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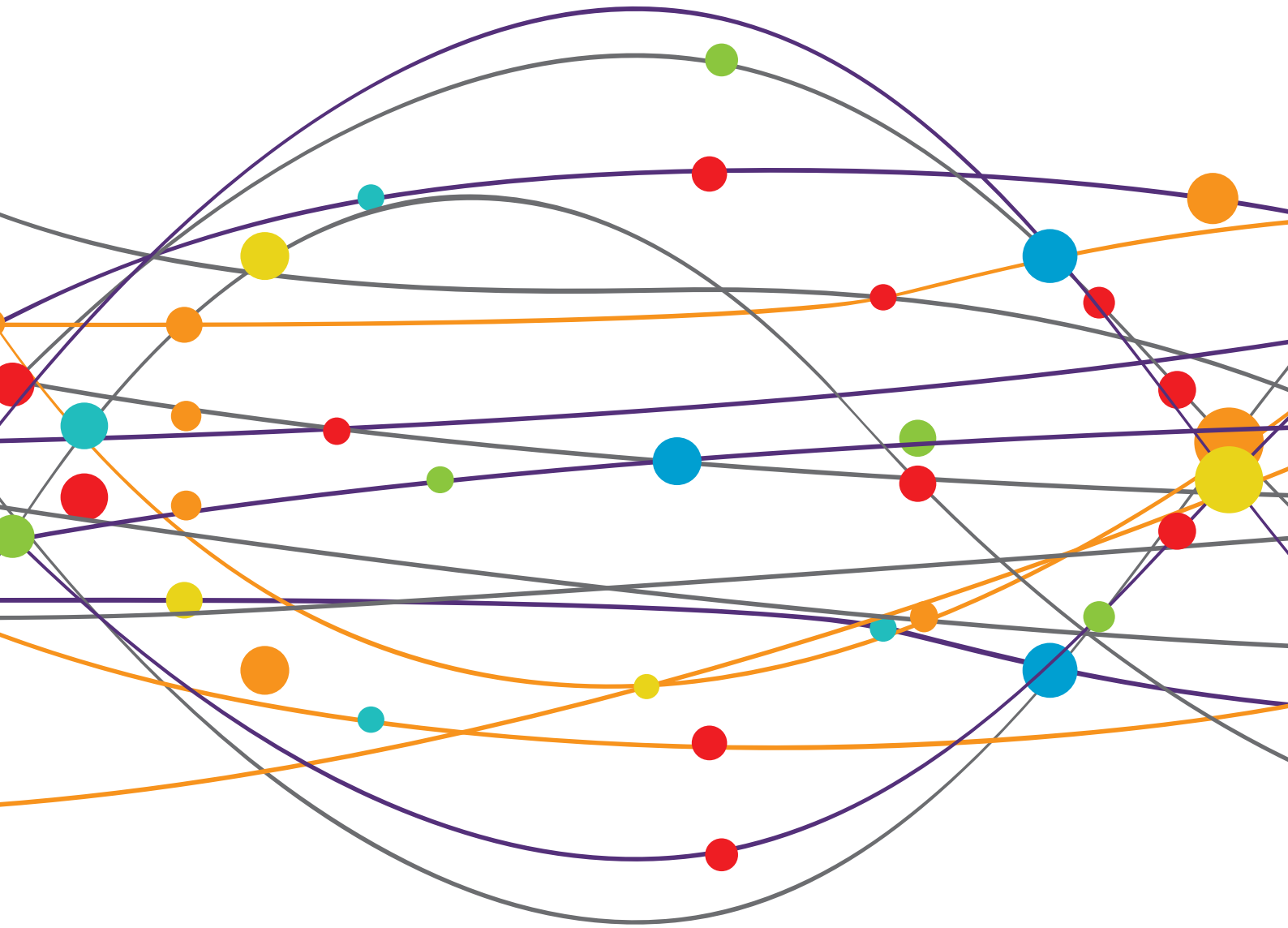

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PEDIATRIC NEUROLOGY EDITOR'S PICK 2021

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REVIEW
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Virtual Reality Enhances Gait in Cerebral Palsy: A Training Dose-Response Meta-Analysis

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Virtual-reality-based training can influence gait recovery in children with cerebral palsy. A consensus concerning its influence on spatiotemporal gait parameters and effective training dosage is still warranted. This study analyzes the influence of virtual-reality training (relevant training dosage) on gait recovery in children with cerebral palsy. A search was performed by two reviewers according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines on nine databases: PEDro, EBSCO, PubMed, Cochrane, Web of Science, EMBASE, ICI, Scopus, and PROQUEST. Of 989 records, 16 studies involving a total of 274 children with cerebral palsy met our inclusion criteria. Eighty-eight percent of the studies reported significant enhancements in gait performance after training with virtual reality. Meta-analyses revealed positive effects of virtual-reality training on gait velocity (Hedge's $g = 0.68$), stride length (0.30), cadence (0.66), and gross motor function measure (0.44). Subgroup analysis reported a training duration of 20–30 min per session, $\leq$4 times per week across $\geq$8 weeks to allow maximum enhancements in gait velocity. This study provides preliminary evidence for the beneficial influence of virtual-reality training in gait rehabilitation for children with cerebral palsy.

Keywords: cerebral palsy, gait, virtual reality, brain injury, rehabilitation

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INTRODUCTION

Gait dysfunctions are prominent in children with cerebral palsy (1, 2). Reduction in gait velocity, cadence and stride length are common spatiotemporal gait characteristics exhibited by children with cerebral palsy (2). Recent experimental and review studies have reported the beneficial influence of virtual-reality training strategies to considerably influence gait performance in children with cerebral palsy (3, 4). According to Aminov et al. (5), virtual reality is a superior rehabilitative approach when compared with conventional therapeutic approaches. The authors suggest that this strategy can allow a patient to (re)learn motor skills while interacting with real-life scenarios in an ecological yet patient-centric manner (6).

The application of this intervention is dynamic as it allows real-time "multisensory" feedback of executed movement to both the performer and the medical practitioner. This further can simultaneously facilitate the motor planning and perception of the performer and allow the medical practitioner to monitor and control the complexity of the virtual-reality task/environment according to each performer's capability (7). Several underlying mechanisms through which virtual-reality training can facilitate motor rehabilitation have been reported. For instance, amplification of sensorimotor representation by augmented sensory feedback (8–12), enhancement of error feedback (13), reduction of cognitive load (14–17), reduction of musculoskeletal coactivation (18), increased arousal (19), and motivation (20) are few of the

reasons by which virtual-reality training might enhance gait recovery (3, 4, 21). Moreover, neuroimaging studies have reported that training with virtual reality can facilitate recovery by instigating cortical reorganization (22) and neural plasticity (23, 24), thus suggesting a strong potential for virtual-reality-based training for recovering gait in children with cerebral palsy.

Recent systematic reviews have reported the beneficial effects of virtual-reality-based training on gait performance in children with cerebral palsy (3, 4). However, to the best of our knowledge, only one study has elucidated the influence of virtual-reality training on gait performance in children with cerebral palsy statistically, i.e., a meta-analysis (3). Chen et al. (3) performed a meta-analysis on eight studies and reported a positive effect size of 0.75 (0.34–1.16) on the ambulation function after training with virtual reality. Although the findings of this study are in line with previous reviews, there were certain limitations. Firstly, the authors did not explore the cause of heterogeneity observed in the analysis, i.e., $I^2 = 59\%$. Secondly, the authors did not describe the specific variables evaluated in the ambulation function, i.e., no information was provided as to what these enhancements were applicable on, for instance, gait velocity, stride length, etc. Thirdly, the authors included some studies in the analysis that, on re-evaluation, were found to not have evaluated any gait parameter at all.

In the present systematic review and meta-analysis, our aim is to develop a state of evidence defining the influence of virtual-reality training on spatiotemporal gait parameters in children with cerebral palsy. Moreover, the importance of determining training dosages in neurological rehabilitation has been emphasized in several studies (25–31). Therefore, as a secondary objective, this present review also aims to elucidate effective training dosages for virtual-reality-based gait training that could be incorporated by medical practitioners during gait rehabilitation for children with cerebral palsy.

METHODS SUMMARY

This review and meta-analysis was performed according to PRISMA guidelines (32). A systematic search of literature was performed across nine academic databases. The inclusion criteria were as follows: (i) Randomized controlled trials (RCTs) and Controlled clinical trials (CCTs), (ii) virtual-reality interventions (any training duration and setting), (iii) spatiotemporal gait parameters evaluated, (iv) gross motor function and/or performance measure evaluated, (v) $\geq$4 PEDro score, (vi) children with cerebral palsy (age range: 6–18 years), (vii) peer-reviewed publications, and (viii) in English, German, Hindi, Punjabi, and Sanskrit languages. For a detailed method section, see the **Supplementary Material**.

Quality and Risk of Bias Assessment

The quality of the reviewed studies was assessed using the PEDro scale by both the reviewers (33).

Level of Evidence Assessment

A level of evidence analysis, i.e., strength of recommendation, was assigned to each outcome measure, i.e., gait velocity, stride length, cadence, stride width, and gross motor function measure. This assessment was combinedly based on the methodological quality and design of the evaluated studies (34).

Data Analysis

A within-group, i.e., pre–post meta-analysis, approach was performed to develop a better quantitative interpretation of the virtual-reality intervention (35). The meta-analyses were conducted using CMA (Comprehensive meta-analysis V 2.0, USA). The meta-analyses evaluated the influence of virtual-reality training on gait velocity, cadence, stride length, stride width, and gross motor function measure score. An analysis for publication bias was performed by Duval and Tweedie's trim and fill procedure (36). The alpha level was set at 5%.

Characteristics of Included Studies

The initial search across the nine academic databases yielded a total of 989 studies, which, upon implementing the inclusion criteria, were reduced to 16 (**Figure 1**). Thereafter, both qualitative and quantitative data were extracted from all the studies (**Supplementary Table 2**). Of the 16 included studies, 5 were randomized controlled trials and 11 were controlled clinical trials.

Participants

A total of 274 participants were analyzed in the 16 incorporated studies. The included studies provided data on 120 females and 154 males. Descriptive statistics relating to the age (mean $\pm$ standard deviation, range) have been mentioned in **Supplementary Table 3**. In the included studies, three studies did not define the gender distribution (37–39).

Risk of Bias

Individual scores attained by the studies using the PEDro scale for each factor have been mentioned (**Supplementary Table 4**). The average PEDro score of the 18 included studies was computed to be (M $\pm$ S.D). 5.7 $\pm$ 1.4 out of 10, indicating, on average, a "good" quality of the studies. Here, one study scored 9 (40), one study scored 8 (41), three scored 7 (39, 42, 43), two scored 6 (44, 45), six scored 5 (37, 46–50), and three studies scored 4 (38, 51, 52). The risk of biasing across the studies has been illustrated in **Figure 2**. Individual scoring by the studies on each parameter has been mentioned in **Supplementary Table 3**.

In this present study, publication bias was analyzed by Duval and Tweedie's trim and fill method. The graph plots the evaluated weighted effect size, i.e., Hedge's *g* values against standard error (**Figure 3**). Here, the absence of publication bias is determined by symmetrical distribution of the studies about the combined effect size. The trim and fill test elucidated any missing studies based on a fixed effect model in the present analysis. However, the method suggests that no studies are missing. Under the random effects model, the point estimate is 0.48 and the 95% confidence interval (CI) is 0.26–0.71 for the combined studies. Using trim and fill, the imputed point estimate is 0.66 and the 95% CI is 0.43–0.89.

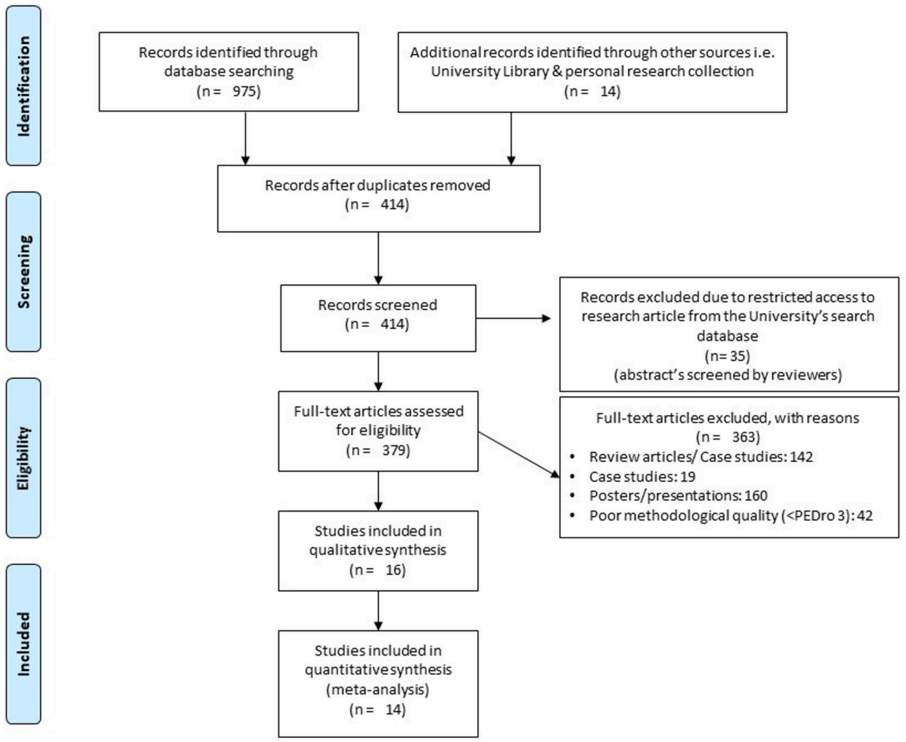

FIGURE 1 | PRISMA flowchart for the inclusion of studies (32).

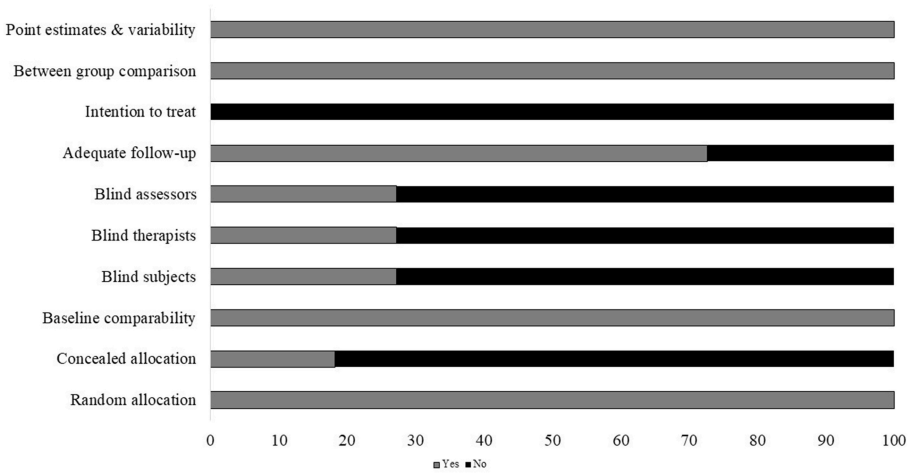

FIGURE 2 | Risk of bias across studies (x axis: %).

Level of Evidence

The analysis of level of evidence based on evidence-based nursing care guidelines revealed a "III Level of Evidence," supporting the beneficial effects of virtual-reality training on gait and motor performance in children with cerebral palsy. This level of evidence was awarded to all the evaluated parameters and dose–response subgroup analyses. The appraisal of level of evidence score was based on the design and scoring of the included studies, i.e., controlled clinical trials.

OUTCOMES

The current qualitative and quantitative evidence from the review suggests the beneficial effects of virtual-reality training

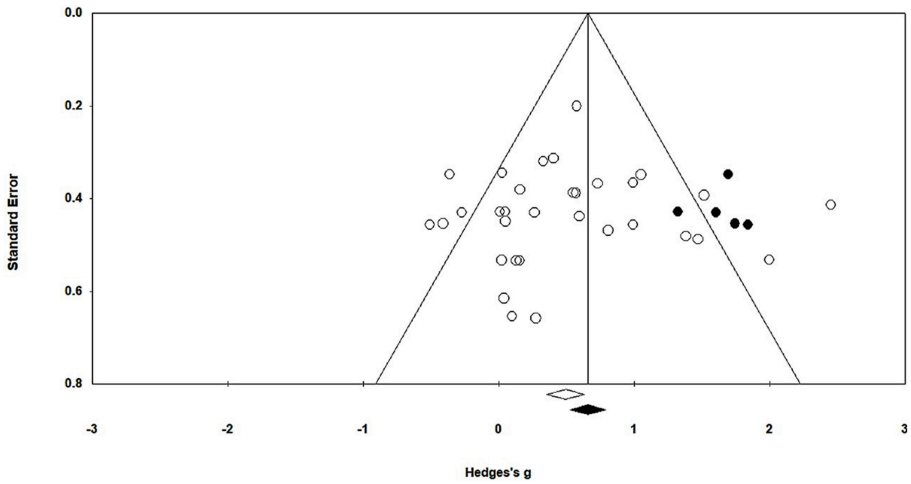

FIGURE 3 | Trim and fill funnel plot for Hedge's g and standardized effect for each value in the meta-analysis. Each of the effect is represented in the plot as a circle. Funnel boundaries represent area where 95% of the effects are expected to lie if there were no publication biases. The vertical line represents the mean standardized effect of zero.

on spatiotemporal gait parameters for children with cerebral palsy. Nine included studies reported significant enhancement in gait performance for children with cerebral palsy after virtual-reality training. Two studies reported no influence of virtual-reality training gait on spatiotemporal gait parameters (see **Supplementary Table 3**) (48, 50).

META-ANALYSIS REPORT

Gait Velocity

Gait velocity was assessed among 13 studies. Additional data were extracted from one study (40). The analysis of studies revealed (**Figure 4**) a *medium* effect size in the positive domain ($g = 0.68$, 95% CI: 0.35 to 1.01) with negligible heterogeneity ($I^2 = 13.1\%$, $p > 0.05$). In the included studies, only one study did not utilize a training intervention with virtual reality (50). Therefore, in a subsequent analysis, we removed this study and reperformed the analysis to elucidate the influence of virtual-reality training on gait velocity. The analysis of studies revealed (**Supplementary Figure 1**) a *large* effect size in the positive domain ($g = 0.76$, 95% CI: 0.44 to 1.07) with negligible heterogeneity ($I^2 = 10.7\%$, $p > 0.05$).

Session Length

20–30 min: A subgroup analysis was performed on nine studies. The analysis revealed a *large* effect size (**Supplementary Figure 2**) in the positive domain ($g = 0.88$, 95% CI: 0.51 to 1.24) with negligible heterogeneity ($I^2 = 12.1\%$, $p > 0.05$).

40–45 min: A subgroup analysis was performed on three studies. The analysis revealed a *medium* effect size (**Supplementary Figure 3**) in the positive domain ($g = 0.26$, 95% CI: −0.24 to 0.77) with no heterogeneity ($I^2 = 0\%$, $p > 0.05$).

Sessions per Week

≤4 sessions per week: A subgroup analysis was performed on six studies. The analysis revealed a *large* effect size (**Supplementary Figure 4**) in the positive domain ($g = 0.78$, 95% CI: 0.09 to 1.47) with negligible heterogeneity ($I^2 = 1.4\%$, $p > 0.05$).

≥5 sessions per week: A subgroup analysis was performed on six studies. The analysis revealed a *medium* effect size (**Supplementary Figure 5**) in the positive domain ($g = 0.69$, 95% CI: 0.42 to 0.97) with negligible heterogeneity ($I^2 = 2.4\%$, $p > 0.05$).

Number of Weeks of Training

≥8 weeks: A subgroup analysis was performed on four studies. The analysis revealed a *medium* effect size (**Supplementary Figure 6**) in the positive domain ($g = 0.69$, 95% CI: 0.25 to 1.13) with negligible heterogeneity ($I^2 = 2.0\%$, $p > 0.05$).

≤7 weeks: A subgroup analysis was performed on eight studies. The analysis revealed a *medium* effect size (**Supplementary Figure 7**) in the positive domain ($g = 0.65$, 95% CI: 0.35 to 0.94) with no heterogeneity ($I^2 = 0.69\%$, $p > 0.05$).

Stride Length

Stride length was assessed among four studies. Additional data were extracted from one study (40). The analysis of the studies revealed (**Figure 5**) a *medium* effect size in the positive domain ($g = 0.30$, 95% CI: −0.40 to 1.01) with no heterogeneity ($I^2 = 0\%$, $p > 0.05$). In the included studies, only one study did not utilize a training intervention with virtual reality (50). Therefore, in a subsequent analysis, we removed this study and reperformed the analysis to elucidate the influence of virtual-reality training on stride length. The analysis of studies revealed (**Supplementary Figure 8**) a *medium* effect size in the positive

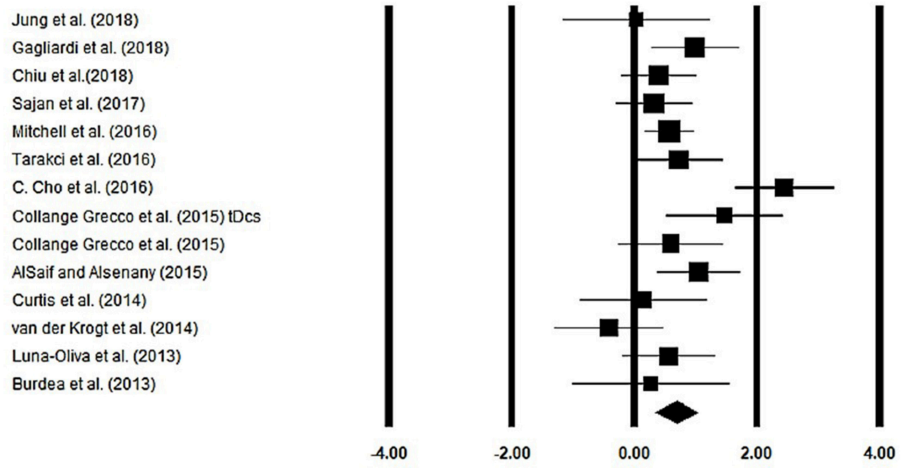

FIGURE 4 | Forest plot illustrating individual studies evaluating the effects of virtual-reality training on gait velocity among children with cerebral palsy. Weighted effect sizes, Hedge's g (boxes), and 95% CI (whiskers) are presented, demonstrating repositioning errors for individual studies. The (Diamond) represents pooled effect sizes and 95% CI.

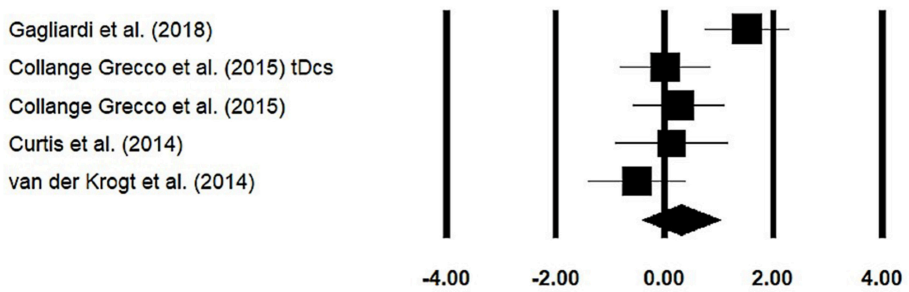

FIGURE 5 | Forest plot illustrating individual studies evaluating the effects of virtual-reality training on stride length among children with cerebral palsy. Weighted effect sizes, Hedge's *g* (boxes), and 95% CI (whiskers) are presented, demonstrating repositioning errors for individual studies. The (diamond) represents pooled effect sizes and 95% CI.

domain ($g = 0.50$, 95% CI: −0.20 to 1.24) with no heterogeneity ($I^2 = 0\%$, $p > 0.05$).

Cadence

Cadence was assessed among two studies. Additional data were extracted from one study (40). The analysis of studies revealed (**Supplementary Figure 9**) a *medium* effect size in the positive domain ($g = 0.66$, 95% CI: −0.52 to 1.84) with negligible heterogeneity ($I^2 = 10.8\%$, $p > 0.05$).

Stride Width

Stride width was assessed among three studies. Additional data were extracted from one study (40). The analysis of studies revealed (**Figure 6**) a *small* effect size in the negative domain ($g = -0.07$, 95% CI: −0.57 to 0.43) with negligible heterogeneity ($I^2 = 4.5\%$, $p > 0.05$). In the included studies, only one study did not utilize a training intervention with virtual reality (50). Therefore, in a subsequent analysis, we removed this study and reperformed the analysis to elucidate the influence of virtual-reality training on stride width. The analysis of studies revealed (**Supplementary Figure 10**) a *small* effect size in the negative domain ($g = -0.23$, 95% CI: −0.53 to 0.06) with moderate heterogeneity ($I^2 = 0\%$, $p > 0.05$).

Gross Motor Function Measure

Gross motor function measure was assessed among six studies. The analysis of studies revealed (**Figure 7**) a *medium* effect size in the positive domain ($g = 0.44$, 95% CI: 0.06 to 0.83) with negligible heterogeneity ($I^2 = 0.54\%$, $p > 0.05$).

Session Length

20–30 min: A subgroup analysis was performed on four studies. The analysis revealed a *medium* effect size (**Supplementary Figure 11**) in the positive domain ($g = 0.56$, 95% CI: 0.05 to 1.07) with negligible heterogeneity ($I^2 = 0.08\%$, $p > 0.05$).

40–45 min: A subgroup analysis was performed on two studies. The analysis revealed a *small* effect size (**Supplementary Figure 12**) in the positive domain

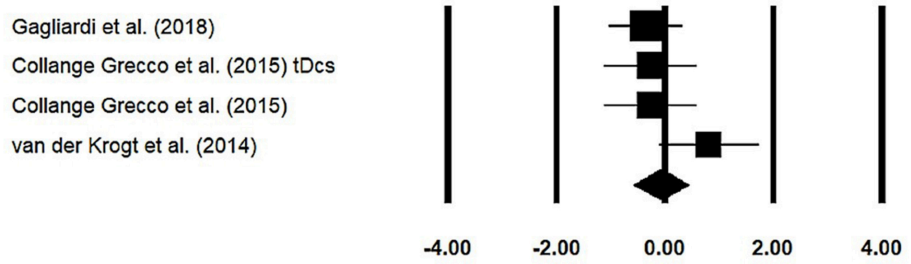

FIGURE 6 | Forest plot illustrating individual studies evaluating the effects of virtual-reality training on stride width among children with cerebral palsy. Weighted effect sizes, Hedge's *g* (boxes), and 95% CI (whiskers) are presented, demonstrating repositioning errors for individual studies. The (diamond) represents pooled effect sizes and 95% CI.

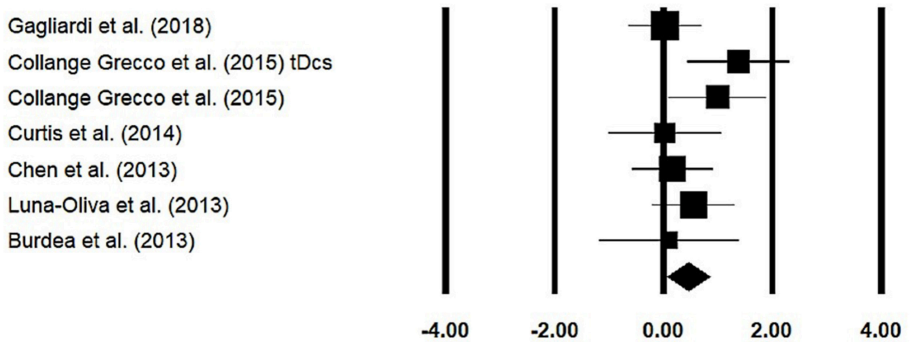

FIGURE 7 | Forest plot illustrating individual studies evaluating the effects of virtual-reality training on gross motor function measure among children with cerebral palsy. Weighted effect sizes, Hedge's *g* (boxes), and 95% CI (whiskers) are presented, demonstrating repositioning errors for individual studies. The (diamond) represents pooled effect sizes and 95% CI.

($g = 0.14$, 95% CI: −0.50 to 0.78) with no heterogeneity ($I^2 = 0\%, p > 0.05$).

DISCUSSION

The primary objective of this present systematic review and meta-analysis was to synthesize the current state of knowledge to determine the effects of virtual-reality training on spatiotemporal gait parameters in children with cerebral palsy. The findings from the current meta-analyses suggest a positive influence of virtual-reality training to enhance gait performance. Spatiotemporal parameters, i.e., gait velocity, cadence, and stride length, which are usually adversely affected in cerebral palsy (53), were enhanced after training with virtual reality, i.e., gait velocity ($g = 0.76$), stride length ($g = 0.76$), and cadence ($g = 0.80$).

Studies have suggested that virtual-reality training can facilitate motor performance by providing a performer with real-time "multisensory" feedback of the executed movement (19, 54, 55). Children with cerebral palsy have been reportedly associated with substantial deficits in sensory perception, which might affect their motor planning and performance (56). Here, the addition of different sensory modalities, for instance, auditory, visual, and proprioceptive feedback, could provide a "sensory deficit" patient with enriched knowledge of performance (movement amplitudes, relative limb position) and result (4, 19, 57–59).

Additionally, the enhancements in spatiotemporal gait parameters could be attributed to substantial changes in force, power, and kinematics at the ankle and knee joints (60). According to Chen et al. (46), virtual-reality-based training can substantially enhance isokinetic muscle strength and the amount of physical activity performed by children with cerebral palsy. This was also demonstrated in our analysis where gross motor function measure was enhanced (0.45) after training with virtual reality. In terms of movement kinematics, we presume that the explicit multisensory (i.e., visual–auditory–proprioceptive) feedback concerning the movement execution within the virtual environment could have allowed the patients to specifically time and control their movement patterns [see guidance hypothesis (61, 62)], thereby promoting a smooth movement pattern with reduced musculoskeletal co-contraction (18). In this review, several studies reported enhancement in gait kinematic scores (45, 50, 63, 64), which usually are adversely affected in children with cerebral palsy.

Furthermore, the patient-centered, closed-loop [tailored difficulty progression (65)] approach of virtual-reality training could be an additional reason for the superior influence of

this rehabilitation intervention as compared to conventional approaches like resistance training (66), rhythmic auditory cueing (67), and robot-assisted training (68). Here, linking the individual performance measures concerning the motor restrictions and cognitive performance with the adaptive modulation of the task to be trained can provide adaptive mechanics in the virtual environment that might facilitate neuroplasticity (69). For instance, Xiao et al. (22) in a neuroimaging study reported the beneficial influence of virtual-reality-based training on motor planning and execution centers. The authors reported enhanced activations in primary, secondary motor, sensorimotor, and premotor cortices in stroke patients after virtual-reality training [also see (19, 70)]. Interestingly, the authors also reported hyperactivity in the ipsilesional somatosensory cortex with virtual-reality training (22). This enhanced activation in the somatosensory cortex of the affected hemisphere could be interpreted as unmasking of (pre)existent movement patterns (functional recovery *via* motor relearning) (70, 71).

Finally, we also elucidated specific virtual-reality training dosages that could be beneficially incorporated to attain maximum benefits in gait recovery in children with cerebral palsy. Fluet and Deutsch (72) had previously emphasized future studies for deducing training dosages in neurorehabilitation. The authors hypothesized that larger training dosages might account for more enhancements in motor recovery. However, as per the current findings of this meta-analysis, this was not the case. In terms of the length of training sessions, higher increments in spatiotemporal gait parameters were noted during training interventions lasting for 20–30 min as compared to 40–45 min of training (gait velocity: 0.88 vs. 0.26, gross motor function test: 0.56 vs. 0.14). Likewise, similar increments were noted for the number of sessions per week, i.e., $\leq$4 sessions allowed higher increments in gait velocity as compared to $\geq$5 weeks (0.84 vs. 0.65). In terms of the number of weeks of training, more number of weeks was observed to allow a greater influence on training, i.e., $\geq$8 weeks allowed better performance as compared to $\leq$7 weeks of training (0.83 vs. 0.65).

Two major limitations persisted in the present review. First, this study was not pre-registered in a prospective register for systematic reviews, such as PROSPERO. Second, a dose–response meta-analysis was performed for some variables with very few number of studies. This could raise concerns regarding the reliability of some outcome measures, i.e., incurring a type II error. We strongly recommend the reader to interpret the results with caution. Nevertheless, our findings are in line with previously published "high-quality" systematic reviews and meta-analyses that report positive or negligible effects of virtual-reality training on gait performance in children with cerebral palsy (3, 4). However, this present review extends the findings of these studies due to several reasons. Firstly, the present review incorporates a higher number of experimental studies that support our conclusion, i.e., 16 studies (274 participants) as compared to previously published review studies (3, 4). This large difference in the number of included studies could be attributed to the higher number of relevant academic databases searched (with multiple languages), i.e., nine, and the inclusion of controlled clinical trials. Secondly, this current review presents robust evidence to suggest training dosage with virtual reality for allowing enhancements in gait velocity and gross motor function test. Thirdly, this study provides evidence for the beneficial effects of virtual-reality training on generalized physical activity and motor output. It is important for the reader to consider that it is not our intention to disregard previously published reviews and meta-analyses. These reviews have addressed different factors for individuals with cerebral palsy (i.e., quality of life, arm recovery, and postural control), which was not the objective of the present review. Therefore, in our opinion, interpretations should be drawn simultaneously from all the reviews to develop a better understanding of the influence of virtual-reality-based training strategies for gait recovery after cerebral palsy. A III Level of Evidence supported the beneficial effects of virtual-reality-based training on gait performance. This weak level of evidence strongly warrants the need for multiple, high-quality, multicentered, randomized controlled trials to support the application of virtual-reality training on gait performance in children with cerebral palsy.

In conclusion, virtual-reality training facilitates gait performance in children with cerebral palsy. The present study reports the influence of virtual-reality-based training on the most commonly evaluated spatiotemporal gait outcomes; this shall allow the clinicians to better interpret the results with previous studies and other interventions and deduce practical implications for the benefit of children with cerebral palsy. The review, based on limited state of evidence, i.e., III Level of Evidence, suggests a training duration of at least 20–30 min, $\leq$4 times per week across $\geq$8 weeks.

AUTHOR CONTRIBUTIONS

SG conceptualized the study, carried out the systematic-review, statistical analysis, and wrote the paper. IG assisted in the systematic-review process and reviewed the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00236/full#supplementary-material

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Ketogenic Diet as a Treatment for Super-Refractory Status Epilepticus in Febrile Infection-Related Epilepsy Syndrome

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Background: Febrile infection-related epilepsy syndrome (FIRES) is a fatal epileptic encephalopathy associated with super-refractory status epilepticus (SRSE). Several treatment strategies have been proposed for this condition although the clinical outcomes are poor. Huge efforts from neurointensivists have been focused on identifying the characteristics of FIRES and treatment to reduce the mortality associated with this condition. However, the role of ketogenic diet (KD) in FIRES is not fully understood.

Methods: We performed a retrospective review of patients who met the diagnostic criteria of FIRES, SRSE, and were treated with KD between 2015 and 2018 at the Department of Pediatrics, Xiangya Hospital of Central South University. The following data were recorded: demographic features, clinical presentation, anticonvulsant treatment, timing and duration of KD and follow-up information. Electroencephalography recordings were reviewed and analyzed.

Results: Seven patients with FIRES were put on KD (5 via enteral route, and 2 via intravenous line) for SRSE in the PICU. The median age was 8. Four patients were male and 3 were female. Although patients underwent treatment with a median of 4 antiepileptic drugs and 2 anesthetic agents, the status epilepticus (SE) persisted for 7–31 days before KD initiation. After KD initiation, all patients achieved ketosis and SE disappeared within an average of 5 days (IQR 3.5), although there were minor side effects. In 6 patients, a unique pattern was identified in the EEG recording at the peak period. After initiation of KD, the number of seizures reduced, the duration of seizure shortened, the background recovered and sleep architecture normalized in the EEG recordings. The early initiation of KD (at the onset of SE) in the acute phase of patients decreased the mRS score in the subsequent period ($p = 0.012$, $r = 0.866$).

Conclusions: The characteristic EEG pattern in the acute phase promoted timely diagnosis of FIRES. Our data suggest that KD may be a safe and promising therapy for FIRES with SRSE, and that early initiation of KD produces a favorable prognosis. Therefore, KD should be applied earlier in the course of FIRES. Intravenous KD can be an effective alternative route of administration for patients who may not take KD enterally.

Keywords: ketogenic diet, febrile infection-related epilepsy syndrome, super-refractory status epilepticus, PICU, EEG

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INTRODUCTION

Febrile infection-related epilepsy syndrome (FIRES) is a rare epileptic encephalopathy of unknown etiology which occurs in patients without active epilepsy or underlying neurological disorders. It is characterized by new onset of refractory status epilepticus (SE) following a short nonspecific febrile illness (1). It is a biphasic disease, with an acute phase of refractory SE that always transforms into super-refractory SE (SRSE, i.e., SE that continues or recurs 24 h or more after the onset of anesthesia). This condition often requires intensive care and management, and it often progresses to a chronic phase which is characterized by refractory epilepsy without an intervening silent period. In the acute phase, the risk of mortality is high. Moreover, survivors are likely to live with a drug-resistant epilepsy and neuropsychological impairment. Nevertheless, few individuals do not experience neurologic sequelae or mild learning difficulties (2). Multiple therapeutic modalities have been reported for this disease, but most of them do not effectively control the high epileptic activity in such patients (3). Therefore, it is imperative to develop FIRES therapies with high efficacy.

Ketogenic diet (KD) contains high-fat, low-carbohydrate, and moderate protein content. It alters the primary cerebral energy supply from glucose to ketone bodies, mimicking the biochemical changes of starvation (4, 5). Although the exact mechanisms of KD's are not well-defined, it is thought that KD has antiepileptic activity, anti-inflammatory effect and neuroprotective activity (6–9) making it a therapeutic target for FIRES. In recent years, several small case studies have highlighted the value of diet in the treatment of FIRES. Results from such studies indicate that diet is not only suitable for acute patient management, but also for long-term management (10, 11). In April 2018, the International Ketogenic Diet Study Group have reached a consensus that KD is particularly useful for FIRES (12).

Despite fatal presentation, high disability and fatality rate in patients with FIRES, this syndrome is rarely diagnosed. Patient with FIRES are often diagnosed using the exclusion diagnosis method, and this explains why patients with FIRES are often diagnosed at a late stage due to lack of early disease markers. Currently, there is inadequate scientific data on the therapeutic efficacy of early initiation of KD for FIRES. Therefore, we aimed to explore strategies for timely recognition of FIRES through EEG recordings. Moreover, the KD therapy was given to 7 patients with FIRES and SRSE to investigate the efficacy and safety of KD as well as determine the effect of early KD treatment on the prognosis of FIRES patients.

MATERIALS AND METHODS

Patients and Approvals

We carried out a retrospective review of 7 patients diagnosed with FIRES (13, 14) and SRSE, and treated with KD at the Department of Pediatrics of Xiangya Hospital in Central South of China between 2015 and 2018. This study was approved by The Central South University Institutional Review Board.

Clinical Data

The following patient data was collected: demographics, general situations, history of seizures or SE, Glasgow Coma Scale (GCS), total hospital length of stay (LOS), ICU LOS, and antiepileptic drugs (AEDs) used before KD initiation and at discharge. Anesthetic agents, SE duration prior to KD, KD ratio, type and duration of KD, presence of ketones, and KD side effects were also obtained. KD therapy, seizure burden and modified Rankin Scale score (mRS) score at follow-up were collected in the outpatient neurology clinics. The clinical data were supplemented by a retrospective review of the electronic medical records (EMC).

Considering that SE has a subtle or no clinical manifestations in FIRES, its diagnosis requires EEG monitoring. Here, two approaches were adopted to define SE. Before hospital admission, SE was defined by continuous clinical or electrographic seizures lasting 5 min or more and/or recurrent episodes without recovery in between episodes (15). After admission, SE was defined by either a single seizure lasting $\geq$30 min or recurrent seizures totaling $\geq$30 min in any 1-h period (16).

All EEG recordings were digital with time-locked synchronized video. Electrodes were positioned in accordance with the international 10–20 system of electrode placement. The EMG activity and ECG were also recorded. All patients were monitored with EEG for 2–15 h immediately after arrival at our PICU. The frequency of EEG monitoring varies, depending on available institutional resources and perceived clinical condition. All EEG recordings were analyzed individually by a board-certified pediatric neurologist and two Asian Epilepsy Academy (ASEPA)-certified electroencephalographers. The following EEG features were analyzed: seizure burden, interictal and periodic epileptiform discharges, background activity, and sleep architecture. Five segments of EEG recordings were reviewed selectively to confirm the specific EEG pattern of FIRES and the effectiveness of KD. We used the first recording at admission to our PICU as the 1# epoch, the last recording prior to initiation of KD as the 2# epoch. The recording at which SE disappeared after KD treatment was recorded as the 4# epoch, and the last recording before discharge as the 5# epoch. The 3# epoch was selected to divide the period between 2 and 4# approximately into two equal parts. All EEG data was stored in original format and was used for review.

Data were analyzed by reviewing of physician notes, laboratory findings, neuroimaging studies, EEG recordings and other supporting data.

Diet Initiation

Baseline laboratory examinations including complete blood counts, serum carnitine, chemistries, electrolytes, lipids, fatty acids were performed for all patients prior to KD initiation. These parameters were evaluated by a collaborative team of neurologists, critical care medicine personnel and nutrition experts to ensure there were no contraindications to KD therapy. KD was administered through two routes without a fasting period. All patients received enteral KD (EN KD)

except 2 patients with severe gastrointestinal dysfunction who were placed on intravenous KD (IV KD). The commercially available ketogenic formula (Jiantong, Kinton medical food. Ltd, Guangzhou, China) with a fat to protein and carbohydrate (in gram scale) ratio of 4:1 were administered via enteral route and mixed with the current formula at the prescribed ketogenic ratio. IV KD comprised of commercially available fat emulsion with medium-chain triglycerides (Lipovenoes MCT, Sino-Swed Pharmaceutical Corp. Ltd, Wuxi, China), dextrose and amino acid solutions (Vamin, Sino-Swed Pharmaceutical Corp. Ltd, Wuxi, China).

