization relates to effective motor response (e.g., gait and balance, as well as any timed sequential or movements) (190, 231).

Machado et al. (94) reported that qEEG changes in coherence and spectral analysis could be associated with a visual-auditory sensory integration impairment, which in ASD children is lateralized to the right hemisphere (94, 232, 233). Hence, several authors have affirmed that RPRs reflect anatomic and functional connectivity abnormalities in brain networks (190, 227, 228, 230, 231).

Do RPRs Reflect Motor Impairment in ASD?

Several authors have suggested that RPRs correlate with motor function independent of the age of the infant, and significantly more so among infants whose parents demonstrated subclinical autistic characteristics. Hence, the RPRs might modify the developmental trajectory of the infant's motor function, and as a result, their assessment could serve as an early marker of atypical development (144, 156).

There exists a consensus that besides the principal features of ASD, delays, and abnormalities in motor development are key components of ASD regardless of multiple etiology and subtypes of the condition (25, 27, 44, 144, 156).

The anomalous frontostriatal cortex in AD individuals with RtPRs variously affects the motor pathways that suppress primitive reflexes. This notion is coherent with findings that ASD impacts various cognitive functions in the same way that dementia is related to dysfunction of the frontal lobes and/or basal ganglia (i.e., frontotemporal dementia or Parkinson's disease dementia). These findings in dementia have suggested functional and anatomic connectivity impairment in ASD and other neurodevelopmental disorders (19, 224).

RETAINED PRIMITIVE REFLEXES CAN BE BIOMARKERS AND A TARGET FOR TREATMENT

Retained primitive reflexes may be one of the earliest markers of abnormal or delayed cortical maturation and by extension, of ASD and other neurobehavioral disorders (231, 234). The rooting and sucking reflexes and many other primitive reflexes are present at birth (43). The inability of an infant to attach to its mother and breastfeed, often seen in children with developmental delays and delays or asymmetry of rolling over at 3–5 months of age, may be the early indicators of ASD (3, 77, 235). Therapists have recommended exercises that stimulate or reproduce primitive reflexes to remediate various neurobehavioral disorders (26, 149, 236).

Methods that have indicated some promise in the treatment of various neurodevelopmental disorders including ASD are ostensibly founded on the theory that attributes the difficulties to RPRs that affect the child's normal growth and development as well as academic and cognitive skills (25, 237–239).

Although limited, some studies indicate a neurodevelopmental basis for a range of difficulties associated with maturation and motor development that manifest in cognitive and social difficulties. The collective research in this area demonstrates that the existence of RPRs has implications for skills such as balance and coordination as well as learning and cognition. The work of Goddard Blythe (240–243) has concentrated on children between 7 and 9 years of age and supports the case for early interventions to improve and develop coordination and balance, especially when such neurological dysfunction may be contributing to cognitive and motor delays or effects. Brown's (244) intervention study appears to support this line of research as does Melillo et al. (25). She found that with children between 4 and 5 years of age, practicing particular movements facilitated their performance of the fine motor activity and academic performance by inhibiting RPRs. Similar findings were reported by McPhillips and Mulhern [(239), cited in (245), p. 69] who indicated the relation between children with reading problems and motor control and balance. Chambers and Sugden's (246) found ineffectiveness in motor skill performance was highly associated with academic performance. Activities of daily living were found to be successfully facilitated by intervention programs that supported fine and gross motor skills thus being significant factors in early childhood learning [(247), p. 50]. The evidence indicates that physical development is fundamental to the development of a child's cognitive abilities.

Programs, based on perceptual-motor interventions have suggested that relatively simtime have not ple training is capable of moderating the facilitation of learning and brain structure (248, 249). In short, the difficulty is that the majority of current programs of this sort at the present time have not undergone rigorous evaluation and scrutiny. These types of interventional strategies invariably employ specific motor activities and exercises. Some of the advocated tasks are adjusted to the individual's needs (250), while others may be generic (251–253). These types of interventions often integrate actions, such as throwing and catching, ostensibly improving vestibular function, fine and gross motor skills, and academic accomplishment.

While some programs have promoted exercises that imitate the actions of fetuses and infants, it has been noted that rehearsing the activities of the early stages of development can inhibit the perseverance of RPRs. This has oftentimes been used as a justification for programs that advocate exercises that simulate fetal and infant activity and infants (238, 239, 254). Claims have been made that movements following primitive reflex patterns will inhibit those reflexes and improve cognitive function and the ability to acquire academic skills (239).

Grzywniak (255) studies the effectiveness of exercises aimed at supporting the development of children with learning difficulties and RPRs. Their symptoms included visual-motor coordination and attentional deficits, hyperactivity, and reduced visual and auditory analysis and synthesis.

While not many studies have thoroughly examined the effects of reducing RPRs on clinical outcomes in developmental disabilities in general and in ASD in particular, of note are Pimentel (256) and Anderson (143, 257), who discussed the connections between RPRs and developmental delays. The comparative studies scrutinized groups with developmental delays and RPRs (144, 160, 164, 238, 258). McPhillips and Mulhern (239) performed a double-blind study of the cognitive effects of reducing the presence of RPRs. A regression analysis study was conducted by de Bildt et al. (259). Melillo (260) and Hyatt et al. (252) produced meta-analyses. While at least eight systematic review articles examined, RPRs alone, or meta-analyses of RPRs in development, none explored the evidence surrounding reflex-based interventions (108, 159, 241, 252, 260–263). Expert review studies over many years included those by Rider (264), Endler (265), Ottenbacher (262), Smith et al. (266), and Mailloux et al. (267). The studies analyzed RPRs of individuals with reflex delays, developmental disabilities, and

difficulties with sensory integration skills, but none provided evidence for effectiveness in the treatment of developmental disabilities of any kind by RPR reduction.

Barret et al. (268) identified three best evidence reviews (144, 238, 242). Goddard Blythe (242) provided a summary of independent studies that examined academic performance associated with developmental exercise programs that demonstrated positive effects on academic performance. Jordan-Black (238) conducted a nonrandomized control study to establish evidence for the effectiveness of reflex-based interventions in improving academic performance, from which she indicated that her system reduces RPRs in particular the asymmetrical tonic neck reflex, and has a significant effect on reading and mathematics abilities of children who had undergone her intervention. Chinello et al. (144) found that the parents of infants demonstrating subclinical traits of ASD were more likely to demonstrate RPRs and were more susceptible to developing ASD later. The study did not measure the outcome of therapeutic intervention. Collectively, these papers demonstrate the best evidence and relevance to RPR reduction and its effect on cognitive function. reflex integration. Only Goddard Blythe (242) and Chinello et al. (144), examined the effects of RPRs in developmental disorders in general and to some extent in ASD. Jordan-Black's (238) study did not address children with ASD.

Although the mechanism of how these exercises can inhibit primitive reflexes and affect or improve neurobehavioral disorders has not been previously described to our knowledge. We speculate that utilizing these exercises can increase the sensory stimulation and feedback to the nervous system that stimulates synaptogenesis and neuroplasticity of more rostral and complex areas of the brain (269–271). We conjecture that this may be associated with inhibition through descending propriospinal connections that inhibit these reflexes that would, under normal circumstances, lead to more complex individualized volitional control of movement that will stimulate growth and cortical maturity. Ultimately, this can lead to the release of "bottom-up interference" that can delay the cortex's maturation and prevent appropriate top-down regulation that will ultimately inhibit primitive reflexes (25, 27, 44, 94).

DISCUSSION

The incidence of ASD has been increasing at epidemic levels, and we think that the driving force behind this increase is a combination of genetic and environmental factors, emphasizing environmental determinants. We think that epigenetic factors related to lifestyle changes over the past two decades, especially reduction of early motor activity and spatial exploration of children, have led, in part, to the significant rise in the incidence of ASD. We surmise that the reduction in the activation of activity and experience-dependent genes that stimulate synaptogenesis and neuronal plasticity of central neurons and glial cells can help increase the size and complexity of the brain during the first 3 years of life. We think that this is the basis of both the maturational cortical delay identified in almost all neurobehavioral disorders, including ASD, and their associated RPRs (272–276). After the first few months of life, the feedback created by primitive reflex-generated movement can lead ultimately to the inhibition of these reflexes and the activation of more complex subsequent postural reflexes (64, 211, 277) resulting in a more complex interaction with the environment, that in turn leads to greater sensory feedback thereby activating genes that allow for the creation of integration and coordination between various cortical networks [for a more detailed analysis cf. (44)]. As these cortical networks become more connected and integrated, they increase the speed of their interaction, and their synchronization improves, allowing more areas to be activated simultaneously (24, 39, 278–282).

Delayed cortical maturity and motor coordination may occur due to the abnormal persistence of primitive reflexes. In that case, the brain will not continue to grow and develop at a normal rate and sequence. As the brain's hemispheres develop at different rates and times (283, 284), with the abnormal, asymmetric persistence of primitive reflexes, a maturational dysfunction in between hemisphere coherence can be produced where one hemisphere may mature at an average rate and the other not (231, 285–288). This can be associated with significant synchronization and temporal coherence dysfunction, decreasing large cortical networks between the two hemispheres from binding temporally and spatially. This can result in a functional disconnection syndrome (88, 102, 135, 137, 279, 289, 290).

We can conclude based on our current understanding that if there is any delay in maturation of the pyramidal tracts, the brainstem, or the frontal lobe, it is reasonable to assume that there might be a delay in the disappearance of the Babinski sign and other primitive reflexes. Many neurologists and pediatricians assume that the Babinski sign and other primitive reflexes that are present at birth and sustained for the first year of life will automatically disappear after that initial period unless brain damage is present (25, 27, 40, 94, 95, 233, 291).

Therefore, primitive reflexes are the tools that can be employed early in neonatal and infant development to evaluate the integrity of the central nervous system. They are brainstem-mediated, automatic movement patterns present in full-term infants at birth. With the maturation of the central nervous system, these primitive reflexes become more challenging to evoke after the first year postpartum when the infant becomes capable of voluntary motor activity. RPRs are not infrequently present in children with ASD, ADHD, or cerebral palsy, and may be early indicators of brain-based deficits (3, 4, 22, 148, 292–295).

CONCLUSIONS

Retained primitive reflexes may occur in the absence of any injury. They could be used as a clinical sign of a maturational delay in cortical development that is thought by many to be highly associated with abnormal functional connectivity seen in many neurobehavioral disorders such as ASD. We have also demonstrated that the Babinski sign may reappear later in life is grossly intact in older individuals without any physical damage but is highly related to cognitive decline. This may be a functional degeneration or loss of frontal lobe function, and we can observe this with "frontal release signs." Therefore, the presence of the Babinski's sign in a healthy aged population may be a "returned" reflex which may be an early clinical sign of frontal lobe dysfunction or

degeneration, or it may represent that the Babinski reflex has been present in an individual throughout his or her lifetime. Even though they may be grossly healthy, these individuals may have struggled with neurobehavioral symptoms. This maturation process commences from the bottom of the brainstem and the primitive reflexes. They help promote bottom-up development, which then promotes growth and maturation of the brain and frontal lobes, which then, in top-down feedback, regulates the brainstem nuclei, coherent with Huglings Jackson, the Principles of Dissolution.

In conclusion, we hypothesize that RPRs in ASD are, in part, associated with maturational delays and imbalances and not necessarily a result of actual structural damage or pathology. They are, in part, a result of environmental influences and are therefore amenable to remediation. We think that the presence of RPRs and the developmental milestones that might be delayed or absent as a result may be the earliest markers of developmentally delayed children, in general, and those with ASDs in particular. Assessing RPRs in ASD then is essential for multiple reasons. Firstly, RPRs may be a possible biomarker for ASD that, jointly with early signs of attentional deficit, eye contact, and other factors, might aid in characterizing the developmental trajectory of the character of ASD in infancy. In line with this approach, it has been stated that slight disparities in motor behavior in early development (i.e., RPRs) might exert an adverse cascading effect on the subsequent development of motor skills and also in numerous other domains (i.e., communication and social behavior and /or object exploration). Secondly, the detection of early motor abnormalities could also be an encouraging avenue for the delineation of subtypes of ASD.

AUTHOR CONTRIBUTIONS

GL, RM, and CM: conceptualization and methodology. SK: software, visualization, validation, and investigation. GL, RM, CM, YM-F, MC-A, and TM: writing—original draft preparation. GL and EC: supervision. GL, RM, CM, YM-F, MC-A, TM, and EC: writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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Acute Neurological Presentation in Children With SARS-CoV-2 Infection

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Background: In the pediatric population, the knowledge of the acute presentation of SARS-CoV-2 infection is mainly limited to small series and case reports, particularly when dealing with neurological symptoms. We describe a large cohort of children with acute SARS-CoV-2 infection, focusing on the neurological manifestations and investigating correlations between disease severity and population demographics.

Methods: Patients aged 0–18 years with a positive molecular swab were recruited between April 2020 and March 2021 from a tertiary Italian pediatric centre. Clinical data, imaging, and laboratory test results were retrieved from our local dataset and statistically analyzed.

Results: A total of 237 patients with a median age of 3.2 years were eligible; thirty-two (13.5%) presented *with* neurological symptoms, including headache (65.6%), altered awareness (18.8%), ageusia/anosmia (12.5%), seizures (6.3%), and vertigo (6.3%), combined in 7 (21.9%) cases. Respiratory (59.5%) and gastrointestinal (25.3%) symptoms were the most common among the 205 (86.5%) patients *without* neurological involvement. Neurological symptoms did not significantly influence the severity of the triage access codes. Moreover, pre-existing medical conditions were not higher in the group *with* neurological manifestations. Overall, fifty-nine patients (25%, 14/59 *with* neurological symptoms) required treatment, being antibiotics, systemic steroids, and heparin those most prescribed.

Conclusion: Our study supports the overall benign course of the SARS-CoV-2 infection in children. Neurological manifestations, except for headache, remain a rare presenting symptom, and disease severity seems unrelated to pre-existing medical conditions.

Keywords: acute, COVID-19, children, neurological symptoms, SARS-CoV-2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the human coronavirus responsible for the Coronavirus disease 2019 (COVID-19) pandemic, which spread worldwide starting from late 2019 to early 2020 (1). Likewise, its "cousins" [i.e., SARS-CoV and the Middle East respiratory syndrome (MERS-CoV)], SARS-CoV-2 can replicate in the epithelial cells and pneumocytes of the lower respiratory tracts, causing either pneumonia or acute respiratory distress syndrome (2–4). However, the clinical spectrum of COVID-19 is largely heterogeneous and disease severity and progression are mainly influenced by host factors, including age, sex, and pre-existing chronic conditions (e.g., hypertension, type 2 diabetes mellitus, and obesity) (5–10). Particularly, current evidence suggests that age itself is the most significant risk factor for severe COVID-19 and its adverse health outcomes (11).

To date, few studies have specifically investigated the acute neurological presentation of COVID-19 in the pediatric population. The rate of asymptomatic children ranges from 4.4 to 23% of all the cases, and may be undermined as many asymptomatic children escape screening (12–16). The most frequent non-neurological manifestations are fever, cough, respiratory distress, rhinorrhea, sneezing or nasal congestion, pharyngitis, vomiting or nausea, abdominal pain, diarrhea, and fatigue (17). Factors associated with intensive care unit (ICU) admission are mainly represented by neonatal age, male gender, lower respiratory tract disease, and pre-existing medical conditions (e.g., chronic pulmonary disease, congenital heart disease, malignancies, and neurological disorders) (14, 15). Mortality rate in pediatric cohorts is low (up to 0.7%) (12–14, 18, 19).

Neurological manifestations of COVID-19 in children are mainly limited to headache, asthenia, and ageusia/anosmia, the latter being particularly difficult to assess in this population and, thus, underreported (16, 20–23). However, more severe neurological complications, including encephalitis, seizures, and cerebrovascular infarct, are described in small series or single case reports (23–32).

Additional research is needed to fully assess the neurological implications of the SARS-CoV-2 infection in children. We report the clinical presentation of a large cohort of children whit acute SARS-CoV-2 infection, describing the neurological features, as well as investigating correlations between disease severity and population demographics.

METHODS

Patients

Patients aged 0–18 years who tested positive for SARS-CoV-2 with a molecular swab at the Emergency Department (ED) or on admission to a ward, were recruited from a tertiary Italian pediatric centre between April 2020 and March 2021. Clinical data including previous medical history, imaging, and laboratory test results were retrospectively collected through our local dataset. Patients' parents/caregivers gave written informed consent. The study was reviewed and approved by Comitato Unico Regionale Regione Liguria, Genova, Italy.

Statistical Analysis

Patients were divided into two groups; those *with* and those *without* neurological symptoms. Categorical variables were compared using the Chi-squared test (X^2) if expected frequencies > 5, otherwise using the Fisher's exact test. The thresholds of *p*-value were set at 0.05 (statistical significance) and 0.01 (high statistical significance). Quantitative variables were reported in terms of mean values and standard deviations (SD) in the case of normally distributed data (determined using the Shapiro–Wilk test) or in terms of median values with 1st and 3rd quartiles (1st–3rd q) in case of skewed distributions. Mann–Whitney *U* test was used to compare two quantitative variables in case of skewed distributions. Each neurological manifestation was further stratified by age in two subgroups: pre-scholar (<6 years) and scholar (>6 years).

RESULTS

Clinical Features

A total of 237 patients (113 females) were recruited (**Table 1**). The median age was 3.2 years (0.8–10.7 years, 1st–3rd q). Two hundred twenty-two (93.7%) patients were tested at the ED, of which 182 (76.8%) were admitted with symptoms suggestive of COVID-19 (i.e., fever, cough, pharyngodynia, rhinitis, headache, vomiting, and diarrhea). Forty-three (18%) individuals were asymptomatic, 15 (34.9%) of them being positive at a scheduled admission to the ward.

Thirty-two (13.5%) patients with a median age of 10.9 years (5.8–13.3 years) presented *with* neurological symptoms including headache (65.6%), altered awareness (18.8%), ageusia/anosmia (12.5%), seizures (6.3%), and vertigo (6.3%). Photophobia, facial paresthesia, endocranial hypertension, and meningitis were

TABLE 1 | Comparison between patients *with* and *without* neurological symptoms.

	Patients *with* neurological symptoms	Patients *without* neurological symptoms	*p*-value
Total, *n* (%)	32 (13.5)	205 (86.5)	
Female, *n* (%)	18 (56.25)	95 (46.34)	0.3436
Ethnicity: Caucasian, *n* (%)	26 (81.25)	143 (69.76)	0.2123
Median age, y (1st–3rd q)	10.9 (5.8–13.3)	2.5 (0.7–9)	**0.0002**
Respiratory symptoms, *n* (%)	21 (65.63)	122 (59.51)	0.5646
GI symptoms, *n* (%)	17 (53.13)	52 (25.37)	**0.0018**
Cardiologic symptoms, *n* (%)	1 (3.13)	6 (2.93)	1
Hospitalized, *n* (%)	14 (43.75)	88 (42.93)	1
Mean admission duration, d	6	4	–
Pre-existing conditions, *n* (%)	7 (21.8)	37 (18.05)	0.62

d, days; GI, gastrointestinal; n, number; y, years. Respiratory symptoms include: cough, pharyngodynia, pharyngitis, rhinitis, respiratory distress, apnoea, and chest pain. GI symptoms include: nausea, vomiting, abdominal pain, and diarrhea. Statistically significant p-value is in bold.

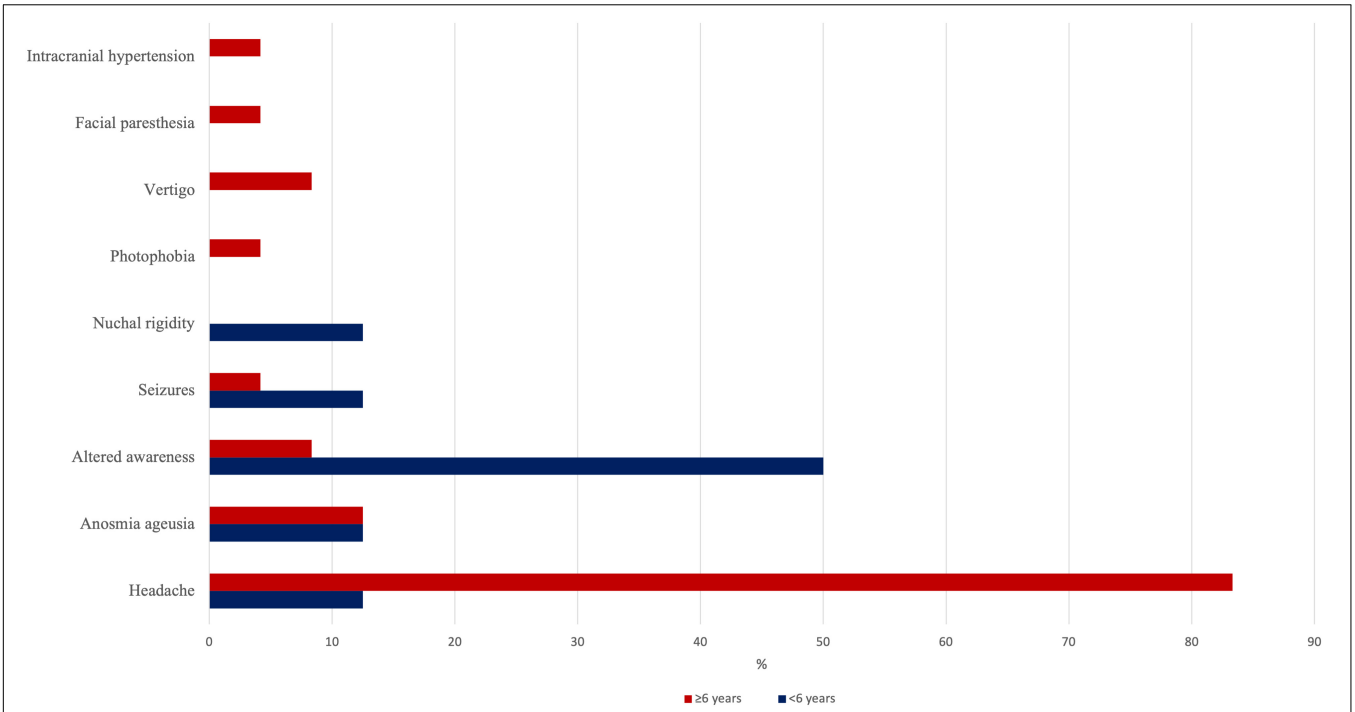

FIGURE 1 | Prevalence of each neurological manifestation in our series, stratified by age subgroups (<6 or >6 years).

found in each patient (**Figure 1**). Seven (21.9%) patients showed two or more associated neurological features. In this group of patients, cough, pharyngitis, rhinitis, and diarrhea were the most frequently associated non-neurological manifestations. Fourteen (43.8%) patients within this group required hospitalization with a median stay of 5 days (mean, 6 days). One patient (7.1%) only required, first, non-invasive and, then, invasive ventilation support due to bilateral pneumonia and respiratory failure. Seven (21.9%) children had pre-existing medical conditions (i.e., trilinear cytopenia, X-fragile syndrome, hyperthyroidism, ischemic stroke, jejunum atresia, asthma, and schizophrenia).

Two hundred and five (86.5%) patients with a median age of 2.5 years (0.7–9 years) did not report neurological symptoms. Eighty-eight (42.9%) of them required hospitalization with a median stay of 5.5 days (mean, 4 days); moreover, 3 (3.4%) patients required non-invasive ventilation support due to low O_2 saturation parameters. In this group, respiratory (59.5%) and/or gastrointestinal (GI; 25.3%) involvements were the most common. Thirty-seven (18.1%) children had pre-existing medical conditions, of which 9 (18%) involved the respiratory tract (i.e., asthma, allergy, and cystic fibrosis).

Of the total cohort, fifty-nine patients (25%, 14/59 *with* neurological symptoms) required treatment. The most prescribed drugs (32/59, 54%) were antibiotics (e.g., amoxicillin-clavulanic acid), systemic steroids, and heparin. Intravenous immunoglobulins, pulmonary surfactant, and inotropic drugs were administered to a patient with symptoms suggestive of meningitis at the ED and who, then, underwent invasive ventilation.

In our cohort, 5 (2.1%) children had a previous history of epilepsy, namely symptomatic epilepsies (1 arachnoid cyst and 1 astrocytoma), genetic generalized epilepsy, developmental epileptic encephalopathy, and epilepsy associated with X-fragile syndrome. In all cases, seizures were well-controlled with a mean of 1.6 (range: 1–3) anti-seizure medications (ASMs). ASMs included valproate (3 patients), clobazam (2 patients), levetiracetam (2 patients), and ethosuximide (1 patient). The only patient admitted with seizure re-exacerbation was an 11-year-old girl affected by X-fragile syndrome under levetiracetam monotherapy; she experienced a focal-onset febrile seizure with spontaneous resolution, no additional investigations were needed, and she was discharged without changes in her treatment regimen.

New-onset seizures occurred in a 5-year-old female with a history of ischemic stroke and jejunal atresia, admitted to the ED for a focal-onset motor seizure. Midazolam was administered with seizure remission. Brain magnetic resonance imaging (MRI) confirmed the previous ischemic lesion plus a post-ictal left fronto-insular perfusion alteration, and the electroencephalogram (EEG) showed left frontotemporal epileptiform abnormalities (**Figure 2**). The girl was discharged after 1 day in good clinical conditions without therapy.