The KD was initiated at a low ratio (about 2–3:1 in EN KD, 1:1 in IV KD) and then gradually advanced to a ratio of 3–4:1. The calorie intake of KD was restricted to 1/3 of the estimated diet energy needs (75% of recommended dietary allowance) initially and increased by 1/3 every 1–3 days (slower in IV KD) to full estimated diet energy. This step-by-step approach was adopted to prevent hypertriglyceridemia or hyperamylasemia induced by high intake or infusion of lipids. All dextrose was removed from fluids and all medications were changed to low-carbohydrate forms. Glucose-free solutions such as anticonvulsants and saline were prepared as required. Electrolytes, minerals, vitamins, and micronutrients were prepared at normal concentrations. The enteral preparation was given 6–8 times per day. IV KD was infused continuously over 20 h and stopped for 4 h. When enteric-related complications such as gastrointestinal bleeding and enteroparalysis were resolved, EN KD was initiated and the calorie intake was increased by 1/10–1/5 of the estimated daily intake, adjusted after each 1–2 days to replace IV KD. Electrolytes, arterial blood gases, serum ketone bodies and glucose were measured once KD was initiated.

Data Analysis

Statistical analysis was performed using the Statistical Package for Social Science (IBM, SPSS Statistics Version 21). Proportions were calculated for continuous variables whereas medians and interquartile ranges (IQRs) were calculated for categorical variables. Pearson correlation analysis was applied for the duration of SE before KD initiation and mRS score at the latest follow-up (the normality test was completed).

RESULTS

Clinical Characteristics

In this series, all 7 patients (4 boys and 3 girls) were transferred from other health facilities and diagnosed with FIRES and SRSE in our PICU. All patients had a normal health and psychomotor development in their history. They did not have familial history of seizure or a known neurologic disease. The ages of patients at disease onset were between 1.5 and 13 years. The demographic data, clinical manifestations and management are described in **Table 1**. Out of 7 patients, 4 had a GCS score<8 at admission. The hospital LOS ranged from 35 to 86 days (median 46; IQR 18.5; mean 50.3 ± 18.5) and ICU LOS ranged from 19 to 61 days (median 32; IQR 15; mean 32.6 ± 14.7). Among them, no clear etiology could be determined despite extensive analyses ranging from microbiologic, metabolic and autoimmune causes, and early brain Magnetic Resonance Imaging (MRI).

All patients experienced a non-specific febrile illness prior to SE. Fever preceded the first seizures with a median duration of 4 days, ranging from 2 to 5 days. Shortly after onset of seizures, within 24 h, the seizures rapidly progressed into SE or became frequent with loss of consciousness between the attacks. An obvious feature from the first EEG segment (2–15 h) was the heavy burden of seizure and most of them were either focal or subclinical with altered consciousness. Out of the 7 patients, 5 had seizures comprising >50% in a 1-h period. Notable EEG parameters are described below and summarized in **Table 2**.

We identified a specific ictal pattern of the seizures in 6 patients. This seizure pattern consisted of sharp (or spike) wave and/or sharp (or spike) slow wave complex of low to moderate amplitude, arising from unilateral or bilateral focal area, spreading to the same hemisphere and/or bilaterally with higher amplitude and faster/slower frequency, sometimes shifting from one hemisphere to the contralateral (**Figure 1**). Interictally, some patients displayed unidentified sleep architecture and diffuse delta-theta background slowing, with or without multifocal sporadic or periodic epileptiform discharges (sharp or spike waves, complex waves). These features might be undistinguishable from non-intermittent ictal activity in severe cases.

All patients experienced SE for 7–31 days (median 12; IQR 11; mean 16.6 ± 9.5) before KD initiation. In spite of the conventional management with a median of four AEDs and two continuous anesthetic agents before initiation of KD, the nearly continuous electrical or electroclinical seizures were poorly controlled or reappeared when anesthetic agents were tapered. Moreover, 2 patients received several anesthetics assistants (Fentanyl or its derivatives, Cisatracurium Besilate, Vecuronium) to control volcanic seizures. Three patients were under full mechanical ventilation due to severe respiratory depression. Similarly, other therapies such as intravenous immunoglobulin (IVIg) (in all patients) and Methylprednisolone (in 5 patients) were ineffective before initiation of KD. Before initiation of KD, most of the patients (except three) has stopped immunotherapy before or early after admission at our hospital. The three patients were continued on a low-dose steroid therapy even after KD initiated.

Outcomes After KD Treatment

All patients achieved resolution of SRSE (**Table 3**) within a median of 5 days (IQR 3.5) after initiation of KD. The time to ketosis (defined as the level of serum β-hydroxybutyrate >1 mmol/L) of 6 of 7 patients ranged from 1 to 6 days, but 1 of 7 patients required 11 days to achieve ketosis after KD initiation. According to the EEG data, the seizure burden reduced, duration of seizure shortened, background recovered, and sleep architecture normalized gradually after KD initiation. Besides, 4 of 7 patients were weaned off general anesthetics within 15 days of KD initiation whereas one of them stopped anesthesia infusion before KD initiation to reduce respiratory secretions.

TABLE 1 | Patient characteristics and treatment.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Antecedent febrile infection	FUO	URTI	URTI	GE	URTI	FUO	FUO
Latent period*	3	5	4	4	2	5	3
Admit GCS score	4	3	9	E4VTM1	E1VTM1	Confusion	Confusion
Duration of SE before KD, *d*	31	12	11	15	11	29	7
No. of AEDs received before KD	5	4	4	5	3	4	4
AEDs received before KD	PHB, CZP, VPA, TPM, OXC	VPA, LEV, OXC, NZP	PHB, VPA, TPM, LEV	PHB, CZP, VPA, TPM, OXC	VPA, TPM, LEV	VPA, LEV, TPM, OXC	VPA, LEV, TPM, OXC
No. of anesthetic agents before KD	2	2	2	2	2	1	1
Anesthetic agents before KD	Midazolam, Propofol	Midazolam, Propofol	Midazolam, Propofol	Midazolam, Propofol	Midazolam, Propofol	Midazolam	Midazolam
Anesthetics assistants before KD	Suf, Cisatracurium Besilate, Vecuronium	/	/	/	Fentanyl	/	/
Other treatments before KD	MP, IVIG, PE	MP, IVIG	IVIG	MP, IVIG	MP, IVIG, PE	IVIG	MP, IVIG
AMV	Yes	No	No	Yes	Yes	No	No
KD ratio	1.2:1→ 4:1	1.5:1→ 3.2:1	2:1→ 2.5:1	1.5:1→ 3:1	0.7:1→ 4:1	2:1→ 3:1	3:1→ 4:1
Type of KD	Parental	Enteral	Enteral	Enteral	Parental	Enteral	Enteral

AEDs, antiepileptic drugs; CLB, clobazam; CZP, clonazepam; F, female; FUO, fever of unknown origin; GCS, Glasgow Coma Scale; GE, gastroenteritis; IVIG, intravenous immunoglobulin; LEV, levetiracetam; M, male; MP, methylprednisolone; NZP, nitrazepam; OXC, oxcarbazepine; PE, plasma exchange; PHB, phenobarbital; Suf, sufentanil; TPM, topiramate; URTI, upper respiratory tract infection; VPA, valproic acid.

**Latent period: the days between the febrile illness and onset of seizures.*

The two patients who received intravenous KD were changed to enteral diet route gradually after resolution of SRSE.

There was no severe acidosis (HCO_3^- <17 mmol/L) or hypoglycemia during KD therapy. Seven patients showed mild anomalies in their laboratory tests (**Table 3**). Of the 7 patients, 3 developed hyperlipemia or experienced diarrhea while 1 of 7 patients developed both conditions. Serum amylase increased to 389.7 mmol/L in one patient who was given IV KD, but normalized after adjustment of the proportion of KD ingredients, carbohydrate and by changing to enteral route. On discharge, all patients were followed up at the pediatric neurology outpatient clinics (**Table 3**). The number of AEDs used at hospital discharge ranged from 3 to 4 drugs. Duration from the time of discharge to the most recent follow-up ranged from 6 to 40 months. No patient remained on KD at the latest follow-up. Of 7 patients, one patient continued the diet for nearly 1 year compared to 6 patients who discontinued KD in 3 months. Among them, one patient had a recurrence of intractable epilepsy after about 2 weeks of treatment. The patient was, therefore, put on therapy of vagus nerve stimulation (VNS). The remaining patients were free of seizure attacks during the acute phase (4 were seizure-free, and one had intermittent seizures). At the time when KD was stopped, 4 of 7 patients had a mRS score ≤3, and the rate increased to 6 of 7 at the latest follow-up. A shorter duration of SE before KD initiation reflected a lower mRS score in the subsequent period ($p = 0.012$, $r = 0.866$). The recovery of phycomotor function was diverse among the patients, 3 cases (patient 2,3,7) were almost cured, 1 case (patient 5) had a significant improvement, and 3 cases (patient 1,4,6) showed a moderate improvement.

DISCUSSION

Although the clinical manifestations and disease course of FIRES have been well-defined in the literatures, this sudden and severe epileptic encephalopathy is still challenging. Patient with FIRES are often diagnosed using the exclusion diagnosis method, and this explains why patients with FIRES are often diagnosed at a late stage due to lack of early disease markers. FIRES is characterized by repeated and prolonged seizures, and patients with FIRES are always in prolonged pharmacologic coma which is often accompanied with medical complications. It is important to develop available diagnostic tools to facilitate prompt diagnosis and design effective therapies to improve the management and prognosis of this condition.

This study found that EEG monitoring is an effective method of diagnosing FIRES based on recordings of explosive seizures (most were subclinical) with high frequency. At the peak hours of SE, we identified a characteristic EEG pattern consisting of focal ictal activity of small to moderate amplitude at the onset of the seizure, which evolved into higher amplitude and faster/slower frequency, spreading unilaterally and/or bilaterally, sometimes shifting from one hemisphere to the contralateral. These features can be used for early recognition and intervention of FIRES.

Currently, there are no effective treatments for FIRES. Several case studies have shown that AEDs and immunological therapy do not satisfactorily control FIRES. And other studies have raised that the prolonged burst-suppression coma may be associated with a more severe course (2). Interestingly, some children with FIRES displayed good response to KD. Nine studies including 26 patients have reported this phenomenon (7, 10, 13, 17–22). The

TABLE 2 | EEG features.

			Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Ictal	Seizure duration/ Average seizure burden	1#	2–40 min/1–2/h	6–33 min/3/h	2–4 min/6/h	0.5–1.5 min/2/h	2–5 min/13/h	1 min/1/h	2–4 min/0–1/h
		2#	1–6min/20/h	Continuously (unable to count)	0.5–5.5 min/8/h	0.5–2 min/3–4/h	2–3 min/40/h	12 min/0–1/h	1.5–3 min/2–3/h
		3#	1–7.5 min/17/h	Continuously (unable to count)	/	/	2–3 min/15/h	3–17 min/2/h	1.5–3.5 min/5–6/h
		4#	No seizure	Several mins/5/h	No seizure	2 min/0–1/h	1 min/10/h	No seizure	No seizure
		5#	No seizure	No seizure	0.5–2 min/2/h	No seizure	2–3 min/5/h	No seizure	No seizure
	Seizure origin	1#	L/R/BO, L/RF, L/RP, RC	RO, RT	L/RF	LO, LT	L/RO	Generalized	L/RT
		2#	L/R/BO, L/RT, L Rolandic	LT, R Rolandic	RO	LO, LT	L/R/BF, L/R/BT, L/R/BO	RT, generalized	L/RT, L/RF
		3#	L/R/BO, L/R Rolandic	L/RP, L/RO, RC	/	/	BF, BC, BT, BO	L/RF	RT, RF
		4#	No seizure	L/R Rolandic, L/RO	No seizure	LO	LF, RO, L/R Rolandic	No seizure	No seizure
		5#	No seizure	No seizure	L/RF	No seizure	RF	No seizure	No seizure
	Spreading/ Generalizing/ Shifting	1#	Yes/Yes/No	Yes/Yes/No	Yes/Yes/No	Yes/No/No	Yes/Yes/Yes	NA/NA/No	Yes/Yes/No
		2#	Yes/Yes/Yes	Yes/No/Yes	Yes/Yes/No	Yes/No/No	Yes/Yes/No	Yes/Yes/No	Yes/Yes/No
		3#	Yes/No/Yes	Yes/No/Yes	/	/	Yes/Yes/No	Yes/Yes/Yes	Yes/No/Yes
		4#	No seizure	No/No/Yes	No seizure	Yes/No/No	Yes/No/No	No seizure	No seizure
		5#	No seizure	No seizure	Yes/Yes/No	No seizure	Yes/No/No	No seizure	No seizure
Inter -ictal	Background	1#	Unidentified	Diffuse continuous δ activity with epileptiform discharges	Unidentified	Diffuse continuous δ activity with epileptiform discharges	Unidentified	Diffuse continuous δ and θ activity	Diffuse continuous θ activity
		2#	Unidentified	Diffuse continuous δ activity with epileptiform discharges	Diffuse continuous δ activity	Diffuse continuous δ activity with epileptiform discharges	Unidentified	Diffuse continuous δ and θ activity	Diffuse continuous δ activity
		3#	Diffuse continuous δ activity	Diffuse continuous δ activity with epileptiform discharges	/	/	Unidentified	Diffuse continuous δ activity	Diffuse continuous δ activity
		4#	Diffuse continuous δ with less θ activity	Diffuse continuous δ activity with epileptiform discharges	Diffuse continuous δ with less θ activity	Diffuse continuous δ activity with epileptiform discharges	Diffuse continuous δ activity	Diffuse continuous δ activity	Diffuse continuous δ activity
		5#	Diffuse continuous δ with θ activity	Diffuse continuous θ activity	Posterior basic rhythm	Diffuse continuous θ with less δ activity	Diffuse continuous δ activity	Diffuse continuous δ with θ activity	Posterior basic rhythm
	Sleep spindles	1#	Yes	Unidentified	Yes	No	Unidentified	Yes	Yes
		2#	Unidentified	Unidentified	No	No	Unidentified	Yes	Unidentified
		3#	No	Unidentified	/	/	Unidentified	Yes	Unidentified
		4#	Yes	Unidentified	No	Yes	Unidentified	Yes	Unidentified
		5#	Yes	Yes	Yes	Yes	Yes	Yes	Yes

L, left; R, right; B, bilateral; T, temporal; C, central; P, parietal; O, occipital; F, frontal; NA, not applicable.

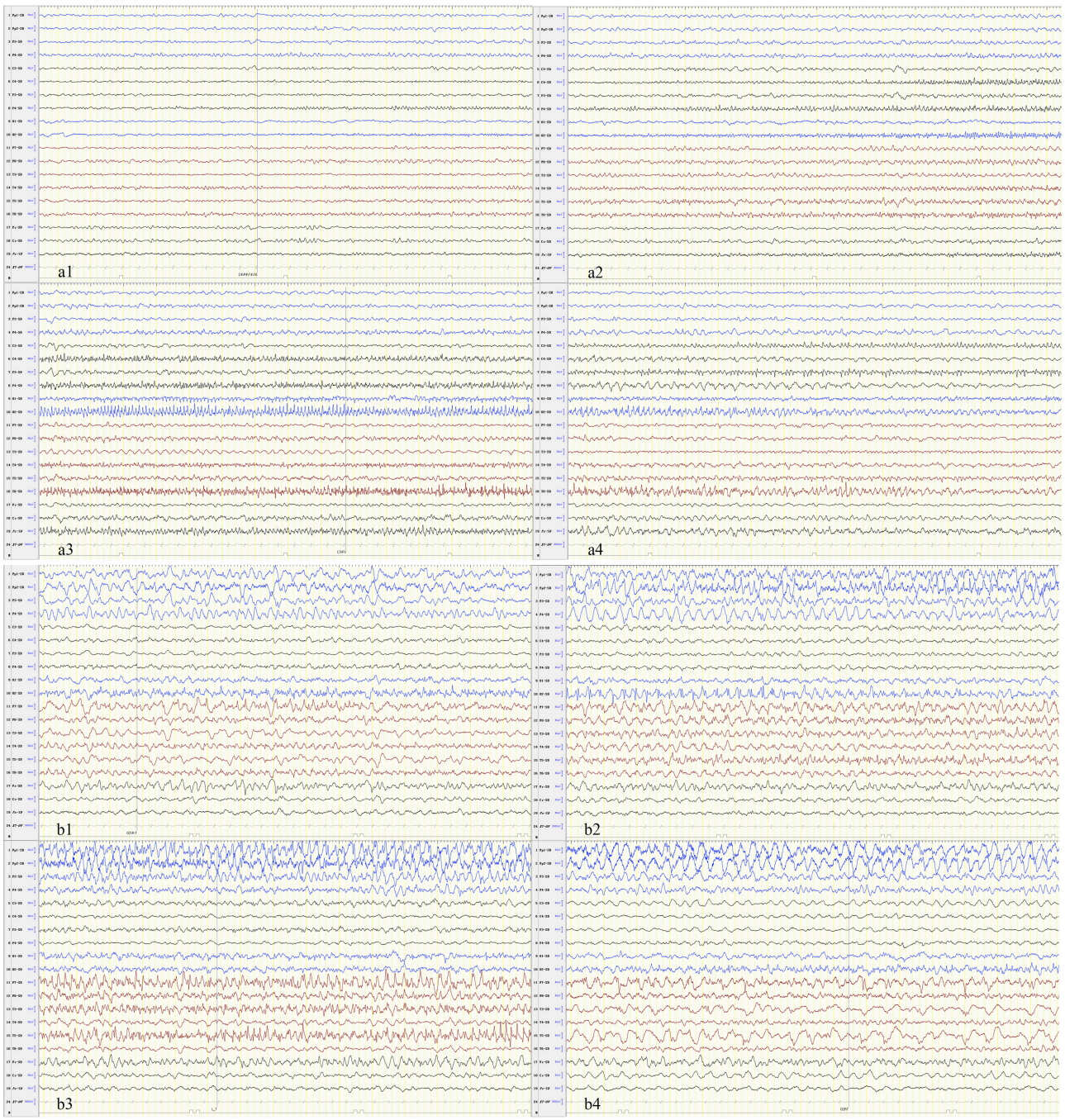

FIGURE 1 | The seizure begins in the focal or multifocal regions with spread to the unilateral or bilateral hemisphere and shifting from one hemisphere to the contralateral. **(a)** Patient 1 and **(b)** patient 6.

effective rate (>50% seizure reduction) was 62%. In this study of 7 children with FIRES and SRSE, we found that immunotherapy and AEDs could not control this condition, but KD treatment produced significant therapeutic efficacy and safety, which is consistent with previous reports.

It is worth noting that the clinical presentation of patients in this study shows that early initiation of KD improves outcomes of FIRES. In all patients, the frequency of seizures at the last follow-up was similar, and seizures were reduced by ≥75% in all patients except one who converted to VNS. However, their functional outcomes were dissimilar. A shorter duration of SE in the acute phase of patients indicated a lower mRS score in the subsequent period ($p = 0.012$, $r = 0.866$). Patients (2, 3, 5, 7) who had an SE duration of <15 days before initiation of KD had a

TABLE 3 | Patient in-hospital and long-term outcomes.

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Clinical outcomes								
Time to ketosis, *d*		3	2	11	1	5	1	3
Length of SE after KD initiation, *d*		6	6	1	0	10	5	4
Length of anesthesia after KD initiation, *d*		23	29	14	11	24	7	NA
No. of AEDs at hospital discharge		4	4	3	4	4	3	3
AEDs at hospital discharge		VPA, TPM, OXC, CLB	VPA, LEV, TPM, NZP	VPA, TPM, OXC	VPA, TPM, OXC, NZP	VPA, TPM, OXC, NZP	LEV, TPM, OXC	VPA, LEV, TPM
Adverse events		Hyperlipidemia, transient hyperamylasemia	Hyperlipidemia, diarrhea	Diarrhea	Hyperlipidemia	Hyperlipidemia	Diarrhea	Diarrhea
Total LOS, *d*		86	48	36	38	63	46	35
ICU LOS, *d*		61	32	19	23	40	33	20
Follow-up								
Duration of follow-up, *m*		13	20	31	40	6	14	11
Duration of KD, *m*		11	3	3	2	3	3	2
Seizure burden	At the end of KD	Monthly seizures (after controlled for 1 month)	No seizure	No seizure	Daily seizures (after controlled for 2 weeks)	Monthly seizures (after controlled for 2 months)	No seizure	No seizure
	At most recent follow-up	Monthly seizures	No seizure	Monthly seizures	Monthly seizures	Monthly seizures	Monthly seizures	No seizure
mRS score	At the end of KD	4	0	0	4	3	4	0
	At most recent follow-up	4	0	0	3	1	3	0

mRS, modified Rankin Scale; NA, not applicable (because the anesthesia had been withdrawn before KD initiation in patient 7).

better prognosis with mRS scores ≤3 at the end of KD and ≤1 at the last follow up. In these patients, it is important to recognize FIRES symptoms early using specific EEG pattern and initiate KD treatment. KD therapy resolved SRSE successfully unlike other treatments (immunotherapy, four or more AEDs, and at least one general anesthetic agent). This indicates that KD can prevent damage from excitotoxicity processes, inhibit secondary processes induced by initial excitotoxicity, prevent multiple complications due to prolonged anesthesia and unconsciousness, increase alertness of patients and decrease the need for invasive respiratory support.

Generally, KD can be administered through enteral route (by mouth or tubes). But patients with conditions such as FIRES often suffer from gastrointestinal problems caused by prolonged use of AEDs, general anesthetics and mechanical ventilation. Some of the gastrointestinal problems include vomiting, severe diarrhea, and intestinal bleeding. Therefore, administration of drugs using enteral route may worsen these complications. In fact, in the presence of these conditions, enteral intake of KD is compromised and ketosis is impaired, making KD diet unsafe and inefficient (23). In this study, KD was given as parenteral nutrition to 2 children with FIRES and SRSE (patient 1 and 5) who required bowel rest due to upper gastrointestinal hemorrhage or gastroplegia. Thereafter, they were converted to enteral diet successfully. This study has demonstrated that KD can be effectively applied to patients with intestinal failure who need to start KD early.

The major limitations of this study should be discussed. This is a retrospective study containing a small number of patients. Considering the severity of patients with SRSE, we were unable to include a group of patients with SRSE and FIRES who were not put on KD treatment during the same period for comparison. Moreover, a combination of treatments comprising AEDs, immunotherapy and KD was used, making it difficult to isolate the exact benefit of a single intervention while excluding other treatments. Nevertheless, considering the rapid response (resolution of SE in clinical course and improvement of EEG features on assistant examination) in acute phase and good prognosis of patients in this study, we conclude that KD treatment is effective for FIRES patients. More studies are needed to reveal the exact mechanism of KD in FIRES, define the optimal timing and propose a protocol of KD application.

CONCLUSIONS

In summary, this study shows that KD has a therapeutic effect on FIRES, especially in the acute phase as it can resolve SRSE. Early identification of FIRES and prompt initiation of KD therapy may improve the prognosis

of this condition. Unique EEG features of FIRES may aid early diagnosis. Intravenous KD is a reasonable alternative for patients who cannot take KD enterally due to intestinal failure.

AUTHOR CONTRIBUTIONS

PP, LM, and JP conceived the study. XD and FH provided the clinical information and PP checked. CC, XW, and JP analyzed the EEG data. PP drafted the initial manuscript which was edited by LM, SG, FY, and JP.

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frontiers in Neurology

BRIEF RESEARCH REPORT
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Dosage Related Efficacy and Tolerability of Cannabidiol in Children With Treatment-Resistant Epileptic Encephalopathy: Preliminary Results of the CARE-E Study

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Purpose: There is uncertainty regarding the appropriate dose of Cannabidiol (CBD) for childhood epilepsy. We present the preliminary data of seven participants from the Cannabidiol in Children with Refractory Epileptic Encephalopathy (CARE-E) study.

Methods: The study is an open-label, prospective, dose-escalation trial. Participants received escalating doses of a *Cannabis* Herbal Extract (CHE) preparation of 1:20 Δ^9-tetrahydrocannabinol (THC): CBD up to 10–12 mg CBD/kg/day. Seizure frequency was monitored in daily logs, participants underwent regular electroencephalograms, and parents filled out modified Quality of Life in Childhood Epilepsy (QOLCE) and Side Effect rating scale questionnaires. Steady-state trough levels ($C_{ss,\ Min}$) of selected cannabinoids were quantified.

Results: All seven participants tolerated the CHE up to 10–12 mg CBD/kg/day and had improvements in seizure frequency and QOLCE scores. $C_{SS,Min}$ plasma levels for CBD, THC, and cannabichromene (CBC) showed dose-independent pharmacokinetics in all but one participant. $C_{SS,Min}$ CBD levels associated with a >50% reduction in seizures and seizure freedom were lower than those reported previously with purified CBD. In most patients, $C_{SS,Min}$ levels of THC remained lower than what would be expected to cause intoxication.

Conclusion: The preliminary data suggest an initial CBD target dose of 5–6 mg/kg/day when a 1:20 THC:CBD CHE is used. Possible non-linear pharmacokinetics of CBD and CBC needs investigation. The reduction in seizure frequency seen suggests improved seizure control when a whole plant CHE is used. Plasma THC levels suggest a low risk of THC intoxication when a 1:20 THC:CBD CHE is used in doses up to 12 mg/kg CBD/kg/day.

Keywords: cannabidiol, Δ^9-tetrahydrocannabinol, cannabis, epileptic encephalopathy, cannabinoid plasma levels

INTRODUCTION

Recent trials with pharmaceutical grade cannabidiol (CBD) or CBD-enriched *Cannabis* Herbal Extract (CHE) support CBD's ability to reduce seizure frequency in children with intractable epilepsy, including those with epileptic encephalopathy (1–5). Yet, there are significant knowledge gaps regarding the use of CBD and other cannabinoids in children, including the pharmacokinetics (PK), pharmacogenetics, and dose-concentration-effect relationships for these compounds (6). The resultant inability to provide evidence-based dosing and therapeutic monitoring of *Cannabis*-based products in children, combined with concerns regarding potential intoxicant effects of Δ^9-tetrahydrocannabinol (THC), leads to a reluctance by many physicians to authorize CHE to these patients.

The age-related developmental changes that influence drug PK and pharmacodynamics (PD) complicate the development of appropriate dosing regimens for pediatric age groups (6). Without an understanding of dose concentration-effect relationship, a dosing regimen is largely empirical and/or anecdotal, and fraught with potential safety concerns.

CARE-E is a multi-center, phase 1, open-label, dosage escalation study using a Health Canada approved and Good Manufacturing Practices certified 1:20 THC:CBD CHE as adjunct therapy to treat children with epileptic encephalopathy. The primary objectives were to assess the safety and efficacy of CBD-enriched CHE, whereas secondary objectives included an analysis of trough steady state ($C_{SS,Min}$) levels of CBD, THC, and cannabichromene (CBC); as well as an assessment of the correlation between cannabinoid levels and therapeutic effect. CBC levels were measured as the CHE used in this study contained 4% CBC by volume. We present results for seven CARE-E participants recruited at the University of Saskatchewan site.

METHODS

Trial Design

The study is a phase 1, open-label, dosage-escalation clinical trial in which participants receive a 1:20 THC:CBD CHE in twice daily dosing. Upon enrollment (Visit 1) participants continue their current anticonvulsant regimen and baseline seizure frequency is determined for 1 month. At Visit 2 CHE dosing is initiated with a CBD dose of 2–3 mg/kg/day. At Visits 3–5 the CHE is increased at 1-month intervals with CBD doses of 5–6 mg/kg/day at Visit 3, 8–9 mg/kg/day at Visit 4, and 10–12 mg/kg/day at Visit 5. At Visit 6 the CHE is weaned over a 1-month period after which the participants have their end of study visit (Visit 7). Care-givers monitor and record seizure frequencies in daily seizure logs. The complete study design and methodology have been described previously (7).

Ethics

Prior to enrollment, written and informed consent was obtained from the child's parents or legal guardian. This study received a No Objection Letter (NOL) from Health Canada, was approved by the University of Saskatchewan Biomedical Research Ethics Board and registered with ClinicalTrials.gov (NCT03024827).

Participants

Inclusion criteria included pediatric patients between the ages of 1 to 10 years with epileptic encephalopathy resistant to standard medical treatment (as per International League Against Epilepsy definition of drug resistant epilepsy) and at minimum one major seizure per week or four major seizures per month (8). Seven participants from Saskatoon who met the inclusion criteria completed the study. All study data were collected and managed using the REDCap electronic data capture tool hosted at the University of Saskatchewan (9).

Efficacy Outcome Measures

Seizures occurring in a cluster were counted as a single seizure due to challenges arising from caregivers individually recording each seizure within a cluster. The data from the seizure logs was entered into REDCap (9) at each visit and underwent an independent audit performed by the University of Saskatchewan Clinical Trial Support Unit. To allow for variations in the length between study visits, the average daily number of seizures between visits was calculated by dividing the number of seizures recorded between each visit by the number of days between visits.

At Visits 2–6, participants underwent a 2-h EEG for assessment of degree of background slowing and spike index. The first EEG was performed prior to starting the CHE and each subsequent EEG was performed prior to a scheduled CHE dosage increase. To ensure consistency in EEG interpretation, an EEG rating scale for background slowing (encephalopathy) proposed by Lüders was used (10). A spike index ranked on a five-point scale ranging from 0 (=No Spikes) to 4 (=Continuous Spiking, defined as spikes occupying more than 70% of the EEG) was also calculated for each EEG.

At Visits 2–7, parents completed a modified Quality of Life in Childhood Epilepsy (QOLCE-55) survey which, in addition

to questions assessing the domains including cognition, physical independence, social engagement, well-being, behavior (11), contained 13 additional items about sleep, verbal and non-verbal communication, interpersonal interactions, and irritability. Each item was rated from 1 (=Very Often) to 5 (=Never) or marked "Not Applicable." The scores for reverse items were inverted and then all scores were transformed using (Score-1)×25. The mean score for each subscale was calculated ignoring those marked "Not Applicable."

Safety Outcome Measures

During the study caregivers recorded a description all adverse events associated with CHE in a participant diary. From Visit 3 to Visit 7, caregivers rated adverse effects previously described with CBD. Sleepiness/Lethargy and Irritability were rated from 0 (=Not Present) to 4 (=Present All The Time). Nausea/Vomiting and Diarrhea were rated from 0 (=Not Present) to 5 (=More Than Once Per Day). At each visit, the information for the preceding month was self-reported and provided to the study nurse.

At Visits 2–6, blood samples were collected for complete cell count and differential cell count, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, aspartate transaminase, alanine transaminase, alkaline phosphatase, and gamma glutamyltransferase, total and direct bilirubin, lipase, albumin, cholesterol, and triglycerides. Elevations in liver enzymes or lipase were considered significant if they were more than three times the upper limit of the normal reference range.

Quantification of Cannabinoids in Plasma and Steady State Trough Levels ($C_{SS,\ Min}$)

To measure plasma trough steady-state ($C_{SS,Min}$) cannabinoid levels, blood was collected on Visits 2–5 into lithium heparin Barricor vacutainers and centrifuged for 10 min in a clinical centrifuge (1,500 rpm) (12). These plasma samples (200 μL) were prepared and analyzed for CBD, CBC, and THC levels according to a validated liquid chromatography-mass spectrometry (LC-MS/MS) method that while developed independently in our lab is similar to a previously reported validated plasma cannabinoid assay (7, 13). All samples were stored at −70°C prior to analysis. Analytical method validation indicated the assay was specific and linear from 0.49 to 125 ng ml^{-1}, for THC and CBD, and 0.98–125 ng ml^{-1} CBC with $r^2 > 0.998$. Matrix effects ranged from 40 to 50% depending upon analyte resulting in extraction efficiencies in a similar range but recoveries were >88%. Intra- and inter-day precision and accuracy of the method was within ± 15%. The samples were analyzed in three batches (October 2017, May 2018, and March 2019). While most samples were analyzed within 3 months of collection, some were analyzed up to 8 months after collection. Stability analysis indicates stability of cannabinoids stored frozen for 3 months but stability beyond this is unknown. Full details of the quantification method of cannabinoids in plasma samples are available as **Supplementary Protocol** provided with this manuscript.

Steady-State Trough Anticonvulsant Levels

During the study, the participants' anticonvulsant medications were not adjusted. The exception was clobazam, which was decreased if it was felt that clobazam side effects were being exacerbated by the known interaction between CBD and clobazam (14). Prior to decreasing the dose of clobazam, trough clobazam, and norclobazam levels were measured.

Trough anticonvulsant levels were measured at Visits 2–6 to identify a possible drug interaction with CBD, a known competitive inhibitor of CYP2C and CYP3A isozymes (15). $C_{SS,Min}$ levels were obtained for valproic acid, lamotrigine, levetiracetam, topiramate, and clonazepam. $C_{SS,Min}$ levels for stiripentol were not obtained as this assay was not available to us through the Saskatchewan Provincial Health Laboratory or its partnering laboratories.

Statistical Analysis

Due to the small sample size reported a formal statistical analysis was not performed. Data are presented from individual participants and as the mean ± standard error of the mean (s.e.m.) (**Figures 1**, **3**) and in a descriptive manner to illustrate trends emerging from the data (**Figure 2**). A formal analysis will be done when all patients are included in the trial.

RESULTS

Demographic Characteristics and Compliance

At time of enrolment, all participants failed at least 2 appropriate anticonvulsants, and none were using the ketogenic diet or had a vagal nerve stimulator. All participants were fully compliant with all study protocols. **Table 1** summarizes study participant characteristics. As per the publishing guidelines of this journal the participants' gender is not included and age at recruitment is provided in ranges (1–3, 4–6, 7–10 years).

Safety and Tolerability Outcome Measures

While all participants reported Sleepiness/Lethargy and Irritability during the study, no scores increased by more than two points. Irritability improved in two participants following a decrease in clobazam dosage. Occasional incidences of nausea and vomiting, diarrhea, increased appetite, difficulty sleeping and spasticity were reported. Changes in the side-effect rating scales were not consistent and, apart from nausea and vomiting, did not correlate with increased doses of the CHE. None of the side effects were severe enough to prompt withdrawal from the study. The side effects rating scale scores are provided in **Supplementary Tables 1A–D**.

No significant changes in complete blood count and differential, electrolytes, renal panels, triglyceride, cholesterol, albumin, or bilirubin levels were observed. All participants had elevated ALP at Visit 1; however, these levels did not increase with the introduction and titration of CHE, with the exception of participant A-07, whose ALP increased to 300 U/L (reference: 30–110 U/L) at Visit 4, but decreased back to 144 U/L at Visit 5.

Participant A-01 had a slight elevation of GGT at 44 U/L (reference 10–35 U/L) seen at Visit 3 only. Participant A-03 had

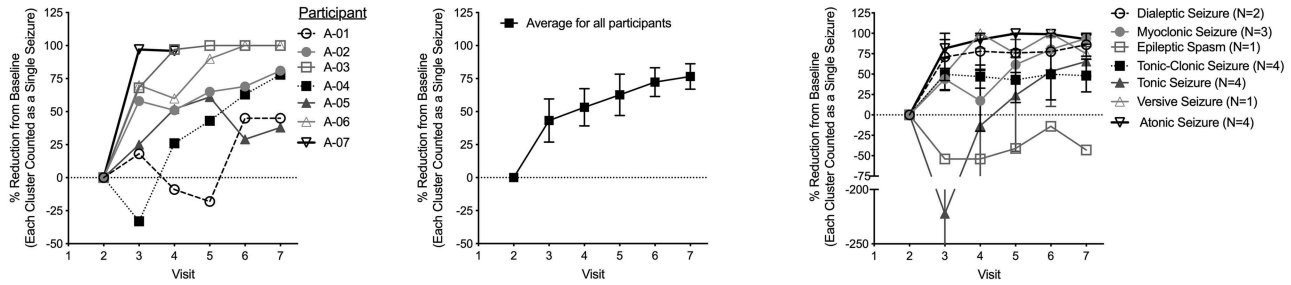

FIGURE 1 | (A) Percentage reduction in daily average seizure frequency as compared to baseline. The value shown at each visit represents the decrease in seizure frequency from baseline during the preceding month. **(B)** Average percentage reduction in daily average seizure frequency from baseline for all seven participants at each study visit. **(C)** Average percentage reduction in daily seizure frequency from baseline for all seven participants broken down into seizure type. Data are shown as mean ± s.e.m.

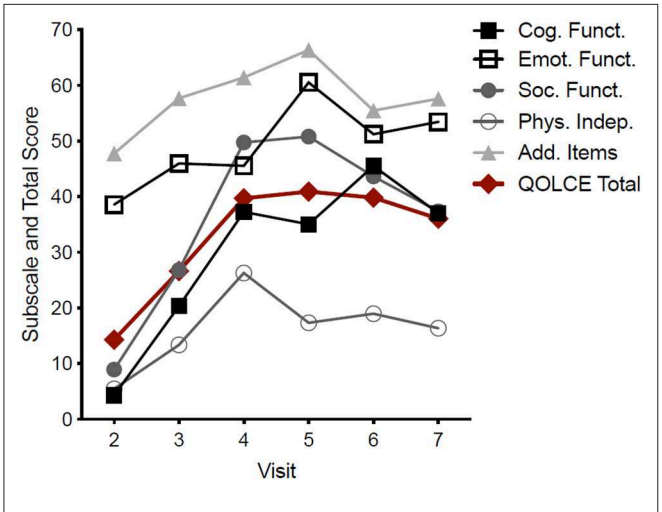

FIGURE 2 | Pooled QOLCE-55 scores and subscores for all seven participants. The values shown at each visit represent the QOLCE-55 total and subscores for the preceding month. Data are mean from seven participants.

a marked elevation of GGT to 738 U/L (10–50 U/L) during an admission to Pediatric Intensive Care for sepsis. GGT decreased to 73 U/L the following month and returned to normal on post-study follow up despite continuing CHE.

Participant A-04 had slight elevations of AST at Visits 3 and 6 (48 U/L and 44 U/L, respectively -reference: 10–40 U/L). GGT was elevated prior to, and remained elevated throughout, the study, reaching a peak of 88 U/L at Visit 4. Participant A-04's serum lipase at 173 U/L (normal: 22–51 U/L) was significantly elevated at Visit 5. As he was asymptomatic and an abdominal ultrasound was normal, he continued to receive CHE. By Visit 6, lipase levels decreased to 83 U/L and returned to normal following the study after valproic acid dosing was decreased and CHE was continued at 10–12 mg/kg/day.

No clinically significant adverse events directly attributed to the CHE were encountered. Two participants had serious adverse events requiring hospitalization, but these were not related to the study drug. During their hospitalizations, both remained on their routine anticonvulsants and CHE.

Efficacy Outcome Measures

The average reduction in daily seizure frequency between visits for each participant is displayed in **Figure 1A**. Over the study period, all seven participants had an improvement in seizure frequency with CHE. One participant (A-04) had a transient worsening of seizures at a CBD equivalent dose of 2–3 mg/kg/day. All participants had a reduction in average daily seizure frequency at a CBD equivalent dose of 5–6 mg/kg/day with six participants having a decrease >25% and four participants having a decrease >50%. After increasing to 10–12 mg/kg/day, the average reduction across all participants was 74% (**Figure 1B**) with all participants having a >25% reduction in daily seizure frequency, five participants having a decrease >50%, and three participants being seizure free. One participant was seizure free on an 8–9 mg/kg/day CBD equivalent dose.

During the final month of the study, when CHE was weaned off completely in the first three weeks, the reduction in seizure frequency was maintained in all participants and continued to improve in three participants (A-02, A-04, A-05) despite no changes to their anticonvulsant regimens.

While there was a reduction in daily seizure frequency between visits for all seizure types recorded, the greatest reduction was seen in atonic and versive seizures while epileptic spasms increased in frequency (**Figure 1C**). The percentage reduction in frequency of reported seizure types compared to baseline for all seven participants at each visit are also provided as **Supplementary Figures 1A–G**.