Imaging and Cardiological Findings

Imaging data were available in 61 (25.7%) patients; twenty-eight (45.9%) performed a chest X-ray in 16 (57.2%) cases showing interstitial pneumonia. Chest CT was performed in 4 patients, resulting in a case of microembolism and two of interstitial pneumonia. Chest MR performed on one patient

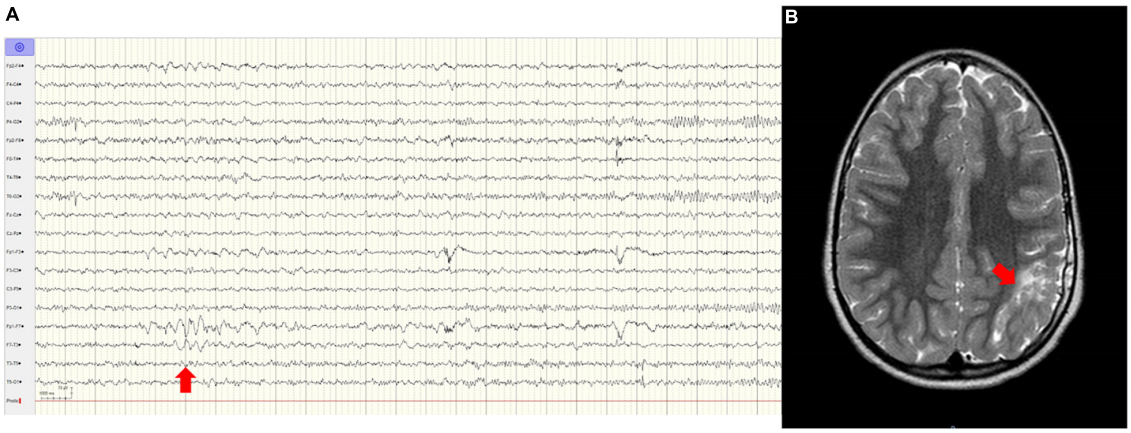

FIGURE 2 | (A) EEG performed at the Emergency Department (ED) showing left fronto-temporal epileptiform abnormalities (arrow). **(B)** T2-weighted axial brain MR, showing the consequences of an old ischemic lesion in the left parietal lobe (arrow).

showed hypoperfusion of the lower lung segments and a pleural effusion flap.

A brain CT scan was performed on the girl with epilepsy and a history of previous ischemic stroke, showing an unchanged ischemic area with left fronto-insular altered perfusion. This last finding was further confirmed with a brain MRI, and an EEG showing left fronto-temporal epileptiform abnormalities, compatible with the clinical presentation of a right-sided motor seizure. Additionally, other two patients performed a brain MRI: in one patient it was normal, whereas in the other case, it revealed *pseudotumor cerebri* in a child with papilledema at the ophthalmological examination.

Cardiac investigations (namely ECG, cardiac or epiaortic vessels ultrasounds) were performed in 13 (5.5%) patients, 8 *without* and 5 *with* neurological symptoms. In the group of patients *without* neurological symptoms, the main abnormal findings were altered cardiac rhythm at the ECG (i.e., tachycardia, and lower atrial rhythm alternating with sinus rhythm), mild mitral insufficiency, and "benign" pericardial effusion at the ultrasound. Conversely, among the 5 patients *with* neurological symptoms, there was a single case of thrombotic atrial formation.

Laboratory Tests Results

Eighty-two (34.6%) patients underwent laboratory test assessment: blood cell count, inflammatory biomarkers including erythrocyte sedimentation rate (ESR), procalcitonin (PCT), ferritin, c-reactive protein (CRP), fibrinogen, D-Dimer, liver and kidney function, coagulation profile, and pro-BNP were evaluated when deemed clinically appropriate (**Table 2**). Median values of ferritin, CRP, fibrinogen, and D-dimer were similar between the two groups of patients (**Table 3**).

Disease Severity-Population Demographics Correlations

Stratification by age range revealed a higher prevalence (46.9%) of neurological symptoms in patients aged between 6 and 12 years, followed by those aged 13–18 years (28.1%). A lower prevalence of neurological symptoms was found in patients younger than 6 years, whose symptoms mainly involve the respiratory or GI tract. Accordingly, the median age in the group *without* neurological symptoms was 2.5 years, compared to 10.9 years in patients *with* neurological symptoms (**Supplementary Figure 1**). The prevalence of each neurological manifestation stratified by age range is shown in **Table 4** and **Figure 3A**.

TABLE 2 | Main laboratory test results in patients tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Parameters (normal values)	Tested patients *n* (%)	Values out of normal age range *n* (%)
PCT (<0.50 ng/mL)	35 (14.7)	8 (22.9)
ESR (1–10 h/mm)	19 (8.0)	14 (73.7)
CRP (<0.46 mg/dL)	82 (34.5)	38 (46.3)
Fibrinogen (180–350 mg/dL)	41 (17.2)	21 (51.2)
aPTT (23.2–33.2 s)	39 (16.4)	2 (5.1)
PT% (63–129)	39 (16.4)	2 (5.1)
PT-INR (0.74–1.25)	34 (14.3)	0 (0.0)
D-dimer (<0.55 ug/mL)	26 (10.9)	19 (73.1)
Pro-BNP (0–125 pg/mL)	9 (3.8)	5 (55.6)
Troponins (0–0.16 ng/mL)	13 (5.5)	0 (0.0)
Glycemia (45–100 mg/dL)	73 (30.7)	23 (31.5)
AST (<40 U/L)	77 (32.4)	28 (36.4)
ALT (<40 U/L)	76 (31.9)	11 (14.5)
CK (0–150 U/L)	41 (17.2)	5 (12.2)
LDH (84–480 U/L)	49 (20.6)	28 (57.1)
Ferritin (20–200 ng/mL)	13 (5.5)	4 (30.8)
Haematocrit (36–44%)	82 (34.5)	5 (6.1)
Haemoglobin (11–13 g/dL)	85 (35.7)	34 (40.0)
Leucocytes (5,800–15,300/mm3)	86 (36.1)	2 (2.3)
Lymphocytes (31.9–73.1%)	84 (35.3)	5 (6.0)
Neutrophils (14.8–54.2%)	85 (35.7)	27 (31.8)
Platelets (150–400/mm3)	84 (35.3)	14 (16.7)

n, number; CK, Creatin Kinase; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; PCT, ProCalciTonin.

TABLE 3 | Comparison of inflammatory markers between patients *with* and *without* neurological symptoms.

	With neurological symptoms (median)	*Without* neurological symptoms (median)
Ferritin, ng/mL	115 (*n* = 3)	133.0 (*n* = 10)
PCT, ng/mL	0.25 (*n* = 7)	0.25 (*n* = 28)
CRP, mg/dL	0.23 (*n* = 12)	0.23 (*n* = 70)
Fibrinogen, mg/dL	327 (*n* = 5)	358 (*n* = 36)
D-dimer, mg/L	1.07 (*n* = 3)	1.21 (*n* = 23)

n, number; CRP, C-Reactive Protein; PCT, ProCalciTonin.

The triage code given on admission at the ED according to the Italian former color code system (red – emergency, yellow – urgency, green – non-urgent, and white – minor issues) was green in 78.1%, yellow in 18.8%, and red in 3.1% of the children *with* neurological symptoms, while patients *without* neurological symptoms got white code in 2.9%, green in 77.6%, and yellow in 12.2% cases (**Figure 3B**). The median stay at the hospital in patients *with* neurological symptoms was 5 days (mean, 6 days), while in the second group, a median of 5.5 days (mean, 4 days) was observed.

Patients *with* neurological symptoms showed a higher frequency of GI symptoms ($p = 0.0018$). A comparison of the occurrence of pre-existing medical conditions between the two groups resulted in a $p = 0.62$ (>0.05).

Long-Term Clinical Course

Four (12.5%) patients of the group *with* acute neurological symptoms reported persisting symptoms, which required new admission to our centre. Two cases were readmitted due to dyspnoea and recurrent bronchospasms, respectively. Two 14-year-old adolescents complained about persistent asthenia: the girl had a history of anxiety disorder under pharmacological treatment, while the boy also reported recurrent headaches and arthralgia of the shoulders and elbows.

DISCUSSION

Rating the prevalence of COVID-19 symptoms in the pediatric population may be subjected to case detection differences, and yet, after more than 2 years since the start of the pandemic, the real impact of SARS-CoV-2 infection on this population stays quite elusive (33). Most of the other published COVID-19 pediatric series have concentrated on the serious multisystem inflammatory syndrome in children (MIS-C) with neurological symptoms or other rare neurological sequelae in children with pre-existing neurological problems.

We analyzed the signs and symptoms of children who tested positive for SARS-CoV-2 with a molecular swab. Notably, in our cohort, the headache was the most frequent manifestation, followed by altered awareness/confusion, and ageusia/anosmia. Even considering the higher age (median, 10.9 years) of patients within the neurological group, ageusia and anosmia were less common in our cohort as compared to the literature (34). Notably, in the group *without* neurological symptoms, the median age at admission was significantly lower, possibly underlying one of the limitations of the current study, as patients aged less than 6 years are expected to have more difficulties in reporting symptoms.

TABLE 4 | Patients *with* neurological symptoms stratified by age range.

AGE	Total pts N° (%)	Headache	Anosmia/ ageusia	Altered awareness	Seizures	Nuchal rigidity	Photophobia	Vertigo	Facial paraesthesia	Endocranial hypertension
0–2 y	5 (15.6%)	0	0	4 (80.0%)	0	1 (20.0%)	0	0	0	0
3–5 y	3 (9.4%)	1 (33.3%)	1 (33.3%)	0	1 (33.3%)	0	0	0	0	0
6–12 y	15 (46.9%)	12 (80.0%)	2 (13.3%)	2 (13.3%)	1 (6.7%)	0	1 (6.7%)	1 (6.7%)	0	1 (6.7%)
13–18 y	9 (28.1%)	8 (88.9%)	1 (11.1%)	0	0	0	0	1 (11.1%)	1 (11.1%)	0

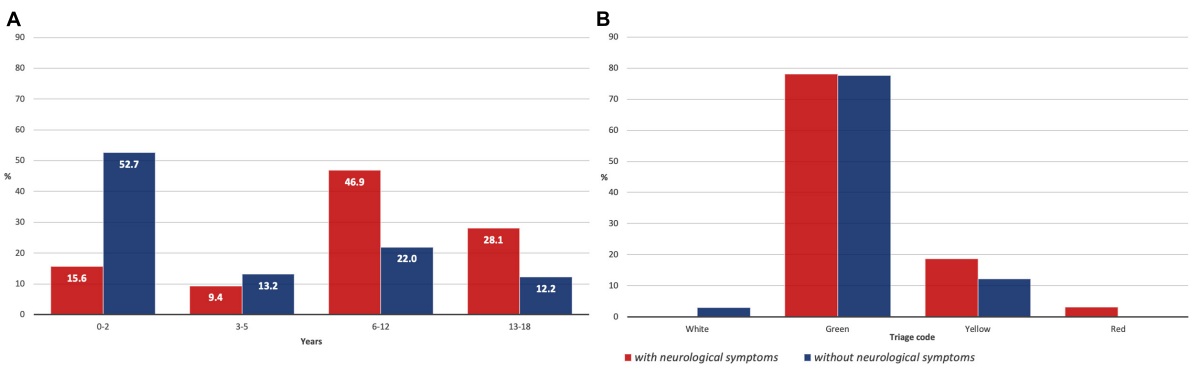

FIGURE 3 | (A) Patients *with* and *without* neurological symptoms stratified by age range; **(B)** Patients *with* and *without* neurological symptoms stratified for priority triage code at the Emergency Department (ED).

Comparison of the occurrence of pre-existing medical conditions between groups did not result in a statistically significant difference (p = 0.62), meaning the occurrence of neurological symptoms could not be affected by previous medical history. Nevertheless, there seems to exist a difference between groups, being respiratory comorbidities the primary pre-existing condition in the subgroup of patients *without* neurological symptoms. Conversely, a high statistical difference (p = 0.0018) was found in the concomitant occurrence of GI symptoms within the neurological subgroup.

Only one red priority code was assigned at the ED, pointing toward a low-grade infection severity in our pediatric population. No patient died, and life-threatening events occurred in one patient only (of the "*with* neurological symptoms" group). This data may significantly differ from the current literature on COVID-19 in children, possibly related to the selection criteria of our series, where only patients with a positive molecular swab were included, thus, skimming patients with MIS-C, which usually occurs 4–6 weeks after SARS-CoV-2 infection (35). Moreover, given the Italian health system structure, ED access occurs earlier than in other countries (e.g., the United States), where it is often delayed (36). Accordingly, early hospitalization of patients affected by MIS-C has been related to a better outcome (35). No differences in treatments, laboratory test results, and mean stay at the hospital were found between the two groups of patients. Only two ascertained cases of long-term neurological symptoms were observed at a one-year-long follow-up involving all the patients *with* neurologic features, thus, highlighting the rarity of these complications in the pediatric population (37).

Few reports of real-time PCR (RT-PCR) SARS-CoV-2-positive children experiencing epileptic seizures are currently available in the literature (29, 38–41). In our cohort, 2 patients were presented at the ED with seizures; one experiencing seizures re-exacerbation despite being previously well-controlled with a single ASM, the other with newly-onset seizure within the context of a malacic region due to a previous ischemic stroke. These findings are in line with the literature, indicating that seizures remain a rare presenting symptom in pediatric patients (42, 43), and that a certain degree of predisposed background is necessary to generate epileptic discharges. RT-PCR, for the identification of specific variants of SARS-CoV-2 was not performed as tests preceded the spread of the Delta variant in Italy and the identification of the Omicron variant. Yet, a higher prevalence of seizures has recently been reported in patients affected with these two SARS-CoV-2 variants (44).

In conclusion, neurological symptoms including peripheral facial palsy, encephalitis, and Guillain-Barrè syndrome are rare acute presenting symptoms in children with COVID-19, while they are more frequent at long-term follow-up and within the context of MIS-C (34, 45–48). Some limitations may be found in the current study, including those about a single-centre experience; moreover, the frequent changes in internal protocols (e.g., swab execution indications, clinical management of patients with SARS-CoV-2, the absence of neonates due to different diagnostic pathways) inevitably influenced data collection. Moreover, in line with previous studies, symptoms may have been underreported in younger children. Our study provides a whole-year picture of the acute symptoms in children tested postive for SARS-CoV-2, suggesting that patients *with* neurological symptoms neither have more severe clinical conditions nor have more pre-existing comorbidities. The course of the infection seems quite benign in children; however, additional research including the characterization of the clinical spectrum related to spreading variants (i.e., Delta and Omicron) is needed to fully assess the neurologic implications of SARS-CoV-2 infection in this population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Unico Regionale Liguria. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

GP and AR: study design and data acquisition, analysis, and interpretation of data, and wrote the manuscript. FB, MB, NB, EA, MSV, and TG: data collection, contribution to the manuscript, and interpretation of the data. AS, AV, EP, PI, and MM: critical revision of the manuscript. PS and GB: study design and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.909849/full#supplementary-material

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BRIEF RESEARCH REPORT
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Real-World Experience Treating Pediatric Epilepsy Patients With Cenobamate

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Introduction: In one third of all patients with epilepsy, seizure freedom is not achieved through anti-seizure medication (ASM). These patients have an increased risk of earlier death, poorer cognitive development, and reduced quality of life. Cenobamate (CNB) has recently been approved as a promising novel ASM drug for the treatment of adults with focal-onset epilepsy. However, there is little experience for its application in pediatric patients.

Methods: In a multicenter study we evaluated retrospectively the outcome of 16 pediatric patients treated "off label" with CNB.

Results: In 16 patients with a mean age of 15.38 years, CNB was started at an age of 15.05 years due to DRE. Prior to initiation of therapy, an average of 10.56 (range 3–20) ASM were prescribed. At initiation, patients were taking 2.63 (range 1–4) ASM. CNB was increased by 0.47 ± 0.27mg/kg/d every 2 weeks with a mean maximum dosage of 3.1 mg/kg/d (range 0.89–7) and total daily dose of 182.81 mg (range 50–400 mg). Seizure freedom was achieved in 31.3% and a significant seizure reduction of >50% in 37.5%. Adverse events occurred in 10 patients with fatigue/somnolence as the most common. CNB is taken with high adherence in all but three patients with a median follow-up of 168.5 days

Conclusion: Cenobamate is an effective ASM for pediatric patients suffering from drug-resistant epilepsy. In addition to excellent seizure reduction or freedom, it is well-tolerated. Cenobamate should be considered as a novel treatment for DRE in pediatric patients.

Keywords: epilepsy, Cenobamate, anti-seizure medication, seizure freedom, outcome, children, pediatrics, adverse effects

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INTRODUCTION

Epilepsy is one of the most common neurological disorders in children (1). Epilepsy is drug-resistant in about one third of the pediatric epilepsy population, i.e., treatment with two or more correctly chosen and dosed anti-seizure medications (ASMs) does not achieve seizure freedom (2). In these patients, the chance of reaching seizure freedom is lower than 15% (3).

Given the increased risk of earlier death, poorer cognitive development, and reduced quality of life of patients with drug resistant epilepsy (DRE), (4, 5) the possibility of a curative approach through epilepsy surgery must be assessed early (6, 7). However, even in individuals with focal-onset DRE, epilepsy surgery is not always feasible as a curative (or palliative) approach. In these patients, further ASM treatment is needed.

Cenobamate (CNB) is a new ASM recently approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of focal-onset seizures in adults. While the exact mode of action of CNB is not known, it has been shown to enhance inhibitory currents at $GABA_A$ receptors (8, 9) and to block excitatory currents by promoting the inactivated state of voltage-gated sodium channels (10).

Results regarding CNB efficacy are promising with placebo-adjustment seizure freedom in ∼20% and seizure reduction of >50% in about half of patients with focal seizures who were concomitantly taking up to three ASM (11). CNB was taken with high retention, suggesting good long-term tolerability (12). CNB is not approved for use in children and adolescents despite the high need for treatment in the pediatric population. Hence, there is no experience for use of CNB in the pediatric epilepsy population with respect to dosing, side-effects, and efficiency. Here we describe our experience with CNB in 16 pediatric patients.

METHODS

We performed a retrospective multicenter study of pediatric patients treated with CNB. All patients up to 18 years of age at initiation of treatment were included. All patients being treated with CNB at the epilepsy centers participating in this multicenter study were included in this study. Data was extracted from medical files using a standardized data sheet. Data on seizure reduction and side effects are based on information provided by parents and patients. For the evaluation of therapy success, seizure frequency was compared to the 4 weeks before therapy initiation and the last 2 weeks under therapy when therapy duration was <3 months. For patients with longer therapy duration, the last 4 weeks were compared. The cumulative data was then imported into SPSS 28 for analysis and evaluation. Descriptive statistics were used to calculate frequencies and percentages. Group data are presented as mean ± standard deviation unless otherwise stated. The study was approved by the local ethics committee (approval no. EA2/084/18).

RESULTS

A total of 16 pediatric patients with DRE (56.3% female, 43.8% male) were treated with CNB at a mean age of 15.05 ± 1.64 years (range 12.08–17.67) at treatment start (**Table 1**). The median age at seizure onset was 39 months-of-age (range 0–108) and the average disease duration was 11.73 years (range 6.33–16.42). Epilepsy causes included abnormalities of cortical development ($n = 5$), residual brain damage following infarction, asphyxia or encephalitis ($n = 4$), genetic diseases ($n = 2$) and autoimmune ($n = 1$). The epilepsy cause was unclear in 25% of cases. At initiation of CNB the patients were prescribed concomitantly a mean number of 2.63 ASM (range 1–4), and had a life-time number of 10.56 ASM (range 3–20). The most common ASM were clobazam ($n = 8$), lacosamide ($n = 6$), and lamotrigine ($n = 5$).

Two individuals had not become seizure free despite epilepsy surgery (P3: lesionectomy; P10 lesionectomy followed by hemispherotomy), and five despite ketogenic diet.

The titration scheme applied to the cohort varied given that there is no experience in CNB titration in pediatric patients. CNB was given once a day orally. The initial dose chosen in pediatric patients was most frequently 12.5 mg (range 6.25–25) and a mean of 0.22 mg/kg/d (range 0.12–0.36) (**Table 1**). The mean body weight of individuals was 62.16 kg (range 32–107). In most individuals CNB was increased by 0.47 ± 0.27 mg/kg/d every 2 weeks (27.9 mg ± 14.83). The mean maximum CNB dosage was 3.1 mg/kg/d (range 0.89–7) with a total daily dose of 182.81 mg (range 50–400 mg) (**Figures 1A,B**). The individual titration until highest dosage is given for each patient in **Supplementary Figure 1**. In most cases, the dosages of other ASM were kept constant at CNB initiation. In eight patients, other ASMs could even be reduced or discontinued after CNB initiation (4, 5, 7–11, 13). In most cases, ASMs were reduced or discontinued to reduce drug burden, as well as to reduce side effects due to possible pharmacodynamic interaction.

Adverse events (AE) occurred in ten cases (62.5%) during 198 CNB up-titration. However, AE were in most cases no severe enough to result in a discontinuation of CNB. In patient 16 CNB dosing resulted in an increase in seizure frequency and 201 prolongation of seizures. For this reason, CNB was discontinued 8 weeks after initiation, at a dose of 50 mg (0.89 mg/kg/d), under which seizure frequency and duration returned to previous levels. In another patient (Patient 4), a transient increase in seizure 205 frequency occurred. However, the up-titration was continued to a dose of 300 mg (5.17 mg/kg/d). This resulted in seizure freedom. In two other patients, AEs were too severe during the follow-up, and CNB was discontinued (P6, P13). In three patients AEs led to a reduction of dosage (P4, P5, P9). Most common adverse effects were somnolence/fatigue ($n = 5$). In two patients, seizure frequency increased during the titration period as delineated above, leading to a discontinuation of CNB in one patient. Three patients reported agitated behavior. Vertigo was observed in three patients. One patient each reported nausea and a balance disorder, diplopia and increased impulsive and agitated behavior, increased appetite resulting in weight gain and impaired sleep quality. One patient reported a rash with scaling first of the hands, then feet, as well as transient red eczema on the hips, which did not lead to discontinuation of CNB. No drug rash with eosinophilia and systemic symptoms (DRESS) occurred.

The effect of CNB treatment was evaluated at a median follow up of 168.5 days (range 56–314). All patients continued to take CNB except for three (81.25%) Seizure freedom was observed in five patients (31.3%) (**Figure 1C**). A seizure reduction >50% was observed in six patients (37.5%), four patients (25%) had a reduction of seizures <50%, and one case had an increase of seizure frequency.

TABLE 1 | Pediatric patients treated with Cenobamate.

ID	Sex	Age at seizure onset (months)	Cause	Age at CNB start (years)	No. of ASM at CNB start (ASM)	No. of ASM at CNB start	CNB titration protocol starting dosage (total, mg/kg/d); delta per 2 weeks (total, mg/kg/d); maximum dosage (total, mg/kg/d)	Outcome	Follow up (days)	Adverse effects	Reduced ASM	Discontinued ASM
1	m	86	FCD	17.67	15	3, CLB, PER, ESL	12.50, 0.12; 33.33, 0.31; 200, 1.87	Free	314%	None	-	-
2	m	48	Unclear	16	12	4, LCM, CLB, CBD, PER	12.500, 15; 25.00, 0.30; 100, 1.2	<50	292%	Somnolence	-	-
3	m	0	Infarction	15.08	6	3, LCM, CLB, BRV	6.25, 0.36; 6.82, 0.1; 75, 1.07	Free	268%	None	-	-
4	m	64	Unclear	12.25	13	2, VPA, OXC	12.50, 0.22; 42.86, 0.74; 300, 5.17	Free	208%	Somnolence, transient seizure increase	-	OCX
5	f	106	Unclear	15.08	8	2, LTG, LCM	12.5, 0.18; 53.33, 0.76; 400, 5.71	<50	199%	Somnolence, vertigo, diplopia, impulsive behavior	LTG	LCM
6*	m	24	Variants LANC3, ALDH7A1, SLC19A3, SZT2 and KCNB1	17	7	3, LCM, BRV, RFM	12.50, 0.13; 33.33, 0.34; 200, 2.03	<50	197%	Increased feeling of hunger, weight gain, poor sleep	-	-
7	w	61	FCD	14.17	11	1, ESL	12.50, 0.25; 43.75, 0.88; 350, 7	>50	193%	None	ESL	-
8	f	93	Unclear	16	10	2, LTG, PGB	12.50, 0.23; 46.15, 0.83; 300, 5.42	>50	180%	Somnolence, impulsive behavior	LTG	PRG
9	f	89	Polymicrogyria,	14.08	9	3, LTG, CBD, PER	12.50, 0.29; 36.36, 0.84; 200, 4.6	<50	157%	Impulsive behavior	-	CBD
10	f	4	FCD	14.79	20	3, LEV, CLB, RFM	12.50, 0.20; 10.53, 0.17; 100, 1.61	>50	151%	Eczema	LEV, RFM	-
11	m	30	del 15q11.2, suspected FCD	13.83	20	4, LTG, VPA, CLB, ESL	12.50, 0.21; 25, 0.42; 200, 3.39	>50	150%	Vertigo	LTG, CLB	VPA, ESL
12	f	5	Infarction	14.83	6	3 LTG, VPA, CLB	6.25, 0.18; 9.09, 0.26; 100, 2.89	>50	125%	None	-	-
13*	f	14	Herpes simplex encephalitis	17.58	7	1, LCM	12.5, 0.34; 25, 0.68; 100, 2.73	Free	107%	Nausea, balance disorder, vertigo	LCM	-
14	m	108	Autoimmun	16	3	2, LCM, BRV	25, 0.32; 33.33, 0.42; 200, 2.53	Free	94%	None	-	-
15	f	0	Asphyxia	14.33	12	3, CLB, RFM, CBD	6.25, 0.20; 10, 0.31; 50, 1.56	>50	81%	None	-	-
16*	f	6	FCD	12.08	10	3, CLB, BRV, CBD	12.5, 0.22; 12.50, 0.22; 50, 0.89	Increase	56%	Seizure increase, somnolence	-	-

*f, female; m, male; ASM, anti-seizure medication; CNB, cenobamate; VPA, Valproate; LEV, Levetiracetam; LTG, Lamotrigine; LCM, Lacosamide; OXC, Oxcarbazepine; CLB, Clobazame; BRV, Brivaracetam; RFM, Rufinamide; CBD, Cannabidiol; PER, Perampanel; ESL, Eslicarbazepine acetate; PRG, Pregabalin; -, no. *Patients in whom CNB was discontinued.*

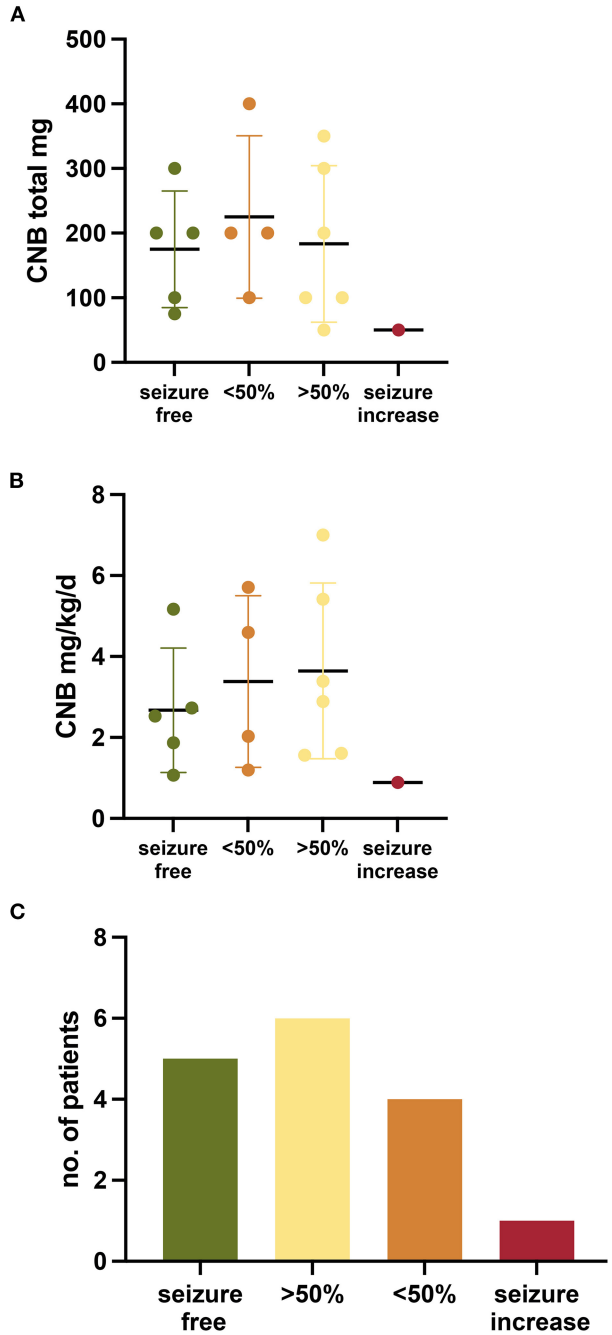

FIGURE 1 | Outcome of patients treated with Cenobamate. **(A,B)** Graph showing the outcome per patient and maximum CNB dosage (mg/kg/d and total) **(C)** Bar chart depicting the outcome of patients treated with Cenobamate.

DISCUSSION

Here we report our experience with CNB in 16 pediatric patients with DRE. CNB was initiated in pediatric patients with body weights between 32 and 107 kg most often at 12.5 mg once a day (0.22 mg/kg/d) and then titrated-up by 0.47 ± 0.27 mg/kg/d every 2 weeks. The individual maximum daily dose varied between 50 and 400 mg, with a mean of about 183 mg. Treatment with CNB resulted in seizure-free or a significant seizure reduction of > 50% in more than two thirds of the patients. These rates of seizure freedom or strong reduction of seizure frequency are in line with data published for adults. No serious adverse events occurred in our cohort. AEs occurred in about two thirds of the pediatric cohort, similar to the rate in adults (50%) (12). Two patients had an increase in seizure frequency, which was transient in one of them, and led to a treatment stop in the second patient. Most frequently, somnolence/fatigue occurred during up-titration, in line with the report of Sperling et al. (12). Less frequently vertigo, nausea, balance disorder, diplopia, increased impulsive/agitated behavior, increased appetite with weight gain and impaired sleep quality were reported. No drug rash with eosinophilia and systemic symptoms (DRESS) occurred. However, with a median follow-up of 168.5 days (range 56–314), these data are only a short time experience. Long-term data on the changes in outcome and complications are needed. Large multi-center prospective studies are necessary to answer these questions.

In conclusion, we report our first experience in treatment of pediatric patients with CNB. CNB showed an excellent effect with respect to seizure control in our small cohort of pediatric patients with DRE. The drug was well-tolerated without severe side effects. The use of CNB should, therefore, be considered in pediatric patients with DRE. Still, data for application of CNB in children prior to the age of 12 years and with a body weight below 32 kg are lacking. Furthermore, these data are only a short time experience due to short follow up duration. Given the need of therapeutic approached in children with DRE, large cohort, prospective studies are needed to determine the dosing schemes necessary for various weight ranges, safety data and efficacy data for use in children of all age ranges.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Charité Universitätsmedizin Berlin. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KM and AK contributed to the conception and design of the study. KM, TB, TK-P, TM, and EB contributed to

acquisition of data. KM organized the database analyzed the data, wrote the first draft of the manuscript, and created figures and tables. All authors discussed the results, revised the first draft, and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

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Development, Reliability, and Validity of the Preschool Learning Skills Scale: A Tool for Early Identification of Preschoolers at Risk of Learning Disorder in Mainland China

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Background: Early identification of children at risk of learning disorders (LD) may mitigate the adverse effects of delayed intervention by guiding children to receive preventive services at an earlier age. However, there is no assessment tool for the early identification of children at risk of LD in Mainland China. Therefore, this study aimed to create a Chinese version of the Preschool Learning Skills Scale and investigate its validity and reliability.

Methods: Firstly, a pilot scale was designed based on literature review and expert review. Secondly, a pre-survey of the pilot scale was conducted. In phase 3, a formal survey was carried out to test the reliability and validity of the scale by involving 2,677 preschool children from 7 kindergartens. Data were collected using a checklist for demographic characteristics, the preschool learning skills scale, the Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P), and Conners' Rating Scales.

Results: The final scale included 38 items under seven factors. The reliability and validity tests confirmed that the Cronbach's alpha, split-half reliability, and test–retest reliability coefficients of the scale were 0.946, 0.888, and 0.941, respectively. The Spearman correlations of factor-total score ranged from 0.685 to 0.876. The results of criterion-related validity showed a direct and significant association between the preschool learning skills scale with the BRIEF-P ($r = 0.641$, $P < 0.001$) and the cognitive problems factor of Conners' Rating Scales ($r = 0.564$, $P < 0.001$). The model had a good fit ($\chi^2/df = 3.489$, RMSEA = 0.047, RMR = 0.024, CFI = 0.912, TLI = 0.900, and IFI = 0.912). Multigroup confirmatory factor analysis supported the structural and measurement invariance on the preschool learning skills scale across gender and grade.

Conclusions: The developed preschool learning skills scale has good reliability and validity, indicating that the scale can be used to identify preschool children at risk of LD and can be recommended for use in clinical research and practice.

Keywords: learning disorder, preschoolers, early identification, scale development, validity and reliability

INTRODUCTION

Specific learning disorder (LD) is a complex neurodevelopmental disorder (1). According to DSM-5, SLD is a general term that refers to a group of disorders, which may involve persistent difficulties in reading (dyslexia), written expression (dysgraphia), and/or mathematics (dyscalculia), albeit not accounted for by low intelligence (IQ), sensory acuity (e.g., visual problems), poor learning opportunities, or developmental delay (e.g., intellectual disability) (2). DSM-5 describes LD as a neurodevelopmental disorder with a biological origin, including the interaction of genetic, epigenetic, and environmental factors (3, 4). LD is a lifelong disease that may have adverse consequences for children and adults at the educational, social, financial, and professional levels (5).

Studies have shown that the prevalence of LD in the general population ranges from 3 to 12% depending on factors such as different assessment tools, boundaries of measurement, cultural background, gender, age, etc. (6–8). The average age at which children are diagnosed with LD is 9 years (grades 3–4 of primary school). This is when the academic demands rise and exceed the individual's limited capacities, and children begin to appear to face academic difficulties in school (9). However, it is generally believed that LD occurs prior to kindergarten and continues into adulthood (10, 11). Delayed intervention may have adverse and lasting consequences on the acquisition of academic skills. On the contrary, early identification of children at risk of LD may mitigate the adverse effects of delayed intervention by guiding children to receive preventive services at an earlier age (9, 12).

Researchers have developed many behavior checklists for the screening of LD, which are primarily suitable for school-age children, mainly focusing on academic skills such as reading, writing, and arithmetic (13, 14). In addition, there are behavioral checklists for specific disorders, such as Dyslexia Screening Instrument, designed to identify children who exhibit behaviors related to spelling, reading, writing, or language-processing difficulties (15, 16). These screening tools are relatively brief and cost-effective measures to justify a more detailed assessment or diagnostic test (17).

However, preschool age is a crucial period for early identification and intervention of LD. In kindergarten, children with mathematics disorders already have deficits in comparing non-symbolic and symbolic Arabic numbers (18). Several early or pioneer literacy skills measured in preschool-age have demonstrated strong relationships with future decoding and reading comprehension achievement, such as phonological awareness, rapid naming, and oral language (19, 20). Meanwhile, effective, early reading instructions can improve reading outcomes of children with LD (21, 22). Therefore, more and more researchers pay attention to the early recognition of LD in preschool children (23). Some checklists have been developed for early screening of LD in Hong Kong and Taiwan, such as the Hong Kong Learning Behavior checklist for Preschool Children (Parent Version) (24). However, there is no specific assessment tool in mainland China for the early identification of Preschoolers at risk of LD.

Thus, this study aimed to construct a brief, easy-to-use scale to specify the characteristics of LD for parents from mainland China to identify preschoolers at risk of LD at an early stage and to test the validity and reliability of the scale.

TABLE 1 | Characteristics of participants ($n = 2,677$).

Participants	Frequency (*n*)	Percentage (%)
Sex		
Boys	1,445	53.98
Girls	1,232	46.02
Age, y		
3.0–3.9	170	6.35
4.0–4.9	947	35.38
5.0–5.9	861	32.16
6.0–6.9	699	26.11
Questionnaire fillers		
Mother	2,090	78.07
Fathers	587	21.93
Father education		
Less than high school	129	4.82
High school graduate	350	13.07
Associates degree	614	22.94
Bachelor's degree	1,206	45.05
Master's degree or above	378	14.12
Mother education		
Less than high school	157	5.87
High school graduate	354	13.22
Associates degree	605	22.60
Bachelor's degree	1,277	47.70
Master's degree or above	284	10.61

METHODS

The Preschool Learning Skills Scale (**Supplementary File 1**) aimed to develop a brief screening measure for the early identification of preschoolers at a risk for LD. The study was ethically approved by the Institutional Review Committee of Nanjing Medical University.

Development of the Preschool Learning Skills Scale

The development of the preschool learning skills scale for parents of preschoolers in mainland China followed many stages (25).

In the first stage, based on an extensive review of relevant literature and published questionnaires, as well as on consultations with parents of children with LD, and specialists and teachers who specialize in LD, the primary cognitive and behavioral manifestations of children with LD in the preschool

Abbreviations: LD, learning disorder; BRIEF, behavior rating inventory of executive function; EFA, exploratory factor analysis; CFA, confirmatory factor analysis; χ^2/df, chi-square goodness of fit values; RMSEA, root-mean-square error of approximation; RMR, root-mean-square residual; CFI, comparative fit index; TLI, tucker-lewis index; IFI, incremental fit index; CR, composite reliability; AVE, average variance extracted.

TABLE 2 | Factor loadings from the exploratory factor analysis of the preschool learning skills scale ($n = 1,540$).

Item	Factors						
	A	B	C	D	E	F	G
Factor A: Attention							
Item 1	0.670						
Item 2	0.582						
Item 10	0.694						
Item 11	0.713						
Item 19	0.650						
Item 24	0.593						
Item 35	0.664						
Factor B: Memorization							
Item 3		0.458					
Item 6		0.672					
Item 7		0.657					
Item 25		0.401					
Factor C: Visual perception							
Item 20			0.432				
Item 22			0.537				
Item 27			0.624				
Item 29			0.696				
Item 34			0.444				
Factor D: Auditory perception							
Item 15				0.517			
Item 18				0.579			
Item 28				0.692			
Item 36				0.593			
Factor E: Motor coordination							
Item 8					0.583		
Item 12					0.616		
Item 17					0.583		
Item 23					0.432		
Factor F: Verbal competence							
Item 4						0.556	
Item 5						0.701	
Item 9						0.598	
Item 13						0.787	
Item 14						0.680	
Item 16						0.605	
Item 21						0.485	
Item 26						0.617	
Item 33						0.701	
Item 37						0.599	
Factor G: Mathematical concept							
Item 30							0.534
Item 31							0.650
Item 38							0.673

TABLE 3 | The reliability of the preschool learning skills scale ($n = 1,540$).

Factors	Cronbach's alpha	Split-half	Test-retest
A: Attention	0.835	0.789	0.927
B: Memorization	0.737	0.752	0.856
C: Visual perception	0.716	0.697	0.774
D: Auditory perception	0.757	0.723	0.856
E: Motor coordination	0.703	0.713	0.917
F: Verbal competence	0.871	0.834	0.939
G: Mathematical concept	0.674	0.638	0.842
Total score	0.946	0.888	0.941

years were determined to identify key components related to LD risk. Finally, a total of 7 dimensions with 71 items were generated from the item pool, which forms the index system framework of the scale, classified into seven categories: attention, memorization, visual perception, auditory perception, motor coordination, verbal competence, and mathematical concept.

In phase 2, a panel of experts in developmental–behavioral pediatrics, psychology, pedagogy, public health, and clinical evaluation ($n = 5$, including the first and corresponding authors) reviewed the bank of items to classify those contents into each key theme to identify gaps and overlaps between items and to figure out the suitability of items for parents of preschool children. The scale was adapted and revised with inappropriate items removed. Finally, 55 items were selected to form the first draft of the preschool learning skills scale. All items were rated on a 5-point Likert scale ranging from never to always.

In phase 3, to ensure the scale's content validity, the questionnaire was reviewed and refined again by a panel of experts ($n = 8$) to determine whether the items comprehensively reflected the key behaviors that could develop into LD in preschool-aged children to establish content validity. Then, the questionnaire was put to an experimental test to render certain that the parents of preschoolers ($n = 20$) understood the items as intended. Furthermore, based on the experimental testing results, the questionnaire was refined by modifying the items which were difficult to understand, had semantic ambiguity, and were prone to ambiguity due to the experimental testing results.

Pre-Survey of the Preschool Learning Skills Scale

A convenience sample of participants selected 657 preschool children from two kindergartens in Nanjing in December 2020. Exclusionary criteria were children who were diagnosed with a neurodevelopmental disorder such as autism, intellectual disability, or other disabilities. The parents of eligible participants electronically signed the consent document and completed the 55-item learning skills scale (Draft 1 of the preschool learning skills scale) and a demographic survey.

Through the pre-survey, 55 items were scientifically refined in the following methods: critical ratio method, frequency distribution analysis, variation coefficient (CV) method,

TABLE 4 | Spearman correlations of interfactor and factor-total score (n = 1,540).

Factors	Factors						
	A	B	C	D	E	F	G
A: Attention	1						
B: Memorization	0.651***	1					
C: Visual perception	0.553***	0.577***	1				
D: Auditory perception	0.571***	0.614***	0.620***	1			
E: Motor coordination	0.512***	0.502***	0.499***	0.567***	1		
F: Verbal competence	0.563***	0.668***	0.665***	0.716***	0.569***	1	
G: Mathematical concept	0.417***	0.490***	0.569***	0.545***	0.488***	0.573***	1
Total score	0.803***	0.804***	0.787***	0.817***	0.712***	0.876***	0.685***

****P < 0.001.*

correlation analysis, Cronbach's alpha coefficient method, and exploratory factor analysis (25). Considering the above six methods comprehensively, if two or more methods excluded an item, the item would be deleted from the scale. Finally, the preschool learning skills scale with 38 items in 7 factors is compiled.

Reliability and Validity of the Preschool Learning Skills Scale

A total of 2,677 preschool children were selected from 7 kindergartens in Nanjing. Exclusion and inclusion criteria were the same as those for the pre-test of the study. The parents electronically signed the consent document and completed the questionnaire survey within 3 days of receipt of the survey (first assessment T1). In addition to the 38-item learning skills scale (Draft 2 of the preschool learning skills scale) and a demographic survey, 600 parents were randomly selected to extra complete the Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) and Conners' Rating Scales (Conners 3-P) for assessment of criterion-related validity.

The reliability of the scale was assessed using Cronbach's alpha, split-half correlation, and test–retest. A random sample of the parents filled in the preschool learning skills scale 2 weeks after the first survey (T2) to assess test–retest reliability.

For the exploratory and confirmatory factor analysis, data from four kindergartens (n = 1,540) were used for the exploratory factor analysis (EFA), and data from three kindergartens (n = 1,137) for the confirmatory factor analysis (CFA). The EFA was performed by IBM SPSS Statistics 26.0. The suitability of the data for factorization was evaluated by the value for the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity (preferably significant) (26). The EFA was done *via* iterative Maximum Likelihood with Promax Rotation to extract the factors due to the correlation of the factors (27). The criterion for loading and cross-loading was set at 0.4. The CFA was performed with IBM SPSS Amos 22.0. The goodness of model fit was evaluated using the following fit indices: the chi-square goodness of fit (χ^2/df) values, with values <5.0 deemed acceptable; the root-mean-square error of approximation (RMSEA); the root-mean-square residual (RMR) <0.05; the comparative fit index (CFI); the Tucker-Lewis index (TLI); and the incremental fit index (IFI) >0.9 (28, 29). The construct validity of the preschool learning skills scale was examined with the standardized regression coefficients and construct reliability (CR) for convergent validity and the correlation coefficient, and the square root of AVE for discriminant validity.

Multigroup Confirmatory Factor Analysis of the Preschool Learning Skills Scale

We performed a multigroup confirmatory factor analysis across gender and grade. Subsequently, using the sample from three kindergartens (n = 1,137), we conducted a multigroup confirmatory factor analysis to examine the measurement invariance of the seven-factor structure across gender: boys vs. girls. Measurement invariance was also tested across age groups: Group 1 (Junior Class of kindergarten), Group 2 (Middle Class of kindergarten), and Group 3 (Senior Class of kindergarten). The measurement invariance was evaluated using the following fit indices: the change in chi-square values ($\Delta\chi 2$) and the fit indices (RMR, RMSEA, CFI, ΔRMSEA, and ΔCFI) (30, 31). Measurement invariance is supported when ΔRMSEA is <0.015 and ΔCFI is <0.02 (32).

RESULTS

Participants

As shown in **Table 1**, the study included 2,677 preschool children: 1,445 boys and 1,232 girls with a mean age of 5.2 years (SD = 0.9, range 3.5–6.8). There was no significant mean age difference between the genders. Most of the questionnaire fillers were mothers (n = 2,090, 78.07%).

The Exploratory Factor Analysis of the Preschool Learning Skills Scale

The appropriateness of factor analysis was measured by The Kaiser–Meyer–Olkin (KMO) measure of the sampling adequacy and the Bartlett test of sphericity. The results showed KMO = 0.966 and Bartlett significance $P < 0.001$, indicating that exploratory factor analysis was appropriate.

Seven factors were extracted from the preschool learning skills scale by the EFA (see **Table 2**). The factor loadings varied from 0.401 to 0.787. The seven factors explained 55.30% of the variance.

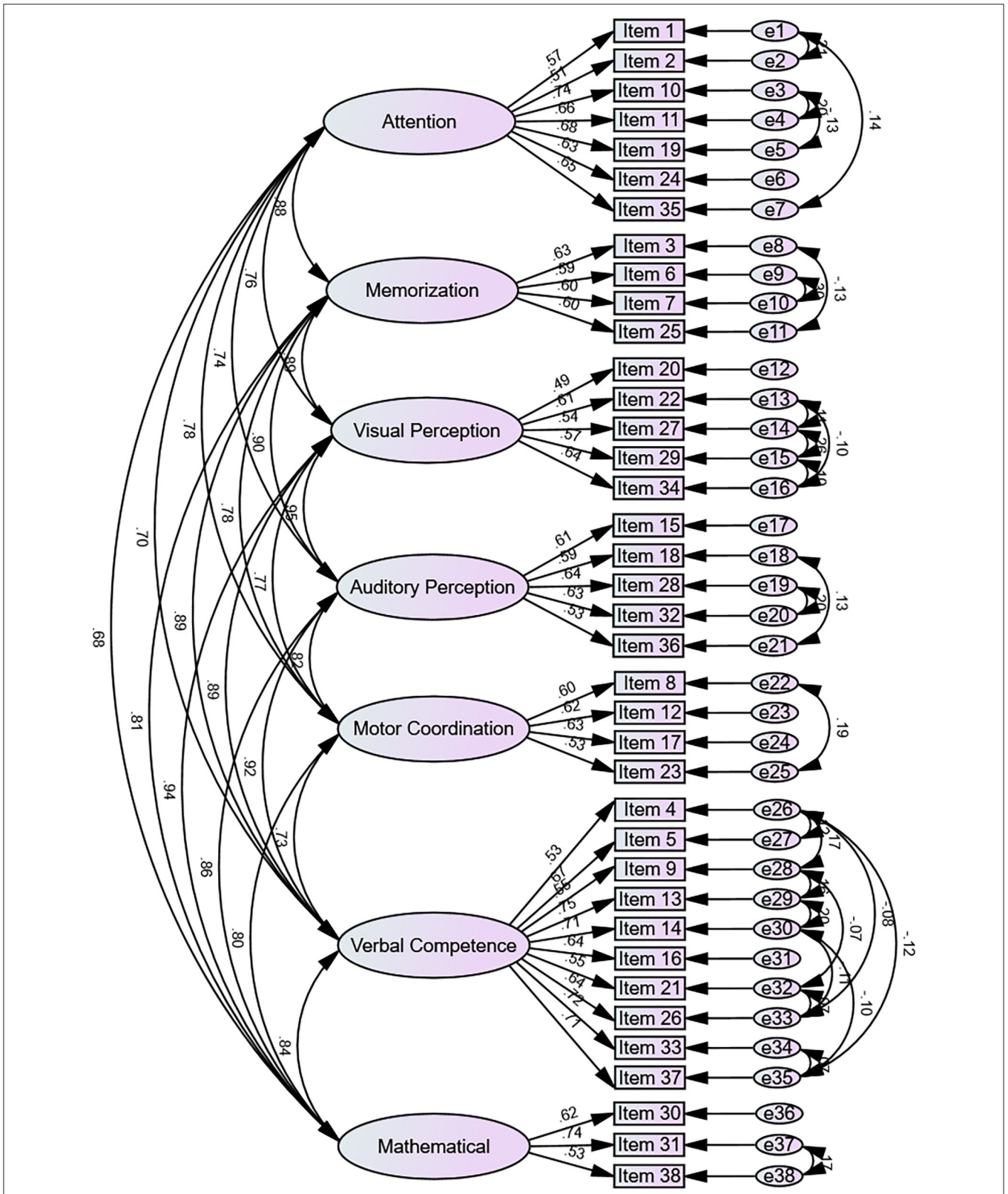

FIGURE 1 | The standardized path coefficients of the preschool learning skills scale ($n = 1,137$).

Reliability and Validity of the Preschool Learning Skills Scale

The reliability results of PLSS are shown in **Table 3** below. Cronbach's alpha coefficient of the preschool learning skills scale was 0.946 and Cronbach's alpha coefficients of the factors ranged from 0.674 to 0.871, all >0.70 except for mathematical concepts, which meant that the scale had good internal consistency reliability. The split-half reliability coefficient was 0.888, and the split-half reliability coefficient of the factors varied between 0.638 and 0.834, indicating that the scale had good internal reliability. The test–retest reliability coefficient was 0.941, and the test–retest reliability coefficient of the factors ranged from 0.774 to 0.939, which reflected excellent test–retest reliability of the scale.

The Spearman correlations of the factor-total score ranged from 0.685 to 0.876. The Spearman correlations of the interfactor varied between 0.417 and 0.716 (**Table 4**); the correlation coefficients among factors A, B, C, D, and F were beyond 0.6, but <0.8, indicating that the correlation between factors had reached an acceptable level. Moreover, all Spearman correlations of the factor-total score were more significant than the interfactor. The results of the correlation analysis showed a direct and significant association of the preschool learning skills scale with the Behavior Rating Inventory of Executive Function-Preschool Version ($r = 0.641$, $P < 0.001$) and the cognitive problems factor of Conners' Rating Scales ($r = 0.564$, $P < 0.001$).

The Confirmatory Factor Analysis of the Preschool Learning Skills Scale

Figure 1 shows the factor structure and model fit of the preschool learning skills scale using CFA. The CFA of the seven-factor model of the preschool learning skills scale showed that the $\chi^2/df = 3.489$, the RMSEA was 0.047, the RMR was 0.024, the CFI was 0.912, the TLI was 0.900, and the IFI was 0.912. These results indicated that the goodness-of-fit index of the model was valid. The standardized regression coefficients of each item of the preschool learning skills scale ranged from 0.490 to 0.747. These values were more than 0.4 (**Table 5**) and CR values also were more than 1.965 ($P < 0.001$), which indicated that the items corresponding to each latent variable were highly representative. The discriminant validity of the preschool learning skills scale was evaluated using correlation coefficients among seven factors (**Table 6**). Correlations ranged from 0.129 to 0.211 among seven factors, all of which were significantly correlated ($P < 0.001$). The correlation coefficients were all less than the square root of the corresponding AVE. These results were satisfactory and indicated adequate discriminant validity in the study.

Measurement Invariance Across Gender and Grade

To examine the measurement invariance of the preschool learning skills scale across both gender and grade, we used a multigroup confirmatory factor analysis approach, which assesses the measurement invariance across two or more groups by using a series of increasingly stringent, nested models.

Table 7 presented that the fit indices for Model 1 (configural invariance) indicated that the seven-factor measurement model of the preschool learning skills scale had an acceptable fit within each gender group (RMR = 0.027; RMSEA = 0.036; CFI = 0.899). Based on the indices of practical fit and the change in RMSEA and CFI criterion recommended, our results concluded that Model 2 (Metric invariance), Model 3 (Scalar invariance), and Model 4 (Strict factorial) fit nearly as well as Model 1 (RMR <0.05; RMSEA <0.05; CFI >0.8; ΔRMSEA <0.015;

TABLE 5 | Standardized regression coefficients of the preschool learning skills scale ($n = 1,137$).