By the time the CBD dose was increased to 10–12 mg/kg/day, all participants -except for participant A-07, who had a normal background activity on the initial EEG– had an improvement in their EEG encephalopathy rating scale with most improving by one point on the rating scale. Participant A-03 had an improvement by two points. During the course of the study, three participants had an improvement in their EEG Spike Index scores. Participants

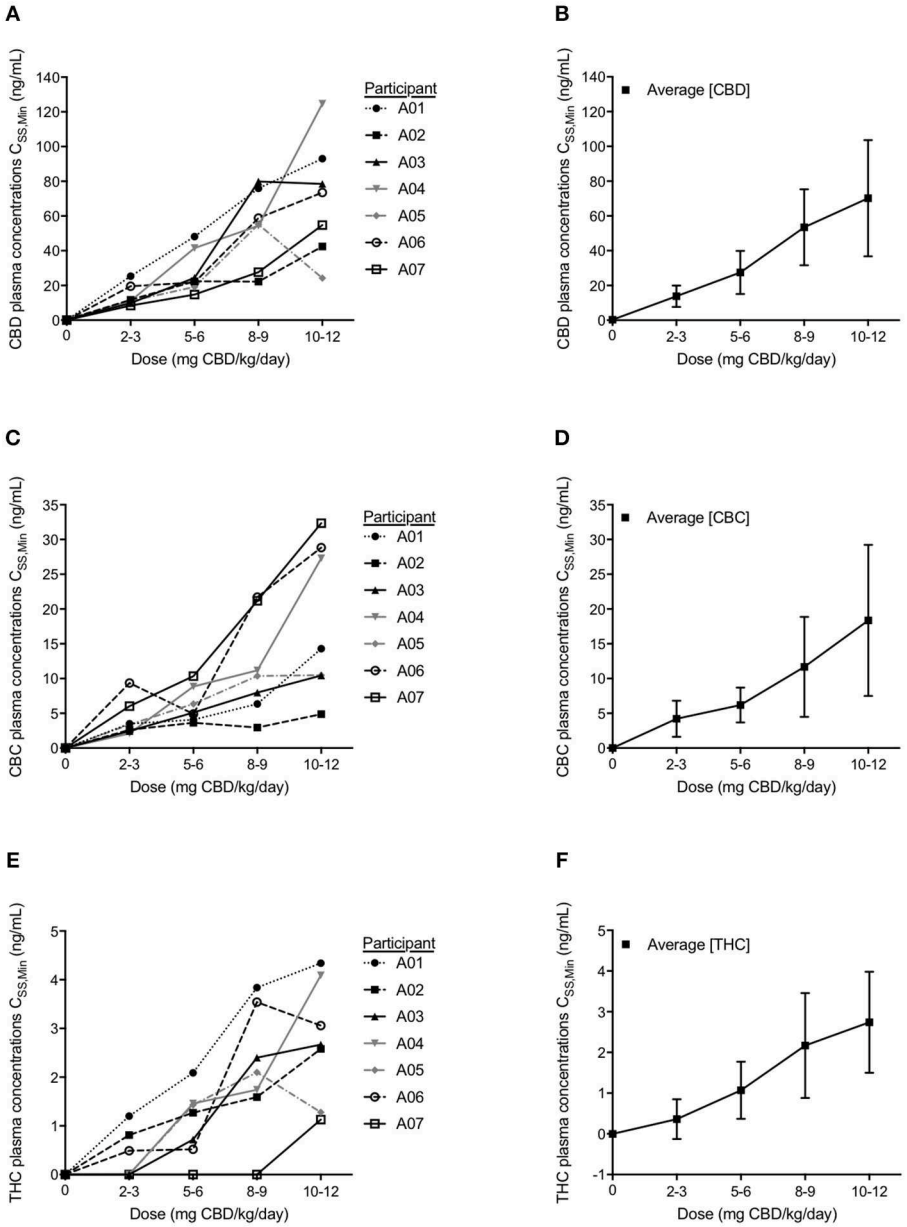

FIGURE 3 | Participant minimum steady state ($C_{SS,Min}$) plasma concentrations and average plasma $C_{SS,Min}$ levels for each cannabinoid of cannabidiol (CBD) **(A,B)**, cannabichromene (CBC) **(C,D)**, and Δ^9-tetrahydrocannabinol (THC) **(E,F)** analyzed with LC-MS/MS. Values shown represent steady state levels after 1 month on the corresponding dosage of CBD measured just prior to a dose administration. Data are mean ± s.e.m.

A-03 and A-04 had resolution of their continuous spike activity in sleep. Full details of EEG results are provided in **Supplementary Figures 2A,B**.

An improvement in the total QOLCE-55 scores was observed in all participants with the greatest improvements were found on the Cognitive, Social and Emotional Functioning subscales (**Figure 2**). While the improvements in the QOLCE scores decreased during the weaning period following Visit 6 the scores remained improved over the baseline scores.

Plasma Cannabinoid Plasma Levels in Relation to Dosage Escalation and Decrease in Seizure Frequency

Cannabinoid $C_{SS,Min}$ plasma concentrations were measured at the end of each subsequent month's dosage escalation (**Figures 3A–F**). With each dosage escalation, CBD and CBC $C_{SS,Min}$ values generally increased proportionally with dose in all participants, except for participant A-04, whose last dose escalation resulted in non-proportional increases in both CBD and CBC $C_{SS,Min}$ values (**Figures 3A–C**).

TABLE 1 | Participant characteristics at time of recruitment into CARE-E including age, epilepsy diagnosis, and concomitant anticonvulsant medications.

Participant ID	Age (years)	Weight (kg)	Epilepsy diagnosis	Predominant seizure types	Number of seizures in baseline month	Concomitant anticonvulsant therapies and daily dosage (mg/kg/day)
A-01	4–6	17.2	Dravet syndrome (SCN1A mutation)	Tonic-clonic, tonic, myoclonic	11	Stiripentol (50 mg/kg/day) Clobazam (1.3 mg/kg/day)
A-02	4–6	14.6	Dravet syndrome (SCN1A mutation)	Dialeptic, myoclonic (in clusters), Tonic-Clonic	343	Clobazam (0.3 mg/kg/day) Stiripentol (63 mg/kg/day) Topiramate (20 mg/kg/day)
A-03	4–6	23.7	Lennox Gastaut syndrome, continuous spike wave in sleep (evolved from cryptogenic infantile spasms)	Dialeptic, atonic, tonic	195	Valproic Acid (29 mg/kg/day) Clobazam (1.2 mg/kg/day) Lamotrigine (5.3 mg/kg/day) Levetiracetam (59 mg/kg/day)
A-04	1–3	11.4	Lennox Gastaut syndrome (Cerebral palsy-perinatal asphyxia)	Epileptic spasms, tonic, myoclonic	1,223	Lamotrigine (4 mg/kg/day) Valproic Acid (53 mg/kg/day)
A-05	1–3	14.3	Lennox Gastaut syndrome (cerebral palsy-perinatal asphyxia)	Atonic (in clusters), tonic, tonic-clonic	56	Lamotrigine (10.2 mg/kg/day) Clonazepam (0.08 mg/kg/day)
A-06	7–10	20.9	Dravet syndrome (SCN9A mutation)	Tonic clonic	10	Topiramate (9.6 mg/kg/day), Clonazepam (0.17 mg/kg/day) Valproic Acid (36 mg/kg/day)
A-07	4–6	18.6	Dravet syndrome (SCN1A mutation)	Atonic, tonic clonic, versive partial	165	Valproic Acid (19 mg/kg/day) Clobazam (1.1 mg/kg/day) Stiripentol (29 mg/kg/day)

Underlying aetiology of epileptic encephalopathy listed in parentheses.

Following a month of CBD at 5–6 mg/kg/day, the four participants with a >50% reduction in average daily seizure frequency, had $C_{SS,Min}$ CBD levels ranging from 14.8 to 24.4 ng/mL. After a month of CBD at 10–12 mg/kg/day, the five participants with a >50% reduction in average daily seizure frequency, had $C_{SS,Min}$ CBD level ranging from 42.5 to 124.7 ng/mL. The $C_{SS,Min}$ CBD levels corresponding with the CBD dosage at which the three participants became seizure free, ranged from 54.8 to 78.9 ng/mL (**Figure 3A**).

In all but two participants (A-04 and A-07), $C_{SS,Min}$ THC levels were detectable at 2–3 mg/kg/day. Even at the highest dose of CHE, the $C_{SS,Min}$ THC levels were low—below 4 ng/mL in all but two participants with the highest level being 4.34 ng/mL (**Figure 3E**).

Effect of CHE on Steady State Levels of Anticonvulsants

Apart from clobazam, $C_{SS,Min}$ anticonvulsant levels did not change significantly and remained within therapeutic limits with the following exceptions. Valproic acid levels for participant A-07 doubled between visits 2 and 6 but remained within therapeutic range (350–700 umol/L). For participant A-06, Valproic acid levels decreased to below therapeutic range at 16 umol/L between visits 4 and 5 suggesting medication non-compliance. $C_{SS,Min}$ clobazam and norclobazam levels of the four participants taking clobazam during the study are provided in **Supplementary Table 2**. For participants A-02 and A-03 these levels are not available for visit 6 due to the samples being misplaced in our hospital central laboratory. Three participants (A-01, A-02, and A-03) experienced side effects felt to be secondary to clobazam prompting a decrease in their clobazam dosage. In all three participants, these apparent side-effects of clobazam resolved with a decrease in clobazam dosing. Co-administration of clobazam did not appear to correlate with higher levels of CBD or CBC at each dosage escalation.

DISCUSSION

CARE-E is an open label dosage finding study designed to assess the safety and efficacy of a CBD-Enriched *Cannabis* Herbal Extract (CHE) in children with intractable epileptic encephalopathy. The study involved measurement of $C_{SS,Min}$ levels of CBD, THC, and CBC and their relationship with safety, tolerability, and efficacy outcome measures in hopes to identify appropriate doses of similar *Cannabis* products in children. This study is the first to report pediatric $C_{SS,Min}$ levels of CBD, THC, and CBC in any pediatric dosage escalation study and provide guidance on initial dosing of CBD-enriched CHEs.

Escalating doses of CBD-enriched CHE from 2–3 mg/kg/day to 10–12 mg/kg/day resulted in no serious adverse events related to the CHE. Parents reported sleepiness/lethargy and irritability in most participants, but these side effects were assessed after starting the study drug and were likely pre-existing. Transient increases in sleepiness and irritability in three participants taking clobazam resolved after clobazam dose was decreased, suggesting these side-effects could be secondary to an interaction between the CHE and clobazam (14). Laboratory monitoring noted significant elevations in GGT, AST, and lipase levels in two participants, both of whom were also taking valproic acid. Participant A-03's marked elevation of GGT was likely secondary

to sepsis, which occurred during the study. Participant A-04's transient and non-significant elevation of AST and significant elevation of lipase levels were likely secondary to a predisposition to hepatic and pancreatic dysfunction from high-dose valproic acid and corroborates observations reported elsewhere (3, 16). The fact that this participant had a preexisting elevated GGT suggests that liver enzymes should be screened prior to starting CHE, especially if the child is already prescribed valproic acid.

The concentrations of CBD, THC, and CBC appeared to increase linearly with dosage in six of the seven participants, suggesting dose-independent pharmacokinetics for these participants within this dosage escalation trial. The greater than proportional increase in $C_{SS,Min}$ CBD with the final dosage increase in participant A-04 may suggest dose-dependent pharmacokinetics with saturation of first-pass metabolism and an increase in the oral bioavailability. Participant A-04 did not exhibit any change in clinical status or in anticonvulsant therapy to explain this disproportional increase in $C_{SS,Min}$. To confirm the possible non-linear pharmacokinetics in children, a dosage escalation study involving a larger sample size and a higher dose beyond the doses used in the current trial will be necessary. The possibility of dose-dependent PK, though, raises a safety concern, which also warrants further investigation in pediatric patients and suggests a need to limit dose sizes and not to simply continue increasing doses until an appropriate effect is observed.

CBD and THC both inhibit enzymes involved in the metabolism of many anticonvulsants including CYP2C and CYP3A isoenzymes (17). While increases in Clobazam and norClobazam levels were seen in some participants taking clobazam, overall co-administration of CHE did not significantly affect $C_{ss,min}$ levels of the other concomitant anticonvulsants. It would have been of interest to measure $C_{ss,min}$ levels of stiripentol given that many children with Dravet syndrome would also be taking this medication and stiripentol is metabolized by CYP2C19 and CYP3A4 isoenzymes. At present it is unknown if there is a pharmacokinetic interaction between CBD and Stiripentol. Although an assay to measure stiripentol levels is described, it is not indicated for therapeutic purposes by the manufacturer or regulatory bodies such as Health Canada, FDA or the EU. As such, it was not available for us for the purposes of this study (18).

The potential intoxicating effects of any THC present in CHE remain a concern for pediatric patients. Oral consumption of *Cannabis* products results in lower peak levels of THC as compared to smoking due to a high first-pass effect and slow erratic absorption from the gastrointestinal tract. However, intoxication can still occur because of greater distribution into the central nervous system and conversion to 11-hydroxy-THC, which is also intoxicating and has a half-life as long as, or longer, than THC (17, 19, 20). The $C_{SS,Min}$ levels of THC increased in a seemingly linear relationship to dosage, and with the exception of two participants at the highest dosage level, these remained lower than levels that have been reported to cause intoxication (19). Tachycardia and conjunctival injection—felt to be reliable markers of intoxication from THC—were not seen during the study. The lack of intoxication seen in our participants whose plasma THC levels exceeded 4 ng/mL may have been due to the reported CBD-mediated attenuation of the intoxicant effects of THC (21).

An overall trend for improvement in seizure control and QOLCE scores was observed with increasing CHE dosage and $C_{SS,Min}$ CBD levels. A >50% reduction in average daily seizure frequency occurred in four of seven participants at a CBD dose of 5–6 mg/kg/day, and all participants had a >25% reduction in seizures at a CBD dose of 10–12 mg/kg/day. In the QOLCE scores, there was a trend toward improvements in cognitive, social, and emotional function in relation to CBD dosage. These data suggest that the initial target dose of CBD should be 5–6 mg/kg/day when a 1:20 THC:CBD whole plant extract is used and can be increased as needed up to 10–12 mg/kg/day with careful consideration of potential non-linear pharmacokinetics at higher doses.

Trembly and Sherman reported that adult patients taking purified CBD had no improvement in seizure control when their plasma CBD levels ranged from 20 to 30 ng/mL, while a significant decrease in seizure control occurred when plasma CBD levels increased above 150 ng/mL (22). While it is challenging to correlate $C_{SS,Min}$ levels of CBD and CBC with efficacy in reducing seizure frequency based on our data, we do note that the $C_{SS,Min}$ CBD levels associated with a >50% reduction in average daily seizure frequency and seizure freedom in this study were lower. Further analysis with larger sample sizes are needed to delineate which $C_{SS,Min}$ level of CBD is associated with optimal seizure control and improved QOLCE scores.

Two recent systematic reviews of clinical trials assessing pharmaceutical grade CBD in children with treatment epilepsy provide insight into the expected outcomes at the CBD doses used in these trials. In pooled data of 17 observational studies, Stockings et al. found that CBD at 20 mg/kg/day resulted in 48.5% of patients having a 50% reduction in seizures and QoLCE scores improved in 55.8% (23). Lattanzi et al. also performed a systematic review of the four clinical trials assessing pharmaceutical grade CBD in children with treatment resistant Lennox Gastaut and Dravet Syndromes. They reported that the pooled average difference in seizure frequency between CBD and placebo with CBD at 10 mg/kg/day was 19.5% while that with CBD at 20 mg/kg/day was 19.9% both in favor of CBD. A seizure frequency reduction of 50% (for all seizure types) was 37.2% with CBD at 20 mg/kg/day and 21.2% with placebo (24).

An "entourage" effect in which the clinical efficacy of cannabinoids when used in combination are greater than when used individually has been demonstrated in several animal models of epilepsy but has yet to be reported for human trials (25–27). While we saw clinical efficacy with regards to reduction in seizure frequency and improvements in QoL scores with CBD doses lower than those reported in studies using pharmaceutical grade CBD, the small number of participants reported require caution when interpreting the results and preclude drawing definite conclusions in particular with regards to possible entourage effect. Additionally, CARE-E was not designed to compare efficacy of CHE to pharmaceutical grade CBD. This can only be addressed in a head to head comparative study.

The preliminary findings presented in this manuscript are however, in keeping with the results of a metanalysis of clinical

studies comparing whole plant Cannabis CHE to pharmaceutical grade CBD in children with refractory epilepsy. This metanalysis found that while there was no significant difference between the CHE and pharmaceutical grade CBD in attaining 50% reduction in seizures, 71% of children taking CHE had improvement in seizure frequency compared to 46% taking purified CBD ($p < 0.0001$). The average CBD dose for children taking CHE was 6 mg/kg/day (28).

The three participants who became seizure free were taking long acting benzodiazepines (clobazam or clonazepam) but clobazam and clonazepam levels did not increase for two of these participants. This suggests that, while CBD and long acting benzodiazepines likely have a synergistic effect, this is not necessarily due to an increase in plasma benzodiazepine levels.

The reported half-life of CBD ranges from 9 to 32 h; however, the influence of age and concomitant anticonvulsants on the half-life remain largely unknown (29). These influences on half-life, however, would not explain the month-long continued improvements in seizure control and QOLCE scores observed in our three participants during the wean-off CHE. Such sustained effects often involve epigenetic changes and therefore it is possible that any long-term beneficial effect of CBD may reflect, in part, an as-of-yet unrecognized epigenetic effect (30). In order to assess if any long-lasting effect might be mediated through an active metabolite of CBD, we will measure participants' plasma levels of CBD, CBC, THC, and their metabolites upon completion of the 1-month weaning period in subsequent participants who are enrolled in CARE-E.

While an improvement in background EEG activity was seen in four participants, there was no correlation between CBD dosing and improvements on the background score. A more complete examination of a potential relationship between the improvement in cognitive functioning seen in the QOLCE scores and improved background activity seen on EEG is warranted. The reduction in spike count following CBD treatment in three participants is reminiscent of the effect observed with broad-spectrum anticonvulsants such as benzodiazepines and valproic acid (31).

Although the reported improvement in seizure control and quality of life are promising, these findings must be interpreted with caution as there are several limitations in this preliminary report, in particular the small sample size and potential reporting bias inherent with open label studies. The lack of a placebo group and self-reporting of outcomes may limit the ability to discern any placebo effect which is seen in many drug trials. Reporting fatigue experienced by caregivers also may be a confounder, in particular for seizure frequency data.

CONCLUSION

The preliminary results of seven participants from the CARE-E study suggest CBD-enriched CHE up to 10–12 mg/kg/day is generally well tolerated. All participants had improvements in seizure frequency, modified Quality of Life in Childhood Epilepsy (QOLCE), and electroencephalogram (EEG) rating scores. Steady state $C_{SS,Min}$ data for CBD, THC, and CBC suggest linear PK, although one participant gave possible evidence of non-linear PK at higher doses. The preliminary data suggest an initial CBD target dose of 5–6 mg/kg/day when using a 1:20 THC:CBD CHE in children with treatment resistant epileptic encephalopathy. $C_{SS,Min}$ CBD levels suggest that dosing with a CHE containing THC and other cannabinoids may be more effective than purified CBD alone. Based on clinical observations and measurement of plasma THC levels, intoxication from THC is unlikely to occur when a 1:20 THC:CBD CHE is used within therapeutic doses. The anticonvulsant effect of CHE persisted after it was weaned off, suggesting an enduring anticonvulsant effect.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the University of Saskatchewan Biomedical Research Ethics Review Board with written informed consent from the parents/legal guardians of all subjects. The parents/legal guardians of all subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the University of Saskatchewan Biomedical Research Ethics Review Board.

AUTHOR CONTRIBUTIONS

RH, RT-W, JA, BA, SC, RL, AL, SM, DM, DN, EP-L, BS, and JT-Z contributed to the design of the study protocol. RH, LH, EL, and PM are site investigators for CARE-E. SC analyzed the data. RH, RT-W, JA, RL, and AL interpreted the data. SV assisted with the development and validation of the plasma cannabinoid assay used in this study. RH drafted the manuscript. All authors contributed to the revision of the manuscript and approved it for submission.

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The Potential of Telemedicine to Improve Pediatric Concussion Care in Rural and Remote Communities in Canada

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Concussion is a form of mild traumatic brain injury that affects thousands of Canadian children and adolescents annually. Despite national efforts to harmonize the recognition and management of pediatric concussion in Canada, timely access to primary and specialized care following this injury remains a challenge for many patients especially those who live in rural and remote communities. To address similar challenges facing patients with stroke and other neurological disorders, physicians have begun to leverage advances in telemedicine to improve the delivery of specialized neurological care to those living in medically underserved regions. Preliminary studies suggest that telemedicine may be a safe and cost-effective approach to assist in the medical care of select patients with acute concussion and persistent post-concussion symptoms. Here we provide an overview of telemedicine, teleneurology, the principles of concussion assessment and management, as well as the current state of concussion care in Canada. Utilizing preliminary evidence from studies of telemedicine in concussion and experience from comprehensive systems of care for stroke, we outline steps that must be taken to evaluate the potential of telemedicine-based concussion networks to improve the care of pediatric concussion patients living in underserved rural and remote communities in Canada.

Keywords: concussion, traumatic brain injury, telemedicine, teleneurology, network

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BACKGROUND

Concussion has emerged as an important public health issue among children and adolescents living in Canada. Over the past 10–15 years, an increasing number of youth are presenting to emergency departments and primary care providers with head injury and concussion, placing significant demands on the Canadian healthcare system (1, 2). With timely medical assessment and proper education and guidance, the majority of pediatric concussion patients will make a complete return to sport and school activities within 1–4 weeks (3). Children and adolescents who sustain head trauma and do not have timely access to proper medical care are at risk of concussions, more severe forms of traumatic brain injury (TBI) or other serious neurological conditions going unrecognized and untreated. They are also at risk of premature return to sports that can lead to additional injury

resulting in more severe or prolonged symptoms, and in rare cases fatal or disabling brain injury (4). Although most pediatric concussion patients can be successfully managed by their primary care provider, certain patients including those with pre-existing conditions, those who develop persistent post-concussion symptoms, and athletes returning to high risk sports often benefit from referral to medically-supervised multi-disciplinary pediatric concussion clinics that have the personnel, expertise, and diagnostic resources to meet their complex needs (5, 6).

Unfortunately, timely access to primary and specialized healthcare is not universally available to all youth in Canada, especially those living in rural and remote communities who can face significant geographic, socio-economic and cultural barriers to accessing these services (7–13). Over the past two decades, telemedicine has emerged as important tool to help address disparities in healthcare access for patients living in medically underserviced regions. With an accumulating base of evidence to support the feasibility, safety, clinical utility, and cost-effectiveness of telemedicine in the management of acute stroke (14–20), use of this technology is now rapidly expanding to deliver care to patients with other neurological disorders including concussion (21–27).

Here, we provide an overview of telemedicine and teleneurology and outline the principles of concussion management and the current state of concussion care in Canada. Drawing from preliminary studies examining the use of telemedicine in concussion and well-established systems of care for stroke, we present a vision for telemedicine-based concussion networks to serve as an innovative approach to help optimize care of pediatric concussion patients living in rural and remote communities in Canada. We also discuss the limitations, barriers and future research directions that must be addressed to support wider adoption and implementation of telemedicine-based concussion care in Canada.

DEFINITIONS

To examine the potential use of new or emerging technologies to improve healthcare delivery, it is important to have a clear understanding of definitions and terminology. Telemedicine was a term first coined in the 1970's, which literally translates to "healing at a distance" (28). The World Health Organization has defined telemedicine as "the delivery of health care services, where distance is a critical factor, by all health care professionals using information and communication technologies for the exchange of valid information for diagnosis, treatment and prevention of disease and injuries, research and evaluation, and for the continuing education of health care providers, all in the interests of advancing the health of individuals and their communities" (29). Telemedicine-based patient care can be provided synchronously and asynchronously. Synchronous or "real-time" care is commonly provided through in-person bidirectional audio-visual videoconferencing between a patient at an "originating" site and a healthcare provider at a "distant" site. Asynchronous or "store-and-forward" care is provided by the transmission of medical information (e.g., clinical data, diagnostic test results) to a remote provider. An example of asynchronous care is an eConsultation whereby a referring provider sends clinical data to a distant specialist who can provide recommendations, test interpretation or arrange an in-person consultation with the patient (30, 31). In addition to contributing to direct patient care, telemedicine-based technology can also be used to facilitate other healthcare activities including education, research and administrative functions (i.e., telehealth).

CURRENT USE OF TELEMEDICINE FOR NEUROLOGICAL DISORDERS

Recent advances in the use of telemedicine to improve care for patients with neurological disorders, termed "teleneurology," have been driven primarily by an increasing worldwide burden of neurological disorders coupled with persistent deficiencies in access to specialized neurological care. The emerging potential of telemedicine to transform care for patients with neurological disorders living in medically underserved communities is best exemplified by its application to those with acute stroke. Two decades ago, Levine and Gorman (32) proposed that telemedicine or "telestroke" could be used to increase timely access to specialized neurological care and thrombolytic therapy for patients with acute stroke presenting to rural and remote hospitals that were not staffed by stroke neurologists. Following this seminal commentary, subsequent work has established a firm base of evidence supporting the feasibility, safety, efficacy and cost-effectiveness of telemedicine-based acute stroke management (19, 20, 33). Research has now demonstrated that clinical outcomes are comparable between remote hospitals serviced by telestroke and comprehensive stroke centers and that telemedicine is also helpful for identifying patients who could benefit from other therapies such as endovascular interventions or emergency neurosurgery (16). Moving beyond initial acute stroke management, telemedicine is now used to facilitate multi-disciplinary care including intensive care monitoring, secondary stroke prevention and rehabilitation (14, 17). Today, telestroke is a well-established component of care for comprehensive stroke centers that can now provide expanded clinical coverage to vast geographic territories through distributed or "hub-and-spoke" regional telestroke networks. Published position statements and expert reviews continue to establish and refine standards for telestroke programs and networks offering even greater opportunity to improve quality of care and patient outcomes as well as identify areas of future research and implementation (15, 18, 20, 34–37).

Building on this experience with stroke, teleneurology has expanded to assist with the care of patients with other neurological disorders such as dementia, headache, multiple sclerosis, spinal cord injury, cerebral palsy, epilepsy and movement disorders (21, 23, 26, 27). Preliminary work has demonstrated the feasibility of academic centers with expertise in pediatric neurology to establish telemedicine-based networks that provide specialized care for youth with epilepsy and headaches (38, 39). In addition, there is now a strong body

of literature to support the use of telemedicine to provide consultative and therapeutic services to patients with mental health disorders (40–42).

Using coordinated provincial and territorial telehealth networks, a growing number of Canadians are now benefitting from improved access to sub-specialty experts via telemedicine-based services such as in-person videoconferencing and eConsultation (30, 43–45). These programs and networks provide the critical technology, expertise, and infrastructure to facilitate further expansion of telemedicine-based neurological care for patients living in medically underserved rural and remote communities throughout Canada.

CURRENT STATE OF CONCUSSION CARE IN CANADA

Concussion is a condition that exists along a clinico-pathological spectrum of TBI and arises from the transmission of biomechanical forces to the brain leading to temporary alterations in neurological functioning (46). Injury mechanisms among children and adolescents can vary by age but often involve falls, sports, motor vehicle collisions or other accidents (47, 48). Although most pediatric patients with an acute concussion will make a complete neurological recovery within 1–4 weeks with proper education and guidance, ∼30% of patients will develop persistent post-concussion symptoms and benefit from additional multi-disciplinary assessment and management (3, 48). Failure to undergo prompt medical assessment following head injury can result in a delay in the diagnosis of severe forms of TBI, spine injuries and serious neurological conditions leading to death or disability. Concussion patients who do not receive proper medical care are also at risk of returning to sports prematurely leading to recurrent injury, more severe or prolonged symptoms and rare but catastrophic brain injury resulting from second impact syndrome or malignant cerebral edema (4).

Over the past 15 years, there has been a significant increase in the number of Canadian youth seeking medical attention for concussion and head injury. A retrospective population-based study in Ontario observed a 4.4-fold increase in the number of emergency department and physician office visits for concussion from 2003 to 2013 among youth 5–18 years of age (2). Similarly, a report from the Canadian Institute for Health Information indicated that over 17,000 youth were evaluated for sport-related brain injuries in emergency departments in Ontario and Alberta in 2016–2017 representing an increase of 28% over the past 5 years (1). Although the factors contributing to this recent increase remains unclear, increased recognition and reporting of concussions as well as increased concern regarding the long-term effects of these injuries likely play an important role. Following implementation of a school-based concussion policy among Ontario schools in 2014, there was a 30% increase in the monthly rate of emergency department visits for concussion and a significant increase in the proportion of concussions reported to have occurred at school (49).

To address the public health concern of concussions in Canada, Parachute, the Public Health Agency of Canada and national sport stakeholders published the *Canadian Guideline on Concussion in Sport* that outlines a standardized clinical pathway and recommendations to help improve concussion education, recognition, and management for sport- and non-sport-related concussion among youth and adults (50). In addition, the Ontario Neurotrauma Foundations (ONF) has published guidelines that outline standards for concussion clinics and provide clinical recommendations for the management of adult and pediatric concussion patients (51–53). Lastly, some provincial governments have demonstrated interest in enacting youth concussion legislation that would mandate that all youth with a suspected concussion undergo medical assessment and medical clearance prior to returning to school and sport activities (54). In March 2018, Ontario became the first province in Canada to enact youth concussion legislation, termed Rowan's Law (55).

Despite the increasing burden of concussion among Canadian youth, there are emerging concerns that some patients are not receiving adequate medical care following these injuries. National and international guidelines recommend that youth with a suspected concussion undergo urgent medical assessment and obtain medical clearance prior to returning to full sport activities (46, 50) However, a retrospective population-based study in Ontario from 2003 to 2013 found that only one third of youth sought medical follow-up or clearance following an initial visit for concussion (56). Although the responsibility to provide medical assessment and clearance for youth with concussion generally falls upon primary care providers, surveys conducted among Canadian family medicine physicians, pediatricians and emergency department physicians demonstrate considerable knowledge gaps with a significant proportion failing to provide proper post-injury guidance and care (57–60).

To address these complex issues, Canada has experienced an explosion in "concussion clinics" that advertise specialized care to this patient population. Despite increasing access to concussion care in some regions of Canada, research suggests wide variability in the personnel and practices among these facilities many of which do not meet ONF standards for concussion clinics (53). In a study examining providers and clinics advertising specialized concussion care on the Internet, only 40% indicated the presence of an on-site medical doctor. Access to healthcare professionals with expertise in TBI (e.g., rehabilitation medicine physicians, neurosurgeons, neuropsychologists, neurologists) were found to be limited at the majority of sites with very few having access to the full complement of specialists needed to provide comprehensive concussion care (61). A notable proportion of clinics were also found to advertise services that were not supported by current empirical evidence (e.g., baseline testing) or were being offered without appropriate on-site expertise (e.g., neurocognitive testing without the presence of an on-site neuropsychologist).

Although access to high-quality primary and specialized concussion care is a concern for youth throughout Canada, it is even more limited for children and adolescents who live in rural, semi-isolated and isolated communities. Access to healthcare is an important social determinant of health and it is well-recognized that patients living in rural and especially remote northern communities in Canada, a significant proportion of who are Indigenous, face numerous geographic, socioeconomic

and cultural barriers to accessing primary and specialized healthcare services (7–13). Factors such as travel distances, access to ground transportation, the high costs of air travel, lack of child care, inability to take time off work, communication barriers and previous negative interactions with the healthcare system can all contribute to disparities in healthcare utilization among people living in rural and remote communities. Primary care within many remote northern Canadian communities is provided by community health centers that are staffed primarily by nurses and supported by periodically visiting nurse practitioners and physicians. Lack of access to advanced diagnostic imaging as well as emergency and specialized services within rural and remote communities makes travel to major urban centers unavoidable for many patients thereby placing an enormous financial burden on patients and the healthcare system. For patients required to travel long distances by air or ground during the harsh winter months, abrupt changes in weather and seasonal road conditions due to storms, blizzards, or frigid temperatures can also prevent safe travel for extended periods thereby contributing to additional delays in receiving timely medical care (8).

In light of these challenges, the Canadian Academy of Sport and Exercise Medicine, the College of Family Physicians of Canada and the Canadian Medical Association has recently recommended that telemedicine and other virtual networks be explored as novel approaches to improve access to concussion care in Canada (62). However, to fully evaluate the potential of telemedicine to improve care for concussion patients in medically underserviced communities requires an in-depth appreciation of the fundamentals of head injury and concussion management.

MEDICAL ASSESSMENT AND MANAGEMENT OF CONCUSSION

Patients who sustain physical trauma to the head or neck can experience non-specific neurological symptoms such as headache, dizziness, sensitivity to light and sound, fatigue, and difficulty remembering and concentrating. They can also present with red flags such as worsening or severe headaches, neck pain, diplopia, seizures, changes in mental status as well as numbness or weakness of the extremities. To provide a medical diagnosis of acute concussion or persistent post-concussion symptoms a clinician must rule out more severe forms of TBI (e.g., subdural hematoma), cervical spine injury, and medical conditions (e.g., stroke, migraine, demyelinating disease, neoplastic or infectious conditions) that can present with non-specific neurological symptoms including red flags. Accordingly, international and national guidelines recommend that all patients with a suspected concussion undergo a medical assessment (46, 50). To complete a comprehensive medical assessment requires a primary care provider to conduct a clinical history, perform a focused physical examination, as well as order and interpret diagnostic tests (e.g., computerized tomography, blood work). The clinical history should include demographic information as well as details related to the mechanism of injury, initial symptoms, as well as the presence of red flags. Pre-existing conditions that effect concussion recovery or management should be collected including a history of previous concussion/TBI, migraine headaches, epilepsy, learning and mood disorders (63). The nature and severity of post-concussion symptoms should also be assessed using a validated and age-appropriate symptom inventory such as those included in Sport Concussion Assessment Tool 5 (SCAT5) or Child SCAT5 (46). The physical examination of patients with a history of head and spine trauma should ideally include a comprehensive assessment of level of consciousness and mental status as well as cranial nerve, motor, sensory, reflex, cerebellar, balance and gait testing. Assessment of vestibulo-ocular functioning including testing of convergence, smooth pursuits, saccades, and vestibulo-ocular reflex should be completed (64). Patients presenting with episodic vertigo should undergo Dix-Hallpike or Supine Roll testing to screen for benign paroxysmal positional vertigo (BPPV). Lastly, assessment of the cervical spine including range of motion and palpation for central and paraspinal tenderness should be performed.

Over the years several standardized concussion assessment tools have been developed including the SCAT (65), Vestibular Oculomotor Screening Tool (VOMS) (66) and the King Devick (K-D) test (67). Although these tools have been shown to be helpful in assisting with screening of athletes with suspected sport-related concussion, they do not include all of the objective physical examination tests that are required to comprehensively assess neurological, cervical spine, and vestibulo-ocular functioning in patients presenting with suspected acute traumatic brain and spine injury or persistent post-concussion symptoms. Additionally, these tools are not sufficient when deciding if an athlete with a suspected concussion is safe to return to sport.

While a medical diagnosis of concussion can usually be confirmed based on the results of the clinical history and physical examination, supplemental tests can be helpful in certain instances. In rare cases where patients present with non-specific or transient concussion-like symptoms, computerized neurocognitive testing performed by a clinical neuropsychologist can be used to screen for objective deficits in cognitive functioning (68). Diagnostic imaging (including plain radiographs, computerized tomography, and magnetic resonance imaging) should be considered in patients with suspected structural brain or cervical spine injuries and should be directed by validated clinical decision-making rules where available (5, 69–72). Patients presenting with post-traumatic seizures should also undergo electroencephalography.

Once a medical diagnosis of concussion has been confirmed, patients should be provided with verbal and written education regarding the signs and symptoms of concussion, warning signs that should prompt a return for repeat medical assessment as well as guidance about how to make a gradual and safe return to school, work and sport-related activities. Athletes should be provided written documentation regarding what sport-related activities they are medically cleared to return to. Following an initial brief period of rest (24–48 h), student-athletes should be advised to follow their respective Return-to-School and sport-specific Return-to-Sport strategies and seek medical follow-up prior to returning to full contact practices and games where

applicable (50). At present, there is no gold standard test to confirm physiological recovery following concussion. In general, patients should be considered clinically recovered when they are asymptomatic at rest (or have returned to their pre-injury neurological status in those with pre-existing conditions such migraine or mental health disorders), are tolerating full-time school, work, and physical exercise without symptoms and have a normal physical examination (5). Physicians should also ensure that athletes returning to sports have successfully completed stages 1–4 of their sport-specific Return-to-Sport strategy (non-contact practice) without any concussion-like symptoms prior to providing written medical clearance for them to return to full contact practices and games. In patients with pre-existing conditions (e.g., previous concussions, ADHD, mental health disorders) and those returning to sports with a risk of repeat head injury, additional supplemental tests such as graded aerobic treadmill testing and neuropsychological testing can be helpful to assist with confirming clinical recovery (73, 74).

Emerging evidence suggests that persistent post-concussion symptoms are mediated by heterogeneous and often overlapping pathophysiological and psychological factors that can lead to exercise intolerance, vestibulo-ocular or cervical spine dysfunction, cognitive impairments, post-traumatic headaches and mental health disorders (75, 76). Patients with persistent post-concussion symptoms should ideally be referred to multi-disciplinary concussion clinics and programs that have on-site access to physicians with expertise in concussion and TBI and who work closely with licensed experts in disciplines such as neuropsychology, neurology, physiotherapy, exercise science, neuro-ophthalmology, and psychiatry (5, 50, 53). Together, these teams can facilitate additional testing, develop individually-tailored targeted rehabilitation programs to address the patient's persistent symptoms and facilitate multi-disciplinary clearance to return to full school, work and sport activities.

CURRENT STATUS OF TELEMEDICINE IN CONCUSSION MANAGEMENT

The preceding discussion on the principles of concussion management and access to concussion care in Canada provide important background to help evaluate the few clinical studies that have examined the use of telemedicine to assist with assessment and management of patients with concussion.

Among these studies, Vargas et al. (24) presented a case report of a 15 year old male who sustained a head injury during soccer and underwent initial medical assessment and normal computerized tomography of the brain at a local emergency department. Two weeks post-injury, the patient underwent consultation by a neurologist via real-time videoconferencing. Clinical history was collected and a physical examination was performed including assessment of cranial nerve and sensory functioning, muscle stretch reflexes, coordination, and gait (the use of a remote examiner or telepresenter was not indicated). The SCAT-2 was used to assess cognition and balance. Scores on the Headache Impact Test-6, Generalized Anxiety Disorder 7-item Questionnaire and Patient Health Questionnaire were reviewed as well as the results from previous computerized neurocognitive testing. Images from the patient's previous CT scan were also reviewed via a remote Picture Archiving and Communication System (PACS) system. Based on the patient's symptom burden and the results of the physical examination and computerized neurocognitive testing, the patient was advised to refrain from further physical activity pending an in-person consultation with a concussion specialist. The authors suggested that telemedicine was useful in identifying a concussed athlete and determining the need for follow-up assessment to inform additional workup and return-to-sport decision-making. In another study, Vargas et al. (25) examined the use of telemedicine to assist with the sideline assessment of collegiate football players with suspected acute sport-related concussion. A prospective cohort of 11 athletes with suspected concussion underwent assessment by a remote neurologist via real-time videoconferencing as well as in-person assessment by a sideline healthcare provider using the Standardized Assessment of Concussion (SAC), K-D Test and modified Balance Error Scoring System (mBESS). The examiners were blinded to each other's examination and return to sport decisions until the end of the assessment. A high level of agreement was found between in-person and telemedicine-based assessments for the SAC (100%), K-D test (assessments were within a difference of 3 s 100% of the time) and the mBESS (assessment were within 3 points 100% of the time). Furthermore, return-to-sport decisions between providers were in agreement 100% of the time. The authors concluded that sideline telemedicine evaluations were safe and effective in determining return to sport status in athletes with suspected concussion. Lastly, the present authors conducted a retrospective review of a pilot telemedicine program established between a provincial multi-disciplinary pediatric concussion program and a remote hospital in northern Manitoba, Canada located ∼760 km away (22). Due to the limitations of performing a complete physical examination via unassisted videoconferencing, all eligible patients were required to have undergone a medical assessment by a physician or nurse practitioner prior to referral and were screened by a single neurosurgeon to determine whether initial assessment via real-time videoconferencing was feasible or whether an in-person assessment was warranted. During the study period, 20 patients (median age 13 years; range 1.8–17) were evaluated through the telemedicine program including 18 (90%) who underwent initial assessment via real-time videoconferencing. The median time from the date the referral was received and reviewed at the concussion program to the date of initial consultation with the neurosurgeon was 2 days. One patient who presented with transient monocular visual disturbance and neuroimaging evidence of an orbital floor fracture following an assault and one patient who was referred from a local emergency department but lived in another remote community underwent in-person initial assessment. Initial assessments using real-time videoconferencing consisted of a clinical history, administration of the Post-Concussion Symptom Scale which was faxed between sites, review of previous diagnostic imaging studies via a secure PACS system and an abbreviated physical examination performed without a remote examiner that included

testing of gross extraocular movements, facial symmetry, tongue movement, pronator drift, cervical spine range of motion, symptom provocation during saccade and gaze stabilization testing, balance testing and immediate and delayed 5-word recall. Among this cohort, 17 patients were diagnosed with acute concussions, one patient was diagnosed persistent post-concussion symptoms and post-traumatic migraine headaches and two infants were diagnosed with head injuries. Patients were managed according to national and international guidelines and were faxed completed copies of the *Canadian Guideline on Concussion in Sport* Medical Assessment and Clearance forms to facilitate a gradual return to school and sport-related activities where applicable. Four patients were referred on for further assessment by other members of the multi-disciplinary team including a headache neurologist (2 patients), vestibular physiotherapist (1 patient), neuro-ophthalmologist (2 patients), pediatric ophthalmologist (1 patient), plastic surgeon (1 patient), exercise physiologist for graded aerobic treadmill testing (1 patient), mobile crisis and adolescent psychiatrist (1 patient). One patient was arranged an MRI of the brain. At the end of the study period, 90% of patients met the criteria for clinical recovery, one remained in treatment and one was discharged to the care of a headache neurologist. Overall, 80% of patients were managed exclusively by telemedicine. The estimated cost avoidance associated with the 66 telemedicine encounters (57 videoconferencing appointments and 9 telephone follow-ups) conducted for this cohort based on regional health authority road travel reimbursement rates was $40, 972.94 or $2048.65 per patient. Delayed follow-up (>1 month) among 16 patients who achieved clinical recovery revealed that no patient experienced recurrent symptoms or a new concussion following discharge from the telemedicine program.

Taken together, preliminary research suggests that the use of telemedicine to assist in the assessment and management of select concussion patients is feasible and may be a useful approach to enhance timely access to safe and cost-effective sub-specialty care for those living in medically underserviced communities. However, there are several limitations and barriers that must be considered and overcome to help optimize its use and adoption throughout Canada.