Item		Factor	Unstandardized estimate	S.E.	C.R.	Standardized estimate
Item 1	<–	A	1.000			0.571
Item 2	<–	A	0.902	0.057	15.751	0.510
Item 10	<–	A	1.203	0.068	17.621	0.739
Item 11	<–	A	1.259	0.076	16.556	0.660
Item 19	<–	A	1.039	0.062	16.864	0.679
Item 24	<–	A	1.028	0.063	16.242	0.629
Item 35	<–	A	1.047	0.058	17.999	0.650
Item 3	<–	B	1.000			0.626
Item 6	<–	B	0.988	0.058	16.964	0.589
Item 7	<–	B	0.965	0.056	17.169	0.598
Item 25	<–	B	0.846	0.052	16.309	0.602
Item 20	<–	C	1.000			0.490
Item 22	<–	C	1.538	0.107	14.318	0.607
Item 27	<–	C	1.187	0.089	13.387	0.539
Item 29	<–	C	1.238	0.089	13.833	0.571
Item 34	<–	C	1.184	0.080	14.758	0.643
Item 15	<–	D	1.000			0.607
Item18	<–	D	0.988	0.059	16.693	0.586
Item 28	<–	D	0.801	0.045	17.958	0.645
Item 32	<–	D	0.854	0.049	17.589	0.628
Item 36	<–	D	0.963	0.063	15.356	0.529
Item 8	<–	E	1.000			0.600
Item 12	<–	E	0.765	0.048	15.798	0.620
Item 17	<–	E	0.925	0.058	15.890	0.625
Item 23	<–	E	0.717	0.046	15.715	0.529
Item 4	<–	F	1.000			0.534
Item 5	<–	F	1.240	0.071	17.516	0.672
Item 9	<–	F	1.094	0.069	15.805	0.548
Item 13	<–	F	1.221	0.070	17.384	0.747
Item 14	<–	F	1.032	0.061	16.909	0.714
Item 16	<–	F	1.072	0.067	15.922	0.636
Item 21	<–	F	0.970	0.067	14.455	0.549
Item 26	<–	F	1.132	0.073	15.449	0.645
Item 33	<–	F	1.184	0.069	17.095	0.723
Item 37	<–	F	1.257	0.078	16.105	0.708
Item 30	<–	G	1.000			0.624
Item 31	<–	G	1.168	0.061	19.069	0.736
Item 38	<–	G	1.171	0.079	14.800	0.535

TABLE 6 | Discriminant validity of the preschool learning skills scale (n = 1,137).

	A	B	C	D	E	F	G
A: Attention	(-)						
B: Memorization	0.211***	(-)					
C: Visual perception	0.131***	0.145***	(-)				
D: Auditory perception	0.162***	0.186***	0.142***	(-)			
E: Motor coordination	0.209***	0.200***	0.142***	0.191***	(-)		
F: Verbal competence	0.148***	0.179***	0.129***	0.169***	0.164***	(-)	
G: Mathematical concept	0.156***	0.178***	0.148***	0.173***	0.198***	0.163***	(-)
Square root of AVE	0.638	0.604	0.573	0.600	0.595	0.652	0.637

***$P < 0.001$.

TABLE 7 | Multigroup CFA Fit indices for the preschool learning skills scale across gender (n = 1,137).

Model	χ2	*df*	Δχ2	Δ*df*	*P*	RMR	RMSEA	CFI	ΔRMSEA	ΔCFI
Model 1 (configural invariance)	3,011.386	1,238.000				0.027	0.036	0.899		
Model 2 (metric invariance)	3,062.051	1,269.000	50.665	31.000	0.014	0.029	0.035	0.898	−0.001	−0.001
Model 3 (scalar invariance)	3,107.914	1,297.000	96.528	59.000	0.001	0.040	0.035	0.897	−0.001	−0.002
Model 4 (strict factorial)	3,251.864	1,360.000	240.478	122.000	0.000	0.041	0.035	0.892	−0.001	−0.007

and ΔCFI <0.02), supporting measurement invariance across gender groups.

Subsequently, measurement invariance across grades was examined. As **Table 8** showed, the configural invariance was supported by the acceptable absolute fit indices (RMR = 0.029; RMSEA = 0.031; CFI = 0.887). In addition, also measurement invariance (at the metric, scalar, and strict factorial level) across grades was present because all absolute and relative fit indices were acceptable. All these results indicated a reasonable level of structural invariance and measurement invariance in all three grade groups of the preschool learning skills scale.

DISCUSSION

Learning disorders refers to a group of disorders characterized by significant difficulties in listening, reading, speaking, writing, attention, memorization, and coordination. These difficulties range from mild to severe. Researchers have made consistent efforts to identify and intervene early to ensure that children receive assistance prior to having poor learning experiences and prevent other problems that may affect their learning abilities (16, 18, 21). As to early identification, it is believed that parents are the ones who closely observe children in parent–child interactions to identify behavioral indicators of LD, such as literacy problems and specific cognitive deficits. Thus, we developed the preschool learning skills scale as a parental checklist to provide information on the characteristics of children with LD at preschool age and as a screening measure to make a more detailed follow-up assessment of children at risk of LD.

Different manifestations of LD can be seen at various ages and as a result of varying learning demands. Delays in speech and language development, numerical and symbolic concepts, motor coordination, and auditory and visual perception are early indicators of children who may have LD (33–35). These indicators may occur concomitantly with attention, memorization, or self-regulation problems. The Hong Kong Learning Behavior checklist for Preschool Children (Parent Version), which was developed by Hong Kong Specific Learning Difficulties Research Team in 2006, identified preschoolers at the risk of learning difficulties in seven aspects: language ability, learning ability, writing performance, attention, memorization, sequencing ability, spatial awareness, and motor coordination (24). However, the scale was specifically validated for the population of Hong Kong, and mainly aimed at the early identification of dyslexia and dysgraphia. The spoken language of Hong Kong includes Cantonese and English, and the scale contains some items to check English ability. Besides, the education system of Hong Kong is different from that of mainland China. The teaching of literacy and handwriting begins in early childhood, much earlier than in the Mainland, so there are many items about the ability to learn Chinese (such as reading or interest in words) and writing performance in that scale. Therefore, the scale is not wholly applicable to the early recognition of LD for Mainland children, but the theoretical framework and some items are still worth our reference.

In this study, we first defined the connotation and characteristics of LD by reviewing the related researches. A literature search was performed using the following terms: "learning ability," "LD," "dyslexia," "mathematical disorder," "kindergarten," and "preschool children" in PubMed, EMBASE, Web of Science, and MEDLINE, CNKI, and Wanfang databases. In the retrieved literature, the behavioral manifestations and characteristics of children with LD were perused, as well as related guidelines, expert consensus, systematic evaluation, and original research on screening and diagnosis of LD. Then, we summarized and compared the scales or tools for early

TABLE 8 | Multigroup CFA fit indices for the preschool learning skills scale across grade ($n = 1,137$).

Model	χ2	*df*	Δχ2	Δ*df*	*P*	RMR	RMSEA	CFI	ΔRMSEA	ΔCFI
Model 1 (configural invariance)	3,830.508	1,857.000				0.029	0.031	0.887		
Model 2 (metric invariance)	3,884.913	1,888.000	54.405	31.000	0.006	0.032	0.031	0.886	0.000	−0.001
Model 3 (scalar invariance)	3,939.602	1,916.000	109.094	59.000	0.000	0.036	0.031	0.885	0.000	−0.002
Model 4 (strict factorial)	4,176.705	1,979.000	346.197	122.000	0.000	0.037	0.031	0.875	0.000	−0.012

identification of LD in domestic and foreign studies, such as the Pupil Rating Scale Revised (PRS) questionnaire, Hong Kong Learning Behavior checklist for Preschool Children (Parent Version), East Asia-Pacific Early Child Development Scales, South African Early Learning Outcomes Measure, etc., to clear and definite the methods, structures, and indicators of early identification of LD. Finally, we studied the localization theory of early identification for LD in Mainland China. Through in-depth interviews with experts in child psychology, developmental-behavioral pediatrics, teachers, and parents of children with LD, their attitudes, views, and experiences toward the early identification of LD in the preschool age were summarized. From these, the preschool learning skills scale, which is the first scale for early identification of preschoolers at risk of LD in Mainland China, establishes the item pool for the initial experimental checklist based on the theoretical framework of the following: attention, memorization, visual perception, auditory perception, motor coordination, verbal competence, and mathematical concept.

Our research conducted psychometric evaluation through the critical ratio method, frequency distribution analysis, variation coefficient (CV) method, correlation analysis, Cronbach's alpha coefficient method, and exploratory factor analysis, suggesting the retention of 38 items to be included in the revised checklist. To evaluate the reliability and validity of the 38-item preschool learning skills scale, we conducted a questionnaire survey among 2,677 preschool children from 7 kindergartens. Overall, our results demonstrate that the developed preschool learning skills scale had good reliability and validity, and showed an excellent fit of the seven derived factors *via* exploratory and confirmatory factor analysis. After establishing a robust factor structure within a sample, it is essential to know whether this factor structure is also applicable across samples or groups, as this "invariance" is the only condition that can be allowed to investigate group differences, for instance, between different genders and ages, and so on (36). Given the difference between the scale scores of boys and girls ($P < 0.001$) and three grades of kindergarten ($P < 0.001$), it is crucial to establish measurement invariance across gender and grade to elucidate if the preschool learning skills scale's sensitivity can identify preschool children at risk of LD. The results of the multigroup confirmatory factor analysis indicated a reasonable level of structural invariance and measurement invariance across gender and grade of the preschool learning skills scale. Therefore, as a screening scale, the preschool learning skills scale was considered to provide information on the cognitive weaknesses of LD and could be used to screen for children at risk of LD for further assessment or preventive interventions.

Nevertheless, further research is needed. Since this study relied on parent reports, it will be necessary for future research to explore the correspondence between parental reports with other reports (such as teachers) and observations of clinicians or educational psychologists (37, 38). More specifically, it will be necessary to further establish the validity of the preschool learning skills scale in the prediction of LD by using multimodal methods and exploring the relationship between the preschool learning skills scale with experimental and behavioral paradigms, such as rapid automatized naming, phonological awareness, letter knowledge, and short-term verbal memorization (19, 39).

Despite these limitations, the preschool learning skills scale has the potential to be a reliable measure that provides a scientific basis for early identification and intervention of LD in preschool age.

CONCLUSIONS

To our knowledge, this is the first study to develop a brief, easy-to-use questionnaire to describe the characteristics of LD in preschool-age for early identification of children at risk of LD. Overall, as an instrument, the developed preschool learning skills scale has good reliability and validity, which indicates that the scale can be used for the early identification of preschool children at risk of LD and can be recommended for use in clinical research and practice. However, study findings are limited to the early identification of preschool children at the risk of LD, and the evaluation of the predictability of the scale is needed. Further research is needed to evaluate the validity of the scale by examining the relationship between the preschool learning skills scale and objective predictors of LD, such as rapid automatized naming, phonological awareness, letter knowledge, and short-term verbal memorization.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee in the

Nanjing Maternity and Child Health Care Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MY: conceptualization, methodology, and writing—original draft preparation. JW: data curation, formal analysis, and writing—original draft preparation. PL: methodology and data curation. YX: validation. YG: data collection. LZ: data collection and validation. NS: language proofreading. YL: funding acquisition. DY: methodology. QH: supervision and writing—reviewing and editing. XC: funding acquisition and writing—reviewing and editing. All authors reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.918163/full#supplementary-material

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Walking alone milestone combined reading-frame rule improves early prediction of Duchenne muscular dystrophy

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Objective: To explore the potential of walking alone milestone combined reading-frame rule to improve the early diagnosis of Duchenne muscular dystrophy (DMD).

Method: To retrospectively describe the genotype and phenotype of Duchenne and Becker muscular dystrophies (BMD) patients with deletions and duplicates in the dystrophin gene. The sensitivity and specificity of the reading frame rule were calculated and compared to that of the combined reading frame rule and walking alone milestone. The diagnostic coincidence rate of two different methods was analyzed.

Result: One hundred sixty-nine male DMD/BMD patients were enrolled, including 17 cases of BMD and 152 cases of DMD. The diagnostic coincidence rate, diagnostic sensitivity, and specificity of the reading-frame rule for DMD/BMD were 85.2, 86.8, and 70.59%, respectively. The sensitivity and specificity of the reading frame principle combined with the walking alone milestone for DMD/BMD were 96.05 and 70.59%, respectively. The diagnostic coincidence rate increased to 93.49%, significantly different from that predicted by reading- frame rule ($P < 0.05$).

Conclusion: The reading-frame rule combined with the walking alone milestone significantly improved the early diagnosis rate of DMD.

KEYWORDS

motor milestone, reading-frame, children, Duchenne muscular dystrophy, prediction

Introduction

Dystrophinopathies, the most common type of progressive muscular dystrophy, is an X-linked recessive neuromuscular disorder caused by mutations in the dystrophin gene (*DMD*). The incidence rate did not vary significantly among countries, regions, or races, with one case occurring in every 3,600–6,000 male births (1).

DMD gene is located on chromosome Xp21.2, with a total length of 2.2 Mb and 79 exons, the largest gene discovered by humans so far. The majority of mutations in *DMD* gene are the deletion/duplication of one or more exons, accounting for about 70–80%. In addition, about 23% of the cases were caused by point mutations in the exon and flanking region of the gene (2).

Children with Duchenne muscular dystrophy (DMD) could not walk independently from 10-15 years old. Their average life expectancy under natural conditions was about 20 years (1). The phenotype of Becker muscular dystrophy (BMD) is relatively mild. BMD usually loses the ability to walk independently after 16 years old, with a life span of more than 30 years. Both DMD and BMD are due to mutations on the DMD gene, but the severity phenotype between the two forms varies considerably. At present, many gene therapy methods for DMD have been gradually applied in clinical practice, such as Ataluren for non-sense mutations in, exon 51 jump and other method, and early treatment can benefit more. Early prediction of DMD and BMD is needed to initiate treatment before the motor loss and joint contracture. "Reading-frame rule" is often used to distinguish BMD and DMD patients (3). Nevertheless, not all patients follow the "reading-frame rule," and about 8% of DMD patients and 34% of BMD patients reportedly do not follow this rule (4).

Quantitative analysis of dystrophy protein in muscle biopsies can help identify DMD/BMD early, but invasive procedures limit its clinical application (5). An early developmental milestone is another variable to predict DMD. Dommelen and colleagues found delays in motor milestones in young males with DMD compared to the control group. Cyrulnik found that 70% of DMD children were delayed in walking alone milestones, followed by crawling (60%) and sitting (38%) (6). Previous studies have shown that gross motor milestones are potentially helpful for early diagnosis of DMD, especially at the age of first walking (6, 7).

This study aimed to evaluate the early predictive value of the reading-frame rule combined with the walking alone milestone for DMD.

Materials and methods

Data collection

A group of unrelated male probands from 208 cases diagnosed in Children's Hospital Affiliated to Zhengzhou University from 2014 to 2021. All the patients had confirmed met the criteria of DMD/BMD.

Inclusion criteria: (1) Male, (2) myopathy (elevated CK with or without proximal limb weakness, (3) genetic testing revealed *DMD* gene with a pathogenic variant.

Exclusion criteria: (1) Female, (2) the cases with point mutation in *DMD*, (3) at the last follow-up, the cases had not lost ambulation and were under 16.

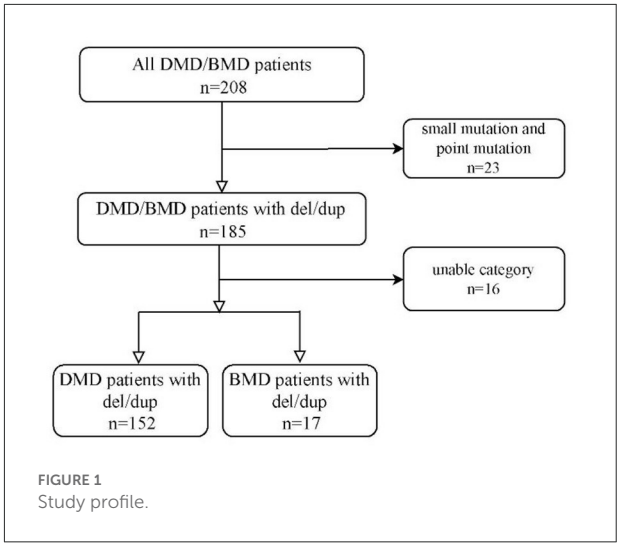

FIGURE 1
Study profile.

Diagnostic principles of DMD and BMD: Patients who lose ambulation and need wheelchairs before 16 years old (<16 y) were classified as DMD, and patients who did not lose independent walking ability at 16 years old (≥16 y) were diagnosed as BMD.

We definite the "criteria for delay of walking alone milestone" as"unable to walk alone until 18 months old."

Two hundred eight cases were registered, 185 (88.9%) cases are deletions/duplications. Twenty-three (11.1%) cases were excluded because of the point mutation. Sixteen cases younger than 16 who could walk independently at the last follow-up were excluded. One hundred sixty-nine cases of DMD/BMD with deletion or duplication were included in our study, Figure 1. We retrospectively reviewed all cases' walking alone milestones and other demographic data.

Statistical analysis

T-test was used to compare DMD and BMD's demographic and clinical data. The Chi-square test (χ^2-test) was used to compare the diagnostic coincidence rate of different methods. Version 25 of the SPSS software (SPSS, Inc.) was used for all statistical analyses, setting the significance at $p < 0.05$.

Ethical consent

The study was approved by the ethics committee of the Children's Hospital Affiliated to Zhengzhou University, Zhengzhou, China.

TABLE 1 Clinical manifestations and investigations of DMD and BMD cases.

	DMD ($n = 152$)		BMD ($n = 17$)		*P*-value
	Mean ± SD	Min–Max	Mean ± SD	Min–Max	
Walking alone milestone (month)	18.03 ± 7.12	14–30	12.88 ± 0.61	12–14	0.00
Onset age (year)	3.9 ± 2.18	0.5–9	6.49 ± 3.06	2.8–12	0.00
Age of diagnosis (year)	6.8 ± 2.83	1.27–14.34	7.16 ± 3.45	2.8–12	0.63
CK(U/L)	16,197.2 ± 8,750.9	4,463.0–57,296.0	8657.5 ± 5933.3	1,199.0–20,048.6	0.001

TABLE 2 The genotype of 169 cases.

	Resource (M/D*)	del/dup**	Reading frame (in/out)***
BMD	1/1	17/0	12/5
DMD	30/7	136/16	20/132
Total	39	169	169

*M, maternal; D, De novo. **Del, deletion; dup, duplication. ***In, in reading-frame; out, out of reading-frame.

Result

Clinical findings

One hundred sixty-nine cases were included in our study. 20 cases had a family history, while the other 149 had no family history or family members refused to provide information. All 169 cases are male. The mean age of onset in DMD and BMD cases was 3.88 ± 2.18 years (range 0.5–9 years) and 6.49 ± 3.06 years (range 2.8–12 years), respectively, but the age of diagnosis delayed, which was 6.80 ± 2.83 (range 1.27–14.34 years) and 7.13 ± 3.45 (2.88 ± 12.12 years), respectively. The mean value of creatine kinase (CK) was 16,197.15 ± 8,750.95U/L (range 4,463–57,396 U/L) in DMD patients and 8,657.45 ± 5,933.32 U/L (range 1,199–20,048.6 U/L) in BMD patients, with the statistical difference ($P < 0.05$; Table 1). In DMD cases, the mean age of independent walking loss was 11.14 ± 1.07 years old (ranging from 8 to 14 years old), while in BMD cases, by our last follow-up time, all patients were older than 16 years old (maximum follow-up to 18 years old) and could walk independently.

The age of walking alone in BMD group was between 12 and 14 months, with a mean age of 12.88 ± 0.61 months, while the age of walking alone in DMD group was between 14 and 30 months, with a mean age of 18.03 ± 7.12 months, Table 1. There were significant differences between the two groups ($P < 0.05$). In BMD group, all cases could walk independently before 18 months, while in DMD cases, 93 cases (61.18%) had an independent walking delay.

Genotype

Thirty-nine of the 169 cases had maternal genetic verification, including 8 with *de novo* mutations and 31 with maternal origin (Table 2). There were 153 cases of deletion, including 17 cases of BMD, 136 cases of DMD, and 16 cases of duplication, all of which were DMD. 17 cases are BMD, including 12 (70.6%) cases in the reading frame and 5 (29.4%) cases out of the reading frame. 152 cases are DMD, including 132 (86.8%) cases out of the reading frame and 20 (13.2%) cases in the reading frame, Figure 2.

One hundred fifty three cases of deletions were as follows: those in a single exon was the most frequent (28.1%), followed by 5 (13.1%), 2 (9.8%), 3 (8.45%), 6 (7.19%), 8 (7.19%), and 7 (6.54%), Figure 3. Therefore, 106 cases (69.3%) had 6 or fewer exon deletions. Deletion of more than 10 exons accounted for only 22 cases (14.38%). In cases of BMD and DMD, there were 5 cases (29.41%) and 17 cases (12.5%) with deletion exons more than 10, respectively (χ^2-test, $P = 0.06$). Figure 3 shows that exons 10–34 (60.1%) were most commonly missing in BMD, followed by 45–51 (32.9%). The most common exon deletion in DMD was 44–55 (54.1%), followed by exon 3–26 (32.38%). Sixteen cases with duplication were DMD, mainly exons 46–54 (30.59%) and 3–7 (20%), Figure 3.

"Reading-frame rule" combined with "walking alone milestone" in prediction for DMD

In 169 cases, 12 cases of BMD are in the reading frame, and 132 cases of DMD are out of the reading frame, in which 85.2% were in agreement with the rule of reading-frame (86.8% for DMD and 70.59% for BMD). Sensitivity and specificity were 86.8 and 70.59%, respectively, assuming that DMD was the case out of the reading frame and BMD was the case in the reading frame. Sensitivity and specificity were 61.18 and 100%, respectively, assuming that the case was BMD when walking alone milestones were younger than 18 months. The case was DMD when the milestone was older than 18 months. The combined sensitivity and specificity were 96.05

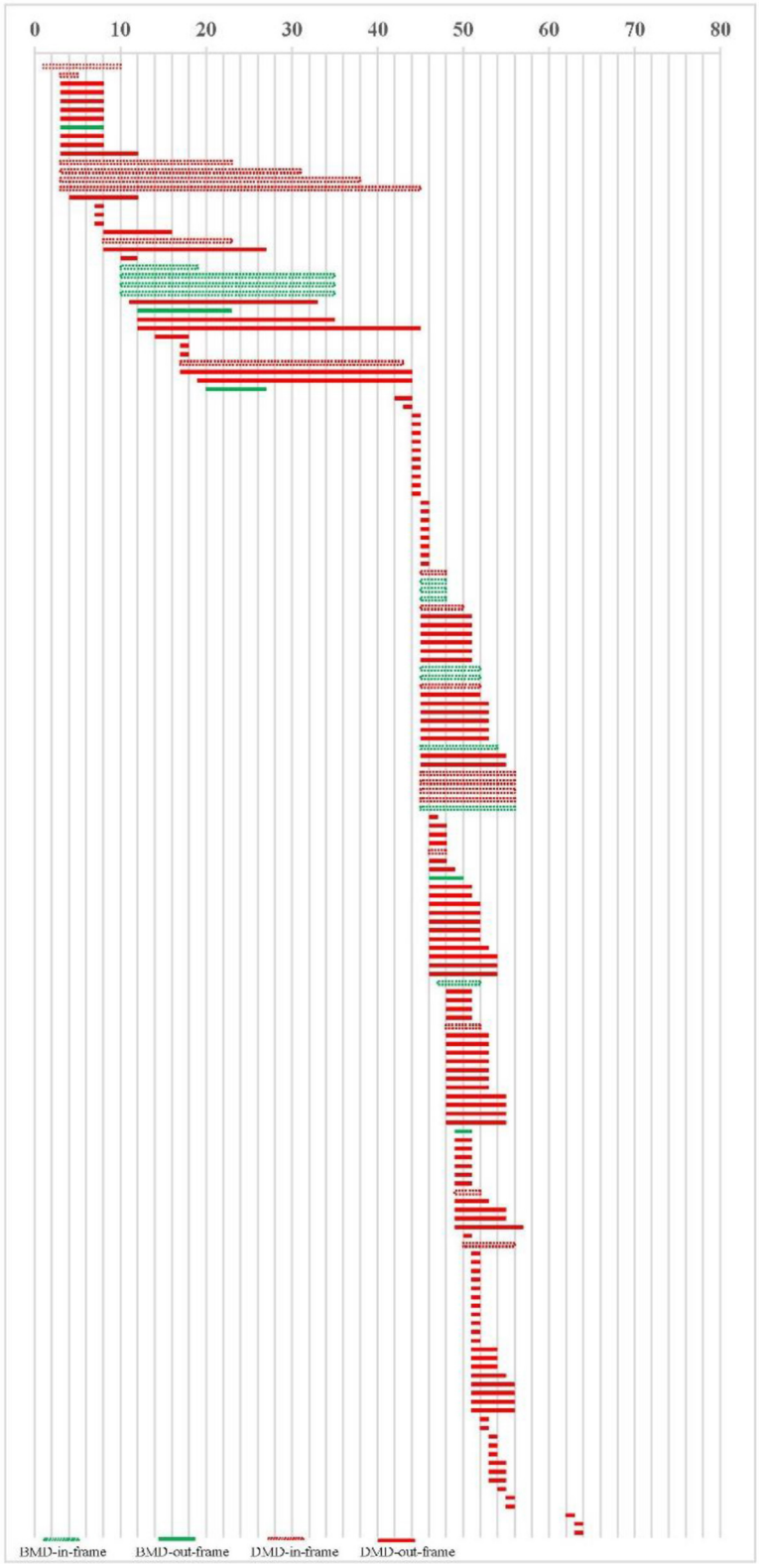

FIGURE 2
Deletion and duplication location in 169 cases.