LIMITATIONS AND BARRIERS

From a clinical perspective, the most important limitation of using telemedicine to assist with the management of concussion patients is the inability to perform a comprehensive physical examination. As stated above, the physical examination is an essential component of the initial medical assessment of patients presenting with head trauma, allowing the physician to rule out more serious forms of TBI, cervical spine injury, and neurological disorders that can present with concussion-like symptoms. Subtle findings on the focused vestibulo-ocular and cervical spine examination can also be helpful in directing treatment of patients with persistent post-concussion symptoms. Although preliminary research suggests that certain aspects of the SCAT can be reliably assessed via in-person videoconferencing (25), complete assessment of cranial nerve (including fundoscopy), motor and sensory functioning, tone and reflexes, as well as assessment of the scalp, jaw and cervical spine cannot be accomplished unless another examiner or telepresenter is available to conduct these tests and present the results to the distant physician (26, 27, 77). Assessment of oculomotor and vestibular functioning including objective testing of convergence, saccades, and vestibulo-ocular reflex requires additional training and experience and therefore is unlikely to be reliably enhanced by use of an inexperienced telepresenter. Despite these limitations, research demonstrates that a small proportion of acute pediatric concussion patients (∼30%) present with objective evidence of vestibulo-ocular and cervical spine dysfunction and that these abnormalities typically resolve with conservative management and without the need for any further diagnostic imaging or therapeutic intervention (78–81). Furthermore, rare patients who present with other post-traumatic co-morbidities (i.e., cranial neuropathies, brachial plexus traction injuries, central cord neuropraxia, BPPV) typically report clinical red flags or symptoms that alert physicians to these conditions. Lastly, those patients who present with a normal physical examination at initial assessment are unlikely to develop new physical examination abnormalities during longitudinal follow-up. Given these present limitations and clinical nuances, the use of telemedicine to assist in the management of concussion patients should be used selectively at this time and only in cases where there are clear geographic and logistical obstacles to providing in-person longitudinal assessment and care. Although the limitations of completing a comprehensive physical examination via telemedicine may be overcome in the future by more experienced telepresenters and new portable technology capable of assessing certain aspects of neurological functioning (e.g., oculomotor and vestibular function), physicians must always exercise a low threshold for requesting an in-person assessment whenever a more comprehensive physical examination, supplemental testing (diagnostic imaging, neuropsychological testing, graded aerobic exercise testing) or multi-disciplinary referrals are needed to optimize patient care. **Table 1** provides preliminary clinical recommendations regarding the potential use of telemedicine in pediatric concussion patients in Canada.

In addition to these clinical limitations, there are a number of other important patient, technological, economic, medico-legal, and administrative barriers that must also be considered and addressed (82). Despite increasing access to specialized care and reducing patient travel, there is concern that the use of telemedicine may alter the patient-physician relationship. Trust, rapport and clear communication between patients and providers are important keys to effective healthcare and may be more difficult to establish via telemedicine vs. in-person encounters. Additional research is therefore needed to assess patient, parent, and healthcare provider experience, comfort and satisfaction with concussion telemedicine programs and to identify ways these services can be optimized to provide user-friendly, culturally-appropriate patient-centered care.

TABLE 1 | Preliminary recommendations regarding the use of telemedicine in pediatric concussion in Canada.

Recommendations for primary care providers

1. Primary care providers including physicians, nurse practitioners and nurses should consider referring pediatric concussion patients to multi-disciplinary pediatric concussion clinics or programs whenever it is felt the patient's needs cannot be adequately addressed by the primary care provider and their existing local resources.

Examples:

- Instances where the primary care provider does not feel qualified to complete a comprehensive medical assessment or provide medical clearance for the patient to return to sports or other activities
- Patients for whom longitudinal medical follow-up is unavailable
- Patients who present with signs or symptoms (e.g., seizures, focal neurological deficits) that may indicate a more serious brain or spine injury or other neurological disorder
- Patients who may require diagnostic tests (e.g., imaging) that are not available near the patient's home community
- Patients who develop persistent post-concussion symptoms (>1 month post-injury)
- Patients with pre-existing conditions (e.g., migraine headaches, mood disorders) that can make it difficult to assess clinical recovery
- Patients who experience an exacerbation of pre-existing conditions (e.g., migraine headaches, mood disorders) and may require multi-disciplinary care
- Patients returning to contact or collision sports or other activities with an elevated risk of head injury
- Patients with a history of multiple concussions, persistent post-concussion symptoms, or abnormal diagnostic imaging findings that require multi-disciplinary return to sport and sport retirement guidance

Recommendations for physicians providing medical assessment and follow-up of pediatric concussion patients

1. Telemedicine (e.g., real-time videoconferencing) may be considered for follow-up appointments in patients presenting with acute concussion or persistent post-concussion symptoms who live in rural and remote communities and have undergone in-person medical assessment by the treating physician.
2. Telemedicine may be carefully considered to assist in the initial medical assessment and follow-up of patients with acute concussion who:
   a. live in rural and remote communities where medical follow-up is unavailable and for whom travel to a multi-disciplinary concussion clinic is difficult
   b. have undergone a previous medical assessment by a physician or nurse practitioner and have a normal physical examination
   c. do not report any signs or symptoms suggestive of a more serious brain or spine injury or other neurological disorder (e.g., focal neurological deficits, neck pain).

Physicians must maintain a low threshold for requesting an in-person assessment in instances where a patient is seen through telemedicine but a comprehensive physical examination, supplemental testing (diagnostic imaging, neuropsychological testing, graded aerobic exercise testing) and/or multi-disciplinary referrals are required to optimize patient care.

Recommendations for other multi-disciplinary healthcare professionals

1. Telemedicine may be considered by neuropsychologists, occupational therapists and psychiatrists to assist in the assessment and longitudinal care of concussion patients who develop persistent cognitive and mood-related symptoms.
2. Telemedicine may be considered by headache neurologists to monitor concussion patients with persistent headaches and who have undergone previous in-person assessment.
3. Telemedicine may be considered by vestibular and cervical spine physiotherapists as well as exercise physiologists to advance treatment plans in patients who have undergone previous in-person assessment.

Expert consensus and additional research will be needed to refine these recommendations in the future.

The potential of telemedicine to bridge gaps in concussion care in Canada is also critically dependent on a number of technological factors that facilitate access to reliable, high quality in-person videoconferencing. The most common systems used include mobile carts or wall-mounted cameras that can be controlled by the distant physician who connects via a secure PC-based software program that meets institutional personal health information and privacy requirements. However, some remote and northern communities may not have access to sufficient bandwidth to support high quality videoconferencing thereby restricting the expansion of telemedicine care to these regions and requiring patients to travel to other communities with these capabilities.

Establishing novel telemedicine sites, even within established telemedicine networks, can be associated with significant upfront capital costs for equipment, credentialing, and ongoing technical support and maintenance. These initial costs are often borne by institutions and healthcare providers while the long-term cost savings of telemedicine programs often benefit the healthcare system and society in general (83). As with other networks and programs, the future growth of telemedicine in concussion will require evidence that these approaches can provide reliable, efficient and cost-effective care. Lastly, there are important medico-legal and administrative barriers for those physicians and healthcare professionals providing patient care through telemedicine. Without a national framework for telemedicine, the Canadian Medical Protective Agency recommends that physicians providing telemedicine-based care consult their provincial or territorial colleges, some of which have published bylaws and polices (84). For those who provide care to patients across provincial and territorial borders, physicians are currently required to become licensed within the jurisdictions in which they and their patient reside. Although there is growing support for adopting a national license for physicians, licensing requirements currently vary across provinces and territories and may be an administrative and financial obstacle for some sub-specialty physicians who wish to offer telemedicine services to patients across their vast institutional catchment areas. Physician reimbursement for telemedicine-based services also varies across Canadian

provinces and territories. The development of billing tariffs that provide remuneration for videoconferencing assessments and telephone follow-ups that are equivalent to that which is earned for in-person visits will be essential to increasing the number of physicians who are willing to add this service to their clinical practice model. In order to decrease physician medico-legal risk and liability, additional research will be needed not only to identify ways that telemedicine may improve concussion care but also ways in which it can be potentially harmful for select patients. Most recently, the Canadian Medical Association, Royal College of Physicians and Surgeons of Canada and the College of Family Physicians of Canada announced the development of a new national taskforce on virtual care that will hopefully address many of these outstanding challenges (85).

FUTURE DIRECTIONS

Given the increasing burden of concussion and mild TBI among Canadian children and adolescents as well as the challenges of accessing specialized medical care among those living in rural and remote regions, it is incumbent on leaders in healthcare to develop innovative and sustainable strategies to address this public health issue and prevent its adverse short and long-term consequences. By overcoming many practical limitations and barriers, telemedicine has emerged as an important platform that can level the playing field and bridge the gaps in care for patients with neurological disorders living in medically underserviced communities. Although preliminary evidence suggests that telemedicine can be used to assist with the sideline assessment of athletes with suspected sport-related concussion (25), this application will likely be of limited use in Canada where national guidelines recommend that all youth with a suspected concussion undergo immediate and permanent removal from their game or practice and be referred for urgent medical assessment by a physician or nurse practitioner regardless of the results of sideline assessment (50). However, by following a roadmap outlined by telestroke, we believe the power of telemedicine can be harnessed to similarly transform and optimize the post-injury medical care of pediatric concussion patients in Canada. Similar to stroke, the model of care that has the greatest potential to be successfully applied to concussion in Canada is the "hub-and-spoke" model. In this system of care, a comprehensive stroke center that meets strict credentialing guidelines serves as the "hub" and provides clinical coverage and consultative services across multiple rural or remote hospitals or "spokes" that do not have access to similar multi-disciplinary expertise and diagnostic resources (16, 17). In more complex systems of care, hospitals that have access to some but not all of the personnel and resources of a comprehensive stroke center act as intermediate sites or "sub-hubs." The authors envision a similar model of care for pediatric (and adult) concussion patients in Canada whereby credentialed university-based regional or provincial comprehensive concussion centers that have on-site access to physicians with expertise in concussion, a dedicated team of multi-disciplinary experts with licensed training in TBI sub-disciplines and appropriate diagnostic and administrative resources would serve as hubs and provide integrated care to healthcare centers and providers across broad geographic catchment areas using existing provincial and territorial telemedicine networks (see **Figure 1**). Patient care would be guided by current national guidelines (Parachute, Ontario Neurotrauma Foundation) and facilitated by the use of national harmonized resources such as the *Canadian Guideline Concussion in Sport* Medical Assessment and Clearance Forms and additional Post-Concussion Education Sheets that are currently being adapted for First Nations, Metis and Inuit youth. Given that many university-based pediatric concussion clinics are frequently involved in research and are linked through national research and clinical guideline working groups (Canadian Traumatic Brain Injury Consortium, Parachute, Ontario Neurotrauma Foundation), these centers and networks would be in an optimal position to conduct collaborative multi-institutional research including randomized controlled trials and cost utility studies to establish a firm base of evidence to support further implementation of these approaches and develop consensus standards and guidelines that would ensure quality control and help secure sustainable funding for these programs. In addition to care for patients with concussion and mild TBI, these networks could also support the multi-disciplinary care of pediatric moderate and severe TBI patients for whom travel is even more challenging due to difficulties with mobility and who require ongoing specialized neuro-physiotherapy, occupational therapy, speech language pathology and neuropsychological services (86) that are unavailable within rural and remote communities.

Driven by a mandate to provide timely access to comprehensive concussion care throughout Manitoba as well as government interest in establishing province-wide youth concussion legislation, the Pan Am Concussion Program in Winnipeg, Manitoba has now established Canada's first provincial pediatric concussion telemedicine program. The Pan Am Concussion Program is a provincial government-funded clinical program that accepts referrals for pediatric sport- and non-sport related concussion patients as well as youth with more severe TBIs who require additional care and rehabilitation. The program serves a geographically and culturally diverse catchment area that includes the province of Manitoba, eastern Saskatchewan, northwestern Ontario and central Nunavut. At this facility, all patients undergo medical assessment and clearance by a neurosurgeon who works with other multi-disciplinary team members including licensed experts in neuropsychology, vestibular and cervical spine physiotherapy, exercise science, neuro-ophthalmology, psychiatry, neurology, and others. Based on encouraging results for a pilot study (22), the Pan Am Concussion has partnered with MBtelehealth to expand the Concussion in the North EConsultation and Telemedicine (CONNECT) Program that provides eConsultation and in-person videoconferencing services for patients who have undergone initial medical assessment and referral by primary care providers across Manitoba. All patients living in rural and remote northern communities who have undergone initial in-person medical

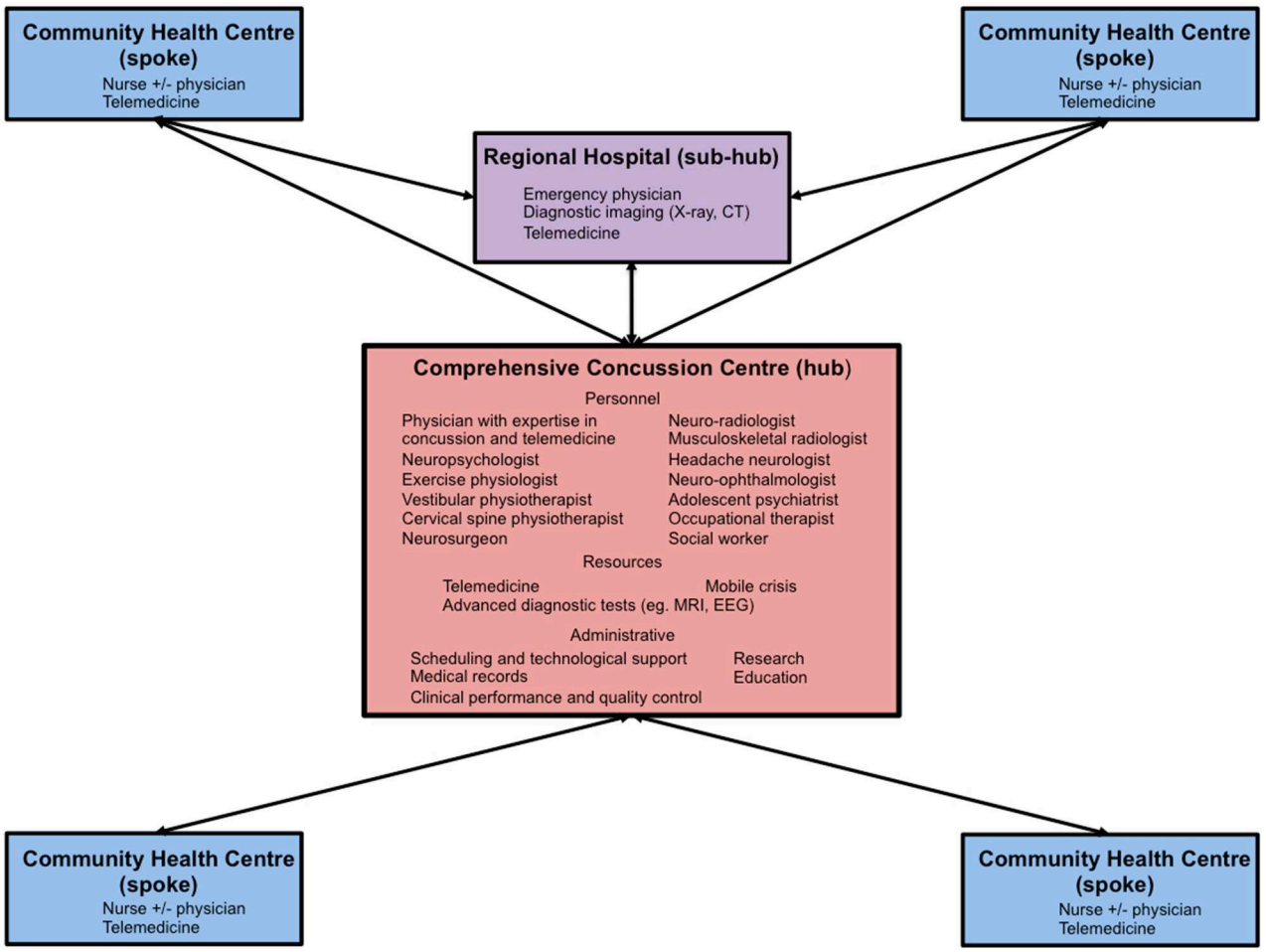

FIGURE 1 | Diagram of a proposed "hub-and-spoke" regional pediatric concussion network. CT, computerized tomography; MRI, magnetic resonance imaging; EEG, electroencephalography.

assessment at the concussion program and who have a normal neurological examination are considered for longitudinal follow-up using telemedicine. Acute concussion patients living in remote or isolated northern communities who have undergone a prior medical assessment by a primary care provider are considered for initial medical assessment and follow-up via in-person videoconferencing as long as there are no clinical concerns for more severe forms of TBI, cervical spine injury or other co-morbidities. In all cases, the neurosurgeon exercises clinical judgment when selecting cases to be assessed and followed through telemedicine and maintains a low threshold to request an in-person assessment in instances where a more complete physical examination or additional testing is required to optimize patient care. For those who do require further advanced diagnostic tests or multi-disciplinary consultations, these appointments are coordinated by the concussion program in order to limit the number of trips away from home. As pointed out by others, the success and sustainability of telemedicine networks is highly dependent on the engagement of clinical champions across the hub and spoke sites (36). Accordingly, our pediatric telemedicine program has benefitted from close working relationships with local healthcare and telemedicine providers within rural and northern communities, organizations that provide physician coverage to these regions (Northern Health Region, Ongomiizwin Health Services), Health Canada's First Nations and Inuit Health Branch, Pan Am Clinic as well as our regional health authorities (Shared Health, Winnipeg Regional Health Authority) and provincial telemedicine provider (MB Telehealth). Moving forward, this program will serve as an important platform to further evaluate the potential of telemedicine to provide safe, timely, effective, equitable, efficient, cost-effective, and culturally-appropriate patient-centered care for pediatric concussion patients living in rural and remote communities in Canada.

CONCLUSIONS

Concussion is common injury among Canadian youth that is placing an increasing burden on the healthcare system. Although

timely medical care is paramount to confirming a medical diagnosis and instituting evidence-based recommendations and care, access to physicians with expertise in concussion remains a challenge for Canadian youth especially for those living in rural and remote communities. Accumulating evidence supports the use of telemedicine to improve access to specialized care for patients with a wide spectrum of neurological disorders. Although preliminary research suggests that telemedicine may be a safe and cost-effective approach to improve care of select concussion patients living in medically underserviced communities, future research is needed to overcome remaining limitations and barriers and build a firm base of evidence to support the development and sustainability of telemedicine-based concussion networks in Canada.

AUTHOR CONTRIBUTIONS

ME and KR: conception and design of the work, drafting the work and revising it critically for intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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Myelin Oligodendrocyte Glycoprotein (MOG) Antibody Diseases in Children in Central South China: Clinical Features, Treatments, Influencing Factors, and Outcomes

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Background and purpose: The clinical and radiological features of myelin oligodendrocyte glycoprotein antibody (MOG-Ab) diseases vary among the patients and studies. In addition, the clinical significance of MOG-Ab for the diagnosis, treatment, and prognosis is not yet established. Therefore, we aimed to evaluate the clinical, radiological, treatments and outcome features of MOG-Ab diseases in Central Southern part of China.

Methods: A retrospective study of children with MOG-Ab disease was carried out from January 2015 to October 2018. Demographics, clinical features, treatments, and outcomes were reviewed. Some of the clinical information was compared including the annualized relapse rates (ARRs) before and after treatment with disease-modifying drugs (DMDs).

Results: Twenty-five patients with MOG-Ab disease were recruited. The onset age ranged from 3 to 12.4 years old. 13 were females and 12 were males. The median follow-up period was 15 months (range 7–63). Most of the cases that aged ≤9 years presented with fever (47.4%), encephalopathy (47.4%), and lesions on white matter and/or deep gray matter (52.6%). While most of those aged above 9 years presented with optic neuritis (ON) (66.7%), and lesions on spinal cord and/or optic nerve (50%). Until the last follow-up, 10 (40%) cases had multiphasic courses while 15 (60%) had a monophasic course, and the mean follow-up time was statistically significant (10.67 vs. 31 months, $p = 0.0001$). DMDs such as rituximab (RTX) or/and azathioprine (AZP) or mycophenolate mofetil (MMF) were used at least once in 56% of the cases. The ARR before and after treatment was 2.4 and 0 respectively ($p < 0.05$). The median Expanded Disability Status Scale scores of our study were 0 (range 0–2). 96% (24/25) of the cases had a full recovery.

Conclusions: MOG-Ab disease among Chinese children share the same clinical characteristics with Caucasians. However, the Chinese children seem to have a

better prognosis than Caucasians. There is an age-dependent phenotypes, as brain involvement is more frequently seen in children younger or equal to 9 years while ON and neuromyelitis optica spectrum disorders are commonly seen in children older than 9 years. DMDs, such as AZA, MMF or RTX, can reduce the ARR.

Keywords: myelin oligodendrocyte glycoprotein (MOG), optic neuritis (ON), demyelinating diseases, disease-modifying drugs, rituximab

INTRODUCTION

Myelin oligodendrocyte glycoprotein (MOG) is a glycoprotein that is localized on the outer surface of the myelin sheath and oligodendrocytes (1). It has been used to induce autoimmune encephalomyelitis in an animal model of multiple sclerosis (MS) (2). The MOG antibody (MOG-Ab) has been identified in pediatric patients with acquired demyelinating syndrome, particularly the acute disseminated encephalomyelitis (ADEM) (3–5). In addition to ADEM, this antibody has also been found in patients with other inflammatory demyelinating diseases (IDDs) such as clinically isolated syndrome (CIS), neuromyelitis optica spectrum disorders (NMOSDs), recurrent bilateral optic neuritis (ON), transverse myelitis (TM), and MS (2, 6).

Patients with MOG-Ab-associated demyelination seem to have unique clinical and radiological features. Nevertheless, the clinical significance of MOG-Ab for the diagnosis, treatment and prognosis is not yet established. The radiological findings associated with these Abs vary among patients and studies. Since its discovery, many cases have been reported in many countries, including China. However, the existing studies from China have very small sample sizes.

This study aimed to evaluate the clinical, radiological, treatments, and outcome features of MOG-Ab diseases. To our knowledge, this study contains the largest sample size in China to date.

SUBJECTS AND METHODS

We retrospectively reviewed the clinical data including the symptoms, physical examination and ancillary examination findings [magnetic resonance imaging (MRI), electroencephalographs (EEG), and cerebrospinal fluid (CSF)], treatments used, and their outcomes. This study included children who were aged 14 years old or younger with a definite diagnosis of MOG-Ab-positive with IDDs at Xiangya Hospital, Central South University. The cohort comprised patients admitted at our center from January 2015 to October 2018. The written and informed consent were obtained from the patients' parents.

For the better assessment of the clinical course of this condition we defined an acute episode as a new neurological deficit lasting <24 h (7). A relapse referred to any new central nervous system (CNS) symptom/sign lasting >24 h in the absence of other causes that was supported clinically or radiologically (8). The criteria for IDDs, including ADEM, NMOSD, CIS, and MS, were in accordance with those of the International Pediatric MS Study Group (9). ADEM-ON was defined as at least one episode of ON after ADEM (10). Any immune-mediated CNS demyelinating disorder not falling into the aforementioned categories was classified as an uncategorized CNS demyelination. All the patients were followed up, and data were collected and analyzed. Patients with a follow-up duration of <5 months were excluded. The dosages and durations of medications used during the acute phase and disease-modifying drugs (DMDs) were reviewed. Complete recovery was defined as a disappearance of clinical symptoms and brain lesions. Almost complete recovery was defined as a disappearance of clinical symptoms with persistence of brain lesions. Partial recovery was defined as a persistence of both clinical symptoms and brain lesions. We compared the annualized relapse rates (ARRs) before and after treatment with DMDs in relapsing patients. The Expanded Disability Status Scale (EDSS) score (rates from 0 to 10) was used to estimate the disability outcome at the last follow-up.

Serum samples from all patients were tested for anti-MOG immunoglobulin G (IgG) and anti-AQP4 IgG by the Chinese branch of the Euroimmun Medical Diagnostic Laboratory using a fixed cell-based indirect immune-fluorescence test (IIFT) employing BIOCHIPs (EUROIMMUN AG, Luebeck, Germany). HEK293 cells transfected with full-length human MOG and AQP4 isoform M1 were used in the anti-MOG and anti-AQP4 IIFTs, respectively. Serum specimens were collected from these patients to test MOG-IgG during the acute attacks or while undertaking follow-up visits from January 2018.

The statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Continuous variables, such as the age and follow-up time, were analyzed with an independent t-test. Categorical variables were analyzed with the chi-square test or Fisher's exact test. $P < 0.05$ (two-sided) was considered significant.

RESULTS

A total number of 54 patients were diagnosed to have IDDs from January 2015 to October 2018 (NMOSD 25 patients; ADEM 17 patients; MS 3 patients; CIS 1 patient; ON or TM 5 patients; Others 3 patients). And the serum samples of all 54 patients were tested for MOG-Ab in which only 25 had positive results.

The Clinical Characteristics

The present study comprised of 25 cases who had first demyelinating symptoms from the age of 3 to 12.4 years. 12 were

males, and male to female ratio was 1:1.08. **Table 1** summarizes the patients' clinical information. Nineteen cases were ≤ 9 years old at the onset of the disease (ranged 3–8.8 years with median of 5.75 years). **Table 2** summarize the demographic and clinical characteristics of those groups.

The initial clinical presentations were as follows: 11 (44%) cases had fever, 9 (36%) had encephalopathy, 7 (28%) had ON (6 bilateral and 1 unilateral), and 7 (28%) had headache. The top four onset symptoms in the group of patients ≤ 9 years were fever (47.4%), encephalopathy (47.4%), headache (26.3%) and seizures (26.3%). The top four onset symptoms in the group of patients older than 9 years were ON (66.7%), fever (33.3%), headache (33.3%) and myelitis (33.3%). Disease onset was preceded by infection in 4 patients; upper respiratory tract infection (n = 3) and mumps (n = 1) which occurred 2 weeks to two months before the onset of the initial attack.

The median follow-up period was 15 months (range 7–63). Until the last follow-up, 10 (40%) patients had multiphasic courses. The median time between the first and second episodes was 10 months (range 3–32). The symptoms during the relapses were different from those of initial presentation. Regarding the initial phenotypes, 48% (12/25) of the cases presented with ADEM, 24% (6/25) with ON and 28% (7/25) with other CNS demyelination. Finally, according to the IDD criteria, 10 patients were diagnosed with ADEM (8 with ADEM, 1 with multiphasic disseminated encephalomyelitis (MDEM), and 1 with ADEM-ON), 6 (24%) with NMOSD (4 patients had a monophasic course while 2 had multiphasic courses), 1 with MS, 1 with CIS, and 7 with uncategorized CNS demyelination (4 patients with monophasic or recurrent ON or longitudinally extensive transverse myelitis (LETM). ADEM was diagnosed more in the group of patients ≤ 9 years old while those aged above 9 years presented more with monophasic or recurrent ON or LETM (**Figure 1**).

The Ancillary Examination Results

Lumbar punctures were performed for 24 patients at first episode. Intracranial pressure was measured in only 20 cases as it was difficult for the other 4 cases. Five of those 20 cases had increased intracranial pressure (>200 mm H_2O in patients number 1, 2, 14, 21, and 24). Elevated levels of CSF proteins and cell counts were found in 25% (6/24) of the cases. Two patients were found to have positive CSF oligoclonal bands (OCB) (**Table 3**).

Four patients (patient number 19, 20, 21, and 25) were found to have anti-N-methyl-D-aspartate receptor (anti-NMDAR) antibodies once. Patient number 19 was found to have anti-NMDAR antibodies on the second episode, of which manifested with headache and seizures. The other 3 patients were found to have antibodies on the first episodes. However, none of the four cases met the diagnostic criteria for anti-NMDAR encephalopathy. 66.7% (16/24) of the cases had an elevated erythrocyte sedimentation rate (ESR) (**Table 3**). Thirteen of the 17 patients whom underwent EEG examinations had abnormal findings. Twelve patients had diffuse background slowing (delta or theta) activity and/or generalized or predominantly frontotemporal slow wave activity, and one displayed both slow wave and epileptic activity. Six children received antiepileptic drugs; levetiracetam (n = 1) and oxcarbazepine (n = 5), and none of them had seizures during the follow-up period.

All 25 cases had positive MOG antibodies in serum but only 6 out 23 cases whom underwent CSF screening had positive results. The relationship between the patients' phenotype and the MOG titer is shown in **Figure 2**. We traced the MOG-Ab titers of 12 cases (7 with a monophasic course and 5 with multiphasic courses). The MOG-Ab titers were low and unchanged during remission for 58.3 and 25% of the cases, respectively. Conversely, the titers were high during remission and relapse for one case each. Only 2 patients who had a monophasic course had a negative MOG-Ab titer at the last follow-up. The MOG-Ab titer was positive even 17 months after the disappearance of the symptoms.

All cases but number 21 had abnormal brain or spinal cord MRI at the onset. The abnormal findings were: sixteen cases had an increased signal on T2-weighted MRI or fluid-attenuated inversion recovery on the brain only (six had lesions on the white matter only, five on the white matter and deep gray matter and/or cerebellum/optic nerve, two on the gray matter and/or white matter, one on the thalamus only, one on the optic nerve only, and one on the subcortical white matter, thalamus, brain stem, and optic nerve), six on both brain and spinal cord (four on the subcortical white matter and spinal cord and two on the white matter, deep gray matter, optic nerve and spinal cord), and two on the cervical spinal cord only. Case number 21 had no lesion on the brain MRI during the first 10 days of the disease onset, but they were observed 20 days later on the subcortical white matter and basal ganglia. The abnormal signal locations on the MRI were split into five categories based on the symptoms (white matter and/or deep gray matter, white matter and spinal cord/optic nerve and/or others, spinal cord and/or optic nerve, gray matter and/or white matter, and cerebellum and/or others). All cases with an abnormal signal on the white matter and/or deep gray matter were ≤ 9 years, and all spinal cord and/or optic nerve abnormalities were found in patients older than 9 years (**Table 2**). When the MRIs from the follow-up period were compared with the initial ones, three children had an asymptomatic new abnormal signal on the brain, the lesions of three children (case number 10, 16, and 21) disappeared, and the lesions of 21 cases decreased regardless of whether relapse occurred.

The Treatments and Outcomes

Acute attacks were treated with methylprednisolone pulse therapy (mPSL) (15–30 mg/kg per day for 5 days), followed by prednisone (1 mg/kg, tapered off within 1–2 months or more) at least once in 24 patients and with intravenous immunoglobulin (IVIG) (0.4 g/kg per day for 5 days) at least once in 22 patients. Other treatments included either methylprednisolone therapy (PSL) (3–5 mg/kg per day for 3–5 days) or intravenous dexamethasone (DEX) (20 mg/m^2 per day for 5 days) followed by prednisone in one case each as well as acyclovir and/or antibiotics (n = 24) as empirical treatment for the suspected CNS infection.

The information regarding the treatment outcomes was available for 49 episodes. 14 documented attacks were treated only with mPSL, 26 with both mPSL and IVIG, 1 with both

TABLE 1 | The demographic and clinical details.

Case number	Sex	Onset age, year	Follow-up time, months	Re-lapse numbers	Onset symptoms	Clinical manifestation	Prodro-mal symp-toms	Lesion location in MRI at onset	Lesion location during the follow-up period	Immuno-suppressive treatment	EDSS at last follow-up
1	F	4.8	24	7	Fever, Headache, Encephalopathy	1st episode of ADEM (acute encephalitis), 7 episodes of rON	None	Subcortical white matter, cervical spinal cord	Optic nerve, cervical spinal cord	mPSL, IVIG, RTX, AZP	0
2	M	10.9	13	0	Myelitis	LETM	URI 2 weeks before onset	Cervical spinal cord	Cervical spinal cord	mPSL, IVIG	0
3	F	3.25	12	0	Myelitis, encephalopathy, seizure	ADEM	None	Subcortical white matter, spinal cord	Subcortical white matter	mPSL, IVIG, RTX	0
4	F	5.8	15	0	Encephalopathy, hemiparesis, external ophthalmoplegia	ADEM	None	Subcortical white matter, cerebellum, cerebral peduncle, spinal cord	Subcortical white matter, cerebellum, cerebral peduncle	mPSL, IVIG	0
5	M	6.7	15	0	Hemiparesis, seizure, fever, myelitis, encephalopathy	ADEM	None	Corpus callosum, subcortical white matter	Corpus callosum, subcortical white matter	mPSL, IVIG	0
6	F	7.2	20	0	Seizure, speech delay, poor cognition	ADEM	None	Subcortical white matter, corpus callosum, thalamus, basal ganglia, and cerebellum	N/A	mPSL	0
7	M	11.5	16	0	BON	BON	URI 2 weeks before onset	subcortical white matter, thalamus	Subcortical white matter, thalamus	DEX, mPSL, IVIG	0
8	M	3	51	1	Headache, unsteadily gait	1st episode of headache and unsteady gait, 2nd episode of unsteady gait	None	Subcortical white matter, cerebellum	Subcortical white matter, basal ganglia, medulla, and cerebellum	mPSL, IVIG, mycophenolate mofetil	0
9	M	10.5	23	1	Myelitis	NMOSD	Mumps 20 days before onset	Subcortical white matter, spinal cord	Spinal cord	mPSL, IVIG, RTX	0
10	M	5.75	12	0	Seizure	CIS	None	Subcortical white matter, white matter adjacent to the posterior horn of lateral ventricle	None	mPSL, IVIG	0
11	F	5.2	13	0	Fever and encephalopathy	ADEM	None	Subcortical white matter, thalamus and basal ganglia, cerebellum, optic nerve, spinal cord	Subcortical white matter, thalamus and basal ganglia, cerebellum, optic nerve, spinal cord	IVIG, DEX, mPSL	0
12	M	6.4	14	0	Myelitis, headache, encephalopathy	ADEM	None	Subcortical white matter, basal ganglia, optic nerve, spinal cord	Subcortical white matter, basal ganglia, Optic nerve, spinal cord	DEX, IVIG, RTX	0
13	F	5.5	25	2	BON	NMOSD	None	Subcortical white matter, thalamus, brain stem, optic nerve	Subcortical white matter, thalamus, brain stem, optic nerve	mPSL, IVIG, RTX	0
14	F	4.5	63	2	Fever, headache	3 episodes of fever and headache	None	Subcortical white matter	Subcortical white matter	mPSL, IVIG, RTX, AZP	0
15	F	7.1	42	2	Encephalopathy	1st episode with ADEM, 2nd episode with UON (L), 3rd episode with headache and psychological and behavioral abnormalities	None	Subcortical white matter	Subcortical white matter, optic nerve	mPSL, IVIG, RTX, AZP	0

(Continued)

TABLE 1 | Continued

Case number	Sex	Onset age, year	Follow-up time, months	Re-lapse numbers	Onset symptoms	Clinical manifestation	Prodro-mal symp-toms	Lesion location in MRI at onset	Lesion location during the follow-up period	Immuno-suppressive treatment	EDSS at last follow-up
16	M	11.8	17	0	Headache, BON	NMOSD	None	Cervical spinal cord	None	mPSL	0
17	F	4.75	8	0	BON	BON	None	Thalamus	Thalamus, right optic nerve	mPSL, IVIG, RTX	0
18	F	8.75	9	0	Fever, Headache	1st episode with fever and headache	None	Subcortical white matter, thalamus	Subcortical white matter, thalamus, brain stem	mPSL, IVIG	0
19	M	8.8	44	3	BON	NMOSD	None	Subcortical white matter	Subcortical white matter	mPSL, IVIG, RTX	2
20	F	7	12	2	Unsteady gait, speech delay	1st episode with ADEM, 2nd episode with headache and myelitis	None	Subcortical white matter	Subcortical white matter, basal ganglia and corpus callosum	mPSL, IVIG, RTX	0
21	F	8.5	8	0	Encephalopathy seizures	ADEM	None	None	Subcortical white matter, and basal ganglia	mPSL, IVIG, RTX	0
22	M	5.4	7	0	Fever, headache	1st episode with fever and headache	None	Cortex, white matter adjacent to the posterior horn of the lateral ventricle	Cortex, white matter adjacent to the posterior horn of the lateral ventricle	mPSL, IVIG	0
23	M	11.6	12	4	UON (R)	5 episodes of rON	None	Optic nerve	Optic nerve	mPSL, AZP	0
24	F	5.75	36	1	Encephalopathy and seizures	MDEM	Fever 2 months prior onset	Subcortical white matter, hippocampi	Subcortical white matter, Hippocampi	mPSL, IVIG	0
25	M	12.4	12	0	Fever, encephalopathy and seizures	ADEM	None	Cortex	Cortex and thalamus	DEX, mPSL, IVIG, RTX	0

M, male; F, female; AZP, azathioprine; ADEM, acute disseminated encephalomyelitis; BON, bilateral optic neuritis; CIS: clinically isolated syndrome; DEX, intravenous dexamethasone; EDSS, expanded disability scale score; IVIG: intravenous immunoglobulin; L, left; LETM, longitudinally extensive transverse myelitis; MOG-Ab, myelin oligodendrocyte glycoprotein antibody; MS, multiple sclerosis; mPSL, methylprednisolone pulse therapy; MDEM, multiple disseminated encephalomyelitis; MMF, mycophenolate mofetil; N/A, not applicable; NMOSD, neuromyelitis optica spectrum disorders; PSL, oral prednisone; PE, plasma exchange; R, right; rON, recurrent optic neuritis; RTX, rituximab; URI, upper respiratory infection; UON, unilateral optic neuritis.

TABLE 2 | Demographics, clinical characteristics and MRI findings.

Item	All patients (%)	Age ≤9 (%)	Age >9 (%)
Number	25	19	6
Female: male	13:12	13:6	0:6
Median age, range (years)	6.6, 3–12.4	5.75, 3–8.8	11.6, 10.6–12.4
Onset symptoms			
Fever	11 (44%)	9 (47.4%)	2 (33.3%)
Encephalopathy	9 (36%)	9 (47.4%)	0
ON	7 (28%)	3 (15.8%)	4 (66.7%)
Headache	7 (28%)	5 (26.3%)	2 (33.3%)
Seizures	6 (24%)	5 (26.3%)	1 (16.7%)
Myelitis	5 (20%)	3 (15.8%)	2 (33.3%)
Hemiparesis	2 (8%)	2 (10.5%)	0
Unsteady gait	2 (8%)	2 (10.5%)	0
Disease spectrum			
ADEM or MDEM or ADEM-ON	10 (40%)	9 (47.4%)	1 (16.7%)
NMOSD	6 (24%)	4 (21%)	2 (33.3%)
ON or rON or LETM	4 (16%)	1 (5.3%)	3 (50%)
MS or CIS or Others	5 (20%)	5 (26.3%)	0
Prodromal symptoms	4 (16%)	1 (5.3%)	3 (50%)
Initial lesions on MRI			
White matter and/or deep gray matter	10 (40%)	10 (52.6%)	0
White matter and spinal cord/optic nerve and/or others	8 (32%)	6 (31.6%)	2 (33.3%)
Spinal cord and/or optic nerve	3 (12%)	0	3 (50%)
Gray matter and/or white matter	2 (8%)	1 (5.3%)	1 (16.7%)
Cerebellum and/or others	2 (8%)	2 (10.5%)	0

ADEM, acute disseminated encephalomyelitis; CIS, clinically isolated syndromes; LETM, longitudinally extensive transverse myelitis; MDEM, multiple disseminated encephalomyelitis; MS, multiple sclerosis; NMOSDs, neuromyelitis optica Spectrum disorders; ON, optic neuritis; rON, recurrent optic neuritis.

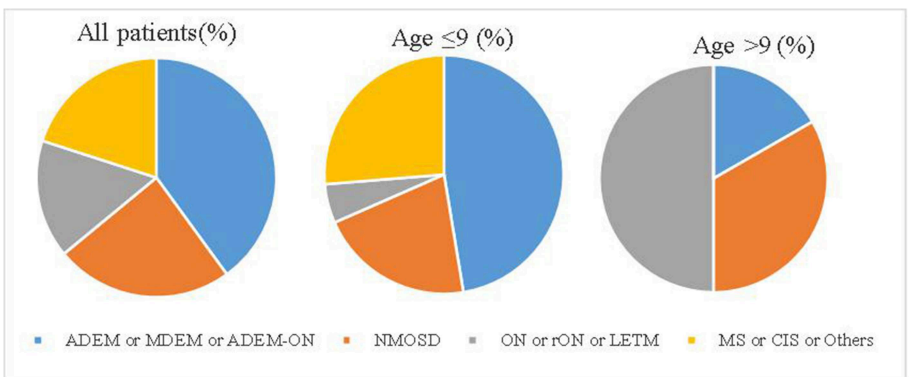

FIGURE 1 | The disease spectrum of each group. ADEM, acute disseminated encephalomyelitis; CIS, clinically isolated syndrome; LETM, longitudinally extensive transverse myelitis; MS, multiple sclerosis; MDEM, multiple disseminated encephalomyelitis; NMOSDs, neuromyelitis optica spectrum disorders; ON, optic neuritis; rON, recurrent optic neuritis.

PSL and IVIG, and 1 with both DEX and IVIG. Conversely, 3 attacks were treated only with PSL, and 4 were not treated at all. Complete or almost complete recovery from acute attacks was noted after 43 episodes (27 were treated with both steroids and IVIG, 10 with mPSL only, 3 with PSL only and 3 were not treated at all) and partial recovery was noted after 3 attacks (2 were treated with mPSL only while 1 with both mPSL and IVIG). Notably, symptoms flared up after withdrawal or tapering of steroids at least once in 4/10 patients.

DMDs were prescribed once in 14 (56%) cases. These included RTX (2 × 750 mg/m^2, days 1 and 15) in 10 cases, azathioprine (AZP) in 1 case (1 mg/kg), both AZP and RTX in 2 cases (case number 1 and 14) and mycophenolate mofetil (MMF) in 1 case (case number 8). **Table 4** shows the efficacy of different DMDs

TABLE 3 | The laboratory findings at first episode.

Case number	Sex	ICP, mmH2O	CSF protein, mg/L	CSF WBC, ×106/L	OCB	Serum MBP-Ab	Serum AQP4-Ab	CSF anti-NMDAR	ESR, mm/h	Other circulating antibodies
1	F	230	140	180	–	–	–	–	23	A-TG/A-TPO+
2	M	205	330	20	–	–	–	–	44	A-TPO+
3	F	130	160	0	–	–	–	–	37	A-TPO+
4	F	150	330	50	–	–	–	–	91	A-TPO+
5	M	150	550	150	+	–	–	–	110	–
6	F	N/A	420	16	–	–	–	–	N/A	A-TG+
7	M	175	220	4	–	–	–	–	3	–
8	M	N/A	460	14	N/A	–	–	–	120	A-TG/A-TPO+
9	M	165	200	4	N/A	–	–	–	9	–
10	M	N/A	240	10	–	–	–	–	79	A-TPO+
11	F	82	540	0	–	–	–	–	67	A-TPO+
12	M	68	550	16	–	–	–	–	37	–
13	F	110	460	46	–	–	–	–	87	–
14	F	205	210	18	–	–	–	–	37	–
15	F	110	100	0	–	–	–	–	20	–
16	M	N/A	N/A	N/A	N/A	N/A	N/A	N/A	71	–
17	F	140	140	14	N/A	–	–	N/A	54	–
18	F	175	110	24	–	–	–	–	16	–
19	M	195	380	14	+	–	–	N/A	33	–
20	F	N/A	250	2	–	N/A	N/A	+/–	19	–
21	F	300	480	18	–	–	–	++	81	–
22	M	155	640	86	N/A	–	–	–	116	Anti-Ro-52+
23	M	190	140	0	–	–	–	–	11	–
24	F	280	410	0	–	–	–	–	14	–
25	M	150	300	50	–	–	–	+	84	A-TP0+

M, male; F, female; AQP4-Ab, Aquaporin-4 antibody; anti-NMDAR, anti-N-methyl-D-aspartate receptor; A-TPO, thyroid peroxidase antibody; A-TG, thyroglobulin antibody; CSF, cerebral spinal fluid; ESR, erythrocyte sedimentation rate; ICP, intracranial pressure; MBP, myelin basic protein; N/A, not applicable; OCB, oligoclonal bands; WBC, white blood cell; "+", positive; "–", negative.

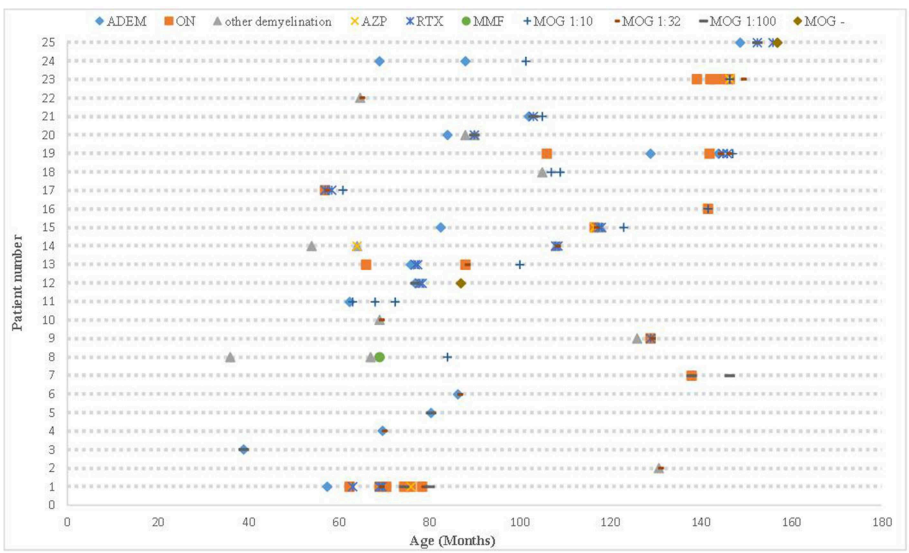

FIGURE 2 | The clinical course, MOG-Ab titers, and DMDs of all patients. ADEM, acute disseminated encephalomyelitis; AZP, azathioprine; DMDs, disease-modifying drugs; MOG-Ab, myelin oligodendrocyte glycoprotein antibody; MMF, mycophenolate mofetil; ON, optic neuritis; RTX, rituximab.

TABLE 4 | Efficacy of different disease-modifying drugs in patients with multiphasic course.

Disease-modifying drug	*N*	Disease duration before treatment (m)	Duration of therapy (m)	ARR before treatment	ARR during treatment
Rituximab	5	3	18	8	0
		11	12	2.4	1.09
		54	7	0.67	0
		39	4	1.23	0
		6	4	6	0
Azathioprine	1	7.3	2.7	8.22	0
Mycophenolate mofetil	1	33	16	0.73	0
Total, median(range)	7	11 (3–54)	7.5 (2.7–18)	2.4 (0.67–8.22)	0 (0–1.09)*

*ARRs, annualized relapse rates; *p-value obtained by independent t-test; m, months.*

in cases with a multiphasic course. The time from initial attack to administration of DMDs was 11 (3–54) months. The ARRs before and after treatment were 2.4 (0.67–8.22) and 0 (0–3.43), respectively ($P < 0.05$). 7/9 cases did not relapse after DMDs treatment. For those relapsed, one patient used AZP initially (ARR was 2.4 before the treatment and 0.24 after the treatment) and then changed to RTX (ARR became 0 after the treatment). The other patient used RTX and then changed to AZP because of an elevated ARR (ARR 4.8 before the treatment and 5.33 after the treatment).

At the last follow-up, all patients were alive, and the final EDSS scores were low (ranging 0–2). The visual acuity (VA) of the 9 patients with a history of ON recovered (VA>1.0) except for the case number 19 who had several black spots in the field of vision (EDSS range 2). The median observation time was 21 months (range 6–42). 96% (24/25) of the cases recovered completely.

Comparison Between Cases With Monophasic and Multiphasic Courses

The mean follow-up time of the patients with multiphasic course was 34.1 months (range 12–63) vs. 13.3 months (range 7–20) for the patients with a monophasic course ($P = 0.0001$). The median time between the first and second episodes was 10 months (range 3–32). The median time between the second and third episodes was 1.5 months (range 1–44). The sex, age at onset, clinical manifestations, ancillary examinations, locations of lesions on MRI and response to treatment did not differ between the two groups. We also compared variables between the cases with NMOSD ($n = 4$) and those with other relapsing IDDs ($n = 6$). And we found that sex, age at onset, phenotypes, seizures, onset of lesion on MRI, ARR and EDSS during follow-up did not differ (**Table 5**).

DISCUSSION

The first evidence for the role of MOG-Ab IgG as a biological marker in children was identified by O'Connor et al. (11) with MOG-Ab-associated demyelination in a subgroup of ADEM patients. In subsequent studies, MOG-Ab IgG was detected in a subset of ADEM, NMOSD, monophasic and recurrent ON and TM patients as well as in those with demyelinating syndromes overlapping with anti-NMDA receptor encephalitis. The clinical features were different between adults and children, and Hacohen and Brenda Banwell proposed that children with MOG-Ab were frequently Caucasian (12). A recent study from Australia showed the clinical courses, treatments and outcomes of 59 cases (33 pediatric patients) with relapsing MOG-Ab-associated demyelination, of whom 73% were Caucasian (13). This study summarizes the clinical features of 25 MOG-Ab-positive Chinese children aged below 14 years.

We found no gender difference between affected males and females (male: female=1:1.08), of which is similar to previous report (12). Fever and encephalopathy were the most frequent onset symptoms in the group of children ≤ 9 years old, whereas ON was the most frequent onset symptom in the group of children older than 9 years. Consistent with our results, other studies demonstrated the same age-dependent phenotypes, with brain involvement more frequently seen in younger children while ON and NMOSD are common in older children (>9 years) (14) and adults (15). We further found that ADEM was the most frequent initial clinical presentation followed by ON. Peschl et al. performed a literature review and compared all studies that analyzed the presence of MOG-Ab in IDDs in which they found that ADEM was the most frequent initial clinical presentation associated with MOG-Ab, followed by ON (16). Four cases in our study (case number 19, 20, 21, and 25) had concomitant anti-NMDAR antibodies in at least one attack. When we compared our 4 current cases with those in our initial study of anti-NMDAR encephalitis (17), we found a reduced mean time to full recovery (1 vs. 4 months) and higher relapse rate (50 vs. 2%). Fan et al. (18) found that the symptoms of MOG-Ab diseases coexisting with anti-NMDAR encephalitis were frequently milder than those of typical anti-NMDAR encephalitis cases, which is in agreement with the findings of this study. Therefore, patients with anti-NMDAR encephalitis should also be tested for MOG-Ab. Our study showed that 6 cases had seizures and 13 cases had abnormal EEGs of which is similar to the recent study by Hennes et al. which showed five out of 34 cases had seizures (19).

Ten cases had multiphasic course up to the last follow-up, and the mean age of these children was 6.45 years. Similar to previous studies, 33.8–54% of children experience clinical relapses (20, 21).

TABLE 5 | Comparison between patients with NMOSD and those with other relapsing IDDs.

	NMOSD	Other relapsing IDDs	All relapsed patients
Number	4	6	10
Female: male	2:2	4:2	6:4
Age at onset, y, median (range)	8.0 (5.5–10.5)	6.1 (3–11.5)	6.85 (3–11.5)
Relapse episodes, median (range)	3 (2–4)	3 (2–7)	3 (2–7)
Phenotype at initial attack, *n* (%)			
ADEM	1 (25%)	3 (50%)	4 (40%)
ON	3 (75%)	1 (16.7%)	4 (40%)
Other demyelinating diseases	0	2 (33.4%)	2 (20%)
Phenotypes in all attacks, *n* (%)			
ADEM	4/12 (33.3%)	4/22 (18.2%)	8/34 (23.5%)
ON	6/12 (50%)	11/22 (50%)	17/34 (50%)
Other demyelinating diseases	2/12 (16.7%)	7/22 (31.8%)	9/34 (26.5%)
Initial lesions on MRI, *n* (%)			
White matter and/or deep gray matter	2 (50%)	3/6 (50%)	5 (50%)
White matter and spinal cord/optic nerve and/or others	2 (50%)	1 (16.7%)	3 (30%)
Spinal cord and/or optic nerve	0	1 (16.7%)	1 (10%)
Gray matter and/or white matter	0	0	0
Cerebellum and/or others	0	1 (16.7%)	1 (10%)
ARR during follow-up, median (range)	0 (0–1.09)	0 (0–3.43)	0 (0–3.43)
EDSS at last follow-up, median (range)	0 (0–2)	0	0 (0–2)
Seizures, n (%)	0	1 (16.7)	1 (10%)

ARRs, annualized relapse rates; ADEM, acute disseminated encephalomyelitis; EDSS; Expanded Disability Status Scale; IDDs, inflammatory demyelinating diseases NMOSDs, neuromyelitis optica spectrum disorders; ON, optic neuritis.

However, Hamid et al. (22) reported that the relapse frequency of MOG-Ab-positive cases was 93% with a long observation period (≥8 years) in adults. Compared with that of the children who had monophasic courses, the mean follow-up time was significantly different in the children who had multiphasic courses (31 vs. 10.67 months, $p = 0.0001$). Therefore, the recurrence rate might increase with the extended follow-up time.

The relationship between the MOG-Ab titers and clinical disease activity remains an area of active investigation, with a recent report suggesting that a high MOG-Ab titer (≥1:1280) predicted a recurrent non-MS course with a sensitivity of 46% and specificity of 86% (20). A large nationwide French study of 197 adults with MOG-Ab (15) observed that the titers were higher at relapse than in remission, but only two patients became seronegative. Similar to these studies, the MOG-Ab titer changed to a higher mean in a relapsing patient and to a lower or constant mean in patients in remission (10/11, 90.9%), and only 2 patients became seronegative. Few patients had increased ICP, CSF protein levels, WBC counts and ESRs, implying the presence of intense intrathecal inflammation during the active phase. According to these findings, MOG-Ab disease in its acute stage should be cautiously differentiated from CNS infection and vasculitis (23). A positive CSF OCB is not common in the patients with MOG-Ab as only 10% of the cases had positive results. However, 36% of the cases had concomitant circulating autoantibodies or autoimmune diseases of which is peculiar from the findings of patients with MS.

Hacohen et al. (12) proposed that children with MOG-Ab might present with four MRI patterns: (1) multifocal hazy/poorly marginated lesions involving both the gray and white matter and typically involving the middle cerebellar peduncles; (2) spinal cord and/or optic nerve involvement with a normal intracranial appearance or non-specific white matter lesions; (3) extensive and periventricular white matter lesions resembling a "leukodystrophy-like" pattern; and (4) cortical encephalitis with leptomeningeal enhancement. According to this proposal and the symptoms of the children in our study, the abnormal signal locations upon MRI were split into the following five categories: white matter and/or deep gray matter, white matter and spinal cord/optic nerve and/or others, spinal cord and/or optic nerve, gray matter and/or white matter, and cerebellum and/or others. The results showed an abnormal signal in the white matter and/or deep gray matter in all patients ≤9 years old and abnormal spinal cord and/or optic nerve signals in all patients older than 9 years (**Table 2**). However, these categories did not predict recurrence, and all lesions decreased or disappeared by the last follow-up.

Our study provides a detailed retrospective analysis of the effects of various DMDs in our patients. Similar to previous studies (24–26), treatment of acute attacks with mPSL and IVIG was effective in most patients. However, 4 out of 10 cases had symptoms that flared up after withdrawal or tapering of prednisone. IVIG was not used alone in our patients. Previous studies showed that breakthrough attacks under AZP were linked to the drug-specific latency period and lack of treatment with oral steroids (24). Treatment with RTX resulted to disease stabilization in some reported cases but was followed by early relapses in several cases; end-of dose relapses occurred 9–12 months after the first infusion (24). The recent multicenter European study (23) showed that AZP, MMF, and RTX were all effective at reducing relapses, although the median EDSS score remained unchanged. In our study, RTX, AZP, and MMF usage for those with multiphasic courses were also effective at reducing relapses (ARR from 2.4 to 0). Seven out of nine cases who used DMDs in addition had no more relapses (median follow-up 6.6 months).Of importance, we found that RTX was effective at reducing the MOG-Ab titer. Consequently, RTX seems to be effective at reducing not only the ARR but also the MOG-Ab titer in a short follow-up time.

In previous studies, severe visual impairment or functional blindness was present at the last follow-up in 36% of all cases, and impaired ambulation due to paresis or ataxia was found in 25% of adult patients (27). However, in our study, the VA of the seven cases out of eight with a history of ON was

almost recovered (VA>1.0) at the last follow-up. 10 cases with multiphasic course in our study had the median EDSS score of 0 (follow-up time was 1–5.25 years). A total of 102 children with relapsing MOG-Ab disease who were all Caucasians had a median EDSS score of 1 or 2.5 in different subgroups (follow-up time was 3–9.1 years) (23). Additionally, 23 Chinese cases with relapsing MOG-Ab disease had a median EDSS score of 1 at the last follow-up (1–8.92 years) (28). The median EDSS score of 13 children from the Partners Pediatric MS Center at Massachusetts General Hospital (29) was 2 (mean follow-up time was 4.88 years), and the score for 9 children from Japan (30) was 0 (4 children had multiphasic course, follow-up time was 8–21 years). Altogether, these findings indicate different prognosis between races and suggest that pediatric Xanthoderms with MOG-Ab disease may have better outcomes than Caucasians.

Our study was limited by a small sample size and retrograde nature of making diagnosis of MOG-positive diseases since all MOG-Ab assay were performed from January 2018.

CONCLUSIONS

Despite the limitations, this *post-hoc* evaluation and analysis of previously collected and published data allowed us to make important observations about MOG-Ab disease in Chinese children who share the same clinical characteristics as Caucasians but have a better prognosis. We have identified age-dependent phenotypes; encephalopathy and lesions on the white matter and/or deep gray matter are more prominent in younger children while lesions on the spinal cord and/or optic nerve are more common in older children. Most of the patients presented with ADEM followed by ON. 40% of the cases had relapses. Treatment of acute attacks with mPSL and IVIG were effective in most of the patients. DMDs, such as AZA, MMF, or RTX can reduce the ARR. Despite of multiple relapses, all patients except one had full recovery.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the research ethics committee of Xiangya Hospital with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the research ethics committee of Xiangya Hospital.

AUTHOR CONTRIBUTIONS

LM and JP conceived the study. LY, CZ, and FH provided the clinical information and LM checked. LY, LW, and JP analyzed the data. LM drafted the initial manuscript which was edited by FY, MK, and JP.

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Prevalence and Risk Factors of Incidental Findings in Brain MRIs of Healthy Neonates—The FinnBrain Birth Cohort Study

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Background: Birth is a traumatic event with molding forces directed to the fetal skull, which may result in intracranial hemorrhages. However, the knowledge on prevalence and risk factors of incidental brain magnetic resonance imaging (MRI) findings in infants is still inconclusive.

Methods: The prevalence and nature of incidental MRI findings were assessed in a birth cohort of 175 asymptomatic infants. The role of delivery method as well as other potential risk factors for intracranial hemorrhages were evaluated. The infants underwent 3T MRI at the age of 2–5 weeks, and the neurological status of the infants with an incidental finding was evaluated by a pediatric neurologist. Information on the delivery method, duration of delivery, parity, used anesthesia, oxytocin induction, and Apgar score was gathered to evaluate their association with the prevalence of hemorrhages.

Results: Incidental intracranial hemorrhages were detected in 12 infants (6.9%), all following spontaneous or assisted vaginal delivery. Vacuum-assistance was found to be a risk factor for subdural hemorrhages with an odds ratio (OR) of 4.7 (95% CI [1.18; 18.9], $p = 0.032$). All infants were evaluated to develop normally by their clinical status.

Conclusions: Incidental intracranial hemorrhages are relatively common among infants born by vaginal delivery. They are often of little clinical significance within the first years of life and have unlikely consequences for later neurodevelopment either. Despite their benign character, investigators should be prepared to share this information with parents competently as the findings can cause parental anxiety, and especially as the popularity of MRI as a research tool is increasing.

Keywords: infant, incidental finding, MRI, subdural hemorrhage, delivery method

INTRODUCTION

Incidental intracranial hemorrhages are common findings in neonatal magnetic resonance imaging (MRI). This is not surprising given the remarkable molding and deformation of the fetal head in the birth canal (1, 2). Despite being common, the relevance of birth-related intracranial hemorrhages is still partially unclear, as are also risk factors behind them.

The estimated prevalence of intracranial hemorrhages in term asymptomatic healthy newborn populations deviate greatly from 8.1 to 46% in previous studies (3–5). Subdural hemorrhages are most common, and it is customary to divide them into two groups according to their location—supratentorial and infratentorial. While in most studies infratentorial hemorrhages form the majority (4–6), one study reported a high prevalence of supratentorial hemorrhages (3). Some studies have observed exclusively subdural hemorrhages (3, 5), while other more infrequently reported findings include subarachnoidal (4) and intraparenchymal hemorrhages (4, 6). Apart from hemorrhages, cysts (e.g., pineal and caudothalamic cysts) are commonly (1.9–57%) reported benign incidental intracranial findings (7, 8).

Neonatal intracranial hemorrhages may appear in multiple locations (3, 4). Subdural hemorrhages, detected immediately after birth, have been described most often to situate posteriorly (3–5, 9). This location differs from that of subdural hemorrhages related with non-accidental head injuries ("shaken baby syndrome"), that typically present in the interhemispheric fissure and over the hemispheres (10, 11). Birth-related intracranial subdural hemorrhages are often widely distributed (forming a thin film) and seldom have indications for clinical interventions (12, 13). However, it remains elusive whether e.g., location of the hemorrhage could play a role in possible consequences.

Vaginal delivery is a risk factor for intracranial hemorrhages (3–6), while the effects of assisted delivery and other obstetric factors remain less clear. Some studies indicate vacuum extraction as a risk factor (14, 15), while others have not detected an association (3, 4, 6). Two studies (sample sizes $n = 88$ and $n = 111$) reported that none of the infants born by cesarean (c-) section had hemorrhages (4, 5), while another ($n = 101$) reported a relatively high prevalence in the c-section group (18%) (3). One study indicated increased birth weight and prolonged delivery to be additional risk factors for intracranial hemorrhages (3).

Incidental findings in MR images seldom have major clinical significance, but as they are common (16), they also likely occur in brain MRI research performed in asymptomatic infant populations, and preparing for their occurrence would benefit from better identifying the risk factors. Further, hearing about these findings may be stressful for the parents (17), which underlines the importance of better understanding both the commonness as well as possible implications of these phenomena.

To these ends, we measured the prevalence of incidental brain imaging findings in healthy infants, characterized the findings e.g., according to their location, evaluated their association with obstetric factors, and assessed their possible effects on neurological development during infancy.

METHODS

This study was reviewed and approved by the Ethics Committee of the Hospital District of Southwest Finland and performed in accord with the Declaration of Helsinki.

Participants

This study was executed as a part of the FinnBrain Birth Cohort Study [www.finnbrain.fi; (18)]. Pregnant women attending their first trimester ultrasound at gestational week (gwk) 12 were recruited at five sites in South-Western Finland. In total, 189 mothers and their infants were recruited to an MRI visit, of which 180 (95.2%) imaging sessions were successfully completed and provided structural MR images, and 175 were included in the subsequent analyses (the rest were excluded due to motion artifacts). The participant families were recruited by a phone call to the mother, at earliest 1–2 weeks after childbirth. The parents gave written informed consent also on behalf of their infant.

The exclusion criteria for the mothers were alcohol or drug abuse; severe psychiatric disorders; medication for psychosis, epilepsy or bipolar disorder. The exclusion criteria for infants included occurrence of any perinatal complications with neurological consequences (e.g., hypoxia), scoring <5 in the 5 min Apgar score; previously diagnosed central nervous system anomaly, an abnormal finding in any previous MRI scan or birth weight <1,500 g. The participating infants were full-term (born between 37 and 43 gwks; with two exceptions born on 36 gwk and two with the information not available, none of which belonged to the incidental finding group). All were born from singleton pregnancies.

Obstetric data were gathered from the Finnish Medical Birth Register of the National Institute for Health and Welfare (www.thl.fi), and included information on duration and mode of delivery, gestational age, use of anesthetics or oxytocin induction during delivery, mother's parity, possible episiotomy, gestational age at birth, child Apgar scores (1 min and 5 min), head circumference, birth weight, birth height and pH of the umbilical blood, among other data that were filled in by the medical staff following delivery at the hospital. Mode of delivery was categorized into (a) vaginal, (b) assisted deliveries (vacuum-assistance), and (c) c-sections (elective or emergency). With anesthetics, two categories were formed: (1) epidural and spinal anesthesia and their combinations and (2) all other anesthetic forms or no pain alleviation. Episiotomy is not a routinely performed procedure during delivery in Finland, as it is in some countries. It was included as one of the obstetric variables as it was considered to be an indirect indicator of increased pressure on the fetus during the second stage of labor.

Image Acquisition

The infants underwent MRI scans in a Siemens Magnetom Verio 3T scanner (Siemens Medical Solutions, Erlangen, Germany) at age 2–5 weeks, calculated from the due date, at the Turku University Hospital. Upon arrival, an experienced radiographer went through the study protocol with the parents and confirmed the lack of contraindications for the scan. Before the scan, the

infants were fed with (breast) milk to help them to sleep and then swaddled into a vacuum mattress to reduce possible limb movement. The scan was performed without anesthetics, and the children were provided with sufficient hearing protection (infant ear wax and custom-sized ear muffs) of ∼42 dB noise reduction. The duration of the whole scanning protocol was ∼40 min (maximum duration 60 min). The family was free to discontinue the study at any point and the scan was aborted if the baby was not soundly asleep and/or still in the scanner.

Axial PD-T2-weighted sequence with 1.0 × 1.0 × 1.0 mm isotropic voxels, TR of 12,070 ms and effective TE times of 13 and 102 ms were used to produce both PD-weighted and T2-weighted images from the same acquisition. The total number of slices was 128. A sagittal 3D T1 sequence with 1.0 × 1.0 × 1.0 mm voxel size (TR 1,900 ms, TE 3.26 ms, TI 900 ms) was also acquired. Additionally, the imaging included field mapping, a set of DTI images and functional imaging for some participants (data not reported here) (19).

All the successful ($n = 175$) brain structural T1 and/or T2 images were evaluated by a radiologist specialized in pediatric neuroradiology (author RP). When incidental findings were detected, the researchers informed the families about the finding(s) in 1–4 weeks' time. All the families with a finding were offered a child neurological examination and consultation by an experienced pediatric neurologist (author TL), and 11 out of 13 families used this opportunity.

Neurological Assessment

The pediatric neurologist's visit consisted of a thorough clinical history and interview of the child's health, developmental milestones and possible abnormal symptoms. A complete somatic examination was accompanied by a neurological examination by an experienced pediatric neurologist using a standardized proforma of the Dubowitz neurologic examination for children below 6 months (20) and the Hammersmith Infant Neurological Examination (HINE) for all children (21). The results of the clinical examination together with the brain MRI findings were discussed with the parents who were given an opportunity to contact the consultant also after the visit.

Statistical Analysis

Statistical analyses were performed with SPSS version 23 (Armonk, N.Y., USA). Descriptive statistics, group comparison analysis and odds ratios for putative risk factors for incidental intracranial hemorrhages were calculated. Statistical significance in the comparisons for the categorical variables was determined with Chi-square test, and for continuous variables, with two-sample t-test (means and SDs) or nonparametric Wilcoxon rank-sum test (medians and median absolute deviation (MAD), scaled by a factor $k = 1.4826$) depending on the normality of the distribution of the data. Multiple comparison corrections were not performed due to small sample size. In the risk factor analysis, statistical significance was calculated by using Boschloo's test (22, 23).

RESULTS

Demographics

All 175 infants were born from singleton pregnancies without any significant prenatal complications as per inclusion criteria. 94 (54%) were males and 81 (46%) females. The mean (SD) maternal age was 30 (4.2) years. Out of the whole sample, 125 (71%) infants were born through non-assisted vaginal delivery, 21 (12%) with instrumentally assisted delivery using vacuum extraction and 29 (18%) with c-section. Epidural or spinal anesthesia was used as analgesia in 95 (59%) deliveries. In all, 72 (41%) of the mothers were primigravida. The study sample was considered to be representative of the Finnish population, as the proportions of different delivery methods as well as other obstetric factors corresponded well with the national statistics on deliveries (24). The demographic data is presented in **Table 1**.

The Prevalence of Incidental Findings

Incidental brain imaging findings were detected in 13 (6 male, 7 female) out of 175 infants, the prevalence of all findings thus being 7.4%, and hemorrhages in 12 infants, making the prevalence of hemorrhages 6.9%. All deliveries within the group of incidental findings were vaginal, of which four were assisted with vacuum [the prevalence in vacuum-assisted deliveries 19% (4/21)].

Description of the Incidental Findings

The findings included subdural and intraparenchymal hemorrhages, cysts and their co-existence. Out of all the incidental findings, subdural hemorrhages had the highest prevalence ($n = 10$, 5.7%). Subdural hemorrhages were mostly located in multiple sites (**Table 2**). The majority ($n = 10$) was located in the posterior fossa (**Figure 1**), but also temporal ($n = 4$) and occipital ($n = 3$) hemorrhages were detected. The temporal subdural hemorrhages coincided mainly with hemorrhages located in the posterior fossa and in the occipital lobe (3 out of 4). Two infants with subdural hemorrhages had also additional parenchymal involvement; one cyst-associated hemorrhage in the caudo-thalamic region (**Figure 1**) and one hemorrhage in the cerebellar region, respectively. Furthermore, one independent cerebellar parenchymal hemorrhage, one caudo-thalamic cyst and one caudo-thalamic cyst combined with a parenchymal hemorrhage were observed. The one infant with an independent caudo-thalamic cyst was excluded from the subsequent analyses due to the high prevalence of these cysts in normal brain development (7, 8), and as birth-related factors are likely uninvolved in their occurrence. The distribution of the hemorrhages is described in **Table 2** and examples of a posterior fossa hemorrhage and a cyst associated with hemorrhage are given in **Figure 1**.

Risk Factors for Hemorrhages

Among the evaluated parameters, vacuum-assisted delivery was found to be a risk factor for subdural hemorrhages with an odds ratio (OR) of 4.7 (95% CI [1.18;18.9], $p = 0.032$) and especially for temporally located hemorrhages (OR = 20.7, 95% CI [2.2;378], $p = 0.0078$) (**Table 3**). Of altogether four temporal subdural hemorrhages, three (75%) occurred with

TABLE 1 | Characteristics of the study subgroups.

	Incidental findings *n* (%) = 13 (7.4)	Hemorrhage *n* (%) = 12 (6.9)	SDH *n* (%) = 10 (5.7)	IPH *n* (%) = 4 (2.3)	No findings *n* (%) = 162 (93)
GENDER					
Male (%)	6 (46)	6 (50)	5 (50)	1 (25)	88 (54)
Female (%)	7 (54)	6 (50)	5 (50)	3 (75)	74 (46)
Birth weight (g) [mean (SD)]	3427 (476)	3448 (490)	3465 (370)	3446 (689)	3531 (432)
Birth height (cm) [mean (SD)]	50.0 (2.1)	50.2 (2.2)	50.8 (1.0)	49.5 (3.8)	50.5 (1.8)
Head circumference (cm) [median (MAD)]	34.8 (1.3)	34.9 (1.2)	34.9 (1.3)	34.8 (0.6)	35.1 (1.3)
Age at imaging [median (MAD)] from due date (days)	21.0 (4.4)* $p = 0.014$	21.5 (4.4)* $p = 0.042$	22.0 (3.0)	19.5 (7.4)	25.0 (7.4)
From date of birth (days)	23.0 (5.9)* $p = 0.025$	22.0 (5.9)* $p = 0.019$	22.0 (5.9)* $p = 0.043$	23.5 (2.2)	26.0 (7.4)
LENGTH OF PREGNANCY					
Weeks [median (MAD)]	40.0 (0.0)	40.0 (0.0)	40.0 (0.0)	43.5 (0.7)	39.5 (0.7)
Days [mean (SD)]	281 (4.4)	282 (4.4)	282 (3.7)	282 (5.9)	280 (8.2)
MODE OF DELIVERY					
Spontaneous vaginal delivery (%)	9 (69.2)	8 (66.7)	6 (60)	4 (100)	116 (71.6)
Assisted delivery (vacuum) (%)	4 (30.8)* $p = 0.04$	4 (33.3)* $p = 0.029$	4 (40)* $p = 0.012$	0	17 (10.5)
Section (%)	0	0	0	0	29 (17.9)
LENGTH OF DELIVERY					
Total (min) [median (MAD)]	462 (312)	458 (303)	418 (266.9)	466 (310)	448 (288)
Cervical dilation (min) [median (MAD)]	416 (285)	400 (245)	383 (252)	435 (274)	415 (274)
Active labor (min) [median (MAD)]	31.0 (17.8)	31.0 (22.2)	34.0 (26.7)	27.0 (13.3)	23.0 (23.7)
Primigravida (%) [median (MAD)]	6 (46.2)	6 (50.0)	6 (60.0)	0 (0.0)	66 (40.7)
Apgar score 1 min [median (MAD)]	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)
Apgar score 5 min [median (MAD)]	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)	9.0 (0.0)
Artery pH [mean (SD)]	7.2 (0.1)	7.2 (0.1)	7.2 (0.1)	7.3 (0.1)	7.3 (0.1)
ANESTHETICS					
Epidural/spinal (%)	8 (61.5)	7 (58.3)	5 (50.0)	3 (75.0)	95 (58.6)
No anesthetics/mild anesthetics (%)	4 (30.8)	4 (33.3)	4 (40.0)	0	65 (40.1)
NA	1 (7.7)	1 (8.3)	1 (10.0)	1 (25.0)	2 (1.2)
Oxytocin induction (%)	5 (41.7)	4 (36.4)	2 (22.2)	2 (66.7)	60 (37.5)
NA	1	1	1	1	2

*SDH or IPH, Hemorrhage; SDH, subdural hemorrhage; IPH, intraparenchymal hemorrhage; SD, standard deviation; MAD, mean absolute deviation (scaled by a factor k = 1.4826); NA, not available. Statistically significant differences between groups of no findings and groups with the indicated finding (all incidental findings, hemorrhages, subdural hemorrhages, and intraparenchymal hemorrhages) are marked with *.*

vacuum-assisted delivery, while the posterior fossa hemorrhages occurred mainly in spontaneous vaginal deliveries. All temporal hemorrhages were observed in infants with primiparous mothers who all had also undergone episiotomy.

No imaging findings were detected in the section-delivered infant group. Birth weight, height, infant sex, duration of gestation or duration of delivery, maternal age, used anesthetics, infant Apgar score at 1 or 5 min, use of oxytocin induction or maternal parity were not related to the occurrence of hemorrhages. The median (MAD) infant age at imaging calculated from the date of birth in the hemorrhage group [23.0 (5.9) days] was slightly lower than in the group with no incidental hemorrhages [26.0 (7.4) days]. Age was not controlled in the risk factor calculations due to small group sizes, but no significant differences were observed in ages between the groups in risk factor analysis.

Condition of the Infants With Incidental Findings

All infants with an incidental finding had been discharged from the maternity ward according to a normal procedure, without any observed long-lasting symptoms. Infants stayed in the maternity hospital, on average, 3.1 days (range 0;6). One newborn, who initially required acute surveillance due to breathing difficulties, was posteriorly diagnosed with cerebellum intraparenchymal hemorrhage. His clinical presentation at birth was deemed to be caused by an infection and he was evaluated as normal upon a neurological consultation visit.

TABLE 2 | Locations of the hemorrhages.

Participant	SDH			IPH	
	Posterior fossa	Occipital	Temporal	Caudo-thalamic	Posterior fossa
1	×	×	×		
2	×	×		×	
3	×	×	×		
4					×
5			×		
6				×	
7	×				
8	×				×
9	×				
10	×		×		
11	×				
12	×				
Total	9	3	4	2	2

SDH, subdural hemorrhage; IPH, intraparenchymal hemorrhage.

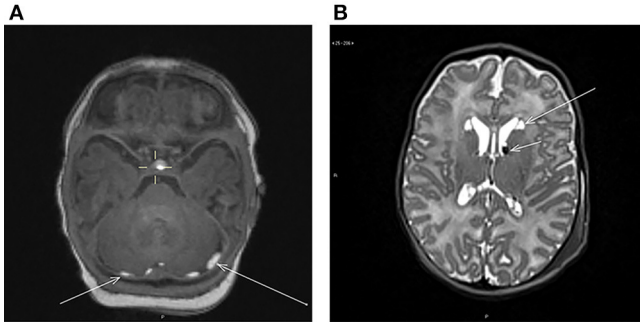

FIGURE 1 | (A) Axial T1-weighted image showing bilateral subdural hemorrhages (arrows) in the posterior fossa behind the cerebellum. **(B)** Axial T2-weighted image showing bilateral cysts at the head of nucleus caudatus (long arrow) and bilateral hemorrhages at the caudothalamic groove (short arrow).

TABLE 3 | Risk of hemorrhages in relation to obstetric variables given as odds ratios (OR, [95% CI]).

	Hemorrhage	SDH	Temporal SDH
Vacuum-assistance	3.4 [0.92;11.7] $p = 0.059$	4.7 [1.18;18.9]* $p = 0.032$	20.7 [2.2;378]* $p = 0.0078$
Primigravidity	1.47 [0.44;4.9] NS	2.3 [0.57;8.5] NS	
Epidural/spinal anesthetics	1.18 [0.34;4.2] NS	0.83 [0.23;3.3] NS	0.66 [0.08;5.7] NS

*Statistically significant differences marked with *. SDH, subdural hemorrhage; NS, non-significant. Statistical significance is calculated with Boschloo's test. Vacuum-assistance is compared to non-assisted vaginal deliveries, primigravidity, and anesthetics are assessed in the whole dataset.*

Follow-Up on Neurological Development

None of the 11 infants who attended the pediatric neurologist's visit had any clinically identifiable, significant neurological symptoms or deficits in their development at the time of the examination (infant age range 7–54 weeks, mean 16.6 weeks). Infant age at neurological examination varied due to scheduling issues but the comparability of the data on neurological development was ensured by using two different, age-appropriate assessment protocols. One family choosing not to book a visit was contacted by phone by the pediatric neurologist. The family explained they did not regard the visit necessary as the child was considered to develop normally in the controls of well-baby clinics.

The general health of all examined children was normal. The neurological assessment of children below 6 months of age ($n = 9$) was performed by the Dubowitz neurologic examination proforma (20). None of the children had clinically significant deviation in the scoring. Four children had one deviant item (mainly mild truncal hypotony) and five children had no deviant items. It has been previously reported that 1–2 deviant items are found in one third of the normal population examined according to Dubowitz infant neurological examination proforma (20). In the Hammersmith Infant Neurological Examination (HINE) (21), cranial nerve function was normal in all children, and the motor milestones were fully normal in 10 and mildly delayed in one child, who also was the only one presenting mild problems with social behavior. The neurological assessment of posture, movement, tone and reflexes were performed and the median optimality HINE score was 65 (range 0.94), while the previously reported optimality score varies between 61 and 74 in normally developing children between 3 and 8 months. No marked delay in developmental milestones or in behavior was detected in the studied children.

Of note, all infants, including the two whose parents declined the pediatric neurologist's visit, were also followed up in Finnish well-baby clinics that perform multiple check-ups during the first year of life.

DISCUSSION

We found that 6.9% of our neonate participants had intracranial hemorrhages as incidental findings in 3 T MRI measurements. None of the hemorrhages proved to be of clinical significance in the follow-up, which supports the hypothesis that they are not a major concern for current delivery treatment guidelines.

Our study reports a lower occurrence of intracranial hemorrhages compared to previous studies, where the prevalence has ranged between 8.1 and 46% (3–6), although the prevalence of 6.9% in our sample is still noteworthy (**Table 4**). The variation in estimates between studies could be partly explained by differences in infant age at time of scanning, as the highest prevalence (46%) was detected when imaging was conducted during the first 72 h postpartum (3). In that same study, all findings were subdural hemorrhages ($n = 46$) (3), while we detected also four intraparenchymal hemorrhages. The former study used 1.5 T magnetic field strength whereas 3 T was employed in ours, partially explaining the detection of intraparenchymal hemorrhages; increasing magnetic field strength improves resolution and facilitates the detection of more

TABLE 4 | Comparison between studies reporting neonatal intracranial incidental findings.

Publication	Magnetic field	Study population	Age at imaging	Prevalence of incidental findings	Incidental finding types	Associated risk factors	MRI indications
Our study	3.0 T	175	2–5 weeks	7.4% (13/175)	10 SDH 4 parenchymal 3 cysts	vaginal delivery, vacuum assistance	None, cohort study of healthy population
Looney et al. (4)	3.0 T	88	1–5 weeks	26% (17/88) (vaginal deliveries only)	16 SDH 1 germinal matrix 2 SAH 5 intraparenchymal (co-existing)	Vaginal birth	None, prospective study of brain development
Rooks et al. (3)	1.5 T	101	72 h	46% (46/101)	All SDH	Vaginal birth	None, healthy asymptomatic infants
Whitby et al. (5)	0.2 T	111	48 h	8.1% (9/111)	All SDH	Vaginal birth, forceps after failed attempt with vacuum	None, healthy asymptomatic infants
Sirgiovanni et al. (6)	1.5 T	240 (152 term, 88 preterm)	1–40 days (mean 10 days)	15% (36/240)	All SDH + 4 co-existing intraparenchymal	Vaginal birth, older gestational age, bigger weight	Neurological symptoms, perinatal asphyxia, abnormal US finding, metabolic symptom, infection
Tavani et al. (9)	1.5 T	24	1–22 days	62% (13/21) (vaginal deliveries only)	17 SDH 1 intraparenchymal 1 intraventricular 7 choroid plexus	Vaginal birth	Known congenital heart disease
Malova et al. (25)	1.5 T	276 (preterm, very low birth weight infants)	–	10% (28/276)	3 developmental venous anomalies 4 arachnoid cysts 6 pituitary abnormalities 15 others	–	Very low birth weight infants in neonatal intensive care unit
Wintermark et al. (26)	3 T	12	1–2 days	17% (2/12)	1 hemorrhage-combination (epidural, subdural, intraparenchymal) 1 sinus thrombosis	–	Asphyxia

SDH, subdural hemorrhage; SAH, subarachnoidal hemorrhage.

subtle incidental findings (3–5). As our scanning time points were later (mainly on weeks 3 and 4 after birth), the discrepancies in prevalence estimates could partly stem from hemorrhage resorption over the first days and weeks of life. In our sample, the age at imaging was lower in the hemorrhage group than in the control group, supporting this notion. Yet, Looney et al. estimated the prevalence of intracranial hemorrhages to be 26% with a magnetic-field strength and imaging time period both close to this study. One explanatory factor for this discrepancy might lie in the thorough prenatal assessments that are routine in Finland, e.g., selecting c-section candidates with care.