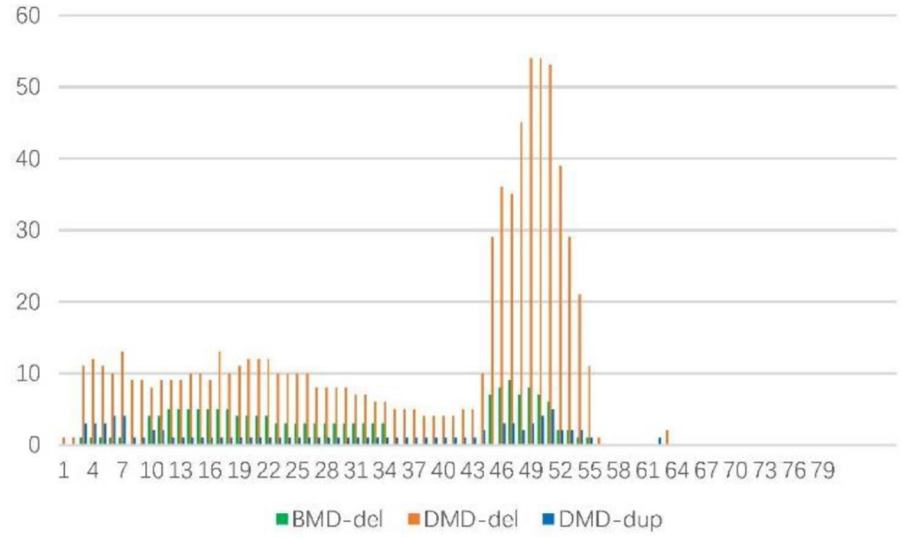

FIGURE 3
Frequency of deletions/duplications of DMD gene exons in 169 cases.

and 70.59%, respectively. The specificity remained unchanged, sensitivity increased by 9.25%, and the diagnostic coincidence rate increased from 85.2 to 93.49% (χ^2-test, $P = 0.014$; Table 3).

Discussion

Predicting DMD as early as possible is the premise of timely treatment

Because DMD pathology is caused by the lack of functional dystrophin, restoring the function or expression of dystrophin is an apparent therapeutic approach. Loss of muscle tissue and function starts at an early age and is currently irreversible. Thus, although restoration of muscular dystrophy protein expression is called upon to slow or even stop the progression of DMD, it will not restore any muscle tissue that has been lost.

The standard way to confirm DMD/BMD recently included serum creatine kinase testing, genetic confirmation, and dynamic assessment of patient scores on motor ability tests (8). The most widely used treatment remains the use of corticosteroids and angiotensin-converting enzyme inhibitors to control the symptoms of cardiomyopathy and rehabilitation and symptomatic support to prolong patient life (1). Current therapies, such as exon 44/45/51/53 skipping and treating DMD with non-sense mutations, have been gradually applied to clinical practice (9–11). Even gene therapy, which leads to high expression of functional muscular dystrophy protein, is not expected to cure when given to patients who have lost most muscle tissue and function. Clemens and others found that DMD patients as young as 4 years exhibited improvements in dystrophin levels and timed motor tests following the 53 exons skipping treatment. Hence, they believed that these cases should be treated before 5 y (12). There are even studies using exon-skipping in patients up to 6 months old (ClinicalTrials identifier: NCT03218995). Pre-treating patients before gene therapy using an exon-skipping approach could potentiate the effect of gene therapy. Such pre-treatment would allow lower and safer vector doses to bring about a higher level of dystrophin expression in the long term (13–15).

Early intervention is essential, but early identification of DMD/BMD is the first step. Some indicators were applied in the early prediction of DMD, and the compound indicators were rarely reported. The reading-frame rule is one of the recognized DMD prediction indicators, and the joint prediction of the walking alone milestone can effectively improve the sensitivity and diagnostic coincidence rate.

Methods for predicting DMD/BMD early and predicting DMD severity developed. CK is a sensitive biomarker because elevated blood levels (10 and 100 times higher than the upper limit) indicate severe muscle damage (16). CK is rather unspecific because plasma levels are also elevated in many forms of other muscle injury and are influenced by other factors, such as muscle mass, age, and muscle activity. Genotype (including reading-frame), modified genes, muscle-specific microRNA, and developmental milestones have been studied to predict DMD/BMD and DMD severity.

In 1988, Monaco et al. proposed the reading-frame rule, and related research supports this hypothesis (17). In the study of Aartsma-Rus, 91% agreed with this rule (3). It had been

TABLE 3 "Reading-frame rule" and "walking alone milestone" in prediction for DMD.

	Sensitivity (%)	Specificity (%)	Diagnostic coincidence rate (%)	*P*-value*
Reading-Frame rule	86.8	70.59	85.2	–
Walking alone milestone	61.18	100	60.09	–
Reading-Frame rule and walking alone milestone	96.05	70.59	93.49	0.014

*P-value: χ^2-test for the total efficiency of joint prediction and the reading-frame prediction.

reported that the reading-frame rule held in 90% of DMD and 94% of BMD cases (18). Later studies suggested that BMD might result in exceptions to the reading frame rule in more cases, perhaps up to 30% (19, 20). However, our study found that the positive prediction rate of the reading-frame rule was 85.2% for all cases, 86.8% for DMD, and 70.59% for BMD. Early clinical treatment is highly urgent for DMD cases, but 12.6% cannot be diagnosed early. Although the reading frame helps predict the severity of skeletal muscle weakness, there is still some phenotypic variability within the prediction.

Some authors reviewed 4000 patients and found that some cases in the reading-frame had DMD phenotype, which was more likely to be in the in-frame deletions starting and/or ending at the extreme ends of the protein (21). In our cases, 20 in-frame deletion/duplication cases had DMD phenotype, as shown in Figure 2. We did not find this feature, which may be related to the small number of our cases. The peak locations for BMD exon deletion are exons 45–51 and 3–26, similar to DMD, exons 44–55 and 3–26. Our study found no difference in the number of exon deletions between DMD and BMD. It is not practical to judge DMD/BMD by exon deletion/duplication site. In cases of BMD and DMD, there were 5 cases (29.41%) and 17 cases (12.5%) with deletion exons more than 10, respectively (χ^2-test, $P = 0.06$). In the study of scholar Juan Yang, among 118 cases of exon duplication of DMD gene in the Chinese population, there were 9 cases of BMD, indicating that exon duplication is more likely to occur in DMD cases (22). No exon duplication was found in our BMD cases, which may be related to the small number of cases in us.

Early gross motor development milestone delay is a clinical characteristic of DMD, but is not necessary for BMD (23). van Dommelen found that between 12 and 36 months of age, differences in the attainment of developmental milestones concerning gross motor activity increased with age (7). Sitting, crawling, and walking alone were considered important milestones in motor development. The most significant lag in DMD is walking alone, which is related to DMD most easily involving the lower limbs (6). Therefore, it is appropriate for us to take the age of first walking to represent the backward development of gross motor. In our study, the walking alone milestone was 12.88 ± 0.61(month) in BMD group, and 18.03 ± 7.12 (month) in DMD group. In DMD cases, 93 cases (61.18%) had an independent walking delay. We believe that the walking alone milestone delay has a limited predictive effect on DMD.

The combined prediction of the reading-frame rule and walking alone milestone has the potential for early diagnosis of DMD

Therefore, we combined the reading-frame rule and gross motor milestone for the above reasons. Our results revealed that combined variables improved the prediction efficiency compared to the isolated reading-frame rule. So, for the first time, we proposed the integration of the two variables to predict DMD. Furthermore, both the genotype and walking alone milestones are very constant and stable, easy for doctors to obtain. Because of the low cost and no harm to patients, it is conducive to promotion. In our study, the reading-frame rule combined with the walking alone milestone increased the diagnostic coincidence rate of DMD from 85.2 to 93.49%. The sensitivity increased significantly, but the specificity did not decrease. Therefore, we believe that the combined index of the above two variables might have application potential for early DMD prediction.

Study strengths and limitations

Our study is a retrospective study, and more detailed motor milestones, early fine motor and assessment of intellectual development level are not available, limiting our prediction age in DMD. We hope to carry out prospective studies in the future. The severity of BMD and DMD is different, and the treatment is different, so early prediction is of great significance. The severity of DMD varies, with some patients living in wheelchairs before the age of 10 and some losing their independent ambulation at the age of 15. Therefore, progress in practical DMD prediction tools is expected to stratify patients accurately and timely.

Conclusion

The reading-frame rule is widely used in DMD prediction. However, its prediction efficiency still needs to be improved. The motor development milestone delay, especially when they are 18 months still cannot walk alone, could predict a considerable part of DMD (7, 24, 25). But the specificity is poor. Our study proposed that the combined prediction of the above two indicators significantly improved the early diagnosis rate of DMD and provided a new tool for earlier diagnosis. Because these two indicators are stable, easy to obtain, and have the potential to be widely promoted in the future. Prospective studies with large samples and multiple regions are still needed to verify it further.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Y-lM and X-yC contributed to conception and design of the study. G-hC, L-fS, YuW, R-lY, and YiW organized the database. W-hZ performed the statistical analysis. Y-lM and W-hZ wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Neurological and psychological effects of long COVID in a young population: A cross-sectional study

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Aim: We evaluated the long-term clinical status of pediatric patients after testing positive for COVID-19. We hypothesized that there are similar symptoms to those that have been described in adults and children and that pediatric patients with neurophysiologic symptoms still present 3–5 months after infection have psychological consequences that interfere with their adaptive functioning.

Method: We recruited 322 COVID-19-positive pediatric patients, between 1.5 and 17 years old, from the outpatient clinic for COVID-19 follow-up. Neurological symptoms were analyzed at onset, after 1 month, and after 3–5 months. A psychological assessment with standardized questionnaires was also conducted to determine the impact of the disease.

Results: At the onset of COVID-19, 60% of the total sample exhibited symptoms; this decreased after 1 month (20%) but stabilized 3–5 months after disease onset (22%). Prevailing long-COVID neurological symptoms were headache, fatigue, and anosmia. In the 1.5–5-year-old subgroup, internalizing problems emerged in 12% of patients. In the 6–18-year-old subgroup, anxiety and post-traumatic stress showed significant associations with neurological symptoms of long COVID.

Conclusions: These data demonstrate that long COVID presents various broad-spectrum symptoms, including psychological and long-lasting cognitive issues. If not treated, these symptoms could significantly compromise the quality of life of children and adolescents.

KEYWORDS

long-COVID syndrome, COVID-19 (coronavirus disease 2019), SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), CNS (central nervous system), psychological effects, children, adolescents, pediatrics

Introduction

Coronavirus disease 2019 (COVID-19), a manifestation of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 (1). During the initial stages of the emergency, Italy witnessed a rapid and massive spread of the infection, which initially led the country to be the most affected by the virus, with many cases and a large number of deaths (2).

Researchers have detailed a range of neurological symptoms in SARS-CoV-2-infected patients (2–5). Due to its local or peripheral presence, in adults the virus could lead to systemic inflammatory responses. Moreover, the increase in cytokine levels has been associated with severe neurological dysfunctions (6). These changes are involved in the pathophysiology of various psychiatric disorders, such as depression, anxiety, post-traumatic stress disorder (PTSD), and obsessive-compulsive disorder (7, 8) in somatic symptoms like body pain and respiratory distress (9, 10) and in neurophysiological alterations such as dysregulation of sleep/wake rhythms and nutrition, the presence of fatigue, and increased inattention and irritability (11, 12).

COVID-19 symptoms can be acute or chronic and persist for a long time. For this reason, *long COVID* or *post-COVID syndrome* is defined as symptoms that persist for > 3 months after onset (13–15). A systematic review and meta-analysis found persistent symptoms in the 80% (95% CI 65–92) of adult population. The most frequent were fatigue, headache, attention deficit disorder, hair loss, and dyspnoea (16). Long-term studies in paediatric patients have reported both neurological problems (headache, fatigue, myalgia, and loss of taste or smell), (13, 17–21) cognitive issues (memory, language, and attention), and neuropsychiatric symptoms (anxiety, depression, and PTSD) (9–11, 22, 23). The persistence of long-term neurological symptoms in adults as well as children and adolescents could be explained by hypometabolism-like brain patterns with long COVID, involving the medial temporal bilateral lobes, brainstem, cerebellum, and the olfactory gyrus right after correction of the small volume (24). However, there is limited information on systematic long-COVID results in the paediatric and adolescent population. Some authors highlight the emergence of psychological symptoms and emotional problems in the child and adolescent population due to the consequences of quarantine (25), highlighting the need to enhance mental health services in the community (26).

The main objective of the study was to detect the presence and type of long COVID symptoms, 3–5 months after onset, to determine similarities with those described by previous studies. We hypothesised that there are similar symptoms to those in adults and children described in the scientific literature. We also hypothesised that patients with neurophysiologic symptoms still present 3–5 months after infection have psychological consequences that interfere with their adaptive functioning.

Abbreviations: CBCL, Child Behavior Checklist; CDI-2 SR, Children's Depression Inventory; CNS, Central Nervous System; COVID-19, Coronavirus Disease 2019; M, mean; MASC 2-SR, Multidimensional Anxiety Scale for Children-2 Self Report; PTSD, Post-Traumatic Stress Disorder; SD, Standard Deviation; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; TSCC-A, Trauma Symptom Checklist for Children-Adolescent.

Methods

This study was conducted between February and November 2021 at the post-COVID outpatient clinic of the maternal-infant ward of the Umberto I University Hospital in Rome, Italy. We included young people between the ages of 1.5 and 17 years who had contracted SARS-CoV-2 and excluded all patients whose parents did not provide informed consent and all subjects who were not present at follow-ups. The cohort consisted of 322 patients aged 1–17 years (167 males, 165 females, mean age = 10 years, range 1.5–17 years) who had recovered from COVID-19. All patients were seen at the onset of the disease and then 1 month and 3–5 months after infection. During the visits, doctors investigated the children's neurophysiologic conditions and completed the COVID-19 symptom checklist. At the last meeting (3–5 months after infection), children and their parents also completed psychological questionnaires to investigate long COVID symptoms. The study included all paediatric patients who came to the clinic for follow-up. They had previously undergone medical examinations at the clinic.

Statistical analyses

Statistical analysis was conducted by using SPSS 18.0 for Windows (SPSS, Chicago, IL, USA). Frequency analyses and descriptive statistics were performed to describe the sample and symptoms. A correlation matrix (Pearson correlation significant at $p < 0.01$ and $p < 0.05$ [two-tailed]) and the chi-square test (significant at $p < 0.05$) were used to determine the associations between symptoms and the size effect between the variables was measured with the phi coefficient (small effect = 0.1; medium effect = 0.3; large effect = 0.5).

Materials

Description of the tools

A COVID-19 neurophysiologic symptom checklist was created to determine the clinical history of each participant. Doctors completed the list after each visit, indicating the

presence/absence of symptoms. Four questionnaires were used for the psychological assessment. The details of each are presented below.

The Multidimensional Anxiety Scale for Children-2 Self Report (MASC 2-SR) (27) is a self-administered questionnaire of 50 items for subjects between the ages of 8 and 18. The tool comprises six scales, which measure the main dimensions of anxiety and a general one that indicates the severity and pervasiveness of the symptoms of anxiety. Standardised T scores have a mean of 50 and a standard deviation of 10. T scores > 65 (1.5 standard deviations [SD]) are considered to be of clinical relevance, between 55 and 64 above average, and < 54 average.

The *Children's Depression Inventory* (CDI-2 SR) (28) is a questionnaire on depressive symptoms for individuals aged 7–17 years. The tool provides a total symptom score and specific scores divided into two categories: emotional problems and functional problems. T scores > 65 (1.5 SD) are considered to be of clinical relevance, between 60 and 64 above average, and < 59 average.

The *Trauma Symptom Checklist for Children-A* (TSCC-A) (29) is a self-assessment questionnaire for children aged 8–16 years, consisting of 44 items and five clinical scales (Anxiety, Depression, Anger, Post-traumatic Stress, and Dissociation). Standardised scores > 65 are considered significant; scores < 65 are not significant.

The *Child Behavior Checklist* (CBCL) (30, 31) is a proxy-report questionnaire for parents to report on their children's behaviour. We used two versions: one for individuals aged 1.5–5 years and one for individuals aged 6–18 years. The profile that emerges from the questionnaires consists of a total scale, a scale of internalising problems, a scale of externalising problems, syndromic scales, and scales oriented to the *Diagnostic and Statistical Manual of Mental Disorders*. The 6–18-year-old version also provides a measure of the children's skills (activity, sociability, and school). For both versions of the CBCL, syndromic scale T scores < 65 are normal, between 65 and 70 are borderline, and >70 are clinical. For the skill scale in the 6–18-year-old version, T scores < 30 are average, between 30 and 35 are borderline, and > 35 are clinical. Scores in the borderline and clinical ranges (> 65) were considered to be alterations from a condition of normality (Figures 2, 3).

Results

Sample

The total sample comprised 322 patients aged between 1.5 and 17 years (mean [M] age = 9.53, SD = 3.73, mode = 10 years), of which 50 were preschool age (age group 1.5–5 years; M = 3.5, SD = 1.39 years) and 272 were school age (age group 6–17 years; M = 10.65, SD = 2.83 years). There were 167 boys and 155 girls. Parents who completed the CBCL (56 fathers and 266 mothers) had a mean age of 43 years (range 26–63 years).

COVID-related neurophysiological symptoms in paediatric patients

At the onset of COVID-19, symptoms occurred in 192 children (60% of the total sample). The remaining 40% of the sample that had no symptoms at onset was recruited only after finding positivity with the swab. After 1 month, 66 patients (20%) showed symptoms, and this level stabilised 3–5 months after the onset of the disease, with 70 patients (22%) showing symptoms.

In the total sample, the prevailing symptom at onset was headache (33.5%), which reduced in frequency and remained

TABLE 1 Symptom course in COVID-19-positive paediatric patients.

Total sample ($n = 322$)

Symptoms	Onset		1 month		3–5 months	
	N	%	*N*	%	*N*	%
Headache	108	33.5	18	5.6	24	7.5
Fatigue	88	27.3	37	11.5	22	6.8
Anosmia	64	19.9	14	4.3	7	2.2
Ageusia	58	18	10	3.1	21	6.5
Myalgia	28	8.7	4	1.2	0	0
Muscoloskeletal pain	10	3.1	0	0	2	0.6
Dysgeusia	6	1.9	2	0.6	0	0
Dizziness	5	1.6	0	0	0	0
Chest pain	1	0.3	10	0.3	2	0.6
Eye pain	1	0.3	0	0	0	0

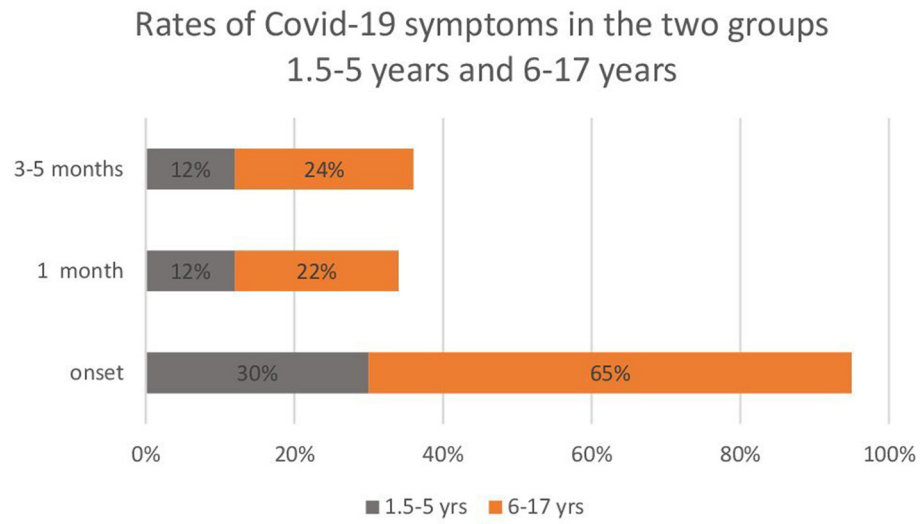

FIGURE 1
Rates of COVID-19 symptoms in the two subgroups of paediatric patients: 1.5–5 years ($n = 50$) and 6–17 years ($n = 272$).

after 3–5 months in 7.5% of the sample. Fatigue (27.3% of patients at onset and 6.8% after 3–5 months) and anosmia (19.9% at onset and 2.2% after 3–5 months) were also prevalent. Ageusia followed a non-linear trend: 18% of patients showed this symptom at onset, followed by a reduction after 1 month (3.1%), and an increase after 3–5 months (6.5%). Based on these data, we hypothesised that the loss of taste also occurs more than 1 month after the onset of COVID-19 symptoms. Other symptoms such as myalgia, dizziness, dysgeusia, and ocular pain tended to regress over time, while chest and musculoskeletal pain were not very prominent at any time (Table 1).

From the analysis of the partial frequencies carried out separately in the two groups (1.5–5 and 6–17 years), the 1.5–5-year-old patients had fewer symptoms than the 6–17-year-old patients. Moreover, symptoms tended to stabilise in fewer pre-school children compared with school-age children (Figure 1).

TABLE 2 Descriptions and percentages of COVID-19 symptoms in the two subgroups 3–5 months after COVID-19 infection.

Presence of smptoms at 3–5 months	1.5 yrs		6–17 yrs	
	N	%	*N*	%
Headache	1	0.3	23	7
Fatigue	1	0.3	21	6
Ageusia	1	0.3	20	6
Anosmia	0	0	7	2
Chest pain	0	0	2	0.6
Musculoskeletal pain	2	0.6	0	0
Dysgeusia	0	0	0	0
Eye pain	0	0	0	0
Dizziness	0	0	0	0
Myalgia	0	0	0	0

Long-term COVID symptoms

In the total sample, 3–5 months after onset of symptoms, neurophysiological symptoms were detected more frequently in the 6–17-year-old patients (Table 2). The descriptive table summarises the symptoms present in the long term in the two groups (1.5–5 and 6–17 years). Headache ($n = 23$, 7%), fatigue ($n = 21$, 6%), and ageusia ($n = 21$, 6%) were more common in children aged 6–17 years. By constrast, in the 1.5–5-year-old group, headache, fatigue, and ageusia were each present in only 1 patient (0.3%). The other evaluated symptoms only occurred in the children aged 6–17 years: anosmia ($n = 7$, 2%), chest pain ($n = 2$, 0.6%), and musculoskeletal pain ($n = 2$, 0.6%).

As detailed in Table 3, in the total sample 34 patients (10%) showed changes in eating habits; 41 patients (13%) presented sleep disturbances; 46 patients (14%) had cognitive, behavioural, and mood problems; and 93 patients (29%) had increased their use of technological tools, with a mean increase of 3 h/day (range 1–5 h).

Considering only the 6–18-year-old group, 36 patients (11%) reported problems falling asleep or waking up at night, and 33 patients (10%) reported dietary changes as an increase ($n = 13$, 4%) or a decrease ($n = 20$, 6%) in food intake. In this group, 43

patients (13%) presented cognitive and behavioural problems, in particular attention disorders (n = 28, 9%). Moreover, 43 patients (28%) showed an increase in the use of technological tools (such as smartphones, tablets, and PCs). In the 1.5–5-year-old group, 5 patients (1%) reported sleep problems, 1 patient (0.3%) reported eating problems, 3 patients (0.9%) showed cognitive or behavioural problems, and 3 children (0.9%) presented an increase in the use of technology.

TABLE 3 Changes in symptoms in the two subgroups 3–5 months after COVID-19 infection.

	Total sample $N = 322$		6–17 yrs $N = 272$		1.5–5 yrs $N = 50$	
	N	%	N	%	N	%
Sleep problems	42	13	37	11.2	5	1.5
Eating changes	34	10.6	33	10.2	1	0.3
Food reduction	21	6.6	20	6.2	1	0.3
Food increase	13	4.1	13	4.0	0	0.0
Behavioral-cognitive	46	14.3	43	13.3	3	0.9
Attention	29	9.0	28	8.7	1	0.3
Academic impairment	5	1.6	5	1.6	0	0
Irritability	5	16.6	4	1.2	1	0.3
Anxiety	3	0.9	3	0.9	0	0
Auditory hallucinations	1	0.3	1	0.3	0	0
Hyperactivity	1	0.3	1	0.3	0	0
Memory	1	0.3	1	0.3	0	0
Obsessions-compulsions	1	0.3	0	0	1	0.3
Technologies	93	28.9	90	27.9	3	0.9

Long COVID psychological conditions

Clinical symptoms and adaptive behaviour

Based on the CBCL completed by the parents on the behaviour of 50 children aged between 1.5 and 5 years (mean age = 3.4; boys = 30 and girls = 20), some patients had above average scores (T scores > 65) for emotional reactivity (n = 4, 8%), anxiety/depression (n = 4, 8%), somatic disorders (n = 5, 10%), closure (n = 3, 6%), sleep problems (n = 4, 8%), and attention problems (n = 2, 4%). Internalisation problems (n = 6, 12%) prevailed over externalisation problems (n = 1, 2%) (Figure 2).

For the 272 children aged 6–17 years (mean age = 10.6 years; 137 boys and 135 girls), the parents reported a decline in overall activity in 147 (54%) children (T scores > 30). There was impairment in recreational activities (sports, games, etc.) (n = 134, 49%), in the social area (n = 36, 13%), and in academic performance (n = 2, 1%). Similarly to the 1.5–5-year-old children, more children had internalising problems with borderline or clinical scores (n = 96, 35%) than externalising problems (n = 8, 10%). Anxiety problems were the most frequent (n = 74, 28%), followed by mood problems (closure and depression; n = 51, 19%), somatic problems (n = 43, 16%), attention problems (n = 20, 8%), oppositional problems (n = 16, 6%), and conducted (n = 6, 2%) (Figure 3).

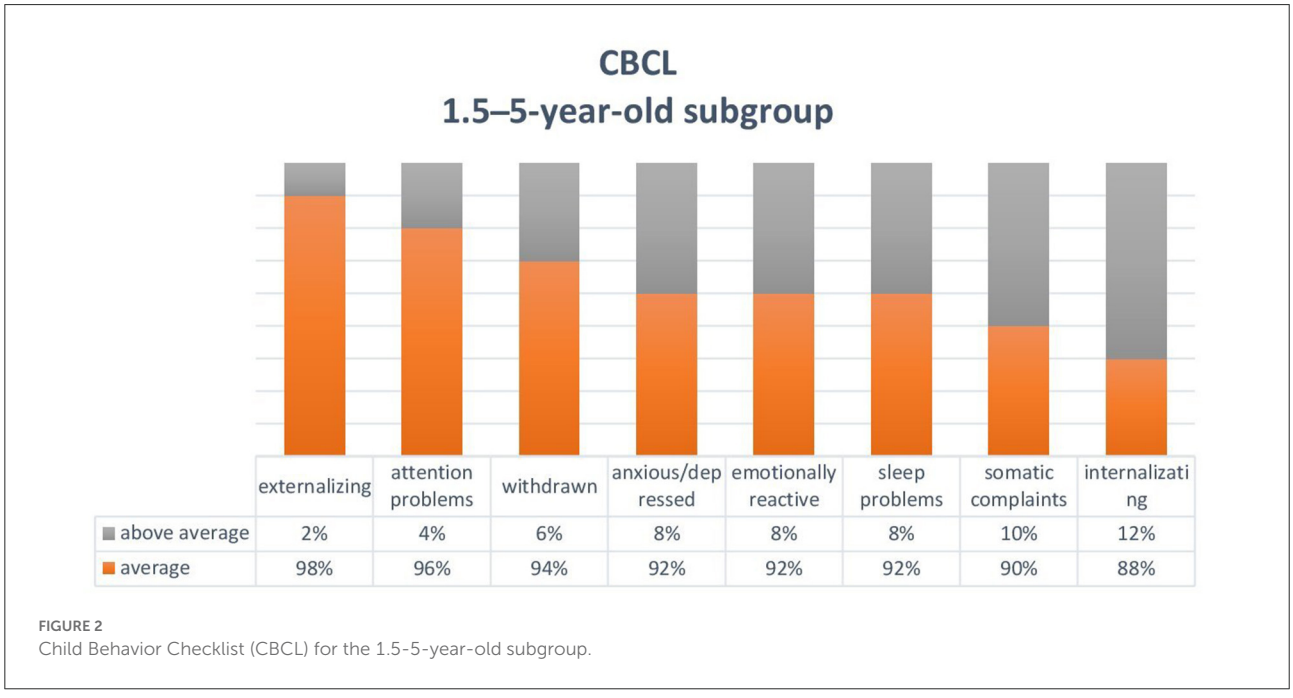

FIGURE 2
Child Behavior Checklist (CBCL) for the 1.5-5-year-old subgroup.

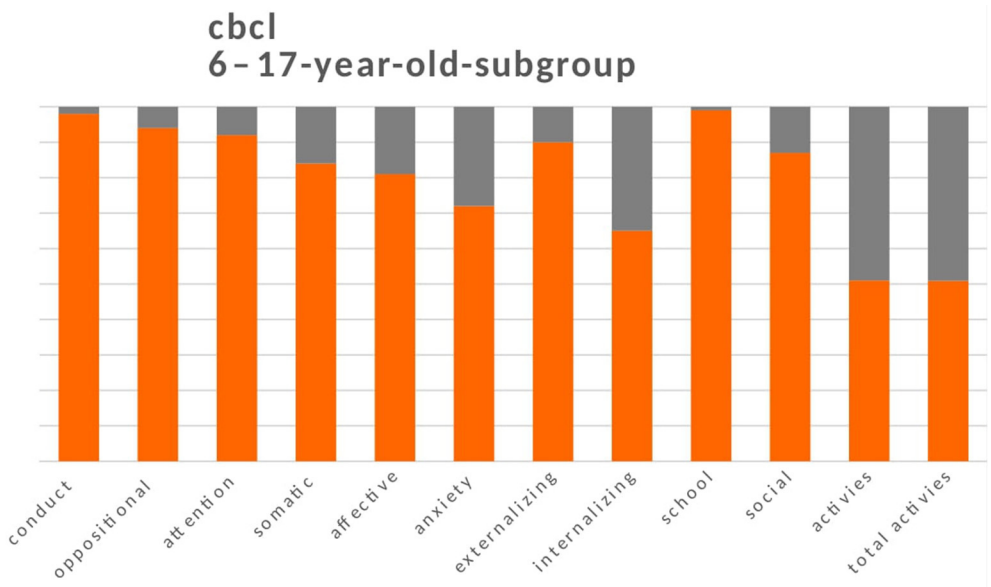

FIGURE 3
Child Behavior Checklist (CBCL) for the 6-17-year-old subgroup.

TABLE 4 Comparison between patients who had symptoms at 3–5 months after COVID-19 infection and those who did not.

Anxiety subscale MASC2	*Total	**Present	***Absent	****P-value	*****Effect size
Group 8–16 years ($n = 226$)					
Separation anxiety	83	25	58	0.259	0.070
Generalized anxiety	65	28	37	**0.000**	**0.246**
Social anxiety	82	31	51	**0.003**	**0.201**
Humiliation	57	24	33	**0.001**	**0.212**
Performance anxiety	90	29	61	0.089	0.113
Obsessions-Compulsions	52	23	29	**0.001**	**0.266**
Physical symptoms	65	29	36	**0.000**	**0.268**
Panic	71	30	41	**0.000**	**0.249**
Tension-Restlessness	67	29	38	**0.000**	**0.254**
Avoidance of danger	47	18	29	0,32	0.142

*number of anxiety symptoms in the 8 to 16 year old patient group.
**number of patients with long COVID neurophysiological symptoms (>3 months) and anxiety symptoms.
***number of patients with anxiety symptoms without long covid neurophysiological symptoms.
****Pearson's Chi-Square Test: significance = p-value < 0.05, indicates the significance between long COVID symptoms and anxiety symptoms.
*****Phi value = 0.1: small effect, = 0.3: medium effect = 0.5: large effect.
Bold value shows the significant relationships between anxiety symptoms (generalized anxiety, social anxiety, humiliation, etc.) and the presence of 3-month neurological symptoms (statistically significant *p*-value) and the effect size of significant scores.

Figures 2, 3 show the sum of the percentages relating to borderline and clinical scores in the two groups of children aged between 1.5 and 5 years and 6-17 years.

Anxious-depressive symptoms

The 6–17-year-old age group was divided into a subgroup of 226 children between the ages of 8 and 16 (mean age = 11.2 years; 117 boys and 119 girls). Some patients showed above average MASC-2 scores (> 55 points) on the social anxiety scale ($n = 52$, 37%) and particularly the performance anxiety subscale ($n = 90$, 40%), followed by feelings of humiliation and rejection ($n = 57$, 25%). There was also separation anxiety in 88 patients (37%), generalised anxiety in 65 patients (28%), obsessive-compulsive symptoms in 52 patients (23%), and fear of danger in 47 patients (21%). There were also high scores in

TABLE 5 Correlations between post-traumatic stress symptoms (Trauma Symptom Checklist for Children-Adolescent [TSCC-A] scales), stressful events, and the presence of neurophysiological symptoms from COVID-190.

	Symptoms onset after covid	Symptoms 1 month	Symptoms 3–5 months	Stressfull events	Anxiety	Depression	Anger	PTSD	Dissociation index	Overt dissociation	Covert dissociation
Symptoms onset after covid	—										
Symptoms 1 month	00.350**	—									
Symptoms 3– 5 months	00.280**	00.579**	—								
Stressfull events	−00.012	00.099	00.027	—							
Anxiety	00.085	00.092	00.111	−00.054	—						
Depression	00.029	00.048	**00.200****	−00.056	00.433**	—					
Anger	−00.079	00.014	**00.147***	00.049	00.385**	00.531**	—				
PTSD	00.100	00.100	**00.174****	−00.062	00.783**	00.673**	00.515**	—			
Dissociation index	00.085	00.022	**00.145***	−00.054	00.547**	00.679**	00.492**	00.601**	—		
Overt dissociation	00.055	00.022	00.070	−00.060	00.537**	00.612**	00.440**	00.580**	00.864**	—	
Covert dissociation	−00.072	−00.060	00.014	−00.042	00.287**	00.217**	00.253**	00.293**	00.287**	00.195**	—

**Correlation is significant at the 000.01 level (2–tailed).

*Correlation is significant at the 000.05 level (2–tailed).

Bold value shows significant correlations between PTSD symptoms (symptoms of anxiety, depression and post-traumatic) and neurophysiological symptoms after 3 months of infection.

the physical dimension ($n = 65$; 28%) with symptoms of panic ($n = 71$; 32%) and tension ($n = 67$; 30%), as responses to the anxious condition.

The CDI-2 revealed the presence of emotional problems in 61 patients (24%), concerning both negative mood–physical symptoms ($n = 39$, 16%) and negative self-esteem ($n = 22$, 9%). There were functional problems with feelings of ineffectiveness in 33 patients (15%) and interpersonal problems in 27 patients (12%).

We compared anxiety scores with the presence or absence of COVID symptoms 3–5 months after infection by using the chi-square test (Table 4). There were significant associations between long COVID symptoms and subtypes of generalised and social anxiety, obsessive-compulsive symptoms, and physical symptoms.

Post-traumatic symptoms

Post-traumatic symptoms were studied with the TSCC-A self-assessment questionnaire in the subgroup of 226 patients aged 8–16 years. Before filling out the questionnaire, doctors asked the parents to report whether their children had been exposed to traumatic events immediately before, during, or after COVID-19 infection. Only six children had been exposed to traumatic events ($n = 3$, hospitalisation of the mother; $n = 2$, death of the grandfather or uncle: $n = 1$, isolation of positive parents).

Twenty-eight patients (12%) scored high on the Post-traumatic Stress scale, 22 patients (10%) had dissociative symptoms (dissociation index), 26 patients had overt dissociation (11%) and 14 patients showed hidden dissociation (6%). Emotions of anxiety ($n = 22$, 10%), depression ($n = 23$, 10%), and anger ($n = 19$, 8%) also emerged, linked to the traumatic state.

Statistical analysis revealed significant correlations between the presence of long COVID symptoms and the clinical questionnaire scores on the PTSD, Depression, Anger, and Dissociation scales. On the contrary, there were no significant correlations between the traumatic events reported by the parents and the questionnaire scales (Table 5).

Discussion

Our findings highlight many common elements regarding long COVID neurological symptoms with other published studies. First, there is great heterogeneity of long COVID neurological symptoms (18, 19, 21, 22). In our paediatric sample, there were *10* initial symptoms, which reduced to six symptoms 3–5 months after the infection. Furthermore, the recurrent symptoms of our sample, such as fatigue, headache, cognitive, and mood problems, were the most

frequent in similar studies (18, 19, 23). Neurological sequelae are similar in adults and children (16, 17), and this and this could be explained by brain patterns similar to long COVID hypometabolism, involving the same areas of the brain (5, 24). However, further studies are needed to confirm this hypothesis. Interestingly, we found a non-linear course for the loss of taste (ageusia), which occurred more frequently 3–5 months after the infection (onset = 58; 1 month = 10; 3–5 months = 21). These data suggest that in some subjects, ageusia might appear later than other symptoms. Unlike other published studies, dividing the sample into two age groups allowed us to highlight a lower onset of neurological symptoms and a more significant reduction over time in the group of younger children (1.5–5 years) compared with older children (6–17 years) (Figure 1). The most relevant aspect of our research concerns psychological problems that, as described by other authors, compromise the daily activities in school-age children (19). The standardised scales we used for the assessment (27–29, 31–35) revealed that a large percentage of children aged 8–16 years developed symptoms of social and separation anxiety (37%), panic (32%), tension (30%), obsessive-compulsive tendencies (23%), and generalised anxiety (28%) related to the presence of neurological symptoms (Table 4). Although small in number, some children had depression and PTSD symptoms. These data demonstrate that long COVID syndrome is not limited to neurological problems but presents various broad-spectrum symptoms that include psychological and long-lasting cognitive aspects. If not treated, these aspects could significantly compromise the quality of life of children and adolescents.

Conclusion

We have found that the most frequent symptoms found in our paediatric sample were similar to those described in the literature and occurred more in the older patients (6–17 years). While neurological symptoms tended to decrease over time, psychological ones were present in greater numbers in 6–17-year-old patients. Recent studies have found similar brain patterns between adult and paediatric COVID-19 populations, but additional longitudinal studies should be conducted in larger cohorts to determine the relationships between COVID-19 infection and the persistence, type, and severity of neuropsychiatric symptoms in different age groups. The results could be useful for planning prevention actions aimed at reducing the risk of chronic symptoms.

Limitations

Due to time limitations we did not compare symptoms with a control group. Furthermore, we have not found studies similar to ours using standardized psychological scales on patients with Long-Covid, and this aspect does not allow us to make a systematic comparison of symptoms with clinical samples similar to ours. Thanks to the use of standardized tools, the study can be replicated.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

CAG conceptualized the study, selected the psychological data collection tools, performed the data analyzes, and drafted the initial manuscript. EB conceptualized the study, selected tools for collecting neurophysiological data, collected the data, and reviewed the manuscript. MM contributed to the conception and design, analysis and interpretation of the data, and drafting of the article. FM, EM, and RN conceptualized and designed the study, planned the inclusion criteria for study participants, and reviewed and revised the manuscript. AS, FL, and AMZ conceptualized and designed the study, supervised the data collection, and critically examined the manuscript for important intellectual content. IA, FA, and FG designed tools for data collection, collected and interpreted the data, and contributed to the drafting of the article. BLC, GB, MC, and GDC made substantial contributions both to the acquisition of the data and to the drafting of the paper. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Cerebrospinal fluid neurofilament light chain predicts short-term prognosis in pediatric Guillain-Barré syndrome

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Introduction: To study cerebrospinal fluid neurofilament light chain (CSF-NfL) levels as a prognostic biomarker in pediatric Guillain-Barré syndrome (GBS).

Methods: Prospective study enrolling 26 pediatric GBS patients and 48 healthy controls (HCs) from neurology units between 2017 to 2021. The CSF-NfL levels were measured by enzyme-linked immunosorbent assay. The children's disability levels were evaluated using Hughes Functional Score (HFS) at nadir, 1 month, and 6 months after onset. The receiver operating characteristic (ROC) curve derived from logistic regression (with age as a covariate) was used to assess the prognostic value of CSF-NfL on the possibility of walking aided at 1 month after symptom onset.

Results: The mean CSF-NfL levels were significantly increased in GBS patients (111.76 pg/mL) as compared to that in HCs (76.82 pg/mL) ($t = 6.754$, $p < 0.001$). At follow- up, the mean CSF-NfL levels after treatment (65.69 pg/mL) declined significantly ($t = 6.693$, $p < 0.001$). CSF-NfL levels upon admission were significantly associated with the HFS at nadir ($r_S = 0.461$, $p = 0.018$). Moreover, the mean CSF-NfL levels in GBS patients with poor prognosis (130.47pg/mL) were significantly higher than that in patients with good prognosis (104.87pg/mL) ($t = 2.399$, $p = 0.025$). ROC curve analysis of the predictive value of CSF-NfL levels with respect to the inability to walk unaided within 1 month showed a significant difference (area under the curve: 0.857,95% confidence interval 0.702-1.000; $p = 0.006$).

Conclusion: CSF-NfL levels were increased in pediatric GBS patients. High CSF-NfL level predicted worse motor function, and was strongly associated with poor short-term prognosis of pediatric GBS. We propose a biomarker for early prediction of outcome in pediatric GBS, which would be applicable for clinical practice and efficacy of treatment in the future.

KEYWORDS

Guillain-Barré syndrome, children, neurofilament light chain, Hughes functional grading, prognosis

Introduction

Guillain–Barré syndrome (GBS) is currently the most common cause of acute flaccid paralysis in children and is thought to be provoked by an aberrant immune response. Potential triggering factors include viruses such as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), Zika virus, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and influenza virus, bacteria such as *Campylobacter Jejuni, Mycoplasma Pneumoniae* and other factors such as vaccinations, surgery, and malignancy (1). Since GBS associated with COVID-19 is being increasingly reported, and given the current impact of the COVID-19 pandemic, it is important to conduct SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) testing with naso- or oropharyngeal swab in all patients with suspected GBS to rapidly isolate cases. The diagnosis of GBS is mainly based on clinical history and presentation, and ancillary investigations such as cerebrospinal fluid (CSF) examination and electrophysiological studies (2). However, making a diagnosis of pediatric Guillain-Barré syndrome can be challenging, and it is highly dependent on ancillary examinations. Electrophysiological studies play an important role in early diagnosis and determining the subtypes of GBS (3). However, the electrophysiological measurements can be normal in the early course of the disease, and hence they might not meet the electrophysiological diagnostic criteria of GBS. Therefore, there is an urgent need for a sensitive and specific biomarker that could identify GBS at the acute stage.

Many studies have shown that patients with GBS differ considerably in their clinical manifestation and prognosis (4, 5). Children with GBS tend to have a good prognosis (6). However, a minority of patients were unable to walk independently 6 months after onset. Therefore, it is critical to identify poor prognoses early on for pediatric GBS patients for implementing effective treatment strategies to avoid irreversible nerve degeneration. Some prognostic methods, such as the modified Erasmus GBS outcome score and Erasmus GBS respiratory insufficiency score can be applied to adult patients with GBS (7, 8), however, whether they are suitable for children with GBS remains unknown. Therefore, it is urgently necessary to discover an objective and valid biomarker that could predict the outcome of GBS in children.

As the most important axonal damage biomarker, neurofilament levels have emerged as important in neurological disorders, such as multiple sclerosis (9), Alzheimer's disease (10), and Guillain–Barré syndrome. Some studies have shown that the CSF and serum concentrations of neurofilament heavy chain levels (NfH) were increased in children with GBS (11), and other studies have demonstrated that high serum neurofilament light chain levels (sNfL) were also increased and associated with poor prognosis in adult GBS (12), however, the correlation of CSF-NfL levels and outcome in children with GBS were not analyzed in these studies. Thus, in the present study, we aimed to: (1) determine the CSF-NfL levels by enzyme-linked immunosorbent assay (ELISA) in healthy children and pediatric GBS patients; (2) analyze the value of CSF-NfL levels in early diagnosis and therapeutic efficacy in pediatric GBS; and (3) examine the association between CSF-NfL levels and clinical features as well as the outcome of Guillain-Barré syndrome in children.

Materials and methods

Subjects

We enrolled 26 pediatric GBS patients (under 14 years of age) who were admitted to our hospital between September 2017 and January 2021. Patients fulfilling levels 1 or 2 of the Brighton criteria of GBS (13) were included in the study. Patients with Miller Fisher syndrome and other causes of neuropathies, such as spinal anterior horn lesion caused by infection of poliovirus or enterovirus 71, acute transverse myelitis, chronic inflammatory demyelinating polyneuropathy, were excluded. The control group consisted of 48 children who visited our hospital because of psychiatric disorders, migraine, and benign intracranial hypertension. They did not present with any known axonal damage or the presence of objective clinical signs after extensive diagnostic evaluation. All the participants provided written informed consent for the use of their CSF for research purposes. This study was approved by the Ethics Committee of the Children's Hospital of Hebei Province.

Methods

CSF samples and analytical methods

CSF samples of GBS patients were collected upon admission and 2 weeks after the treatment. All samples were collected into a polypropylene tube and stored at −80°C until the analysis. Samples were analyzed by two investigators blinded to clinical data using the Nf-light kit (ELISA kits: Uman Diagnostics NF-light®). Sample processing was carried out according to the manufacturer's instructions and protocol. All samples were measured in duplicates. The mean intra- and inter-assay coefficients of variation for mean CSF-NfL level (111.76 pg/mL) were 1.9and 4.5%, respectively.

Clinical data and ancillary investigations

(1) Limb disability was assessed by Hughes Functional Scale (HFS) at nadir, 1 month, and 6 months after onset. Patients with HFS $\geq$ 3 were categorized as poor outcomes, and patients with HFS <3 were categorized as good outcomes. Other clinical manifestations, such as facial paralysis, paraesthesias, autonomic dysfunction, and mechanical ventilation were also analyzed. (2) The pleocytosis and protein levels of CSF were measured within

TABLE 1 CSF-NfL in patients with GBS upon admission and healthy controls.

Variables	GBS patients ($n = 26$)	Healthy controls ($n = 48$)	Statistic values	*P-value*
Age, years, mean±SD	6 ± 3.4	7 ± 2.6	$t = 1.561$	0.123
Male, *n* (%)	14(53.8)	30(62.5)	$\chi^2 = 0.524$	0.469
NfL,pg/mL, mean±SD	111.76 ± 26.33	76.82 ± 17.96	$t = 6.754$	<0.001

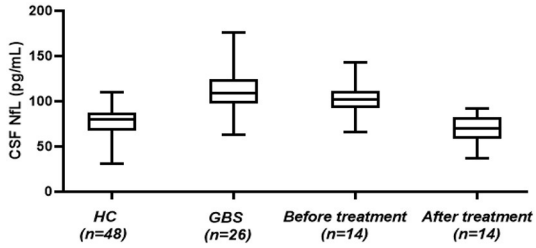

FIGURE 1
CSF-NfL levels in GBS patients and healthy controls. Box plots indicate median and IQR with whiskers extending 1.5 times the IQR. CSF-NfL, cerebrospinal fluid neurofilament light chain; GBS, Guillain–Barré syndrome; HC, healthy control.

4 weeks of weakness onset. (3) Electrophysiologic examinations were performed at least twice and patients were classified into acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN) according to the Hughes electrodiagnostic criteria (14).

Statistical analyses

Statistical analyses were performed using the IBM SPSS Statistics 24. Categorical data were shown as proportions, and continuous data were shown as mean ± SD or medians with IQR. Differences in proportions were tested by χ^2 tests. The continuous variables were tested by the *t*-test or Mann–Whitney U test. The associations between basal characteristics and CSF-NfL levels were analyzed using Pearson correlation or Spearman's correlation coefficient. The receiver operating characteristic (ROC) curve derived from logistic regression (with age as a covariate) was used to evaluate the prognostic value of CSF-NfL on the probability of walking dependently. Statistical significance was set at 0.05.

Results

CSF-NfL in pediatric GBS patients and healthy controls

A total of 26 children patients (mean age 6 years; 14 males) were recruited. The mean CSF-NfL level for the GBS patients upon admission was 111.76 ± 26.33 pg/mL. Forty-eight healthy age-matched controls (mean age 7 years; 30 males) were included, and their mean CSF-NfL level was 76.82 ± 17.96 pg/mL. CSF-NfL levels were significantly higher in the GBS patients than in healthy controls ($t = 6.754$, $p < 0.001$) (Table 1; Figure 1).

At follow-up, 14 patients were recruited, and the mean CSF-NfL levels before and after treatment were 101.85 ± 19.12 pg/mL and 65.69 ± 16.71 pg/mL, respectively. The CSF-NfL levels after treatment declined significantly ($t = 6.693$, $p < 0.001$) (Figure 1).

The CSF-NfL levels were not significantly different between GBS patients after treatment and the healthy controls ($t = 2.069$, $p = 0.053$) (Figure 1).

Relationship between CSF-NfL levels and baseline clinical features in pediatric GBS

CSF-NfL levels upon admission were significantly associated with the Hughes Functional Score (HFS) calculated at nadir ($r_s = 0.461$, $p = 0.018$) (Figure 2A). Meanwhile, CSF-NfL levels were correlated with CSF protein levels ($r_s = 0.392$, $p = 0.048$). Other clinical features and CSF-NfL levels are summarized in Table 2.

There was no association between CSF protein levels and prognosis in GBS patients ($r_s = 0.002$, $p = 0.991$), and receiver operating characteristic (ROC) curve analysis of the predictive value of CSF protein levels showed no statistical difference (area under the curve [AUC]: 0.594, 95% confidence interval [CI] 0.363–0.825; $p = 0.470$).

In this study, the mean CSF-NfL levels in GBS patients with good or poor prognosis were 104.87 ± 24.18 and 130.47 ± 24 pg/mL, respectively, furthermore, this difference was significant ($t = 2.399$, $p = 0.025$) (Figure 2B). A correlation between CSF-NfL and age in adults has been reported previously. We performed an analysis of covariance, with age as a confounder. The mean age in the GBS group was 6 ± 3 years and 7 ± 3 years in the control group. Age and CSF-NfL levels were significantly associated in the control group ($r = 0.0.841$, $p < 0.001$), which was not the case for the GBS patients ($r = 0.119$, $p = 0.561$). Analysis of covariance suggested no influence of age on CSF-NfL concentrations ($F = 2.522$, $p = 0.126$), whereas the age-adjusted group difference was preserved ($p = 0.009$). ROC curves derived from logistic regression (with age as a covariate) were used to analyze the potential predictive value of CSF-NfL levels with respect to unable to walk unaided (Hughes grade of 3 and more) within 1 month, which showed a

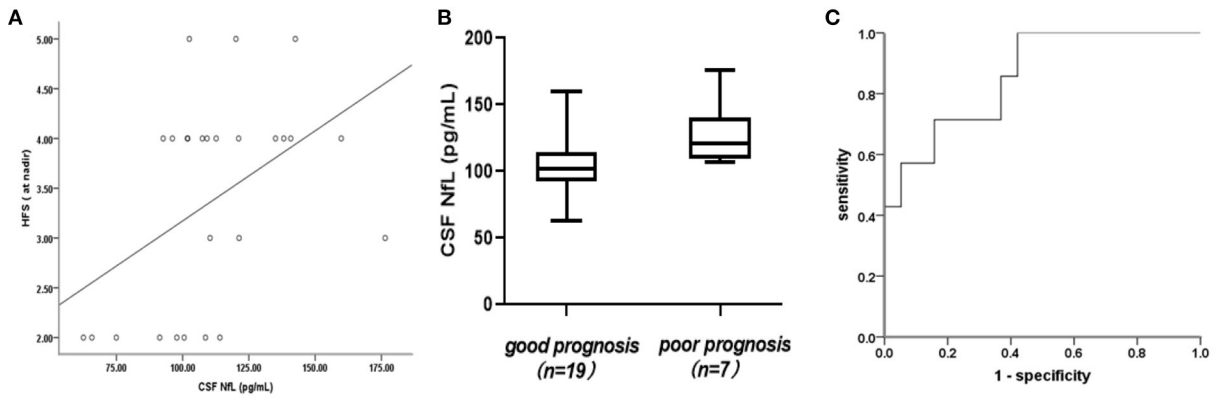

FIGURE 2
(A) Association of cerebrospinal fluid neurofilament light chain (CSF-NfL) concentrations upon admission with the Hughes Functional Score (HFS) calculated at nadir with a Spearman's correlation coefficient r_s of 0.461 ($p = 0.018$). Each dot in the scatter plot represents a sample. **(B)** CSF-NfL levels on admission in patients who were good or poor prognoses at 1 month after symptom onset. Box plots indicate median and IQR with whiskers extending 1.5 times the IQR. **(C)** Receiver operating characteristic (ROC) curve derived from logistic regression (with age as a covariate) was used to analyze the potential predictive value of CSF-NfLlevels with respect to unable to walk unaided (Hughes grade of 3 and more).

statistically significant difference (area under the curve [AUC]: 0.857,95% confidence interval [CI] 0.702–1.000; $p = 0.006$) (Figure 2C).