Vaginal delivery and vacuum-assistance were the only significant risk factors for intracranial hemorrhages, from a number of obstetric variables evaluated in our study. In Finland, the rate of c-sections almost doubled between the years 1998 and 2005, from 6.8 to 11.3% (27), with the latest rate of 15.9% for sections (6.7% for emergency sections) (24), and a similar trend in the popularity of c-sections can be seen worldwide (28). The current reasoning behind selecting c-section as the delivery method more frequently is to ensure the safety of the neonate. However, this has not lead to improvements in neonatal outcomes in the short term, but instead has increased admissions to neonatal intensive care units (NICUs) (27). Cesarean sections are known to predispose neonates to respiratory distress and tachypnea (29). Therefore, it is not warranted to opt for c-section over vaginal delivery based on risk of intracranial hemorrhages.

When instrumental assistance is required during delivery, vacuum-assistance is currently the preferred method. The most common indications for the use of vacuum are problems with recording fetal heart rate and prolonged second stage of labor, and vacuum assistance is associated with primiparity, regional analgesia, smaller birth weight, and induction of labor acting as contributory factors (30, 31). However, again, the potential occurrence of intracranial hemorrhages is not an adequate indication for modifying delivery practices, as they are not shown to compromise infant health. Further, prolonged labor may be the more important common denominator here. The role of the actual instrument assistance remains elusive, as previous studies have provided discordant estimates on whether assisted delivery increases the risk for subdural hemorrhages in the first place, or if spontaneous vaginal delivery is the strongest determinant (3–6). In this study, vacuum-assistance of delivery increased the risk for

hemorrhages, although the hemorrhages were clearly associated with all vaginal deliveries when compared to c-sections, which is line with prior studies (3–6, 9), albeit exceptions exist (32).

Many limitations in extant studies weaken the comparison of consequences between different delivery modes, including incomplete reporting of events during labor that may substantially contribute to the choice of delivery method. Future prospective studies are needed to address this issue.

The neurological assessment of all children was normal measured by 1–2 standardized systems (20, 21). However, the age for the clinical examination (average 3–4 months) is not generally the most ideal for prognostic evaluation of infants. Nevertheless, it was considered as ethically most sound to invite the families as soon as possible in terms of the study protocol. Therefore, two different assessment methods for two different age groups were used to detect any possible deviant signs or symptoms in neurological development. Altogether, neither marked delay in developmental milestones nor abnormal behavior was detected in the studied children. Supporting earlier notions, our study suggests that the clinical consequences of these findings may be marginal (33).

Incidental findings are becoming continuously more frequent as the usage and accuracy of MRI increase. This is especially relevant in research scans using high resolution MRI sequences as a common practice, as the prevalence of incidental findings is higher in them compared to when MRI is performed as a part of clinical screenings, with lower resolution (4.7 and 1.7%, respectively) (34). Current challenges regarding the incidental findings include scarce information on their consequences on the individual level, and lack of best practices on both sharing the information with the participants as well as in guaranteeing that the study participants understand the possible repercussions of participation beforehand (35).

We followed a recommended protocol in the handling of incidental findings (36), including consulting a specialist and discussing the findings with multiple researchers. One suggested way of handling incidental findings is to evaluate the net benefit received by the participant and thus not to disclose the findings with unlike net benefit (36). However, we found that it was a participant's right to become aware of the findings, if willingness had been shown to receive this kind of information, when inquired in advance. Still, when giving people information they did not particularly ask for, the message needs to be accurate, and enough support and guidance should be given. In the future, when proceeding to the MR imaging of older children within the FinnBrain Birth cohort study, the correct handling of the incidental findings will be even more important, as incidental findings may include potential malignancies, etc. (37).

Parental distress can have grave effects on parent-child bonding and attachment behavior (38, 39), which emphasizes the importance of providing parents with knowledge about the clinical relevance of the findings. In this study, the opportunity to discuss the incidental MRI findings with an expert clinician was offered to all families. As no family contacted the clinic for more information after the consultation visit, this approach was regarded as sufficient to reduce anxiety in parents. In general, after receiving normal results from the neurological examination, sufficient effort and resources should be placed on informing parents about the positive prognosis of the child. Furthermore, all feelings of self-accusation and failure should be avoided, and the fact that hemorrhages are quite common in completely normal deliveries without any complications should be highlighted.

Our study design provided an opportunity to evaluate asymptomatic intracranial findings in infants within a representative sample of the Finnish term-born population and likely provided an accurate estimate of the prevalence without deviation due to selecting individuals based on symptoms. Finland, among other Nordic countries, has the lowest neonatal mortality rate (28) and qualitative differences between countries in peripartum care may contribute to differences also seen in the occurrence of hemorrhages (although this aspect was not in the scope of the current study). The sample size is acknowledged to be modest for epidemiological evaluations and does not encompass the age range immediately after birth nor does it include repeated measurements. Further, the neurological follow-up examination of the infants with incidental findings was performed only once and at a relatively young age, so that future developmental problems could not be excluded. The inclusion of additional risk groups such as infants with peripartum complications or even subgroups of healthy neonates with excess crying (maybe due to pain) would be beneficial for future studies. The relatively late scanning time point is also a limitation of this study. Our imaging scheme does not include SWI (susceptibility weighted imaging) or T2* weighted sequences which would improve the detection of intraparenchymal microhemorrhages. SWI sequence does not show hypoxia, which is a more direct sign of brain damage, and which on the other hand is shown in T1 weighted images in addition to subdural hemorrhages (40). Thus, we regarded T1 weighted sequence to serve our purposes better in detecting major intracranial changes as the imaging time was limited. Magnetic resonance imaging was performed at ca. Three weeks postpartum, when delivery-associated hemorrhage volumes may be diminished due to resorption (3, 5). In two previous studies, the scanning was performed within 48–72 h from birth, with the finding percentage ranging between 8.1 and 46% (3, 5), while one study has quite a similar age period to ours and incidental findings in 26% of infants (4). The limitations of our study appear to correspond with those of the previous studies. Thus, in the future, a prospective research setting considering these modifying or confounding factors beforehand is needed to acquire more specific data concerning incidental brain findings related to birth. Future studies could address how fast incidental hemorrhages disappear after birth, to further delineate, e.g., the apt timing for follow-up scans. Also, the effect of variation in blood coagulation on the appearance of incidental findings could be addressed in future. Regarding possible relevance of location or size of incidental intracranial hemorrhage on child outcomes, larger study populations are required.

CONCLUSION

Minor intracranial hemorrhages are detected frequently in infant MR scans. Vaginal delivery and vacuum assistance seem to increase the prevalence of hemorrhages. Most hemorrhages are benign with little clinical significance within the first years of

life and have unlikely consequences for later neurodevelopment either. Investigators should take into account the possibility of detecting such hemorrhages and plan how to address them with apt expertise and careful communication with the families.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available because of restriction imposed by the Finnish law and the study's ethical permissions do not allow sharing of the data used in this study. Requests to access the datasets should be directed to the Principal investigator of the FinnBrain Birth Cohort Study HK (hasse.karlsson@utu.fi).

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

HK and LK devised the project and main conceptual ideas. HK, LK, JT, and NS designed the infant imaging study setting. SL, JT, and NS collected the imaging data. VK, SL, JT, and NS contributed to the analysis of the data. VK, SL, AC, ES, JT, NS, TL, and RK participated to writing the manuscript. TL performed the neurological examinations. RP reviewed the MR images and evaluated the incidental findings. JS, HM, and JT designed the imaging scheme, provided the technical details, and contributed to the analysis tools. HK, NS, and JT supervised the project. All authors provided critical feedback, helped shape the manuscript, and accepted it in it's final form.

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REVIEW
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C_2H_2-Type Zinc Finger Proteins in Brain Development, Neurodevelopmental, and Other Neuropsychiatric Disorders: Systematic Literature-Based Analysis

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Neurodevelopmental disorders (NDDs) are multifaceted pathologic conditions manifested with intellectual disability, autistic features, psychiatric problems, motor dysfunction, and/or genetic/chromosomal abnormalities. They are associated with skewed neurogenesis and brain development, in part through dysfunction of the neural stem cells (NSCs) where abnormal transcriptional regulation on key genes play significant roles. Recent accumulated evidence highlights C_2H_2-type zinc finger proteins (C_2H_2-ZNFs), the largest transcription factor family in humans, as important targets for the pathologic processes associated with NDDs. In this review, we identified their significant accumulation (74 C_2H_2-ZNFs: ∼10% of all human member proteins) in brain physiology and pathology. Specifically, we discuss their physiologic contribution to brain development, particularly focusing on their actions in NSCs. We then explain their pathologic implications in various forms of NDDs, such as morphological brain abnormalities, intellectual disabilities, and psychiatric disorders. We found an important tendency that poly-ZNFs and KRAB-ZNFs tend to be involved in the diseases that compromise gross brain structure and human-specific higher-order functions, respectively. This may be consistent with their characteristic appearance in the course of species evolution and corresponding contribution to these brain activities.

Keywords: brain development, structural abnormality, KRAB domain, mutation, neural stem cells, transcriptional regulation

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INTRODUCTION

Neurodevelopmental disorders (NDDs) are multifaceted pathologic conditions caused by skewed development of the central nervous system (CNS) and subsequent morphological and/or functional abnormalities (1). Manifestations associated with NDDs include, but are not limited to, neuropsychiatric problems, cognitive impairment, motor dysfunctions, language/speech abnormalities, and affective deficits (2). Intellectual disability (ID), autism spectrum disorders (ASDs), motor diseases including developmental coordination disorder, communication, speech and language disorders, attention-deficit/hyperactivity disorder (ADHD), and various genetic disorders, such as Down syndrome and fragile-X syndrome, all fall into the NDD entity (1).

Neuropsychiatric disorders like schizophrenia, major depressive disorder (MDD), and bipolar affective disorder (BAD) are also considered as part of the NDDs (1). There are significantly overlapping clinical symptoms between different types of NDDs (3), suggesting the presence of commonalities shared among them. The pathologic mechanisms developing NDDs emerge during the early stage of brain development organized *in utero* and in childhood, and this is largely due to significant involvement of genome deficits in various key genes required for normal brain development (2). Thus, identifying causative mutations/genetic abnormalities greatly facilitates our understanding of the overall pathogenesis and neuropathological processes of NDDs.

One of the major requirements for normal brain development is the precise coordination of neural stem cell (NSC) activity throughout the embryonic period to early childhood (4). NSCs are self-renewing multipotent cells that give rise to three distinct types of CNS cells: neurons, astrocytes, and oligodendrocytes (4). Differentiated neurons are critical for virtually all brain activities including the coordination of sensory and motor systems, cognitive functions, and mood maintenance (5). On the other hand, astrocytes and oligodendrocytes, also known as glial cells, support proper functioning of the differentiated neurons (5). During the early stage of embryonic brain development, NSCs originate from the neuroepithelial stem cells of the embryonic neural tube (6). NSCs undergo three major stages: (1) proliferation and renewal of the lineage, (2) migration to appropriate brain areas, and (3) differentiation into neurons, astrocytes, or oligodendrocytes; precise transitioning between these stages is critical for normal brain development (7). For example, transitioning from proliferation to differentiation and subsequent induction of the programmed cell death are crucial for the formation of normal anatomical structure of the developing brain by maintaining appropriate cell numbers (8, 9). Importantly, many of these NSCs activities are orchestrated and driven by the spatio-temporal expression of the groups of genes responsible for fine-tuning of transcriptional activity (10). Thus, dysregulation in any processes supported by these key genes impacts proper NSC activities, resulting in the development of NDDs (11, 12).

Transcription factors (TFs) are a family of protein molecules that drive gene transcription by binding directly/indirectly to the upstream genome regulatory elements of protein-coding genes (13). Accumulated evidence indicates that TFs are pivotal for brain development by influencing the ability of NSCs to differentiate into different neural cell lineages and the subsequent formation of various brain areas and substructures (14). Some TFs are also key for the precise neural cell migration to their final brain destinations (14, 15). Among such TFs, the C_2H_2-type zinc finger proteins (C_2H_2-ZNFs) are highlighted to play significant roles in the regulation of NSCs activities and subsequent brain development (16–19). Many of their family members also participate in the pathogenesis and pathophysiology of NDDs (20). In this article, we will discuss the biological activities of C_2H_2-ZNFs in brain development and their pathologic contribution to NDDs.

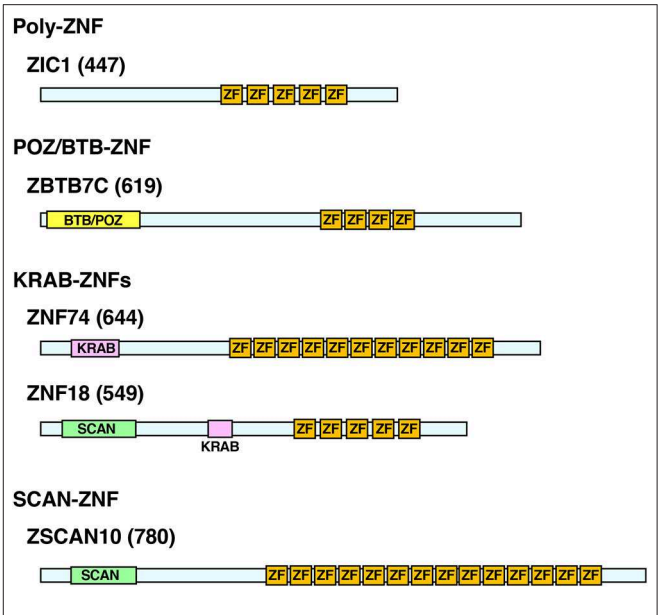

FIGURE 1 | Protein structure of the representative human C_2H_2-ZNFs. Protein structure of ZIC1 (Poly-ZF), ZBTB7C (POZ/BTB-ZNF), ZNF74, ZNF18 (KRAB-ZNFs), and ZSCAN10 (SCAN-ZNF) are shown as representatives of respective subtypes. Some C_2H_2-ZNFs have multiples of the same or different domains as indicated in ZNF18. Location and length of the respective domains are based on the data from the UniProt (https://www.uniprot.org/) and/or the National Center of Biotechnology Information (NCBI) Conserved Domain (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Numbers in brackets indicate amino acid numbers. ZF, C_2H_2-type zinc finger; KRAB, KRAB domain; POZ/BTB, POZ/BTB domain; SCAN, SCAN domain.

C_2H_2-ZNFs

C_2H_2-ZNFs form the largest TF family in the animal kingdom with significant expansion of their members through species evolution (20). The family consists of ∼800 members in humans (20, 21). In addition to C_2H_2-type zinc fingers (ZFs), these proteins contain other functional domains, such as BTB (BR-C, ttk, and bab)/POZ (Pox virus and Zinc finger), KRAB (Krüppel-associated box), and/or SCAN (SRE-ZBP, CTfin51, AW-1, and Number 18 cDNA), and are classified into four subtypes depending on the possession of these domains: (1) poly-ZNFs without any other domains, (2) BTB/POZ-ZNFs, (3) KRAB-ZNFs, and (4) SCAN-ZNFs (20, 21) (**Figure 1**).

ZFs are small peptide domains forming a secondary structure supported by a zinc ion, which makes ionic bonds to the cysteine and/or histidine residues of the finger (22). The C_2H_2-type ZF is composed of up to 30 amino acids with the consensus sequence $\underline{C}X_{2-4}\underline{C}X_{12}\underline{H}X_{2-8}\underline{H}$ (X refers to any amino acid), which forms one α-helix and two β-sheets, respectively, in the carboxyl- and amino-terminal portions (23–25). These secondary structures of the C_2H_2-type ZF fold into a stable three-dimensional assembly through hydrophobic interactions and enclosure of a zinc ion (26, 27). In C_2H_2-ZNFs, multiple ZFs are usually present in tandem and are connected by linkers with conserved amino acid sequences (28). C_2H_2-type ZNFs bind genome DNA at their cognate recognition sequences

located in the regulatory region of their target protein-coding genes by forming various modes of contacts to target DNA double helices with their ZFs. DNA-bound C_2H_2-type ZNFs then recruit cofactors, chromatin remodeling proteins and the RNA polymerase II together with other TFs, and modulate the transcription rates of downstream coding sequences (29). In addition to the primary role as DNA-binding factors, some C_2H_2-type ZNFs use their ZFs for interacting with other proteins or double-stranded RNAs, which may be important for their communication with other proteins/RNAs also attracted to the multi-molecule transcriptional complex formed on DNA (28, 30, 31).

Among the functional domains of C_2H_2-type ZNFs, BTB/POZ, and KRAB domains have transcriptional regulatory activity (mainly repressive but sometimes enhancing) by attracting various repressive cofactor molecules, such as the histone deacetylases, corepressor complex, heterochromatin protein 1 (HP1), and/or the KRAB-associated protein-1 (KAP1). In contrast, the SCAN domain does not have such activities (21, 32). About seven percent (%) of the human C_2H_2-type ZNFs have a BTB/POZ domain, while 43% harbor a KRAB domain and 7% contain a SCAN domain (33). Sixty-seven percent of them have only ZFs without any of these domains (33). Some C_2H_2-type ZNFs have multiples of the same or of different domains (29) (**Figure 1**).

C_2H_2-ZNFs are highly expressed in the developing brain, and control early patterning of the CNS (16). They significantly contribute to the regulation of brain morphogenesis, influencing the proliferation, migration, and cell fate of NSCs or one-step committed neural progenitor cells (NPCs), and their differentiation into neuronal cells (see below). Their implications to brain disorders still remain elusive, but recent clinical studies have identified various mutations in the coding sequences of many *C_2H_2-ZNF* genes in patients with NDDs (20). Hence, we will first discuss the physiologic roles of C_2H_2-ZNFs in normal brain development by focusing on their involvement in the actions of NSCs or NPCs (**Table 1**). We will then describe their involvement in the formation of some structural components of the CNS by introducing experimental and clinical evidence. Further, we will discuss their pathologic contribution to particular forms of NDDs.

ROLES OF C_2H_2-ZNFs IN NORMAL BRAIN DEVELOPMENT AND THEIR INVOLVEMENT IN BRAIN MORPHOLOGICAL ABNORMALITIES

Embryonic brain development or morphogenesis begins with neurulation, the invagination of the neural plate to form the neural tube (4). Upon closure of the neural tube, the neuroepithelial cells residing in the ventricular zone shift from proliferative to neurogenic, and are committed into the radial glial progenitor cells (RGCs), which serve as the primary NPCs for generating neurons and glial cells (8). Neocortical development relies on different NPCs depending on their localization, such as apical progenitors (APs) and basal progenitors (BPs: also known as intermediate progenitors: IPs), which are, respectively localized in the apical surface and the basal side of the ventricular zone (117). Neurogenesis starts at E9-E13 in the mouse embryo in which RGCs go into two modes of cell division: "symmetric" to produce two daughter cells that retain the properties of RGCs, and "asymmetric" dividing into one daughter cell with the property of RGCs and one differentiated neural cell (8). The transition from symmetric to asymmetric division of RGCs is extremely critical for determining the numbers of residing neurons and subsequent brain size, whereas intrinsically coordinated cell cycle progression in these cells plays a role in balancing their proliferating *vs.* differentiating properties (9). Disruption of these processes thus leads to abnormal brain development (9).

Various C_2H_2-ZNFs are significantly involved in the above indicated process of neurogenesis organized by RGCs. The birth of cortical neurons is severely reduced or lost in *Gli3*-mutated mice (9). Gli3 is a poly-ZNF functioning in the sonic hedgehog (Shh) signaling and controls the cell cycle of RGCs by changing the length of the G1 phase (9, 42). Inactivation of Gli3 shortens the length of their entire cell cycle and causes delays in the formation of cortical neurons and the process of cortical lamination (9, 42). Several mutations in the *GLI3* gene are reported in patients with Greig cephalopolysyndactyly syndrome, Acrocallosal syndrome, and Pallister-Hall syndrome, which develop various morphological abnormalities in CNS and polydactyly (43).

The Zeb family of C_2H_2-ZNFs (Zeb1 and Zeb2), which are poly-ZNFs with one atypical homeodomain, is essential for normal brain development. Among them, Zeb1 is required for neocortical development (81). Its peak expression reaches during the period of neocortical development, persists at high levels throughout the embryonic neurogenesis and then decreases postnatally (81). Zeb1 acts as a transcriptional repressor and regulates proliferation, migration, and differentiation of RGCs by affecting the division mode of these cells (81). It promotes and accelerates maturation of the generated neurons and their ability to develop electrophysiological properties (81). Interestingly, inactivation of Zeb1 significantly decreases trans-differentiation from mouse embryonic fibroblasts into functional neurons in an *in vitro* system (81). Zeb2 (also known as Smadip1, Aip1, and Zfhxib) is essential for the transition of RGCs to Bergmann glia cells and astrocytes in mouse cerebellum (118). In humans, the *ZEB2* mutation is associated with Mowat-Wilson syndrome, a genetic disorder characterized by ID, epilepsy, and motor defects (119–121).

The Zic-type poly-ZNFs (Zic1, Zic2, Zic3, Zic4, and Zic5) are expressed in the specific regions of neuroectoderm during the early embryonic phase in mice, and they have essential roles in CNS development (18, 51, 54). Specifically, Zics are pivotal for regulating the proliferation and the differentiation of NPCs in the medial forebrain and cerebellum (122), and are involved in the neurulation process and neural tissue formation (63). They are essential for the neural tube formation, particularly the neural plate closure (122). Zics expressed in the neural tube seem to play a role in the formation of the neural crests as well (122). They also contribute

TABLE 1 | The C_2H_2-ZNFs involved in brain development, NDDs, and/or other neuropsychiatric disorders.

Name	Additional domains	Biologic activities	Pathologic implications	References
Poly-ZNFs				
ADNP2	Homeobox	Expressed in oligodendrocytes	Schizophrenia	(34, 35)
BCL11A (ZNF858A)		Controls migration of cortical neurons	ID, ASDs, seizures, dyspraxia, childhood apraxia of speech, severe speech disorder, brain malformation, and microcephaly	(17, 36, 37)
BCL11B (ZNF858B)		Controls hippocampal neurogenesis and development of corpus striatum		(38)
FEZF1 and FEZF2		Involved in cortical development and promote differentiation of neural stem cells Specifically expressed in subcortical projection neurons and regulates cell fate of residing neurons	Autism spectrum disorders and intellectual disabilities	(39–41)
GLI3		Controls progression of cell cycle in RGS cells through regulating the G1 phase length	Greig cephalopolysyndactyly syndrome, acrocallosal syndrome and Pallister-Hall syndrome	(9, 42, 43)
GLIS1		Promotes generation of the induced pluripotent stem cells	ASDs and Parkinson disease	(44, 45)
GLIS2		Regulates neuronal differentiation		(46)
TSHZ3	Homeobox	Influences synapse development by impairing cortico-striatal connectivity	Autistic traits, intellectual disabilities and speech disturbances	(47)
ZIC1		Controls cerebellar size	Dany-Walker malformation	(18)
ZIC2		Regulates migration of forebrain neurons, CR cells, and pallial-derived neurons	Holoprosencephaly and schizophrenia	(48–50)
ZIC3		Participates in neural crest formation, neurulation, and maintenance of NPCs	Hydrocephalus	(51–53)
ZIC4		Controls cerebellar size	Dany-Walker malformation	(18)
ZIC5		Mediates neural crest development and formation of the neural tube		(54)
ZNF148		Crucial for the development of corpus callosum	Underdevelopment of corpus callosum and aberrant neuron proliferation, microcephaly, and intellectual disabilities	(19)
ZNF292	Coiled coil		ID and ASDs	(55, 56)
ZNF385B		Mediates neuronal apoptosis	ASDs and ID	(57)
ZNF407			ID, ASDs and cognitive impairment.	(58)
ZNF462		Expressed in the ventricular zone and hippocampus Essential for hippocampal formation	ASDs	(59)
ZNF507			Schizophrenia	(60)
ZNF521		Promotes early neuronal differentiation	Anxiety and schizophrenic behavior	(61, 62)
ZNF536		Highly expressed in the developing CNS Promotes neural differentiation	MDD and BD	(63)
ZNF711	Zfx/Zfy transcription activation region	Activates the genes essential for brain development	ID	(64, 65)
ZNF774			ASDs	(66)
ZNF804A*		Implicates in brain connectivity (in the hippocampus and the dorsolateral prefrontal cortex) Implicates in episodic and working memory	Schizophrenia, ID, and ASDs	(67, 68)
ZNF865			ID and cerebral ataxia	(69)
POZ/BTB-ZNFs				
ZBTB7C		Highly expressed in the granular layers of the dentate gyrus and the pyramidal layer of the hippocampal gyrus	ID	(70)
ZBTB16			ASDs	(71)
ZBTB20		Highly expressed in the forebrain Involved in hippocampal neurogenesis, neuronal differentiation and neuronal connectivity Promotes astrocytogenesis	Macrocephaly (autistic features) Intellectual disabilities and autism	(72)

(Continued)

TABLE 1 | Continued

Name	Additional domains	Biologic activities	Pathologic implications	References
ZBTB21			Down syndrome	(73)
ZBTB32			MDD	(74)
ZBTB45		Highly expressed in the developing brain Regulates differentiation of glial progenitor cells, glial cells and oligodendrocyte precursors		(75)
ZmC_2H_2-1			Stress intolerance	(76)
KRAB-ZNFs				
PRDM15 (ZNF298)	SET	Acts in neural cell fate decision	Down syndrome and BD	(77, 78)
ZBTB11	Integrase H2C2		ID	(79)
ZBTB18		Coordinates corticogenesis and promotes radial cell migration	ID, microcephaly and corpus callosum anomalies.	(80)
ZEB1	Homeobox	Controls neuron differentiation Maintains integrity of the blood brain barrier	Schizophrenia	(81, 82)
ZEB2	Homeobox	Regulates the transition of radial glia to Bergmann glia	Mowar-Wilson syndrome	(83)
ZKSCAN4	SCAN		Schizophrenia	(67)
ZNF8		Transcriptional regulation	ASDs	(84)
ZNF18	SCAN	Regulates neuronal activity and/or development	Congenital form of ASDs	(85)
ZNF30			Microcephaly, intellectual disabilities, and poor speech development	(86)
ZNF34			MDD	(87)
ZNF41			XLMR and cognitive defects	(88)
ZNF74		Regulates of synaptic transmission	Schizophrenia and intellectual disabilities	(89, 90)
ZNF81			XLMR and autistic symptoms	(91)
ZNF181			Microcephaly, intellectual disabilities, and poor speech development	(86)
ZNF182			XLMR and autistic symptoms	(91)
ZNF302			Developmental delay, microcephaly, and intellectual disabilities	(86)
ZNF354C		Regulates gene expression during early embryonic brain development	Schizophrenia and depression	(92, 93)
ZNF439			Amyotrophic lateral sclerosis	
ZNF496	SCAN	Upregulated during the differentiation of P19 neural precursor cells	Epilepsy and hyperactivity Microcephaly and abnormal corpus callosum	(94, 95)
ZNF517			ASDs	(96)
ZNF519			Microcephaly, lissencephaly, and ID	(97)
ZNF528			ID	(98)
ZNF534			Epilepsy and ID	(99)
ZNF541			ID	(100)
ZNF546			ID	(101)
ZNF559			ASDs	(102, 103)
ZNF568		Maintains neuron stem cells and regulate neurogenesis	Microcephaly	(104)
ZNF589			ID	(105, 106)
ZNF599			Microcephaly, ID, and poor speech development	(86, 107)
ZNF673			ID and learning disabilities	(108)
ZNF674			ID and the X-linked cognitive disabilities	(108, 109)
ZNF713			ASDs and frontotemporal dementia	(110)
ZNF717			ID and polymicrogyria	(101, 111)
ZNF746	Coiled coil	Regulates neuronal death	Parkinson disease	

(Continued)

TABLE 1 | Continued

Name	Additional domains	Biologic activities	Pathologic implications	References
ZNF778			ASDs and cognitive impairment	(112)
ZNF780B			ID	(101)
ZNF860			Schizophrenia	(113)
ZNF862	HATC-C, RNase H-like		ID, language development, and information processing	(114)
SCAN-ZNFs				
ZNF24/ZNF191		Controls the transition stage from proliferation to differentiation in NPCs		(115)
ZSCAN10		Controls pluripotency of embryonic stem cells	Schizophrenia	(92)
ZSCAN31 (ZNF323)		Involves in early stages of brain development	Schizophrenia	(116)

**ZNF804A has only one ZF.*

significantly in forebrain development, as mutations in *Zic1*, *Zic2,* and *Zic3* result in an inadequate division of forebrain, which fails to develop into two hemispheres (123). Indeed, Zic1, Zic2, and Zic3 are expressed in the NPCs residing in the septum and cortical hem, the sites of generation of the Cajal-Retzius (CR) cells. Mice defective in these Zics demonstrate a reduction in the number of CR cells in the rostral cortex and develop altered localization of the CR cells and cortical lamination defects that resemble the changes noted in type II (cobblestone) lissencephaly (124). Zic1 and Zic4 are also involved in cerebellar morphogenesis (123). Simultaneous deletion of the *ZIC1* and *ZIC4* genes due to their close proximity in chromosome 3 results in a congenital brain anomaly called Dany-Walker malformation (DWM) in humans, which is characterized by hypoplasia of the cerebellar vermis and other brain abnormalities, and develops delayed motor development and cognitive problems in the affected individuals (18).

Several C_2H_2-ZNFs are implicated in the etiology of microcephaly as well. Microcephaly refers to a reduction in brain circumference and diminution in brain volume (125). The majority of cases with microcephaly are congenital forms in which the processes of neuronal proliferation, migration and/or death are affected (125). *De novo* deletions in the 19q13.11 region encompassing four *KRAB-ZNFs* (*ZNF30*, *ZNF81*, *ZNF302,* and *ZNF599*) are identified in two unrelated cases of microcephaly (86). Both patients demonstrated mild to severe ID and speech disturbances (86) and, in mice, microcephaly developed when the *Znf568* gene was knocked out (104). Znf568 is the KRAB-ZNF essential for NSC maintenance and brain size regulation (104). Znf568 is expressed in NSCs of fetal mouse brain (104). It is also expressed in the adult NSCs residing in two neurogenic niches, the subgranular zone (SGZ) and the subventricular zone (SVZ) of the hippocampal dentate gyrus (104). Mice defective in *Znf568* develop a significantly smaller brain compared to wild type mice (104). Reduction of the brain size in these mice is mainly due to defective neuronal migration and subsequent abnormal cortical layering (104). Further, particular single nucleotide variants in the *ZNF568* gene are associated with the head size in humans (104).

ZNF519 is a poly-ZNF highly expressed in brain and is involved in the etiology of microcephaly and lissencephaly (126). The latter is a developmental malformation of the brain cortex (smooth brain without normal convolutions) caused by improper neuronal migration (126). Investigation on a four-generation Pakistani consanguineous family exhibiting congenital microcephaly (Jawad syndrome) and remarkable learning deficits mapped the causative gene(s) to the chromosome 18p11.22-q11.2, which harbors six candidate genes including *ZNF519* (97). The potential contribution of ZNF519 to the development of lissencephaly is also supported by the evidence that its expression is downregulated in mice with *Lis1*, *Dcx,* or *Ywhae* knockouts, whose gene mutations are causative for lissencephaly in humans (126). Zfp462, a poly-ZNF involved in the pluripotency and differentiation of embryonic stem cells by regulating the expression of Sox2, Oct4, and Nanog TFs in mice (127), modulates the expression of the genes specific to neuronal differentiation (59). It is predominantly expressed in the embryonic cerebral cortex particularly in the ventricular zone and hippocampus (59). Homozygotic *Zfp462* knockout is lethal in mice, whereas the heterozygotic mice exhibit developmental delay with low brain weight and anxiety-like behavior with excessive self-grooming (59). ZNF148 is a poly-ZNF associated with congenital brain structural defects in humans. ZNF148 is highly expressed in the developing fetal brain in humans and is crucial for the development of the corpus callosum (19). Four patients harboring *de novo* truncating mutations in the *ZNF148* gene shared core syndromic features including abnormal development of corpus callosum, microcephaly, ID, short stature, and facial dimorphisms (19).

ZNF521 is the KRAB-ZNF acting as one of the intrinsic factors for driving commitment of NSCs to NPCs (61). It also promotes proliferation of these cells and delays their differentiation (61). ZNF24/ZNF191 is a KRAB-ZNF also harboring one SCAN domain (128). ZNF24/ZNF191 is expressed in NPCs, and is required for the maintenance of their proliferation potency by promoting cell cycle progression (115). Accordingly, ZNF24/ZNF191 expression is pronounced during early brain development and its expression decreases after all differentiation occurs (115).

INVOLVEMENT OF C_2H_2-ZNFs IN ID

ID is one type of the generalized NDDs characterized by significant impairment of intellectual (such as learning and reasoning) and adaptive functioning (activities for daily living, such as communication and independent living) (129). It is a heterogeneous disorder with regard to its clinical and genetic characteristics (130). Some C_2H_2-ZNFs are involved in the development of ID, particularly the form called X-linked intellectual disability (XLID) (131). This genetic disease is inherited in an X-linked repressive fashion, and thus, affected boys demonstrate more obvious phenotypes than girls (131). Several *KRAB-ZNF* genes, such as *ZNF41*, *ZNF81*, *ZNF148*, *ZNF673,* and *ZNF674*, residing on chromosome X are reported as novel causative genes for XLID with strong association to particular phenotypes among the other ∼200 candidate genes (109, 132). Several unrelated ID patients displaying similar manifestations and developmental delays shared the same mutations in the *ZNF674* and *ZNF673* genes (108, 109). Four patients harboring *de novo* truncation mutations in the *ZNF148* gene demonstrated overlapping clinical manifestations including ID, microcephaly, and mal-development of the corpus callosum (19). Two mutations in the *ZNF711* gene, which is also located on chromosome X and encodes a poly-ZNF protein, were identified in 11 XLID patients from two families, some of whom additionally demonstrated autistic features (64). ZNF711 has a role in brain development by binding to the PHD finger protein 8 (PHF8) that is the histone demethylase highly expressed in neurons, and failure of ZNF711 to bind to PHF8 affects normal neuronal migration (64, 65). Mutations in the *PHF8* gene, which is also located on chromosome X, cause Siderius-type XLID, characterized by facial dysmorphism, cleft lip/palate, and occasionally microcephaly and ID (133, 134).

INVOLVEMENT OF C_2H_2-ZNFs IN ASDs AND DOWN SYNDROME

ASDs are a group of pervasive NDDs demonstrating heterogeneous manifestations mainly characterized by deficits in social cognition, communication, and restricted behavior with repetitive phenotypes (135). They can range from mild social cognitive impairment to debilitating cognitive abilities (135). Accumulating evidence indicates the significant contribution of C_2H_2-ZNFs in the pathogenesis and pathophysiology of ASDs and autistic features. These include BCLLA, FEZF1, FEZF2, GLIS1, POGZ, TSHZ3, ZBTB16, ZBTB20, ZNF8, ZNF18, ZNF81, ZNF182, ZNF292, ZNF385B, ZNF407, ZNF462, ZNF517, ZNF548, ZNF559, ZNF626, ZNF713, ZNF774, ZNF778, ZNF804A, ZNF827, and many of them are KRAB-ZNFs (**Table 1**). Below, we explain the contributions of some of these C_2H_2-ZNFs in the development of ASDs and autistic features.

A 335.4 Kb-size microduplication located in the Xp11.2p11.3 segment of chromosome X, which includes KRAB-ZNF-expressing *ZNF81* and *ZNF182*, was identified in a patient demonstrating developmental retardation, autistic features, and delayed growth and speech (91). Elevated ZNF182 expression was identified in another ASD case displaying hyperactivity, learning and visual-spatial difficulties, and microcephaly (136). The latter patient harbored a 1.3 Mb-size micro-duplication in Xp11.23p11.3 that includes *ZNF182* (136). These two cases suggest that elevated expression of ZNF182 with the dosage nature of its encoding gene contributes to the development of their ASD phenotypes. *ZNF292* is also a potential target gene for ASDs (55, 56). One study employing a large cohort of the ASD probands obtained from the Autism Clinical and Genetic Resourced in China (ACGC) indicated *ZNF292* as a novel autism risk gene, as the patients harboring various mutations in this gene demonstrated ID and severe language impairment (55). Another study using a large ASD cohort collected from several countries identified four unrelated individuals who had deletions of the *ZNF292* gene (56). Homozygotic and compound heterozygotic mutations in the *ZNF18* gene were identified in the Autism Genetic Research Exchange (AGRE) cohort consisting of ∼1,000 multiplex ASD families (85). ZNF18 is a KRAB-ZNF with one SCAN domain, and is upregulated upon depolarization in mouse neuronal cells, suggesting its potential activity-dependent roles (85). The *ZBTB20* gene is also involved in the development of ASDs in addition to other types of NDDs including 3q13.31 microdeletion and microduplication syndrome, Primrose syndrome and ID (72, 137–139). ZBTB20 is a BTB/POZ-ZNF mainly expressed in the developing forebrain neocortex and is involved in cortical neurogenesis, hippocampal neuronal differentiation and connectivity, and promotes astrocytogenesis (140). Four unrelated individuals with *de novo* inactivating mutations in the Krüppel-like factor 7 (*KLF7*) gene exhibited autistic features along with ID (141). KLF7 is a poly-ZNF, and is essential for neurogenesis and is involved in neuronal differentiation and morphogenesis (141, 142). *Klf7*-knockout mice showed impaired axon projection in several brain regions including the cerebral cortex and hippocampus, and exhibited reduced dendritic branching in hippocampus (142). The pogo transposable element with ZNF domain (*POGZ*) gene is also a plausible candidate for ASDs, as *de novo* missense or nonsense mutations in this gene were identified in at least eight independent ASD patients (143–145). POGZ, a unique poly-ZNF harboring the transposase domain at its C-terminus in addition to nine ZFs (145), is highly expressed in the human fetal brain and is involved in mitosis and regulation of neural proliferation (145). POGZ is also implicated in the development of NDDs and microcephaly, as several *de novo* loss-of-function mutations in this gene were identified in seven patients showing these manifestations (146).

Several C_2H_2-ZNFs have etiologic roles in the manifestations associated with Down syndrome. Down syndrome is a common chromosomal disease caused by the chromosome 21 trisomy or its various rearrangements, and develops ID and constellations of morphological abnormalities (147). Some patients also demonstrate the manifestations reminiscent of ASDs (148). The Tc1 mouse model of Down syndrome shows elevated expression of Znf295 (also known as Zbtb21) in the brain cortex, and its human ortholog is located on chromosome 21, thus dosage abnormality in this BTB/POZ-ZNF may contribute to the development of some neurological manifestations associated

with Down syndrome (149). Since *ZNF298* is located on chromosome 21q22.3 and duplication of this segment is strongly associated with the development of Down syndrome (150, 151), dosage abnormality in *ZNF298* appears to contribute to the development of some manifestations of this disease (152). ZNF298 is a poly-ZNF and has a SET [Su(var)3-9, Enhancer-of-zeste, Trithorax] domain in its N-terminal portion (152).

INVOLVEMENT OF C_2H_2-ZNFs IN NEUROPSYCHITARIC DISEASES INCLUDING SCHIZOPHRENIA, MDD, AND BAD

Schizophrenia is a complex NDD characterized by psychotic symptoms, such as hallucinations and delusions, accompanied by variable degrees of loss of insight (153). Interplay between genetic, biological, environmental, and psychological factors are supposed to play roles in the development of these manifestations (89, 153). *ZNF74* was identified as a candidate gene for modifying the development of schizophrenia in particular patient groups (89). *ZNF74* encodes a KRAB-ZNF, is highly expressed in the developing brain and is located on chromosome 22q11, a gene segment previously identified as a positional candidate locus for the susceptibility to schizophrenia as part of the 22q11 deletion syndrome (154). ZNF74 is highly expressed in the developing fetal brain in humans (19). Several polymorphisms identified in *ZNF74* are significantly associated with age-at-onset of schizophrenia, although no statistical difference was detected for their frequencies between the patients and control subjects (89). Systematic meta-analysis on the psychotic diseases including schizophrenia, BAD, and ADHD identified several gene variants in *ZNF804A*, the zinc finger DHHC-type-containing 8 (*ZDHHC8*) and the zinc finger with KRAB and SCAN domain 4 (*ZKSCAN4*) genes (67). The *ZNF804A* variants, particularly rs1344706, located in the intronic sequence of this gene are highly associated with the development of schizophrenia and its various manifestations (155). *ZNF804A* expresses a poly-ZNF harboring just one ZF, and its reduced expression is likely important for the development of schizophrenia in part by changing the expression of the genes involved in neural cell adhesion, neurite outgrowth, and synapse formation (155). Although *ZDHHC8* is located on chromosome 22q11 and was initially identified as a potential candidate gene for schizophrenia, it turned out not to be involved in this disease in later studies (156, 157). The *ZKSCAN4* gene, also known as *ZNF307* or *ZNF427*, expresses a KRAB-ZNF that harbors a SCAN domain in its amino-terminus (158). This gene is located on chromosome 6p21p22.1, which was previously identified as one of the schizophrenia-associated gene loci (159). Several *ZKSCAN4* polymorphisms were strongly associated with schizophrenia in the Chinese Han population (160), although underlying molecular mechanisms are not known.

Mood disorders, such as MDD and BAD, are among the most common brain disorders caused by various abnormalities in the brain (e.g., imbalance of neurotransmitters), and particular genetic backgrounds precipitate these diseases (161). Several C_2H_2-ZNFs are involved in their pathogenesis. A novel point mutation in the *ZNF34* gene that replaces proline at the amino acid position 17 to arginine (P17R) was identified in a multi-generationally affected family with early-onset MDD (87). The mutation P17R is located in the KRAB-A domain of ZNF34, which is required for the repressive transcriptional activity of this protein, suggesting defective transcriptional regulation by the mutant protein appears to contribute to the development of MDD. *ZNF34* is also associated with BAD; *ZNF34* mRNA was differentially expressed in the postmortem brain samples obtained from patients with BAD (162). *ZNF34* also contains common variants precipitated in this disease (163). Further, *ZNF34* is located on chromosome 8p24.3, which is included in the region shown to be associated with BAD (164, 165). One *ZNF536* polymorphism (rs77554113) is correlated with remission rates of MDD patients who are under anti-depressant treatment, indicating its potential roles in MDD-related pathophysiologic processes (166). ZNF536 is a poly-ZNF highly expressed in neuronal cells and known to suppress neuronal differentiation (21, 63).