Discussion

Guillain-Barré syndrome (GBS) is one of the most common causes of acute flaccid paralysis in children, and is characterized by progressive, relatively symmetrical limb weakness with or without paresthesia (15). The prognosis of GBS tends to be good after effective treatments, however, about one-fifth of the patients are unable to walk independently at 6 months after symptom onset, and about 7% of patients die (16). Many studies have shown that pediatric GBS has a good prognosis (17, 18). In the present study, seven (7/26, 26.9%) and 19 patients (19/26, 73.1%) showed poor and good prognoses, respectively, at 1 month after symptom onset, whereas none of the patients was unable to walk independently at 6 months after symptom onset. Therefore, a convenient and reliable biomarker to predict the clinical prognosis of pediatric GBS is essential for patients.

Neurofilament light protein (NfL) is one of the most important intracellular neuronal skeletal proteins and is secreted into CSF during axonal damage (19). In the literature to date, the potential value of NfL as a biomarker for axonal damage has been discussed in multiple neurological disorders, such as amyotrophic lateral sclerosis (20), chronic inflammatory demyelinating polyneuropathy (21), cerebral vasculitis (22). Patients with these conditions have elevated NfL levels as compared to healthy individuals. This is consistent with the results of our study, wherein mean CSF-NfL levels of pediatric GBS patients were significantly higher than those of healthy controls. Moreover, at follow-up, mean CSF-NfL levels after treatment significantly declined along with the amelioration of the disease. These findings indicate that CSF-NfL levels might become an objective biomarker for early diagnosis and therapeutic efficacy.

CSF albuminocytological dissociation phenomenon could further assist the diagnosis of GBS (23). CSF protein elevation reflected the damage of the blood-nerve and blood-brain barrier and the subsequently increased permeability. GBS patients with higher CSF-NfL levels had more severe demyelination and axonal damage than GBS patients with lower CSF-NfL values, which also manifested as a more severe motor disability. In the present study, CSF-NfL levels were significantly correlated with protein levels and the Hughes Functional Score (HFS) at nadir. All these findings signify that higher CSF-NfL levels could be used to predict worse motor function and more clinical severity.

Higher CSF-NfL levels in adult GBS had also been associated with a poorer clinical prognosis (24). In the present study, CSF-NfL levels in GBS patients with poor prognosis were significantly higher than that in patients with good prognosis. Further, ROC curve analysis demonstrated the potential predictive value of the CSF-NfL levels. This showed that higher CSF-NfL levels were strongly correlated with poor short-term prognosis of GBS, and served as an independent factor associated with the inability to walk. Therefore, higher CSF-NfL levels could also alert physicians in the early stage of the disease to ensure active and effective treatments to improve the prognosis and shorten the course of the disease.

TABLE 2 Relationship between CSF-NfL levels and basal characteristics in pediatric GBS.

Basal characteristics ($n = 26$)	CSF-NfL levels pg/mL	Statistic values	*P-value*
Age, years, mean±SD	6 ± 3.4	$r = 0.119$	0.561
Gender, *n* (%)		$r = 0.289$	0.152
Male	14(53.8)		
Female	12(46.2)		
Preceding event, *n* (%)		—	0.256
Respiratory infection	16(61.5)		
Diarrhea	1(3.8)		
None	9(34.6)		
From onset to nadir, days, median (IQR)	7.5(3.75–12)	$r_s = 0.249$	0.220
Hughes score at nadir, grade, median (IQR)	4(2–4)	$r_s = 0.461$	0.018
Neurological symptoms, *n* (%)			
Facial paralysis	5(19.2)	$r = 0.023$	0.910
Bulbar paralysis	6(23.1)	$r = 0.107$	0.603
Neuropathic pain	17(65.4)	$r = 0.080$	0.697
Autonomic dysfunction, *n* (%)	7(26.9)	$r = 0.215$	0.292
Mechanical ventilation, *n* (%)	14(53.8)	$r = 0.023$	0.911
EMG variants, *n* (%)		$r = 0.187$	0.359
AIDP	23(88.5)		
AMAN	3(11.5)		
Proteins in CSF, g/L, median (IQR)	0.93 (0.68–1.31)	$r_s = 0.392$	0.048
Pleocytosis, 10^6/L, median (IQR)	4(2–9.25)	$r_s = 0.124$	0.546
Treatment, *n* (%)		$r = 0.087$	0.671
IVIg	23(88.5)		
IVIg + PLEX	3(11.5)		
Hughes score at 1 month after onset, grade, median (IQR)	1(1–3)	$r_s = 0.433$	0.027
Hughes score at 6 month after onset, grade, median (IQR)	0	$r_s = 0.107$	0.602
Duration of hospitalization, days, mean ± SD	25.08 ± 11.2	$r = 0.243$	0.232
Prognosis, *n* (%)		$r = 0.440$	0.025
Good	19(73.1)		
Poor	7(26.9)		

GBS, Guillain-Barré syndrome; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; IVIg, Intravenous immunoglobulin; PLEX, plasma exchange; r, Pearson correlation; r_s, Spearman's correlation coefficient.

Our study was also subject to some limitations. First, the association of CSF-NfL levels and electrophysiological subtypes was not evaluated because of a smaller number of cases. Secondly, this was a small prospective study that recruited 26 pediatric GBS patients. We will further conduct some prospective clinical studies on the prognosis of children with GBS based on the present study.

In conclusion, CSF-NfL levels are increased in pediatric GBS patients, higher CSF-NfL predicted worse motor function and were strongly associated with poor short-term prognosis of pediatric GBS. We proposed a biomarker for early prediction of outcome in the pediatric GBS, applicable for clinical practice and future treatment efficacy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

This study was approved by the Ethics Committee of the Children's Hospital of Hebei Province. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LZ and YZ collected serum and CSF samples. JL acquired the electrophysiological data. ZZ completed the statistical analysis. MJ designed the experiments, interpreted the results, and drafted the initial manuscript. KL reviewed the data and revised the manuscript. SS revised the initial draft and wrote the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Neurodevelopmental disorders and anti-epileptic treatment in a patient with a *SATB1* mutation: A case report

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SATB1 variants causing developmental delay with dysmorphic facies and dental anomalies have been reported in a small cohort. Most patients present epilepsy as a main clinical feature in neurodevelopmental disorders; however, its treatment is unknown. Here, we present a Chinese patient with a *de novo* truncating variation in *SATB1* who presented with mild developmental delay. We disclose the detailed anti-epileptic pharmacological treatment that enabled a favorable outcome. Our study provides important information that may aid clinicians in the prognosis and treatment of rare neurological developmental disorders caused by gene mutations.

KEYWORDS

***SATB1*, epilepsy, anti-epileptic drugs, neurodevelopmental delay, protein-truncating variants**

Introduction

Variants of *SATB1* cause clinically overlapping but distinct neurodevelopmental disorders, including intellectual disability, muscle tone abnormalities, hypotonia, spasticity, epilepsy, behavioral problems, facial dysmorphisms, and dental abnormalities. Genotype-phenotype relationships associated with each pathophysiological mechanism have been identified (1). In the limited number of reported clinical cases, motor and speech delays have been the most prevalent neurological manifestations (92 and 89%, respectively). Epilepsy accounts for 61% of all reported nervous system-related phenotypes and is the only symptom with a relatively comprehensive pharmacological treatment. Studies on most neurodevelopmental disorders have proved that the control of early epilepsy is crucial for slowing the progression of neurological impairment and restoring normal neurological function (2, 3). Details of the specific epileptic condition and therapeutic interventions in patients are not available in existing

reports of *SATB1* variants, thus lacking the clinicians' and patients' perspectives of the treatment experience. This study reports a Chinese patient with a pathogenic *SATB1* mutation manifested as epilepsy, growth retardation, and facial dysmorphisms. The treatment process of epilepsy and the growth and development history of the patient are elaborated. Our study provides relevant clinical and pharmacological information for the prognosis and treatment of neurological rare developmental disorders caused by gene mutations.

Case description

The proband was a 7-year-old girl. She was referred to the Medical Genetics Clinic of Shanghai Children's Medical Center, Sanya Women and Children's Hospital, presenting with global developmental delay. She was the only child of non-consanguineous Han Chinese parents. The patient had no abnormalities on prenatal examination and was born naturally at full term. Her parents were physically healthy and had no relevant family history.

The girl sought medical advice for the first time at age 2. She began to roll her eyes up and frequently stopped moving for several seconds, mostly on stimulation. In 2 months, she had a major seizure that lasted approximately 5 min, with convulsions affecting her whole body, her eyes rolling up, and her mouth foaming. Facial features were not recognizable, and dental abnormalities were not evident (**Figure 1A**). She was diagnosed with epilepsy at the local hospital. The EEG (Electroencephalography) showed sharp, sharp-slow, and spinous slow waves in the bilateral occipital areas, but were more prominent on the right side. There were more asynchronous, sharp, slow, and spike waves in the bilateral frontal, middle, and central regions. The patient started anti-epileptic drug therapy. Valproic acid was used for 2 months at a dose of 250 mg (25 mg/kg) twice daily. Seizures occurred several times during this period, and EEG did not improve significantly. For the following 9 months, the dose of valproic acid was reduced to 100 mg (10 mg/kg), and 80 mg oxcarbazepine (8 mg/kg) was introduced, both administered twice daily for better control of the epileptic activity. With the increase in body weight, the doses of valproic acid and oxcarbazepine were adjusted. At the age of seven, when this case was reported to us, the regimen consisted of 160 mg valproic acid (9 mg/kg) and 180 mg oxcarbazepine (10 mg/kg) twice daily. Epileptic activity had been well controlled since the combined use of valproic acid and oxcarbazepine. No seizures or other epileptic activity were noticed, and the electroencephalogram at the age of seven showed only scarce sharp waves during sleep (**Figure 2**).

The Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) was used to evaluate the general intellectual ability of the patient when she was 78 months old. The full-scale intelligence quotient was 78, which is at the lower limit of the normal range. The scores of verbal comprehension, perceptual reasoning, working memory, and processing speed subtests were 81, 79, 85, and 79, respectively. Detailed neurological features are shown in **Table 1**.

To detect disease-causing mutations, genomic DNA was extracted from the peripheral blood samples of the patient and her parents using the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Whole-exome capture was performed using an Agilent SureSelect V6 enrichment capture kit (Agilent Technologies, Inc., Woburn, MA, United States), according to the manufacturer's instructions. The captured library was sequenced using the Illumina HiSeq 2500 System (Illumina, Inc., San Diego, CA, United States). Original sequencing data were assessed using FastQC (version 0.11.2) for quality control. The Burrows Wheeler alignment tool v0.2.10 was used for sequencing data alignment to the Human Reference Genome (NCBI build 37, hg 19). Single-nucleotide variants and small indels were identified using the Genome Analysis Toolkit. All variants were saved in VCF format and uploaded to the Ingenuity Variant Analysis (Ingenuity Systems, Redwood City, CA, United States) and TGex (Translational Genomics Expert) platforms for biological analysis and interpretation, as previously reported (4). Variants detected by next-generation sequencing were confirmed by Sanger sequencing.

Whole-exome sequencing revealed a novel frame-shift variant, c.2128_2129del, p.Leu710Valfs × 42, in the last exon of *SATB1* (NM_001195470.2). Sanger sequencing confirmed the variant as well as the wild-type status of her father and mother (**Figure 1B**). The control population database (gnomAD) and our local control cohort database did not have reports on the detected variant. The frame-shift variant is classified as a pathogenic variant according to the ACMG guidelines for variant interpretation.

Discussion

SATB1 (OMIM × 602075) encodes a transcription factor involved in T cell development and maturation (5). The pathogenicity of *SATB1* variants was initially reported upon the identification of *de novo* variants in two large neurodevelopmental disorder cohorts. These variants suggested a role for this gene in neurodevelopment (6, 7). Accurate genotype-phenotype correlations and disease mechanisms were recently identified during the clinical evaluation of a 42-patient cohort (1). Although the broad phenotypic spectrum of *SATB1* mutations has been described (including neurodevelopmental delay, intellectual disability, muscle tone abnormalities, epilepsy, behavioral problems, facial dysmorphisms, and dental abnormalities), the treatment and prognosis of these patients have not been reported previously.

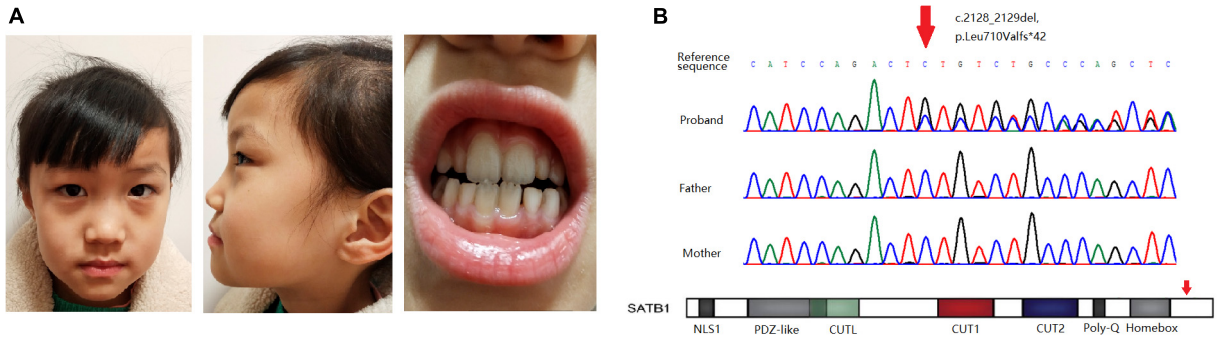

FIGURE 1
(A) Facial features and dental abnormalities are not recognizable in the patient. **(B)** Sanger sequencing of the variant in the pedigree and location of the variant in the gene.

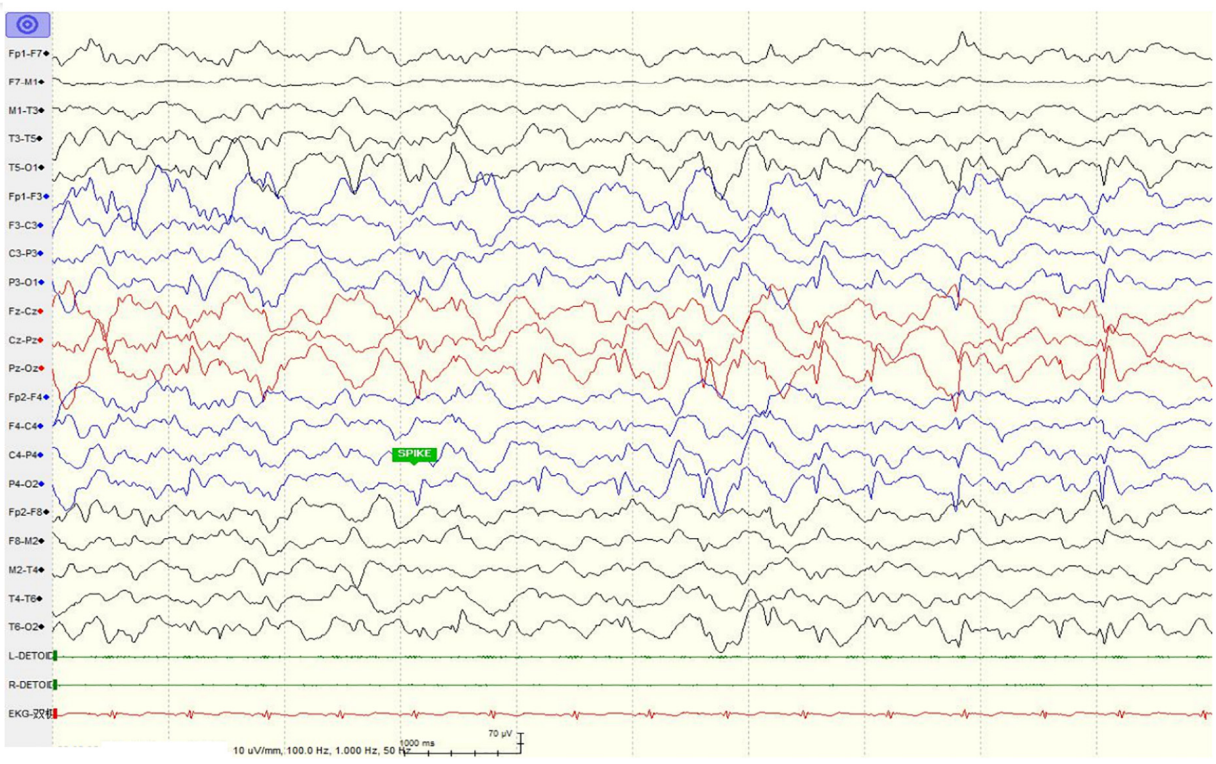

FIGURE 2
Electroencephalogram of the patient showed only scarce sharp waves during sleep at age seven after anti-epileptic drug treatment.

Epilepsy is the only disease symptom for which systematic drug regimens are available, as more than 25 antiseizure medications are currently used. The association between epilepsy and neurodevelopmental disorders is well established. Children, especially newborns, infants, and young children, with developmental epilepsies have an increased risk of cognitive, neurobehavioral, and psychiatric disorders (8–10). This has important implications for treatment, especially in children with epileptic encephalopathy, in whom early and successful treatment of seizures and interictal epileptiform activity may be crucial for positive neurodevelopmental outcomes (11, 12). In children with *SCN1A* seizure disorders, who are at a high risk of sudden unexplained death in epilepsy, seizure control is critical. In addition, prolonged acute seizures

TABLE 1 Neurological clinical features of patients with SATB1 gene variants.

	Our patient	Previously reported patients by den Hoed J, et al. (1)	
		Null variants (1)	Missense variants
Intellectual disability	Yes	9/10	20/22
Developmental delay	Yes	12/12	23/24
Motor delay	Yes	11/12	23/25
Speech delay	Yes	10/12	22/24
Dysarthria	No	1/10	5/9
Epilepsy	Yes	2/10	20/26
Hypotonia	No	5/12	23/25
Spasticity	No	0/12	10/24
Ataxia	No	2/10	4/14
Behavioral disturbances	Yes (anxiety, mild ASD-features)	7/12	17/22
Sleep disturbances	No	3/11	9/18
Brain imaging abnormalities	No	2/7	17/24
Regression	No	1/12	5/24
Facial dysmorphisms	Yes (subtle)	7/11	17/24
Dental/oral abnormalities	Yes (widely spaced teeth)	6/11	11/24

may cause permanent injury. In Dravet syndrome, cognitive deterioration may occur, especially when seizure control is incomplete (3, 13). A beneficial effect of immunotherapy combined with anti-epileptic drugs on seizure frequency and cognition has been observed in patients (14). A strong link between seizure control and improvement in neurological function has been observed in many common and rare epilepsy syndromes (15, 16). The combination of low-dose valproic acid and oxcarbazepine showed satisfactory effects on the control of epilepsy in our patient; her neurodevelopment also improved since she was seizure-free. Satb1 was expressed in midbrain dopaminergic neurons and acted as a dopaminergic neuron-specific regulator (17, 18). The attenuation effect of dopaminergic neurotoxicity by valproic acid was assumed to be effective (19). Oxcarbazepine might effectively reduce seizure frequency when used as an add-on for drug-resistant epilepsy (20), but the mechanism by which valproic acid and oxcarbazepine combination works in patients with SATB1 variants needs further investigation. Treatment process and prognosis of our case could provide reliable reference for management of patients with same gene mutation.

A clear genotype-phenotype correlation has been observed: individuals carrying missense variants were more severely affected than individuals carrying protein-truncating variants (1). Functional assays using cells expressing pathogenic variants of SATB1 harboring missense mutations in the CUT1 and CUT2 DNA-binding domains demonstrated altered transcriptional activity compared with the wild-type protein, which could explain this genotype-phenotype correlation (1). Although the *de novo* frame-shift variant detected in our patients was located in the last exon of the gene, it is likely to escape nonsense-mediated mRNA decay, as a variant located at a more distal position has been reported (p.N736fs × 8). Our patient showed a mild phenotype consistent with previously reported patients presenting protein-truncating variants as a distinct group given the haploinsufficiency of these mutations (**Table 1**). Diagnosis in patients with mild to moderate developmental delay without evident facial dysmorphism and dental/oral abnormalities is difficult; thus, genetic testing in these patients is quite effective for diagnostic purposes.

Conclusion

We report a *de novo* protein-truncating variant of *SATB1* in a Chinese patient with epilepsy, developmental delay, and dysmorphic features. The clinical features of our patient are consistent with previously reported genotype-phenotype correlations. The anti-epileptic drug treatment of the patient is described in detail, providing important information for the control of epilepsy in patients with *SATB1* variations, which is crucial for their neurodevelopment.

Data availability statement

The data presented in this study are deposited in the GSA for human repository, accession number SubHRA003891.

Ethics statement

The studies involving human participants were reviewed and approved by Sanya Women and Children's Hospital Managed by Shanghai Children's Medical Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

YY, CL, JeW, and RY contributed to the conception and design of the study. CL and WL organized the database. YY and DW performed the statistical analysis. RY and YY wrote the first draft of the manuscript. CL, JaW, and LC wrote sections of the

manuscript. All authors contributed to the manuscript revision and read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Case report: Incidence and prognostic value of brain MRI lesions and elevated cerebrospinal fluid protein in children with Guillain-Barré syndrome

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Background: Guillain-Barrè syndrome (GBS) is an acute immune-mediated disorder affecting peripheral nerves and nerve roots with a variable clinical course and outcome. Epidemiologic analyses have revealed that the incidence of the syndrome increases linearly among the age. The clinical diagnosis of GBS is based on the family history, physical and neurological examination, electrodiagnostic exams, and cerebrospinal fluid analysis with the classical presence of albumin-cytologic dissociation. Prognosis is associated with the severity of clinical signs and the type of peripheral nerves involved.

Methods: This study aims to clarify which clinical features can be used for prognostic purposes. We evaluated the correlation between (1) brain MRI lesions and grade of disability; (2) brain MRI lesions and elevated cerebrospinal fluid (CSF) protein; and (3) increased levels of CSF protein and grade of disability. Statistical analysis extracted from these data indicated a good correlation to be a prognostic indicator in children affected by GBS. We found little evidence regarding laboratory tests, imaging, and prognosis. We enrolled 12 continuous patients who met the Brighton criteria for GBS in this retrospective study. Each patient was clinically evaluated at the time of disease onset to assess the GBS disability score and after 2 weeks.

Results: We estimated Pearson's correlation index to evaluate the possible correlation between MRI and disability and CSF protein levels and disability. The correlation coefficient was 0.92 and 0.85, respectively. In addition, we developed a graph to see the trend of the disability values, proteins in the CSF, and damage assessed with MRI in the 12 patients. It seems that these parameters have a parallel trend and a good correlation in each patient. Finally, we calculated the correlation between MRI and CSF protein values, with

an *r*-value of 0.87. The values suggest a correlation among the MRI score, CSF protein, and prognosis.

Conclusion: The MRI and CSF laboratory parameters can be important tools for the clinician not only for diagnosis but also to evaluate the possible worsening of general conditions or the need to prepare measures to support life parameters. Patients who need ventilatory support could be established early from patients who have less severe GBS and can begin rehabilitation earlier. We suggest MRI should be performed routinely in children with GBS to be able to estimate the evolution of the clinical condition.

KEYWORDS

Guillain–Barré syndrome, CSF, childhood, MRI, clinical outcome, neurology-clinical

Introduction

Guillain-Barrè syndrome (GBS) is an immune-mediated rapidly developed polyneuropathy whose etiology and pathogenesis are not yet entirely comprehended. Guillain–Barré syndrome (GBS) manifests clinically as acute flaccid paralysis, marked by the symmetrical weakness of the limbs, and hyporeflexia or areflexia, which arrives full harshness within 4 weeks (1).

Sensory symptoms, such as paraesthesia or insensibility, begin distally and have a symmetrical extension.

Among pediatric patients in the zenith phase of the syndrome, 75% are unable to walk unsupported, 30% are quadriplegic, 35–50% show cranial nerves involvement, and 15–20% have respiratory failure and/or autonomic dysfunctions (2, 3). Mortality, or severe disability due to GBS, occurs in ∼20% of patients (4).

Epidemiological analyses have documented that the incidence of GBS increases with age. In children from 0 to 9 years, it occurs with an incidence of 0.62 cases per 100,000 person-year (py) (5, 6).

Diagnosis of GBS is based on the patient's family history, physical and neurological assessment with sensorial and motor disturbances, electrodiagnostic exams, and cerebrospinal fluid (CSF) which reveals the classical pattern of albumin-cytologic dissociation.

The prognosis of the GBS is linked to the severity of clinical signs and unclear to the presence of autonomic nerve dysfunction (7). Predictors of poor outcomes are defined as a GBS Disability Scale score ≥3 after either 2 weeks or 6 months (8).

We evaluated the correlation among CSF analysis, imaging, and the severity of the GBS in the acute phase to predict a short-term outcome.

Aim of the study

We aimed to evaluate CSF analysis and imaging to determine the severity of the GBS in the acute phase and to predict a short-term outcome.

- We evaluated the possible correlation among MRI radiological classification, the values of proteins in the CSF, and the prognosis of children with GBS.
- The correlation between the increasing values of the radiological classification of the clinic with the GBS disability score and the prognosis was evaluated.

TABLE 1 Levels of GBS disability score.

Score	Description
0	Healthy state
1	Minor symptoms and capable of running
2	Able to walk 10 m or more without assistance but unable to run
3	Able to walk 10 m across an open space with help
4	Bedridden or chairbound
5	Requiring assisted ventilation for at least part of the day
6	Dead

TABLE 2 MRI imaging contrast enhancement pattern.

I	No enhancement
II	Anterior roots>posterior roots
III	Anterior roots=posterior roots
IV	Only anterior roots enhance

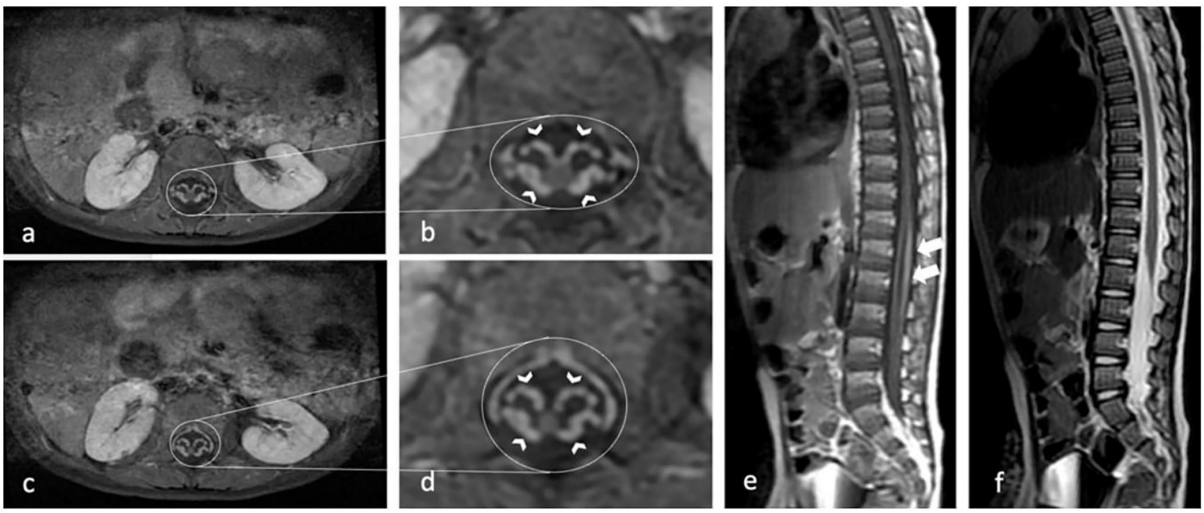


FIGURE 1
Contrast-enhanced axial T1-weighted MR image shows marked enhancement of the anterior and posterior nerve roots (arrow heads) in the conus medullaris and cauda equina **(a–d)**. Sagittal T1-weighted MR image **(e)** show mild thickening and moderate contrast enhancement of nerve root in the conus medullaris and cauda equina (white arrows). Sagittal T2-weighted MR image **(f)** show mild thickening of cauda equina.

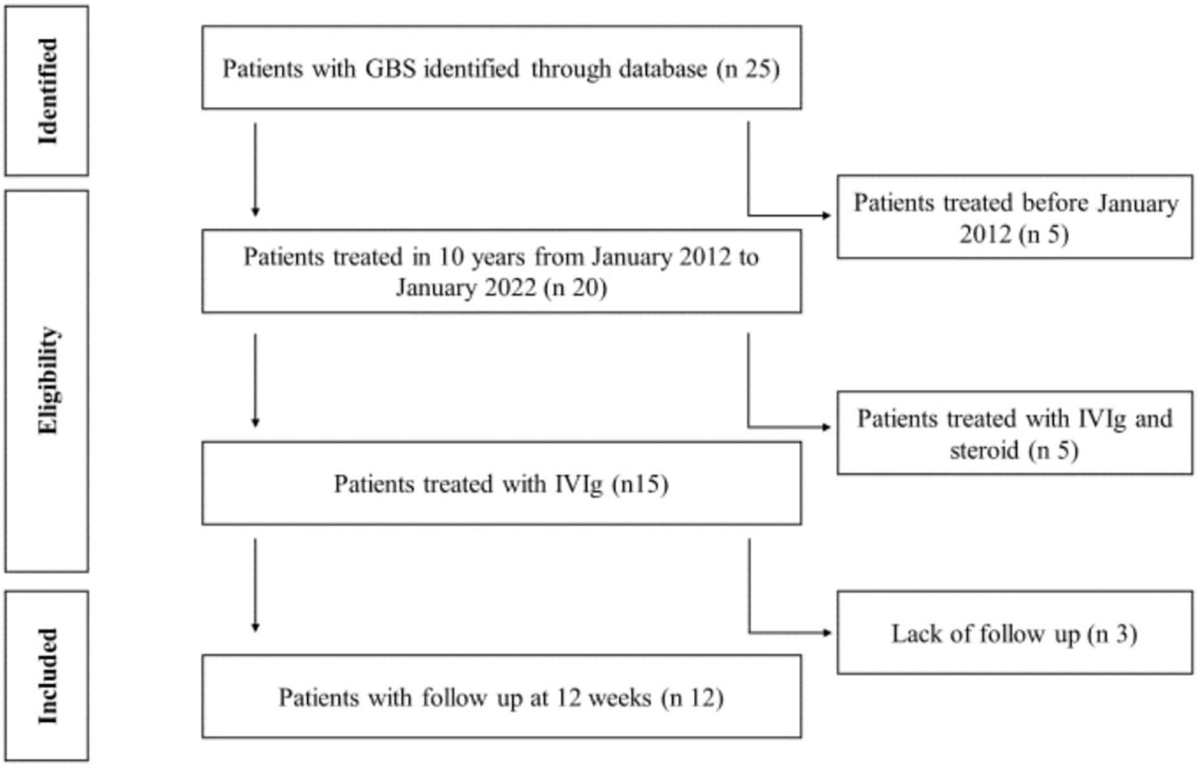


FIGURE 2
Patients recruitment.

TABLE 3 The grade of disability and other clinical features associated to GBS.

Age (Years)	M/F	GBS disability score	Functional deficit	Respiratory deficit	Cranial nerves	Autonomic system
14	M	4	Bedridden		Eyelid ptosis	Ptosis and mydriasis
1.8	F	1	Lower limbs reduced reflexes			
2.5	F	2	Unable to run			
8	M	4	Bedridden			
5	F	2	Reduced activity and play			Sphincter incontinence
1	M	5	Requiring assisted ventilation for part of the day	Jugulum, subdiaphragmatic epigastrium tirage		
13	F	2	Reduced activity and play			
10	F	2	Reduced activity and play			
6	M	3	Able to walk 10 m		Inability to frown	
10	M	4	Chairbound			
7	M	5	Requiring assisted ventilation	Respiratory failure	Nasal voice and dysphagia.	
4	M	4	Chairbound			

- In addition, we evaluated the correlation between increasing CSF protein levels and symptomatology and prognosis.

Materials and methods

A systematic revision of the current literature was conducted using Cochrane, EMBASE, and MEDLINE. In addition to official websites of highly qualified journals which were expected to publish studies related to this topic, for example, the New England Journal of Medicine, The Lancet, PLOS Medicine, Neurology, and Pediatrics were also searched for relevant studies. The words used to search were: "Child; Guillain-Barrè; Neurology; Prognosis; Disability; Laboratory; MRI; cerebrospinal fluid." We found little evidence regarding laboratory tests, imaging, and prognosis. Following, we cite the most recent evidence regarding prognosis and imaging and prognosis and CSF.

Althubaiti et al. in a recent publication on the prognosis value of MRI in children with GBS advise that brain and spinal MRI is a recommended supportive test but a predictive value for clinical and therapeutic outcomes in the short or long term has not yet been proved (9).

Regarding the proteins in the CSF, we want to mention the most recent evidence proposed by Kasser et al. In their letter to the editor, the authors affirmed CSF protein levels seemed to be potentially associated with the need for mechanical ventilation (10).

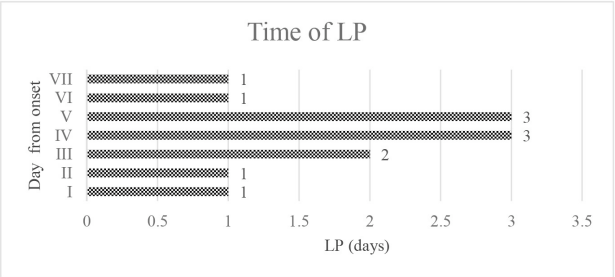
GBS disability score

The Guillain-Barré syndrome (GBS) disability score is a widely accepted scoring system to evaluate the functional level of patients with GBS. It was originally described in Hughes et al. (11) and since then, diverse variations have emerged in the literature.

The patient's level of disability is reported operating on a scale from 0 to 6: Grade 0 is assigned to the asymptomatic patient; Grade 1 is associated with movement reduction but capable of performing manual work; Grade 2 is assigned to those who walk but are unable to perform work with their hands; Grade 3 concerns patients who need support for walking; Grade 4 is assigned to patients who are bedridden. The last 2 degrees of the disease in which there is respiratory failure or death (Table 1). The GBS disability score is not only a clinical parameter but also provides a prognostic value. Revealed disability at the onset and the acute phase of the disease were signs of poor long-term prognosis.

The Erasmus GBS and van Koningsveld et al. (8–12) evaluated the association between clinical severity at onset and short-term prognosis. They introduced three variables that were predictive of poor outcome at 6 months, and inability to walk

TABLE 4 Time (days).



Lumbar puncture (LP) performed 3 days after symptoms onset.

TABLE 5 Clinical presentation.

Variable	Patients (*n*)
Male/Female	7/5
Age (years)	6,8
Trigger events	
-Gastrointestinal infections	3
-Airway infections	8
-Vaccination	1
Time from onset to admission (day)	4
Sensory disturbance	2
Hyporeflexia or areflexia	10

independently: age, preceding diarrhea, and GBS disability score at 2 weeks after entry.

Brain MRI lesions

Magnetic resonance (MR) imaging also contributes to the diagnosis of GBS by demonstrating anterior and posterior intrathecal spinal nerve roots and cauda equine. Gorson et al. (13) showed a correlation between the severity of enhancement of the nerve roots and the severity of the clinical grade. Yikilmaz et al. (14) described the MR features in children with GBS and introduced a radiological classification based on the patterns of contrast enhancement, as reported in Table 2.

Figure 1 shows one of our patients' axials with a marked enhancement of the anterior and posterior nerve roots and a GBS disability score of 4.

Elevated CSF protein

We evaluated the chemical-physical characteristics of the CSF in each child, examining appearance, color, number of cells, glucose, proteins, presence of immunoglobulins, and albumin. We examined all these parameters one by one for their correlation with the GBS disability score and therefore with the prognosis.

In this study, the levels of CSF proteins were evaluated according to the increases of 1, 2, and 3 times the basal cut-off value of 45 mg/dl.

Enrolled patients

The manuscript attempts to evaluate whether a clinical score (the grade of disability of patients), an imaging score (spinal MRI enhancement), and a grade of albumin-cytologic dissociation in CSF are linearly correlated to give a prognostic value of the disease.

We retrospectively enrolled 12 continuous children affected by GBS and diagnosed at the Clinical Pediatrics Department for 10 years from January 2012 to January 2022 (Figure 2). The children were from 1 year to 14 years old, with a mean age of 6.8 years. The gender ratio was 7 boys to 5 girls. Furthermore, all cases examined met the Brighton criteria validated for children (15). Table 1 reports the grade of disability and other clinical features associated with GBS signs observed in the children. We evaluated the following characteristics of the population included in this study:

- Clinical: each patient was clinically evaluated at the time of disease onset assessing the GBS disability score and after 2 weeks. In addition, all the children treated in our center were placed in instrumental clinical follow-up by carrying out checks at 2 weeks to 1 month, 3 months to 6 months, and 1 year from the onset of the disease. In this study, we collected clinical data at onset and 2 weeks after onset (Table 3).
- Imaging: all patients enrolled in this study underwent MR of the brain and spinal cord. Images were evaluated and classified by the pediatric neuroradiology team and assessed by Yakilmaz radiological classification (14).
- Laboratory: lumbar puncture (LP) was performed on average 3 days after symptom onset (Table 4), and all enrolled patients had CSF analyzed.

Patients selected during this period were treated according to guidelines with standard IVIg administration of 1 g/kg daily for 2 days. In addition, patients with clinical follow-up at 2 weeks were included in the study. Data were collected and a statistical analysis was done to determine the presence of a possible correlation among these three variables.

Statistical analysis

We performed a statistical analysis to measure the potential relationship between the clinical progress of children

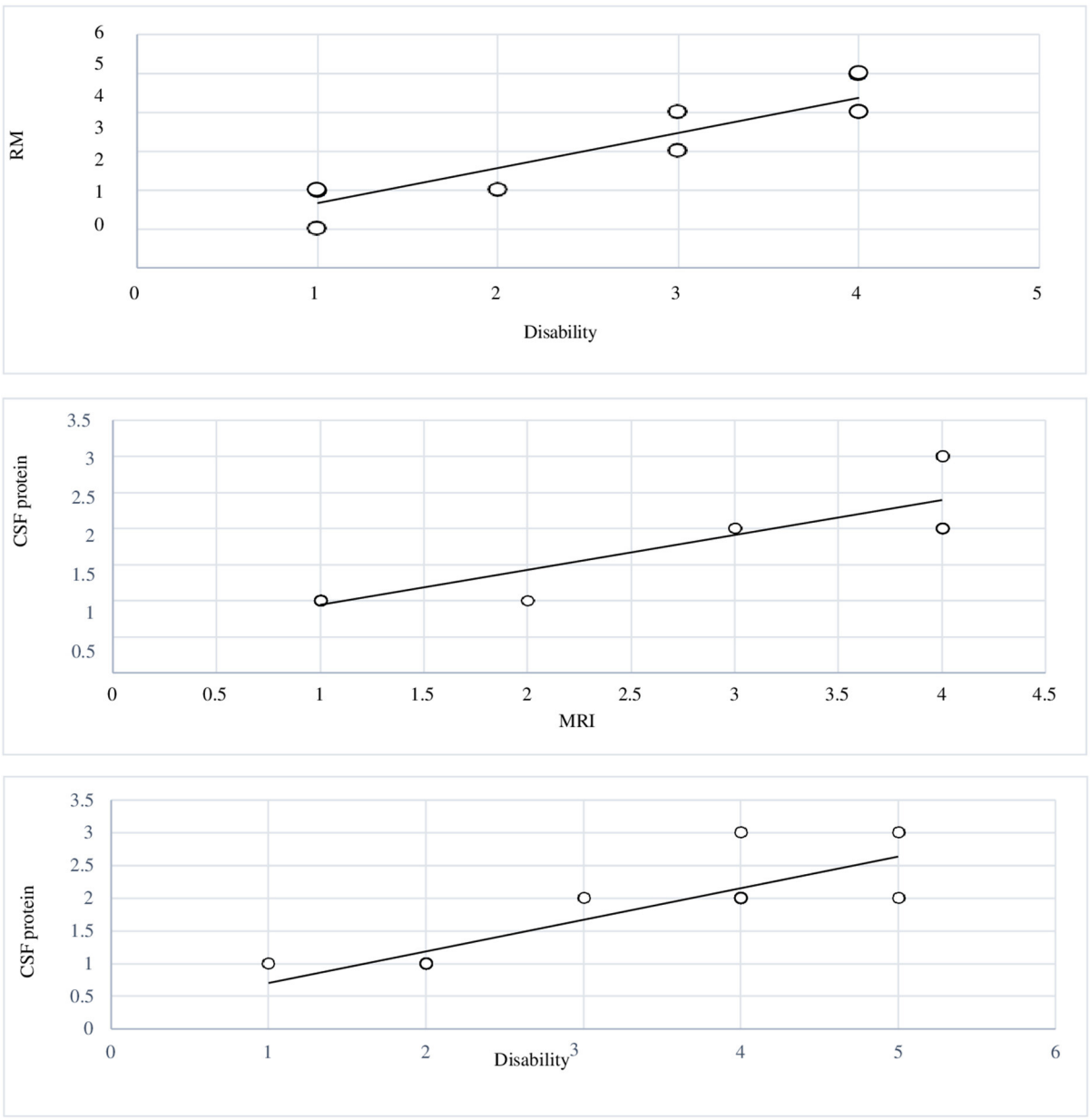


FIGURE 3
Graphic 1 showing the correlation between MRI brain lesions and levels of disability: Pearson's correlation index coefficient was 0.92. Graphic 2 The graphic showing the correlation between levels of disability and elevated CSF protein; r-value 0.85. Graphic 3 reports the correlation between MRI brain lesions and elevated CSF protein; r-value 0.87.

with GBS, MRI imaging, and CSF protein levels. We measured the correlation index between the clinical and assessed and the GBS and MRI scores according to Yakilmaz Classification. We observed that the CSF parameters that correlated with GBS disability score at 2 weeks were protein levels in the CSF, when these were increased 1, 2, and 3 times the cut-off value of 45 mg/dl as Kerasnoudis et al. reported (16).

Results

We performed an extensive literature search and found no publications evaluating a possible correlation among the clinical severity of GBS, imaging, and laboratory values of CSF proteins combined in the same population.

Two children showed notable signs of respiratory impairment and were intubated. Three children showed

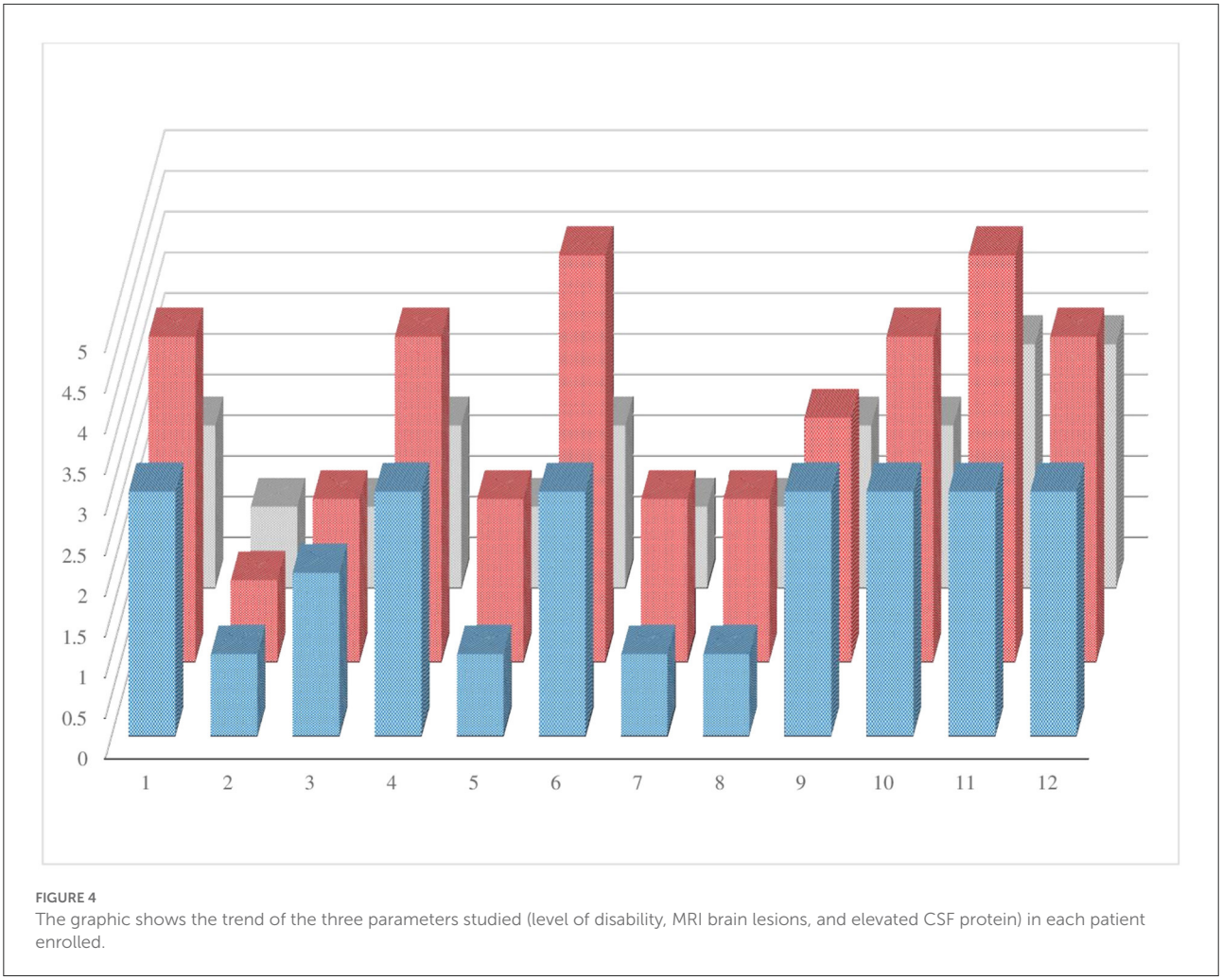


FIGURE 4
The graphic shows the trend of the three parameters studied (level of disability, MRI brain lesions, and elevated CSF protein) in each patient enrolled.

involvement of the cranial nerves. Children with respiratory disturbances involving the cranial nerves had a worse clinical course (Table 1). Trigger events were represented by gastrointestinal infections, high airway infections, and vaccination of 3, 8, and 1 cases, respectively (Table 5).

We executed the statistical analysis to measure the potential relationship among the clinical assessment of children with GBS, MRI imaging, and CSF protein levels.

It seems that these parameters have a parallel trend and a good correlation in each patient. The values of r calculated for all correlations are close to unity and therefore indicate a strong correlation value between them. We developed a graph to see the trend of the disability values, proteins in the CSF, and damage assessed with MRI (Figure 4).

Correlation between MRI lesions and grade of disability

The graph (Figure 3) shows the correlation between brain MRI lesions according to Yikilmaz et al. classification (11) and grade of disability. Pearson's correlation index coefficient was 0.92 ($R^2 = 0.8573$).

Correlation between increased CSF protein and grade of disability

We assessed the chemical-physical characteristics of the CSF in each patient: appearance, color, the number of cells, glucose, proteins, presence of immunoglobulins, and albumin.

We examined these parameters one by one and the correlation with the GBS disability score and the prognosis.

The single parameter showing the more robust correlation with prognosis was the increase of the proteins in the CSF.

The graph (Figure 3) shows the correlation between levels of disability and increased levels of CSF protein. Pearson's correlation index coefficient was 0.85 ($R^2 = 0.7342$).

Correlations between MRI brain lesions and increased CSF protein

Our pediatric neuroradiologists team evaluated and classified magnetic resonance images following the Yakilmaz radiological classification (14). This classification presented in the study was made for descriptive purposes and the potential prognostic use was not investigated.

The graph (Figure 3) reports the correlation between MRI brain lesions and increased levels of CSF protein. The correlation index coefficient had a value of 0.87.

Correlation among the three parameters observed in this study

The graph (Figure 4) was performed to evaluate the trend of the three parameters studied (level of disability, MRI brain lesions, and increased levels of CSF protein) in each of the 12 patients. In the graph, the variables considered have a parallel trend in the population studied.

Conclusion

Staging the pediatric patient affected by GBS assumes an important value both in the general evaluation of the patient and in the prognosis. Currently, prognostic scores are based exclusively on clinical parameters, such as the GBS disability scale and the Erasmus GBS scale. There is no clinical-prognostic score that takes into consideration radiological or laboratory parameters.

To fill this gap, we tried to evaluate the correlation between the imaging staging of GBS on MRI and the chemical-physical examination of the CSF of 12 pediatric patients treated.

In our evaluations, we were able to evaluate a good correlation between them.

We assessed that MRI should be considered an essential and effective exam in the diagnostic and prognostic evaluation process in GBS. The involvement of the anterior and posterior nerve root is associated with severe clinical conditions and, consequently, with a worse prognosis (17).

Brain MRI then should be performed routinely in children with GBS to estimate the possible evolution of the clinical condition.

Regarding the CFS analysis, we observed that among the various elements of cerebral liquor, the best indication for clinical condition and prognostic evaluation were related mainly to the high level of CFS protein, it has also been reported in the literature (18, 19).

The MRI and CSF laboratory parameters can be important tools for the clinician not only for diagnosis but also for having an estimate of the possible worsening of general conditions or the need to prepare measures to support life parameters.

We believe it is important to establish how early the need for ventilatory support in the most severe patient is or to be able to decide to start physiotherapy in less severe cases. The need for this study also arises to give indications to the clinician in the sub-acute phase of the disease.

Our study aimed to predict outcomes in GBS by the use of acute phase clinical features, laboratory analysis, and imaging. We have not found in the literature other studies that use laboratory, imaging, and clinical parameters to stage GBS together.

On the other hand, our systematic search of the literature did not reveal studies that simultaneously evaluate the laboratory aspects of CSF and imaging and their relationship to the clinical conditions of children with GBS.

Especially considering the ongoing International GBS Outcome Study, the potential association among CSF protein, imaging, and disability score may be worthy of further exploration (20).

The study has several limitations in our opinion: the population has a heterogeneous age and this could vary the response to the disease of different patients. In the literature, the early age of onset is a negative prognostic factor (11). The sample is numerically small, and a larger sample of patients coming from different centers would be needed for the next studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of the University of Catania, Italy (Ethical Committee Catania 1 Clinical Registration No. 95/2018/PO). Written informed consent was obtained from the parents.

Author contributions

PP, FP, AF, and MR worked with and helped gather patient data. FG, TT, RR, AG, GC, and ADN drafted the present manuscript. AB, GB, and ADM were the radiologist consultant. PP, ET, MS, and AG were responsible for revising the work

critically for important intellectual content. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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