DISCUSSION

Brain development is organized by the sophisticated coordination of the proliferation, differentiation, migration, and cell death of its component neural cells (4). This is accomplished, mainly, by the intrinsic program of the self-renewing cell lineages, NSCs, and NPCs, through coordinated regulation of their transcriptional network by numerous TFs and transcriptional regulatory molecules (4). Importantly, these processes are under the influence of the individual's genetic background as well as the vulnerability to extrinsic factors, such as infectious agents, toxic substances, and various maternal conditions including immunity (2). Skewing any part of this regulatory network causes NDDs, leading to the development of various degrees of social, emotional, cognitive, and motor deficits (2).

Our literature-based analysis on the brain development and NDDs revealed that numerous C_2H_2-ZNF proteins (74, ∼10% of all human member proteins) are essential or involved in these conditions (**Table 1**). Indeed, many of them play critical roles in the proper functioning of NSCs, such as their potencies of proliferation and commitment into differentiated neural cell lineages. We found that different C_2H_2-ZNFs act on specific functions of these self-renewing cells at the particular developmental stages and their residing brain areas, and defective actions of C_2H_2-ZNFs develop characteristic morphological and/or functional abnormalities depending on their actions, expressed timing and residing cells.

Although there are substantial numbers of exceptions, defective poly-ZNFs (e.g., BLI3, ZEBs, and ZICs) tend to be associated with the NDDs with gross abnormality in brain morphology and/or structure, whereas dysfunction of the C_2H_2-ZNFs harboring additional domains, such as KRAB and SCAN (e.g., ZNF18, ZNF34, ZNF81, ZNF427, ZNF673, ZNF804A, and ZBTB20) are linked to the development of NDDs with abnormality in higher-order brain functions, such as cognitive deficit, memory loss, and emotional changes, represented by

ID, ASDs, schizophrenia, MDD, and/or BAD. C_2H_2-ZNFs are found throughout the organisms from yeasts to humans, whereas their numbers have exponentially expanded following the species evolution, particularly in vertebrates including humans (33). Poly-ZNFs tend to present from lower to higher organisms and mediate the fundamental functions shared by most of them, such as embryonic/fetal development, organogenesis, and limb formation (21). On the other hand, KRAB-ZNFs and SCAN-ZNFs, which appeared in the animal kingdom from vertebrates and mammals, respectively, show their numbers have significantly expanded in higher organisms, with the former demonstrating this trend more obviously (33). It is likely that the addition of these domains to C_2H_2-ZNFs, particularly the KRAB domain, appears to be required for supporting the functions specific to higher organisms, for example, sophisticated cognitive functions unique to humans (21). These pieces of evolutionary evidence on C_2H_2-ZNFs may explain our successful identification of specific C_2H_2-ZNF subtypes in particular forms of NDDs. For example, we found high accumulation of KRAB-ZNFs in ID, ASDs, and psychotic diseases that are associated with dysfunctions in higher-order brain functions, whereas defective poly-ZNFs appears to be linked to gross morphological brain abnormalities, including microcephaly, lissencephaly, and local hypoplasia/anomaly. This is also consistent with the previous finding that the characteristic expression of KRAB-ZNFs in the human brain compared to other primates including chimpanzees appears to be required for driving human-specific brain functions (16).

About two thirds of the KRAB-ZNF proteins are reported to bind retrotransposon sequences incorporated in the genome DNA, and act as protecting agents against reactivation and subsequent genome migration of these mobile elements (167). Retrotransposons cause various genetic diseases with their property of genome mutagenesis and chromosomal rearrangement (168). On the other hand, they are major driving forces for species evolution, participating in the development of a sophisticated gene regulatory network characteristic found in higher organisms by providing new regulatory elements and/or TF-binding sites through insertion of their long terminal repeat promoters (169). Thus, dense involvement of KRAB-ZNFs in neurobiology and neurogenesis might have been established in part through insertion of the regulatory elements originated from retrotransposons that harbor binding sites for KRAB-ZNFs into relevant key genes. Because retrotransposons facilitate the development of non-inherited gene regulatory diversity in brain neurons through their genome migration and subsequent mutagenic property in these non-dividing cells (170, 171), it is possible that dysfunction of the KRAB-ZNFs might influence this unique process mediated by retrotransposons by impacting their reactivation and further increase phenotypic variation of the NDD patients.

In conclusion, we performed the literature-based analysis on the roles of C_2H_2-ZNFs in brain development and pathogenesis of NDDs. We found that numerous C_2H_2-ZNFs play important roles in these physiologic and pathologic processes. We hope that this literature assessment will encourage the researchers' focus on C_2H_2-ZNFs in helping us extend our understanding of brain physiology and pathophysiology.

AUTHOR CONTRIBUTIONS

NA-N wrote the manuscript draft. RM created the table and edited the text. TK edited and created the final manuscript.

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Sporadic and Familial Variants in NF1: An Explanation of the Wide Variability in Neurocognitive Phenotype?

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Background: Cognitive impairment is the most common neurological manifestation in NF1 and occurs in 30–70% of NF1 cases. The onset and severity of each specific cognitive deficit varies greatly from child to child, with no apparent external causes. The wide variability of phenotype is the most complex aspect in terms of management and care. Despite multiple research, the mechanism underlying the high heterogeneity in NF1 has not yet been elucidated. While many studies have focused on the effects of specific and precise genetic mutations on the NF1 phenotype, little has been done on the impact of NF1 transmission (sporadic vs. familial cases). We used a complete neuropsychological evaluation designed to assess five large cognitive areas: general cognitive functions (WISC-IV and EVIP); reading skills ("*L'Alouette,*" ODEDYS-2 and Lobrot French reading tests); phonological process (ODEDYS-2 test); visual perceptual skills (JLO, Thurstone and Corsi block tests) and attention (CPT-II), as well as psychosocial adjustments (CBCL) to explore the impact of NF1 transmission on cognitive disease manifestation in 96 children affected by NF1 [55 sporadic cases (29♀, 26♂); 41 familial cases (24♀, 17♂)].

Results: Familial and Sporadic form of NF1 only differ in IQ expression. The families' socioeconomic status (SES) impacts IQ performance but not differently between sporadic and familial variants. However, SES is lower in familial variants than in the sporadic variant of NF1. No other cognitive differences emerge between sporadic and familial NF1.

Conclusions: Inheritance in NF1 failed to explain the phenotype variability in its entirety. IQ differences between groups seems in part linked to the environment where the child grows up. Children with NF1, and especially those that have early diagnoses (most often in inherited cases), must obtain careful monitoring from their

early childhood, at home to strengthen investment in education and in school to early detect emerging academic problems and to quickly place them into care.

Trial Registration: IDRCB, IDRCB2008-A01444-51. Registered 19 January 2009.

Keywords: NF1, child, cognitive profile, sporadic, familial, hereditary, SES

HIGHLIGHTS

- Familial and Sporadic form of NF1 differ in IQ expression
- SES impacts IQ performance but not differently between sporadic and familial variants
- SES is lower in familial variants than in the sporadic variant of NF1
- No other cognitive differences emerge between sporadic and familial NF1
- Inheritance in NF1 failed to explain the phenotype variability in its entirety.

BACKGROUND

Clinical Features of NF1

Neurofibromatosis type 1 (NF1 or also von Recklinghausen's disease), is a tumor predisposition syndrome characterized by the development of typical cutaneous and ophthalmologic manifestations including cafe-au-lait spots, freckling, dermal neurofibromas and Lisch nodules. NF1 patients may also develop endocrine (early-onset puberty, growth retardation), neurological (learning disabilities, epilepsy), ophthalmological (optic glioma), skeletal (bone dysplasia, scoliosis), cosmetic disfigurement or organ compression due to plexiform neurofibromas and vascular complications (high blood pressure) (1–5). NF1 patients are at increased risk of developing various tumors or characteristic malignancy (malignant peripheral nerve sheath tumor or other malignancies such as intracranial astrocytomas, gastrointestinal stromal tumors, pheochromocytomas, juvenilemonocytic leukemia, leukemia, glioma, rhabdomyosarcoma, and breast cancer) (1, 3, 6).

The NF1 phenotype (clinical presentation) is highly variable in expression (7, 8). First, clinical manifestations are progressive and age dependent. They appear gradually during childhood, from cafe-au-lait macules at birth, to skinfold freckles, then lisch nodules and latter neurofibromas (2). Most of the complications persist into adulthood. Second, even if penetrance is complete in children over 8 years old (9), clinical features range from a very mild manifestation to a very severe form of the disease depending of individuals.

Cognitive impairment is the most common neurological manifestation in NF1 and occurs in 30–70% of NF1 cases (10). Studies characterizing the neuropsychological phenotype of NF1 children have highlighted some strong well-established features (11–17). Clinical studies have revealed a left shift in average IQ, ranging from low to normal IQ. Severe intellectual disability (IQ $<$ 70) is however unusual, occurring in only about 5% of patients (10, 18–20). Children with NF1 are also at increased risk for difficulties with specific cognitive functions: attention, executive function, reading, expressive and receptive language, language cues interpretation, working memory, visual spatial perception, psychomotor skills (10–14, 21–26). Many school-age NF1 children also experience marked difficulties in learning and academic areas and also presented with learning disorders (reading, mathematics/arithmetic, and written expression). Impaired, poor performance on reading or spelling tasks, deficits, defects in visual-spatial and visual-perceptual skills are therefore common (12). The onset and severity of each specific cognitive deficit varies greatly from child to child, with no apparent external causes. The authors tried to link radiologic specific features in NF1 such as T2 hyperintensities to cognitive impairment (27). In almost 75% of cases, NF1 children present with T2-hyperintensities (4) located mainly in the basal ganglia, thalamus, brainstem and cerebellum, which usually resolve in early adulthood and probably reflect intramyelinic oedema (28). Although the presence and number of T2-hyperintensities do not seem to be related to possible cognitive disorders, some authors find a correlation between IQ scores and their thalamic (29) or cerebellar (30) localization, with an improvement of IQ score with resolving T2-hyperintensities (31). As T2-hyperintensities do not explain the specific cognitive deficits encountered in NF1, authors try to correlate finer structural cerebral changes as higher cerebral volumes or global altered diffusion without further success. Thus, the neuronal substratum of NF1 cognitive phenotype remains unclear.

These cognitive problems do not worsen with age but do not resolve either. They were among the most common manifestation to negatively affect quality of life in NF1 (32–36), leading to significant impacts upon scholastic performance, vocational and professional guidance (37, 38).

Diagnosis

Despite advances in understanding the genetics of NF1, clinical diagnoses are often made based on physical characteristics [National Institutes of Health Consensus Development Conference Statement –NIH, 1988 (39) for formal diagnostic criteria for NF1, later reaffirmed in 1997], including cutaneous, ophthalmologic, and orthopedic features (1, 40, 41) see **Box 1**.

Genetic

NF1 is one of the most common childhood neurogenetic disorders worldwide, affecting approximately 1 in every 2,500 to 3,500 individuals (42, 43). It is caused by mutations in the NF1 gene, a classic tumor suppressor gene (44) on chromosome 17 (17q11.2) which encodes neurofibromin that is largely expressed in the nervous system (45–47). The most of NF1 gene lesions

BOX 1 | National Institutes of Health (NIH) diagnostic criteria for neurofibromatosis type 1 (NF1) (39).

The clinical diagnosis is based on the presence of two or more major disease features out of the following:

- Six or more café-au-lait macules >5 mm in greatest diameter in pre-pubescent individuals, and >15 mm in post-pubescent individuals
- Two or more neurofibromas of any type or one plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Optic glioma
- Two or more iris hamartoma (Lisch nodules)
- Distinctive bony lesion such as sphenoid dysplasia, or thinning of the long bone cortex with or without pseudoarthrosis
- A first-degree relative (parent, sibling, or offspring) with NF1 based on the above criteria

inhibit the expression of intact neurofibromin. Neurofibromin belongs to a family of proteins that act as negative regulators of the ras oncogene and serves as a tumor suppressor (43, 48, 49). Disruption of neurofibromin explains why NF1 patients are at risk for developing tumors.

NF1 is an autosomal dominantly inherited disease, which means that when one of the parents has the disease, there is a one in two chance of transmitting NF1 to an offspring (1). However, although NF1 is an autosomal dominant condition, about 50 percent of NF1 cases are due to new mutations (42, 50) resulting from a *de novo* mutation. The NF1 gene exhibits a very high new mutation rate [between $3–5 \times 10^{-5}$ and $1.4–2.6 \times 10^{-4}$, (51, 52)], among the highest observed in humans, 10 times higher than is typical for human disease gene loci (53). The NF1 gene is one of the largest human genes, with about 350 kilobases of genomics DNA, containing 61 exons to encode 2,839 amino acids and producing an 11–12.5 kilobase messenger RNA containing an open reading frame of 8,454 nucleotides (45, 54, 55). However, neither the size nor the complexity of the NF1 gene are sufficient to account for this unusually high new mutation rate (56).

Sporadic cases (consequence of new mutations) occur in the absence of a NF1 family history. It may be difficult to distinguish clinically mosaic NF1 individuals from NF1 individuals with an inherited NF1. It has been noted that more than one sporadic case can be observed in several families, each with a distinct NF1 mutation (57, 58). There are also some rare cases of parental mosaicism for an NF1 mutation, comprising germline mutation (59–61). In this situation, the parents do not carry the disease but one of them carries the genetic anomaly in some of their reproductive cells. Such individuals (considering as having a mosaic NF1) have a weaker but unquantifiable risk of passing on generalized NF1 to an offspring. Parental mosaicism is also considered as a sporadic case (62, 63). It should be noted that the frequency of NF1 mosaicism in the population may be underestimated since some mosaic individuals may have no clinical evidence of NF1 (63).

Given that the development of congenital syndromes and cancer predisposition are associated with advanced maternal or paternal age (64), research has been done on NF1 (65–67) but has lead to conflicting reports and lesser association between age and *de novo* mutations. As in several other autosomal dominant disorders, paternal age has been mentioned to explain sporadic cases. However, although the average age of fathers of children with sporadic NF1 was more often higher than fathers in the general population (65, 66), effect of age to *de novo* mutation is small or non-existent (68). In addition, several studies have suggested that 90% of spontaneous mutations in NF1 originate in the paternal genome (46, 69). Kaplan et al. (63) found that mutated allele (R1968X, recurrent variant present in 1 to 2% of individuals with NF1) is of maternal origin.

Care Management

The wide variability of phenotype both between individuals with NF1 and within NF1 families is the most complex aspect in terms of management and care. Clinical features and presentation of NF1 are extremely variable (7, 8) and involve many of the body systems. The complications of NF1 differ depending on the individual concerned (presence vs. absence of each possible symptom) and variation in their expressions (minimal, mild, moderate, or severe) is considerable and highly heterogenic. In addition, clinical presentation is unpredictable even within the same family (1, 52), including the age of disease onset and the severity of clinical symptoms (70, 71). For example, Upadhyaya et al. (58) report the genetic analysis of a unique family with NF1, in which the three affected members had a different heritable and pathological mutations in their NF1 genes and exhibited different clinical evidence of NF1. In familial form, the severity of manifestations in a child cannot be expected by a parent's clinical course (or other family members) and the risk of having a seriously ill child is 1 in 12 (1). Members of the same family and even more, identical twins with NF1, often exhibit variable syndrome expression. Thus, twin studies, which have been a respected tool for studying genetic disorders, were not conclusive in the NF1 literature reports. Numerous studies have presented monozygotic twins, both affected by NF1 but differing in their phenotypes (62, 72). Kaplan et al. (63) also presented monozygotic twin discordant for autosomal disorder NF1 (unaffected twin that show no clinical manifestations) who is therefore considered as a mosaic even if the distribution of the mutant allele among different cells and tissues seems insufficient to induce clinical manifestations of NF1. Detjen et al. (62) did not detect any mitochondrial DNA differences between individuals of the same twin pair, highlighting that mitochondrial DNA polymorphisms [an obvious candidate for an extrachromosomal phenotype modifier (73)] do not seem to contribute to the phenotypic variability in NF1.

Copy number variants research (CNVs) also failed to understand phenotypic variability (74). In their study, the authors analyze CNVs in 11 pairs of monozygotic twins with several phenotypic discordances and concordances to identify genetic factors potentially affecting disease manifestation but found no differences in CNVs that could justify discordant NF1 characteristics (74).

A recent study (68) found that there is no significant effect of parental age on the incidence of NF1 or the coexistence of different NF1 symptoms, or on their level. The authors also did

not find any relationship between sex and clinical symptoms, although previous study have showed that sex can be a key influence for neural dysfunction in NF1 (75).

With the exception of deleting the entire NF1 gene which is associated with a very severe form of disease (76), clinical manifestations are irrespective of the causative genetic alterations (77): most studies thus showed no relationship between the particular NF1 mutation type (whether they are missense/non-sense, point mutations, splicing, micro- or gross-deletions, micro- or gross-insertions, duplications, etc.) and the expression of clinical manifestations in NF1 individuals. Cognitive deficits encountered in NF1 are no exception to this rule and such uncertainty and lack of knowledge on why complications of NF1 differ depending on the individuals concerned is exactly the same for cognitive impairments (78).

This very high variability of the NF1 phenotype, including for individuals carrying the same NFl gene mutation, suggests that other factors (other modifying genes, epigenetic influences, second hit somatic mutations in the NF1 gene, hormonal milieu, but also environmental factors or chance) might be involved in the clinical expression of the NF1 phenotype and might be the reason behind this phenomenon (71, 79, 80). Little is however known about such relative contributions.

The management of NF1 children is therefore mainly based on follow-up, in order to detect possible complications such as behavioral or cognitive deficit as soon as possible since early care significantly improves skills and abilities. This uncertainty is a source of stress and anxiety for families and establishing prognostic factors could help professionals responsible for the follow-up of these patients.

Aims and Objectives

Despite multiple and serious research, the mechanism underlying the wide variability in NF1 has not yet been elucidated. Prospective identification and screening of such individuals is currently not possible. There is currently no way to measure, predict or know which NF1 patients will develop one or several symptoms but not others (and why), or which NF1 patients will develop complications or not. However, identifying factors that modify the NF1 phenotype may greatly help to improve patient counseling.

Brain development is based on the ongoing interaction of innate biological determinants and environmental determinants that will modulate the organization, structuring and functioning of the brain. In our study, we therefore ask if the mode of transmission (and consequently the environmental determinants) can influence the cognitive phenotype in NF1 patients.

Indeed, while many studies have focused on the effects of high specific and high precise genetic mutations on the NF1 phenotype, little has been done on the impact of NF1 transmission (sporadic vs. familial cases). This question of transmission establishes a solid framework to study environmental factors, the social level in which the child evolves, the detection of disease and diagnosis (which may be earlier in family forms), the difficulties of informing parents of the clinical status of their child and the consequences of such a disclosure [given that pessimism is less common in familial than in sporadic NF1 (36)], etc.

The issue of influence between sporadic or familial onset on cognitive profile of affected NF1 individuals was not extensively discussed. Learning disabilities, which are very frequent in NF1, is however one of the chief factors in the deterioration of quality of life in NF1 patients (10, 36), both in children and adults since they lead to poor school academic performances, preclude individuals to higher education and graduate school, lower the education level and restrict individuals' choices and their professional future. They also cause great damage to self-image, the development of assertiveness and independence.

In this study, we used a large set of cognitive performance abilities (attention, reading, intellectual, and visual-spatial skills), as well as psychosocial adjustments to explore the impact of NF1 transmission (sporadic vs. familial cases) on cognitive disease manifestation in 96 children affected by NF1 [55 sporadic cases (29♀, 26♂); 41 familial cases (24♀, 17♂)].

METHODS

Participants

The participants included 55 children with sporadic NF1 expression and 41 children with the familial NF1 variant, all aged between 8 and 12 years old. Patients with a family history of neurofibromatosis type 1 or a first-degree relative with one criteria of neurofibromatosis type 1, according to the NIH criteria, were classified as having the familial variant. Patients without any familial history of neurofibromatosis type 1 were classified as having the sporadic variant. Seventy-five of them had been previously included in a published study on the cognitive profile of NF1 (81). NF1 subjects were recruited from the existing NF1 patient population at six French national NF1 referral centers (Children's Hospitals of Lyon, Montpellier, Nantes, Paris, Toulouse, and Tours). Inclusion criteria were (1) age between 8 and 12 years and (2) a confirmed clinical diagnosis of NF1 according to the NIH criteria (39). MRI examination was not required for inclusion but if it had been done, symptomatic optic glioma was considered to be an exclusion criterion. Children with a known major medical, neurological or psychiatric disorder that could potentially affect cognition (epilepsy, brain tumor, hydrocephalus, head injury, autism, or intellectual disability with an IQ below 70) or with uncorrectable hearing or visual impairment were excluded.

All parents and children gave their informed oral and written consent, after the nature and objectives of the study were thoroughly explained. Approval to conduct this study was granted by the French Ministry of Health's Hospital Programme for Clinical Research (PHRC 2008, Toulouse University Hospital, no. 08 113 01), Occitanie Regional Council (APRTC no. 09004813), and the local Ethics Committee (CPP Southwest, France) in accordance with the Declaration of Helsinki convention.

Procedure

Participation in the study was offered to parents by a pediatric neurologist through a clinic for follow-up. A leaflet describing the

characteristics of the study, a recruitment letter, a consent form and a demographic/health screening questionnaire were directly given or sent to them later.

All the children were received for two half-day sessions. They underwent a medical examination to exclude ADHD and other neurological and psychiatric diseases, and to confirm the NF1 diagnosis. Then, all the children underwent the same complete five-part individual, neuropsychological evaluation conducted by certified clinical neuropsychologists, using a comprehensive and large protocol designed to assess five large cognitive areas: general cognitive functions; reading skills; phonological process; visual perceptual skills and attention. Each area was composed of several tests which were given in a specific and the same order as part of a neuropsychological battery. However, the order of administration of the five large cognitive areas was randomly changed between subjects to minimize the order bias of the neuropsychological assessment results (81).

Measures

This study fits in with a larger project [methodology described in Chaix et al. (81). Each participant was assessed with a comprehensive battery of standardized psychometric tests including (i) all subtests of the Wechsler Intelligence Scale for Children—Fourth Edition [WISC-IV (82)]; and the French version of the "Peabody Picture Vocabulary Test-Revised" [EVIP (83)] for the cognitive assessment; (ii) the "*Alouette*" French reading test [revised version (84)], the ODEDYS-2 test (85) and the ORLEC battery (86) for the reading and phonological skills assessment; (iii) the Judgment of Line Orientation test (87), the Thurstone test and the Corsi blocks for visual perceptual assessment; and (iv) the Conners Continuous Performance Test-Second Edition (88) and the parent form Child Behavior CheckList (CBCL) questionnaire (89) for the attention and psychosocial skills assessment. All the assessments were conducted in French. French norms were used to calculate scores for all children wherever available.

The issue of the socio-cultural characteristics (occupation and educational level of parents) has also been raised.

Cognitive abilities were assessed with the French-language version of the WISC-IV (82) designed for children between the ages of 6 to 16. In this psychological assessment, four primary Index make up the Full Scale IQ score (Verbal Comprehension Index -VCI, Perceptual Reasoning Index -PRI, Working Memory Index -WMI, and Processing Speed Index -PSI Scores; themselves calculated from several subtests). The core 10 subtests include Comprehension, Similarities, and Vocabulary subtests for the VCI; Block design, Picture concepts and Matrix reasoning for the PRI; Digit span and Letter-number sequencing for the WMI Coding and Symbol search for the PSI. Raw scores were converted to age-scaled scores (standard scores for subtests M = 10, SD = 3; standard scores for index and full scale IQ: M = 100, SD = 15). All subtests and indexes have demonstrated good reliability and validity and are considered good measures of general intelligence.

Reading disorders were evaluated with the "*L'Alouette*" French reading test (84), the most widely used reading test in French-speaking countries. The "*L'Alouette*" test is currently used as the "gold standard" test by health care professionals (especially speech therapists) and researchers to screen for reading level (good or poor readers, dyslexia) among children and adolescents. **Reading text assessment** was completed by word recognition procedures, measured by the "Word Reading" subtest from the ODEDYS-2 test (85). **Phonological processing** (phonological memory and phonemic awareness) was measured with three tests from the ODEDYS-2 battery of tests (85) (1) pseudoword repetition task (phonological short-term memory), (2) phonemic deletion task (phonemic awareness), and (3) blending task or acronyms task (phonemic awareness). **Reading comprehension efficiency** for both sentences and text was assessed through a standardized reading comprehension test, the ORLEC battery (86). The first subtest (L1) consists of text to be read aloud. The second subtest (L3) is silent reading comprehension test with a forced-choice sentence completion test.

Receptive lexical skills were determined by the French version of the "Peabody Picture Vocabulary Test-Revised" (EVIP) (83). EVIP is intended to provide a quick estimate of the receptive vocabulary ability of children from the ages of 2.6 to 18 years old.

Sustained attention and impulsivity capacities were measured by the Conners Continuous Performance Test-Second Edition (88). CPT-II is a computer-administered neuropsychological task used to evaluate the attentional functioning (sustained attention and impulsivity) of individuals aged at least 6 years old. It is commonly used in research and by clinical means for discriminating inattentive, hyperactive, and impulsive behavior difficulties in children via target vs. non-target stimuli designed to have minimal language and memory demands. The CPT-II leads to 12 outcome measures but we only analyzed the four main scores: Omissions (number of non-responses to target), commissions (number of responses to non-target stimuli), hit reaction time (measure of response speed consistency), and perseverations (measure of response inhibition).

Psychosocial adjustments were assessed with the parent form of the Child Behavior CheckList (CBCL) questionnaire (89). The CBCL is a parent-report measure of behavioral and emotional problems for children aged 6 to 18 years used in both clinical and research practice. It lists internalizing and externalizing symptoms of 113 child behaviors.

Visual perceptual abilities were assessed with the Judgment of Line Orientation test (JLO) (87) and the Thurstone test. The JLO test measures a person's ability to match the angle and orientation of lines in space using a task that consists of matching two angled lines that appear at the top of a page, to the angles of two lines among a standard fan-shaped array (semicircle) of 11 lines (separated 18 degrees from each other) appearing at the bottom of the page. The Thurstone's Identical Form Test measures certain capacities of visual and spatial perception. For this test, the visual-spatial component is predominant. **Visual-spatial memory** was assessed by determining forward and backward spans with the Corsi blocks-tapping test.

Socioeconomic status of the parents (SES): Both parents of the participants were asked about their profession and level of education. We coded the professions into eight categories according to the classification of professions and socio-professional categories established by the National Institute

of Statistics and Economic Studies (*Institut national de la statistique et des sciences économiques,* INSEE) in 1982. This statistical classification, that allows professions to be classified, exists at an aggregate level of eight positions (1. Farmers, farm operator; 2. Artisans, traders and entrepreneurs; 3. Employees and higher intellectual professions; 4. Intermediate professions; 5. Employees; 6. Workers 7. Pensioner; 8. Other individuals without activity professional) but is also developed in 24 and 42 positions (with correspondence between the three nomenclatures). This nomenclature allows the grouping of individuals into homogeneous social categories according to their professional activity, on the basis of three main criteria: the hierarchical position within the profession performed (completed by the level of diploma required to practice this profession), the status (employee or self-employed), and the nature of the activity (agricultural or non-agricultural).

To this nomenclature, we added a classification of the level of education in 5 gradients: (1) Without diploma or *"Brevet des colleges"*; (2) CAP or BEP; (3) General, technological or vocational baccalaureate (around 18 years); (4) Diploma 2 years after baccalaureate; (5) Graduate and postgraduate diplomas (Bachelor's degree, Master's degree).

Statistical Analysis

As a first screening step, statistical tests were conducted for every numerical variable to compare the two sub-groups: sporadic and familial. Both Student and Wilcoxon-Mann-Whitney rank-sum tests were run as a way to ensure the relevance of the results. In order to study the effect of socioeconomic status on the previous results, 5-factor ANOVAs were conducted for each numerical variable. These analyses included the NF1 form as well as the level of education and the profession of the two parents. To make it easier to interpret the results, no interaction factors were included in the models. As the mother's level of education appeared as the SES variable with the highest effect, further investigation focused on this factor jointly with the NF1 form. Two-factor ANOVA with interaction were then conducted to assess the potential cross effect of these two factors. Every analysis was performed using the R software (version 3.5.2, released 2018-12-20).

RESULTS

General Characteristic of the Population

The 96 children included in the main analysis were part of this exploratory analysis. Demographic (age, sex) and socioeconomic status variables (level of education and profession of the two parents) are presented in **Table 1**. The population is roughly balanced between the sporadic NF1 variant (55) and the familial variant (41). This proportion allowed statistical comparison between the two groups to be considered. The two groups were homogeneous with no differences in terms of age or gender. Factors that could influence cognitive ability (socioeconomic status of the father and mother: parental educational level and profession) were also analyzed and were similar across groups concerning father data, but significantly different regarding the mother.

TABLE 1 | Demographic and Social Characteristics of the Sporadic and Familial Groups.

	Sporadic transmission *N* = 55	Familial transmission *N* = 41	*P*-value
Demographic characteristics			
Age in years [Mean (SD)]	9.8 (1.4)	10.2 (1.3)	0.2431
Gender [Boys/Girls]	26/29	17/24	
Social characteristics			
Educational level of father (*N*/%)			**0.2081**
Without diploma	9 (16.4%)	4 (9.8%)	
CAP or BEP	11 (20%)	5 (12.2%)	
Baccalaureate	14 (25.5%)	19 (46.3%)	
Two years after baccalaureate	9 (16.4%)	4 (9.8%)	
Graduate and postgraduate diplomas	7 (12.7%)	8 (19.5%)	
Missing	5 (9.1%)	1 (2.4%)	
Educational level of mother (*N*/%)			**0.0212***
Without diploma	9 (16.4%)	14 (34.1%)	
CAP or BEP	10 (18.2%)	2 (4.9%)	
Baccalaureate	13 (23.6%)	12 (29.3%)	
Two years after baccalaureate	15 (27.3%)	4 (9.8%)	
Graduate and postgraduate diplomas	6 (10.9%)	8 (19.5%)	
Missing	2 (3.6%)	1 (2.4%)	
Profession of father			**0.7501**
Farmers, farm operator	2 (3.6%)	3 (7.3%)	
Artisans, traders and entrepreneurs	4 (7.3%)	4 (9.8%)	
Employees and higher intellectual professions	14 (25.5%)	6 (14.6%)	
Employees	10 (18.2%)	10 (24.4%)	
Worker	10 (18.2%)	10 (24.4%)	
Intermediate professions	8 (14.5%)	4 (9.8%)	
Without activity	3 (5.5%)	3 (7.3%)	
Missing	4 (7.3%)	1 (2.4%)	
Profession of mother			**0.0298***
Employees and higher intellectual professions	15 (27.3%)	5 (12.2%)	
Employees	21 (38.2%)	18 (43.9%)	
Worker	1 (1.8%)	4 (9.8%)	
Intermediate professions	10 (18.2%)	2 (4.9%)	
Without activity	8 (14.5%)	11 (26.8%)	
Missing	0 (0%)	1 (2.4%)	

*$p < 0.001$.

Clinical and Neuropsychological Results, Differences Between Sporadic and Familial Variants

Numerical variables ($n = 49$) are presented in **Table 2**. For each variable, mean and standard deviation are displayed as well as the *p*-values of the Student and the Wilcoxon-Mann-Whitney

TABLE 2 | Clinical Characteristics of the Sporadic and Familial Groups.

	Sporadic		Familial			
	Mean	***SD***	**Mean**	***SD***	***P*-value (Student)**	***P*-value (Wilcoxon)**
Efficiency (WISC-IV)						
FSIQ	93.1	13	85	11.5	0.0016*	0.0015*
PRI	92.1	12	85.2	10.5	0.0034*	0.0048*
Block design	8.3	2.8	6.7	2.8	0.0076*	0.0038*
Picture concepts	9.3	2.4	9	2	0.5594	0.7389
Matrix reasoning	9	2.4	7.4	2.7	0.0039*	0.0051*
VCI	100.1	13.5	94.5	13.5	0.0464*	0.0159*
Vocabulary	9.9	2.4	8.9	2.8	0.0595	0.0289
Similarities	10.5	3.2	9.3	2.9	0.0747	0.0261
Comprehension	9.7	2.6	9	2.3	0.2221	0.1448
WMI	90.2	14.1	82.1	14.2	0.0073*	0.0085*
Letter-number sequencing	8.9	3	7.1	2.6	0.0033*	0.0038*
Digit span	7.9	2.7	6.9	2.6	0.0898	0.0851
PSI	94.9	11.7	91.9	13.5	0.246	0.1909
Coding	10.6	12.5	8.8	2.9	0.2976	0.5576
Symbol search	9.1	2.6	8.3	3.1	0.1869	0.2025
Reading speed (Alouette)						
CTL (SD)	−0.5	1.1	−0.7	1.1	0.3656	0.36
CM (SD)	−1.6	2.4	−1.9	3	0.5554	0.6566
Reading strategies (ODEDYS)						
Irregular words reading	−0.3	1.2	−0.4	1.1	0.444	0.3891
Pseudowords reading	−0.9	1.2	−1.1	1.3	0.5068	0.6349
Regular words reading	−0.6	1.5	−0.8	1.9	0.6078	0.8027
Pseudowords reading (duration)	−0.6	1.4	−0.7	1.7	0.6987	0.9117
Irregular words reading (duration)	−0.2	1.4	−0.6	1.7	0.3086	0.4958
Regular words reading (duration)	−0.5	1.6	−0.8	1.7	0.4255	0.5228
Phonological process (ODEDYS)						
Phoneme blending	−0.7	1.1	−1	1.2	0.2475	0.1964
Pseudoword repetition	−1.4	2	−1.7	2.2	0.4488	0.4971
Phoneme suppression	−0.3	0.9	−0.2	1	0.7705	0.5626
Reading comprehension (Lobrot)						
Sentence comprehension (quartile score)	2.3	1	2	1.1	0.1648	0.1177
Text comprehension (quartile score)	2.4	1	2.1	1.1	0.262	0.1891
Lexical level (EVIP)						
Score (SD)	114.3	13.6	110.4	16	0.2204	0.1606
Attention level (CPT-II)						
Commission	54	29.1	58.5	25.3	0.4235	0.5895
Omission	61.8	26.1	61.3	25.7	0.9231	0.8949
Perseveration	54	23.2	56	27.5	0.713	0.8275
Hit reaction time	65.3	30.4	63.7	26.2	0.7812	0.5473
Hit reaction time standard error	63	30.6	67	25.2	0.4887	0.675
Psychosocial (CBCL)						
Totals problems	−3.4	1.3	−3.4	1.6	0.8675	0.9289
Internalizing	−1.4	1.7	−1.3	1.7	0.8166	0.8705
Externalizing	0	1	−0.3	1.2	0.2656	0.2121
Aggressive behaviors	0	1	−0.3	1.1	0.2232	0.1328
Somatic complaints	−1.1	1.4	−1.4	1.8	0.2724	0.5586
Attention problems	−2.1	1.7	−1.7	1.6	0.2892	0.2908
Throughout problems	−0.8	2	−0.2	1	0.0533	0.319

(Continued)

TABLE 2 | Continued

	Sporadic		Familial			
	Mean	***SD***	**Mean**	***SD***	***P*-value (Student)**	***P*-value (Wilcoxon)**
Social withdrawal	−1	1.7	−0.7	1.5	0.4654	0.3882
Delinquent behaviors	−0.2	1.1	−0.4	1.7	0.5103	0.8937
Social problems	−1.4	1.7	−1.2	1.9	0.6092	0.4508
Anxiety/Depression	−1.2	1.6	−1.1	1.6	0.6417	0.5471
Visuoperceptual abilities						
JLO score	−1	1.2	−1.4	1.2	0.0964	0.0955
Thurstone score	0	0.9	−0.2	0.9	0.2048	0.2492
Forward span (Corsi)	0.1	1.3	0	1.4	0.6743	0.8572
Backward span (Corsi)	0.5	1.2	0.5	0.9	0.7652	0.9844

*$p < 0.001$. *FSIQ, Full Scale IQ; VCI, Verbal Comprehension Index; PRI, Perceptual Reasoning Index; WMI, Working Memory Index; PSI, Processing Speed Index.*

TABLE 3 | *p*-values for individuals effects (NF1 group, level of education mother and father, profession mother and father) from a 5-factor ANOVA performed on each significant numerical variable.

	Transmission	Educational level of mother	Educational level of father	Profession of mother	Profession of father
FSIQ	0.0003*	0.0135*	0.1643	0.7697	0.5235
PRI	0.0016*	0.0261*	0.2156	0.4637	0.9465
WMI	0.0044*	0.711	0.3492	0.9438	0.3373
VCI	0.0056*	0.0778*	0.6654	0.9303	0.6537
Matrix reasoning	0.0028*	0.6458	0.8606	0.4742	0.5788
Block design	0.0056*	0.0802*	0.0854	0.1498	0.6718
Letter-number sequencing	0.0035*	0.5546	0.4163	0.7115	0.3456

*$p < 0.001$.

tests. The variables are ordered depending on the test or cognitive processes evaluated.

The differences between sporadic and familial variants were investigated in three ways.

First, a screening consisted of performing two sample statistical tests (**Table 2**) which highlights, on the one hand, the consistency of the results between the two tests, and on the other hand, some variables (ordered below depending on the increasing *p*-values of the Student tests) with significant differences between the sporadic and familial variants: FSIQ, Letter-Number Sequencing, Perceptual Reasoning Index, Matrix Reasoning, Working Memory Index, Block Design, and Verbal Comprehension Index obtained an uncorrected *p*-value lower than 5%. As we are in a multiple testing context, adjusted *p*-values with Bonferroni correction were also calculated. This correction resulted in *p*-values systematically higher than 5%. This means that our results have to be considered with moderation and not as highly significant results. But, the fact remains that the lowest *p*-values are associated with the numerical variables showing differences between the two variants.

Secondly, the effect of SES variables together with the NF1 variant was studied in 5-factor ANOVAs. The results are presented in **Table 3** for the IQ variables with differences between both groups (with a *p*-value for the NF1 variant factor lower than 5% in **Table 2**). It appeared that the numerical variables with the lowest *p*-values for the NF1 variant remained nearly the same as in the first step (FSIQ, PRI, Matrix Reasoning, etc.). In addition, more interestingly, the level of education of the mother was the SES variable with the lowest *p*-value. Let's note also that the Bonferroni correction applied on these *p*-values would give 0.0147 (0.0003*49) for the *p*-value of the NF1 variant related to FSIQ. Thus, the effect of the NF1 variant on the FSIQ is significant when SES is taken into account.

Thirdly, as the level of education of the mother seemed to be the most important SES variable, we focused on the cross-effects of this factor with the NF1 variant. To address this problem, we ran 2-factor ANOVAs with interactions. The results, presented in **Table 4**, show that the interaction effect is never significant. This means that the effects of NF1 variant and the level of education of the mother occur independently: the effect of one factor does not depend on the level of the other factor.

DISCUSSION

In the current study, we wanted to elucidate if NF1 transmission has an impact on the wide variability on cognitive phenotype in NF1 children. The only difference from a broad battery of neuropsychological tests -including psychometric, reading (text and word), phonological, visual-spatial, reading comprehension

TABLE 4 | *p*-values for individual effects (NF1 group, level of education mother) and interaction from a 2-factor ANOVA including interaction performed on each IQ variable.

	Transmission	Educational level of mother	Interaction
FSIQ	0.0021*	0.015*	0.6528
PRI	0.0045*	0.0227*	0.3516
Block design	0.0125*	0.065*	0.326
Picture concepts	0.5473	0.046*	0.1148
Matrix reasoning	0.0059*	0.6793	0.9964
WMI	0.0133*	0.6012	0.2884
Letter-number sequencing	0.0073*	0.8355	0.3677
Digit span	0.138	0.7085	0.3205
VCI	0.0298*	0.1469	0.2034
Comprehension	0.166	0.4325	0.0636
Similarities	0.0403*	0.0051*	0.2901
Vocabulary	0.0557	0.5358	0.715
PSI	0.2936	0.0283*	0.5703
Symbol search	0.2027	0.1724	0.2258
Coding	0.3785	0.4323	0.2128

*$p < 0.001$.

(sentences and text), receptive language, attention and psychosocial assessments- was in the IQ scores.

IQ Differences Between Sporadic and Familial Form of NF1

We detected a highly significant difference between sporadic and familial NF1 cases in all index scores -except Processing Speed Index (PSI)-, Full Scale IQ (FSIQ) and three subtests: Matrix Reasoning, Block Design, and Letter-Number Sequencing.

For the FSIQ, PSI, and WMI, children affected by the familial variant of NF1 were lower than average (standard scores around 80 to 85 for indexes and between 6 and 7 for subtests) while children affected by the sporadic variant of NF1 were average (90 to 95 for indexes and around 9 for subtests) suggesting that patients with sporadic NF1 adapt better to the disease than familial cases.

Our results were in accordance to those of Coutinho et al. (90) that have found better scores in FSIQ, VCI and WMI (the details of the subtests has not been carried out) in children with sporadic NF1 than children with familial NF1. However, our results differ from those of Lehtonen et al. (91), Hyman et al. (10), and Ferner et al. (20) who found similar IQ scores between sporadic and familial variants. Several reasons can be put forward to explain such discrepancies in the results. First, these three studies were designed to compare cognitive profiles between NF1 patients and controls and not between the sporadic and familial variants of NF1. Thus, the authors did not specify if both groups were taken from comparable populations (if number, percentage, age, sex, number of borderline IQ is comparable between groups, if the main confounders were identified and taken into account in the design and analyses to minimize the risk of bias, etc.). Secondly, differences can be due to the sample age. In our study, children are between 8 and 12 years old, while in Hyman et al. (10) and Lehtonen et al. (91), children were older (8 to 16.75 years and 6 to 16 years, respectively) and in Ferner et al. (20), the 103 patients with NF1 (51 sporadic NF1 cases and 52 familial NF1 cases) are between 6 and 75 years (mean age 27.6 y/o; SD 18.2). Genetic influences—that explain significant parts of the observed variation in cognitive functioning, both for children and adults (92, 93)—tend to increase in significance with age, while environmental influences decrease in significance across development (94–96). Brant et al. (94) especially show that the environmental factors that have an influence on variance in intelligence are very minor from age 12 onwards. There is a great similarity of the pattern of contributing factors from between ages 12 and 16, suggesting that the etiology of individual changes in intelligence development is extremely constant by early adolescence. Another and final explanation to such a discrepancy is that, in these three previous studies, mental retardation is not excluded and 6.2% of children with NF1 in Hyman et al. (10), 6% in Lehtonen et al. (91) and 8% in Ferner et al. (20) have an FSIQ <70. The inclusion of extreme cognitive profiles is probably a bias (controlled in our study) that leads to different results.

Over the last two decades, there have been a number of studies, summarized in the systematic review of Lehtonen et al. (12), that have studied the general intellectual functioning of children with NF1. The majority of studies have shown that, although children with NF1 have IQs in the normal range, their IQ is often lower (around 90s) than their peers or than their unaffected siblings (10, 19, 20, 97). However, some studies failed to prove differences in IQ between children with NF1 and norms (29, 98–100). In addition, Lehtonen et al. (12) pointed out a disagreement to the IQ profile of children with NF1: while some studies demonstrated that children with NF1 scored less on all subtests of the WISC, some others detected some significant differences in only some subtests (Block design or Digit span, for example.) between children with NF1 and their siblings. Difference in proportion of transmission (proportion of sporadic vs. familial NF1 in the final sample) variant could perhaps explain these contradictory results.

We found a difference between sporadic and familial NF1 children regarding Block Design (8.3 vs. 6.7, respectively), Matrix Reasoning (9 vs. 7.4) and Letter-Number Sequencing (8.9 vs. 7.1). Block designed[1] and Matrix reasoning[2] (moderately correlate each other; $r = 0.55$) are known to be a good measure of general and fluid intelligence abilities. They measure non-verbal reasoning, visual processing and abstract, visual perception and organization, visual-spatial ability (and visual-constructional ability for Block Design). Letter-Number Sequencing[3] measures attention span, short-term auditory memory processing,

[1]BD (core Perceptual Reasoning subtest) require children to put together red-and-white specially designed blocks in a pattern according to a displayed model. The subtest is timed.

[2]MR (core Perceptual Reasoning subtest) require children to complete a matrix or serial reasoning problem by selecting a missing picture from five response choices. The subtest is untimed.

[3]LNS (core Working Memory Subtest) require children to repeat in a predetermined order to the examiner a series of numbers and letters that they just heard. The subtest is untimed.

sequential processing and mental manipulation (101–104). Those three subtests may be influenced by concentration and attention.

Differences between sporadic and familial groups of NF1 children in those three subtests are very interesting. All three are considered to be the hallmark phenotypic characteristics of patients with NF1: children with NF1 are known to have serious difficulties in visual-spatial abilities, memory and attention (11, 12, 23, 25, 81, 91, 105).

Altogether, it is therefore legitimate to ask whether IQ difference -largely previously proved between NF1 children and peers or unaffected siblings- persist if the modality of transmission is taken into account. Are differences maintained between children affected by a sporadic variant of NF1 and peers and siblings? Do children with the familial variant of NF1 constitute a "bias" or an explanation to the wide variability in cognitive profile of NF1? More research is needed to detail this specific topic. The mode of transmission of NF1 also seems essential to be taken into account in future studies about the cognitive profile of NF1 subjects.

What Is the Role of Socioeconomic Status (SES) for Such IQ Differences in NF1?

Today, the concept that the cognitive performance of an individual depends approximately equally on his/her genetic heritage and his/her environment is a consensus. Recent genome-wide meta-analyses and research studies have identified genomic loci and genes linked to variation in intelligence (106–111). However, it is also known that the socio-economic background of the child places constraints on their IQ (95, 112). First, indices of the families' SES (education, occupation and income of parents) have been proved to moderate the heritability of their children's intelligence (113–115). The heritability of IQ is higher for children who are raised in high SES environments (115). The results of Turkheimer et al. (114) especially demonstrate that the proportion of IQ variance due to environment and genes change non-linearly with SES: in disadvantaged families, the contribution of genes is close to zero and the environment (SES) explains 60% of the IQ variance, whereas is it the reverse in wealthy families. Secondly, in the general literature, the SES environment has been shown to account for variance in cognitive functioning in childhood in many studies. The effects of the environment on IQ, especially the link between the socio-economic level of parents (socioeconomic status and parental education) and the cognitive performance of children is therefore well-established (96, 115). Of course, the level of education of the child's family environment is involved (especially that of the mother): parents from high SES environments indeed offer more occasions for activities and learning experiences to boost and encourage children's intellectual development (115). But differences in intellectual outcomes could also be attributable to the family income, nutrition, sleep, stress, availability of parents, maternal, and paternal involvement, etc. -that have a direct impact on the child's cognitive development and that is directly connected to the child's environment. For example, concerning income, Noble et al. (116) followed a cohort of 1,099 individuals aged 3 to 20 years. Authors highlighted that income relates most strongly to brain structure (especially in regions supporting language, reading, executive functions and spatial skills) among the most disadvantaged children: small differences in income were associated with large differences in brain surface area in these children, whereas in higher income families, the same differences in income involved smaller differences in surface area). Thirdly, some recent studies tend to highlight the link between IQ and epigenetic mechanisms [temporary (or not) genetic changes supported by environment]. For example, in times of high stress, physiological changes in the organism can modify genes. These modifications can impact a set of features that can have knock-on effects affecting the child development. Kaminski et al. (117) have especially found a relationship between the epigenetic modifications of one specific gene and IQ, indicating experiences have an impact on the genetic mechanisms involved in complex processes such as intelligence. Authors thus show that individual differences in IQ are linked to differences in brain activity and epigenetic changes, which are both under environmental influences.

Altogether, studies have found a strong relationship between IQ and SES in the general population, suggesting our experiences/environment not only affect our quality of life, the wiring of our brain, but the very way our cognitive function evolves.

In the NF1 children population, Hyman, Lehtonen and Ferner's studies have examined predictors of the lowering of general cognitive ability and have only found an association with socioeconomic status. SES has also been found to correlate with general intelligence in Lorenzo et al. (118, 119).

In our study, we have found a strong link between (1) sporadic/familial form, (2) IQ and (3) SES family background, especially the mother's education level.

Firstly, children with familial NF1 had a significantly lower SES than children with sporadic NF1, which is consistent with other NF1 studies (90, 91, 118). Lorenzo et al. (118) especially found in a population of 43 children with NF1 (25 sporadic cases and 18 familial cases) that 68% had mothers who completed a university or postgraduate degree in sporadic cases group compared to 28% in the familial cases group. Coutinho et al. (90) similarly found lower SES in children with the familial transmission than in children with the sporadic transmission (41% vs. 19%). Such distribution does not appear to be an unexpected outcome: the sporadic vs. familial NF1 variant has an impact on the social level in which the child evolves (91, 118). NF1 frequently leads to learning disabilities, poor school academic performances (23), lower education level (less likely to graduate from school, less likely to complete tertiary education), and restrict individuals choice and their professional future (individuals with NF1 are and thus fall into lower socio-economic groups) (120).

Secondly, and as previously shown in the general population (115, 121) and in the NF1 population (10, 90, 118), we found that SES was, in turn, associated with IQ achievement. NF1 children from greater SES backgrounds (here children affected by the sporadic variant) had greater cognition scores than those raised from lower SES backgrounds (here children affected by the

inherited variant). Our results are in accordance with Lorenzo et al. (118) and Hyman et al. (10). However, both studies have addressed the issue of the relationship between NF1 in its entirety, IQ and SES (10, 118, 119), without addressing the specific question of the relationship between NF1 variants, IQ and SES. Our results are also in line with Coutinho et al. (90), who found that children with the familial transmission had a lower FSIQ and tended to have a lower SES compared to those with sporadic NF1. However, the authors did not discuss this association (Cause and effect? Consequence? etc.). Our findings therefore increase those of these four previous studies in the comprehension of this trend, highlighting that the disparity recognized between the sporadic and familial variants is likely due to the impact that the NF1 transmission modality has on the SES environment of the family.

In addition, we also demonstrated that there is no significant interaction between group (transmission forms: sporadic vs. familial) and the relationship between the mother's education level and the IQ of the children. In other words, the mother's education level has an impact on the IQ of the NF1 child, irrespective of the transmission mode (sporadic or familial). Having a low SES has a snowball effect on other variables -as cognitive variables- but effect is irrespective of inherited variant. However, as familial NF1 leads more frequently to a low SES, familial NF1 children are most often affected.

Toward a More Complex and Multi-Factorial Approach to Explaining Specific Cognitive Phenotypes in NF1

Another important finding is the absence of differences for tests exploring the usually affected cognitive domains in NF1 (language, visual-spatial domain, executive functions, attention) between the two forms of NF1. We indeed used 10 tests leading to 49 measures, completed by four SES measures. We only found a single test and only seven measures out of 49 where there is a difference between the sporadic and familial NF1 variants. The majority of cognitive functions are therefore not different between the two groups. Consequently, we can argue that transmission (sporadic vs. familial) alone failed to explain the wide variability in phenotype NF1 expression.

Our results were consistent with those of Coutinho et al. (90). Although authors found that children with sporadic NF1 performed better than those with familial NF1 in a large battery of neuropsychological tests (Reading Comprehension tasks, Rey Complex Figure Copy, Spatial Memory, JLO, Imitation of Hand Positions), differences were canceled when FSIQ and SES were taken into account (except for JLO). Our results were also consistent with those of Lehtonen et al. (91) and Erdogan-Bakar et al. (122) where the heritability status of NF1 did not lead to any differences in the performance of the children with NF1 (sporadic vs. familial NF1 groups) on any of the measures (visual-spatial, working memory, spatial memory, executive function, attention, etc.). Note, however, that these two studies were not designed to observe this effect (this is here an ancillary result), so the groups were not controlled and adjusted in terms of number, age, sex, IQ, SES, etc. Our results therefore reinforce, confirm and extend these previous ones with equivalent groups (no bias), and a study especially designed to reply to this question.

The causes of NF1 cognitive phenotype and its variability have been explored with genetic, brain imaging or histological studies (27) but none have successfully explained them until now [for e.g., (10, 18, 21, 27)]. Snippets of explanation are sometimes pointed (UBOs, visual-spatial abilities, etc.) but findings are inconsistent across studies. The IQ variability could be explained by the transmission (sporadic vs. familial) and SES status, while another variability typology (motor impairment, social deficit, executive function impairment, etc.) could be explained by another cause. It is therefore possible that the wide variability in NF1 can be explained by a multitude of causes and not just one, which would partly explain why studies fail to explain phenotype variability in NF1 when they address this question from just one perspective.

Overall, cause-and-effect relationships to explain phenotype variability in NF1 are not always easy to establish and more global approaches are probably needed. Multi-causality is also a possible explanation that should be investigated: either as interrelated causes that interact in a particular order to produce the effect; or as the interaction of multiple risk factors, including environmental, economic, lifestyle and genetic predisposition factors.

CONCLUSION

Altogether, we therefore highlighted (1) that there is an IQ difference between children affected by the sporadic variant compared to the familial variant, (2) that such difference is linked to SES status of the child family, (3) that there is no difference between groups on the impact of SES on IQ, (4) but that there is significantly lower SES in familial NF1 families than in sporadic NF1 families, and (5) that IQ differences between groups seems consequently in part linked to the environment where the child grows up.

The question of NF1 transmission institutes a robust framework to study the impact of environmental determinants and their repercussions on health, care, disease development and prognostics. Inequalities in health reflect the inequalities that can generally be seen within a society. The findings from our study have clinical implications with regard to the management of NF1: children with NF1, and especially those that have early diagnoses (most often in inherited cases), must obtain careful monitoring from their early childhood, at home to strengthen investment in education and in school to early detect emerging academic problems and to quickly place them into care. Our findings also have implications in research that leads to taking care of the effects of inherited and sporadic cases of NF1 in the evaluation of cognitive and behavioral assessments, considering this variable with great interest in developmental studies since it is largely determined by the environment in which the child grows up.

On the other hand, our results imply that the inherited variant of NF1 (familial vs. sporadic) does not explain specific deficits in NF1 (reading, visual-spatial, attention, psychosocial functions). We would strongly encourage research to advance further in

the understanding of phenotypic variability in NF1, since this is a major hindrance to the prognosis, monitoring, and care of such patients. However, we believe that researching a unique and common cause to the set of variabilities is not the right solution. This variability exerts on many components (presence or not of physical characteristics, cognitive functions impairments, level of the symptoms, which one and which intensity, presence or not of UBOs, their location, their numbers, etc.) and maybe there is one cause behind each type of component or clusters of components. Appropriately identifying the responsibilities behind each variability has a real and significant interest for health care in NF1.

LIMITATION

Further research on larger NF1 populations is needed, and shall include genetic data recovery to allow genotype-phenotype analyses and their correlation to the neurobehavioral phenotype.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are not publicly available due to the nature of the data (interventional research protocol involving the human person) but are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study is registered with ClinicalTrials.gov number NCT02397967. The study was approved by the local ethics committee (CPP Sud-Ouest Outre-Mer 1) and conducted in accordance with the Declaration of Helsinki. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YC is the principal investigator of the study, conceived the idea for the study, and was a major contributor in writing the protocol. YC, MB, and EB analyzed and interpreted the data, conceived the first working plan based on results, and wrote the manuscript. SD carried out the statistical analysis, wrote the statistical sections of the manuscript, and reviewed the final manuscript. SL was involved in study coordination and quality monitoring. NF-M was involved in study coordination and carried out the neuropsychological tests. SI carried out the neuropsychological tests. PC and FR included patients. VL-C contributed to the writing of the protocol and reviewed the final manuscript. All the authors read and approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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Application of Machine Learning Using Decision Trees for Prognosis of Deep Brain Stimulation of Globus Pallidus Internus for Children With Dystonia

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Background: While Deep Brain Stimulation (DBS) of the Globus pallidus internus is a well-established therapy for idiopathic/genetic dystonia, benefits for acquired dystonia are varied, ranging from modest improvement to deterioration. Predictive biomarkers to aid DBS prognosis for children are lacking, especially in acquired dystonias, such as dystonic Cerebral Palsy. We explored the potential role of machine learning techniques to identify parameters that could help predict DBS outcome.

Methods: We conducted a retrospective study of 244 children attending King's College Hospital between September 2007 and June 2018 for neurophysiological tests as part of their assessment for possible DBS at Evelina London Children's Hospital. For the 133 individuals who underwent DBS and had 1-year outcome data available, we assessed the potential predictive value of six patient parameters: sex, etiology (including cerebral palsy), baseline severity (Burke-Fahn-Marsden Dystonia Rating Scale-motor score), cranial MRI and two neurophysiological tests, Central Motor Conduction Time (CMCT) and Somatosensory Evoked Potential (SEP). We applied machine learning analysis to determine the best combination of these features to aid DBS prognosis. We developed a classification algorithm based on Decision Trees (DTs) with k-fold cross validation for independent testing. We analyzed all possible combinations of the six features and focused on acquired dystonias.

Results: Several trees resulted in better accuracy than the majority class classifier. However, the two features that consistently appeared in top 10 DTs were CMCT and baseline dystonia severity. A decision tree based on CMCT and baseline severity provided a range of sensitivity and specificity, depending on the threshold chosen for baseline dystonia severity. In situations where CMCT was not available, a DT using SEP alone provided better than the majority class classifier accuracy.

Conclusion: The results suggest that neurophysiological parameters can help predict DBS outcomes, and DTs provide a data-driven, highly interpretable decision support tool that lends itself to being used in clinical practice to help predict potential benefit of DBS in dystonic children. Our results encourage the introduction of neurophysiological parameters in assessment pathways, and data collection to facilitate multi-center evaluation and validation of these potential predictive markers and of the illustrative decision support tools presented here.

Keywords: dystonia, machine learning, deep brain stimulation, decision support systems, decision trees

INTRODUCTION

Deep Brain Stimulation (DBS) of the Globus pallidus internus (GPi) is a well-established management for isolated idiopathic or genetic dystonia both in adults (1–4) and children (5). In childhood, acquired dystonias are more common than idiopathic/genetic dystonias, comprising ∼80% of patients referred for consideration of DBS (5). There are many reports of successful outcomes in acquired dystonias, but benefits are generally more modest and the variability in outcome is much greater than in idiopathic/genetic dystonias (1, 4, 6). Studies of DBS for acquired dystonia are sparse and generally limited to small numbers. For example, a recent meta-analysis and systematic review of DBS for childhood dystonia yielded individual patient data from a total of only 125 patients with acquired dystonia across 72 articles (7, 8).

Appropriate family counseling is essential to manage expectations before a young person undergoes functional neurosurgery, which is not without risk (9, 10) but predictive markers of DBS outcomes in acquired and complex dystonias are lacking (11) and families are asking for more information to help guide this decision (12, 13).

We have previously reported a relationship between neurophysiological measures of corticospinal tract and sensory pathway integrity and outcome from DBS in a group of children and young people with dystonia (or dystonia-dyskinesia) (6). In that study, abnormalities of either Central Motor Conduction Time (CMCT) or Somatosensory Evoked Potentials (SEP) were associated with less reduction in dystonia at one-year follow-up, as measured using the Burke-Fahn-Marsden Dystonia Rating Scale-motor score (BFMDRS-m) and (for SEPs) using the Canadian Occupational Performance Measure (COPM) (6, 13). This was the first study to investigate the role of neurophysiological tools as potential predictive markers which could help to guide counseling of families (6). However, there were a number of limitations: in particular, although the overall sample was large, the numbers of children with abnormal CMCT and/or SEP who proceeded to DBS and already had 1-year outcome data were small. The current study builds on this previous work by reporting findings from a larger group of young people and by leveraging techniques developed in the machine learning community to investigate the most optimal clinical decision tool that synthesizes various clinical features and assesses their accuracy in predicting outcomes.

METHODS

Data were reviewed retrospectively from all 244 children with medically refractory dystonia who attended King's College Hospital between September 2007 and June 2018 for CMCT and/or SEPs as part of their assessment for possible pallidal Deep Brain Stimulation via the Complex Motor Disorders Service at Evelina London Children's Hospital. This was an extension of a previously published dataset (6) comprising 180 children. The neurophysiological studies were performed as part of a standard clinical work-up, along with detailed imaging and a multi-disciplinary clinical assessment by a pediatric neurologist, nurse specialist, physiotherapist, occupational therapist, speech and language therapist and clinical neuropsychologist. Ethical approval for the retrospective analysis was obtained (London-Harrow National Research Ethics Committee, London, UK (17/LO/0439).

Data Acquisition

The young people were examined by a consultant pediatric neurologist with expertise in movement disorders (JPL) and dystonia was classified in line with the Albanese dystonia classification (14), taking into account the clinical characteristics and etiology (**Table 1**). Baseline dystonia severity was assessed using the BFMDRS-m. For those proceeding to DBS ($n = 133$), outcome was expressed as percentage improvement in BFMDRS-m from baseline to 1 year post-operatively. The Canadian Occupational Performance Measure (COPM) was used as an additional outcome measure (13), although was not available in all patients.

CMCT was assessed using Transcranial Magnetic Stimulation (TMS) and the F-wave method and interpreted in relation to established norms, as published previously (6, 15). CMCT reaches adult values by age 3 years for upper limbs (16) and by age 6 years for lower limbs (17). A CMCT was considered abnormal if it was prolonged or the MEP to that limb was absent. For the purposes of statistical analysis, any cases in whom a prolonged CMCT was obtained which could have been physiological, due to immaturity, were excluded from the analysis [see (15) for discussion]. Some children with high MEP thresholds were unable to tolerate the stimulus to high enough intensity to determine whether a normal latency MEP was present. These data were excluded along with any other traces which were

TABLE 1 | Distribution of patients across different etiological groups.

Aetiological group	Number of patients with satisfactory SEP data (% with respect to all those with satisfactory SEPs)	Number of patients with satisfactory CMCT data (% with respect to all those with satisfactory CMCT)
Isolated Idiopathic/Genetic Early onset generalized isolated dystonia. No evidence of structural or degenerative pathology	17 (11.0) (of whom 6 DYT1 positive)	22 (11.5) (of whom 8 DYT1 positive)
Complex Idiopathic/Genetic Early onset generalized dystonia with associated features (Normal cranial MRI). No evidence of structural or degenerative pathology	16 (10.3) (of whom 5 DYT11 positive and 1 TIFF1 positive)	21 (11.0) (of whom 4 DYT11 positive and 1 TIFF1 positive)
Acquired non-degenerative due to perinatal brain injury (Cerebral Palsy)	70 (45.2)	82 (42.9)
Acquired non-degenerative due to metabolic condition	16 (10.3)	19 (9.9)
Acquired non-degenerative due to other cause	29 (18.7)	35 (18.3)
Acquired Degenerative due to Neurodegeneration with Brain Iron Accumulation (NBIA) or mitochondrial disease	7 (4.5)	12 (6.3)
Total	155	191

Only the patients with technically satisfactory data are included.
155/162 (96%) of children tested for SEPs had technically satisfactory data. 157/191 (82%) of children tested for CMCT had technically satisfactory data.

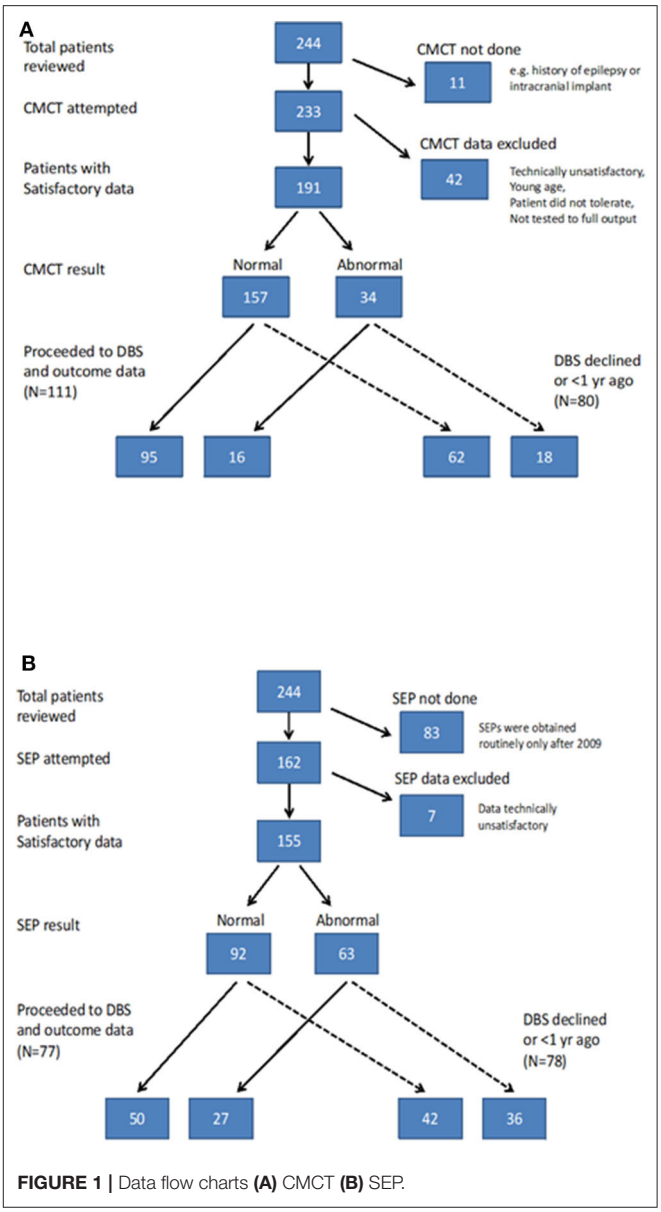


FIGURE 1 | Data flow charts **(A)** CMCT **(B)** SEP.

technically unsatisfactory, as in previous reports (6, 15) (see Flow chart in **Figure 1A**).

SEPs were obtained from all four limbs, using stimulation of the median nerve at the wrist and posterior tibial nerve at the ankle. Upper limb SEPs were recorded over ipsilateral Erb's point, the 7th and 2nd cervical vertebra and contralateral centro-parietal scalp overlying sensory cortex at C3′ and C4′ (2 cm posterior to C3 and C4). Posterior tibial nerve SEPs were recorded over ipsilateral popliteal fossa and midline scalp at Cz′ (2 cm posterior to Cz) and, more recently (from October 2014 onwards), additionally using a Cz′-Cc derivation (18). The upper lumbar components were recorded where possible (T12/L1—iliac crest). Filter band-pass was 1–500 Hz and the sampling rate 2 kHz. At least two averages of 250 artifact-free trials were recorded to determine reproducibility. The components were labeled according to their polarity and peak-times in adults and the data were compared with published pediatric norms (19–21). Cortical potentials were classed as abnormal if they were delayed (peak times greater than published mean for age-group + 2.5 standard deviations), absent or of abnormal waveform (i.e., clear time-locked cortical activity was present but waveforms were poorly formed or broadened) (see **Figure 1**). Technically unsatisfactory recordings were excluded from further analysis (see Flow chart in **Figure 1B**).

For each individual, there was a maximum of 4 limbs of SEP data and 4 limbs of CMCT data, but not all individuals had satisfactory data recorded from all 4 limbs. To simplify the analysis, a "binary coding" was assigned to the SEP and CMCT data for each child. Thus, if CMCT to one or more limbs was abnormal, that child was considered in the "abnormal CMCT" group. Likewise, if the cortical SEP from one or more limbs

was abnormal, that child was considered in the "abnormal SEP" group, corresponding with previous reports (6).

MR examination was performed on an Achieva 1·5 Tesla MRI system (Philips, Best, Netherlands), under general anesthesia. Images were acquired using an 8-channel head coil, according to the local "DBS protocol," to include those sequences required by the neurosurgeons for electrode targeting in the event that the patient went forward for DBS surgery (6, 15). The MRI scans were interpreted by a consultant neuroradiologist and the findings classified, for the purposes of this study, on the anatomical location of abnormalities (6, 15) with the rationale to identify abnormalities in the target nucleus (globus pallidus internus) and to identify patterns of imaging abnormality which would be expected to be associated with dysfunction of the Corticospinal tract (**Table S-1** and **Figure S-2**).

Neurosurgical Procedure

Surgery was performed under isofluorane general anesthesia, in view of the young age of the children. Stereotactic MRI was performed pre-operatively under anesthesia with a Leksell G Frame in place to determine co-ordinates targeted in the postero-latero-ventral GPi. Bilateral electrodes were implanted in each case. The electrodes used are all Medtronic 3389 circumferential electrodes: contacts 0.5 mm apart and 1.5 mm in length. Final electrode placement was confirmed by post-operative stereotactic CT scan, under the same general anesthetic, fused with the intra-operative in-frame pre-surgical MRI. The pulse generator was then inserted (Soletra and Kinetra until 2008, and Activa RC pulse generators thereafter, Medtronic, Minneapolis, MN, USA).

Accuracy of electrode placement within our service has been studied previously (22). Mean Euclidean distance between final electrode tip position and target position was 2.2 mm with no difference in accuracy between isolated genetic/idiopathic and acquired dystonia cases. No correlation was found between outcome at 1 year and Euclidian distance between target and actual position (22).

Analysis of Imaging and Neurophysiology Parameters in Relation to Outcome

Of the 244 children, 133 (54.5%) went forward for DBS and had 1-year outcome data available. All these children had cranial MRI, 111 (83.4%) had satisfactory CMCT data and 77 (57.8%) had satisfactory SEP data (**Figure 1**) [Note SEP recordings were incorporated into the assessment pathway more recently than CMCT, hence the lower numbers (see **Figure 1B**)]. Baseline statistical analysis of these parameters in relation to outcome was performed in SPSS, as per McClelland et al. (6), to allow comparison with the previous report. Differences between groups were investigated using Mann-Whitney test for percentage change in BFMDRS-m (non-normally distributed data) and independent samples *t*-tests for COPM scores (normally distributed data) (see **Supplementary Material**).

The main purpose of the current report is to investigate the potential application of a Machine Learning approach to the prediction of outcome from DBS based on the following parameters: sex, etiology, baseline severity, cranial MRI, CMCT, and SEP. Data from all children proceeding to DBS and with 1-year outcome data ($n = 133$) were included in the Machine Learning analysis, including initially those who had outcome data, but in whom no satisfactory CMCT or SEP data was available. For the purpose of the ML analysis CMCT was classified as Normal/Abnormal/Not available (NA) and SEP was classified as Normal/Abnormal/NA.

Machine Learning for Clinical Decision Support

With the large increase in the amount of data that is now collected, and increasing computational power, a large suite of techniques [commonly termed as "Machine Learning" (ML)] have been developed that have led to disruptive changes in a wide variety of industries. Unlike other industries, adoption of ML-based solutions in routine clinical practice is slow due to the unique challenges of healthcare delivery. One such challenge is the need for clinically interpretable algorithms. Many ML techniques are black boxes which are not suitable, especially in a clinical problem setting where existing evidence is sparse. It is therefore important that any ML-based algorithm developed is clinically interpretable. One of the most widely explored fields within ML, and the one that is most relevant to clinical decision support systems, is supervised learning.

Supervised Learning

Supervised learning uses previous examples with known outputs (or labels) to determine the most optimal decision boundary that can then be used to classify unseen data. This can best be explained with the help of **Figure 2A** which shows two classes (red crosses, and green circles) and the classification task is to determine a decision boundary that can help identify if a new case belongs to the red class or the green class. A linear classifier will seek to determine a straight line that can best separate the two classes. Mathematically, this is equivalent to finding the values of α and β (through optimization using algorithms such as gradient descent) of a linear equation ($\alpha(BR) + \beta(HR)$) that leads to the most "optimal" classification. There are several ML-based classification algorithms, each with their own optimality criterion. Logistic regression (LR) is one of the most commonly used methods for supervised classification. LR is generally simple, easy to implement and interpretable. There are, however, two drawbacks of using an LR-based classification in our case. Firstly, it is a linear classifier, attempting to find a linear combination of different predictors. The example in **Figure 2** shows a situation where a linear classifier will not be able to correctly classify all cases as the separation between classes is non-linear. For LR-based classification algorithms, such cases can be handled by introducing new, non-linear features but this increases the chances of over-fitting. Secondly, LR is not ideally suited to using categorical features and requires techniques such as hot-encoding (a machine learning technique that converts categorical features into numerical values so that algorithms can work as intended, but which may also increase the chances of overfitting) (23).

Decision Trees

Consequently, we propose using decision trees (DT) as the most appropriate supervised classification method in our case. DTs offer the possibility of combining features non-linearly thereby enabling more complex boundaries to be drawn in the feature

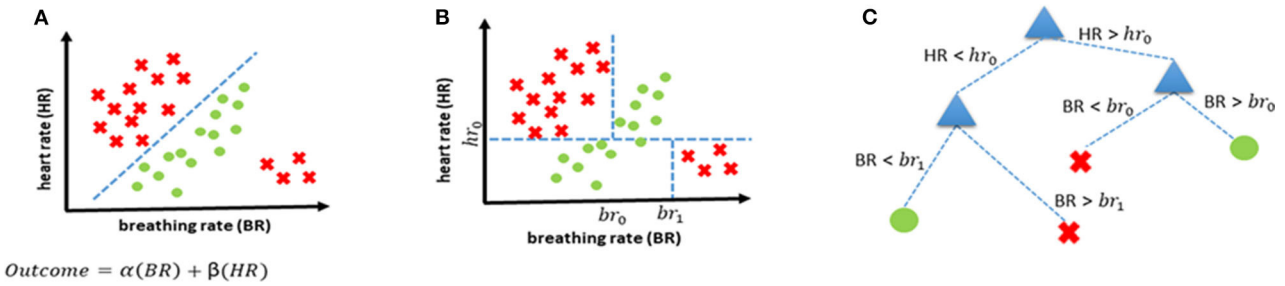


FIGURE 2 | Examples of decision boundaries and trees. **(A)** Linear decision boundary for classification, and the corresponding linear function representing the straight line that separates the two classes. **(B)** Non-linear decision boundary achieved using a decision tree. **(C)** Decision tree corresponding to the decision boundary shown in **(B)**.

space. They also handle categorical features without the need for any hot-encoding. Lastly, they are easily interpretable as every decision can be precisely explained. The same scenario that was introduced earlier in **Figure 2A** is shown to be handled more elegantly with a decision tree-based classifier (**Figure 2B**) and the corresponding decision tree is shown in **Figure 2C**.

In the current study, up to six possible clinical features (sex, etiology, baseline severity, cranial MRI, CMCT, and SEP) were investigated in order to determine the most useful combination for predicting DBS prognosis (favorable or unfavorable).

Training, Validation, and Performance Evaluation

In this study, we used k-fold cross validation. This method is used routinely as an internal validation technique where the data are divided into k groups (each patient's data is randomly allocated in one of the k groups) and the algorithm is then trained on the data from all groups except one. The trained algorithm is then tested on the group that was not part of the training set (i.e., out-of-sample testing). This process is repeated k times. This effectively allows us to use all the data for testing while ensuring that the same data is not used for both training and testing to avoid over-fitting. The metrics we used to assess the performance of the algorithms are based on True Positives (TP: number of patients with favorable prognosis that are correctly predicted), True Negatives (TN: number of patients with poor prognosis that are correctly predicted), False Positives (FP: number of patients with unfavorable DBS outcomes who were wrongly predicted to have a favorable prognosis), and False Negatives (FN: number of patients with favorable DBS outcomes who were wrongly predicted to have an unfavorable prognosis) and these are:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

In order to benchmark the performance of the algorithms, we defined a majority class classifier as the reference. A majority class classifier always outputs the class that is in majority irrespective of the input. The accuracy of such a classifier will be equal to the proportion of the majority class, and any classifier that results in an accuracy greater than the majority class is deemed good. For example, if 48 out of 80 patients show a positive response to a treatment (without taking into account the variables in question), then the majority (60%) of patients improve and the majority class classifier performance is defined as 60% (i.e., one would predict that 60% of patients would show a positive response). The aim of the analysis is to test whether any additional classifiers improve the accuracy of the outcome prediction above this baseline level.

We also used receiver operating characteristics (ROC), where applicable, to determine a range of sensitivity and specificity values as the threshold (BFMDRS-m score in this case) for decision making is changed. As the overall accuracy of any decision tree on the test set (i.e., out-of-sample accuracy) is dependent on the allocation of patient data in various groups during k-fold cross validation, we used multiple iterations and then computed the mean of the resulting accuracy, and where applicable, we have also reported the error bounds (one standard error) of our estimates.

Considering the six clinical feature variables (sex, etiology, baseline severity, cranial MRI, CMCT and SEP), we investigated all possible combinations of these six variables to construct decision trees (DT) and evaluated the corresponding performance with k-fold cross validation. In total, there were $(2^6 - 1)$ i.e., 63 different combinations that were investigated.

All the analysis was carried out in MATLAB (24). For fitting a decision tree model, we used the "fitctree" provided in MATLAB. The hyperparameters in the algorithm were learnt using "Bayesian Optimization."

RESULTS

Standard Feature-Outcome Associations of Imaging and Neurophysiology Parameters

The results of CMCT and SEP data for the cohort as a whole are in keeping with the previously reported smaller dataset (6) and are reported in the **Supplementary Material**. Of the 244 children, 133 went forward for DBS and had 1-year outcome data

available. All these children had cranial MRI, 111 had satisfactory CMCT data (compared with 89 in previous cohort) and 77 had satisfactory SEP data (compared with 51 in previous cohort) (6). Statistical analysis of these parameters in relation to outcome was also concordant with the previously reported smaller dataset and is shown in **Figures S-3**, **S-4**. The remainder of the results focus on the ML analysis.

Machine Learning Analysis

Figure 3A shows the overall distribution of percentage improvement in BFMDRS-m after 1 year follow-up across the 133 patients. In literature pertaining to DBS outcomes, a ≥20% change in BFMDRS-m at 1 year has been reported as a cut-off for defining improvement (25, 26), although this scale was not developed for use in children (8) and is of limited use in acquired dystonia or dyskinetic cerebral palsy (13, 27, 28). In the current dataset, which is dominated by patients with acquired dystonia, the majority of patients do not reach this level of change. However, we initially investigated which DT leads to the highest accuracy based on this criterion, to allow comparison in the context of the wider literature.

Figure 3B shows the optimal DT that was determined. This suggests that etiology alone can provide the highest accuracy when determining whether DBS can lead to ≥20% change in BFMDRS-m at 1 year. According to this DT, any patient who has an isolated genetic or idiopathic dystonia or a complex genetic or idiopathic dystonia (see **Table 1**) is likely to benefit from DBS. In this case, the majority class classifier had an accuracy of 80.5% (i.e., 80.5% of the patients will not achieve ≥20% change in BFMDRS-m). Using the DT resulted in out-of-sample accuracy of 85.5% (an improvement over the majority class classifier).

As noted above, there is already good evidence that individuals with isolated genetic or idiopathic dystonias (previously termed primary) are likely to show improvement with DBS, including a recent meta-analysis of DBS for dystonia in children (8). The area in which predictive factors are particularly needed, however, is with respect to acquired dystonias, in whom the degree of benefit is more variable between individuals and harder to predict (8, 15, 29). Apart from a small study in 10 patients with dystonic-dyskinetic cerebral palsy (28), the above 20% improvement cut-off has not been validated in pediatric studies. Previous studies have suggested that improvements in BFMDRS-m more modest than 20%, are still beneficial to patients and their families (4, 13, 27, 30). Relatively small reductions in dystonia can bring meaningful benefit in function and quality of life (see discussion later on other scales).

Consequently, further investigation focused on the 96 acquired dystonia cases, and any improvement, defined as >0% change in BFMDRS-m was considered as a positive outcome while the remaining cases were considered as negative (other thresholds were also investigated as described later). The majority class classifier for this analysis had an accuracy of 60.42% (i.e., 58 of the 96 children with acquired dystonia proceeding to DBS had a positive outcome by this definition). **Figure 4A** shows the overall accuracy after investigating all 63 possible combinations of clinical variables (sex, etiology, baseline severity, cranial MRI, CMCT, and SEP) and a corresponding table listing the top 10 best performing combinations based on classifier accuracy. It is worth noting that as the assigning of patients in k groups during k-fold cross validation is random, the overall accuracy will vary each time the k-fold cross validation procedure is repeated. **Figure 4A** therefore shows the mean (over all iterations) and **Figure 4B** lists the top 10 best performing combinations based on mean accuracy. Looking at performances using individual features, we can see that only a decision tree using CMCT or SEP (as an individual feature) outperforms the majority class classifier accuracy, yielding accuracies of 66.21 and 63.39%, respectively.

Because the performance of any decision tree depends on which data samples are assigned in which folds (the smaller the data size, the more likely it is to have results sensitive to which folds data are assigned to), we decided to consider the top 10 best performing decision trees as opposed to picking a single best performing tree. These 10 combinations performed fairly similarly, and all have CMCT present as a feature. We can also see baseline dystonia severity is the second most common feature that most consistently appears (along with CMCT) in the top 10 best performing combinations. We therefore chose the decision tree (index 36) that uses CMCT and baseline dystonia severity for further analysis, for two reasons. Firstly, both CMCT and baseline severity are the two features that appear the most consistently in the top 10 best performing combinations. Secondly, baseline severity is a continuous variable which then allowed us to demonstrate how we could select a cut-off threshold and its implication on sensitivity and specificity for personalized clinical decision making.

The decision tree showed equivalent performance regardless of the order in which the variables were included. For the purposes of illustration, we used the following DT (**Figure 5**):

> Assess child by CMCT. If CMCT is abnormal, DBS is less likely to be effective at reducing dystonia. If CMCT is normal, assess the child's baseline BFMDRS-m to determine severity. If the child's condition is very severe (based on a specific cut-off chosen automatically by the decision tree—see below), DBS is more likely to be effective in reducing dystonia severity, as measured using BFMDRS-m. For children with lower baseline severity (i.e., less than the threshold identified automatically), DBS is less likely to be effective in reducing dystonia, as measured using BFMDRS-m.

However, severity is a continuous variable, so what value should be chosen as a cut-off? As the BFMDRS-m cut-off used in the DT is varied, so will the resulting accuracy and the likelihood of missing a positive effect. We consequently devised a Monte Carlo simulation technique to help inform case-specific decisions given patient, family and clinical preferences and the current data (see **Figure 5**). Furthermore, we removed all the cases where CMCT data were unsatisfactory (18 cases out of the total of 96 cases with acquired dystonia) to remove any potential bias in results (see Discussion). In the Monte Carlo simulation, we sampled 100 cases (with substitution) from the total pool of acquired dystonia patients (78 cases after removing those who did not have CMCT available) for 1,000 times and computed the sensitivity and specificity for every possible threshold of the BFMDRS-m in steps of 0.5. **Figure 6A**

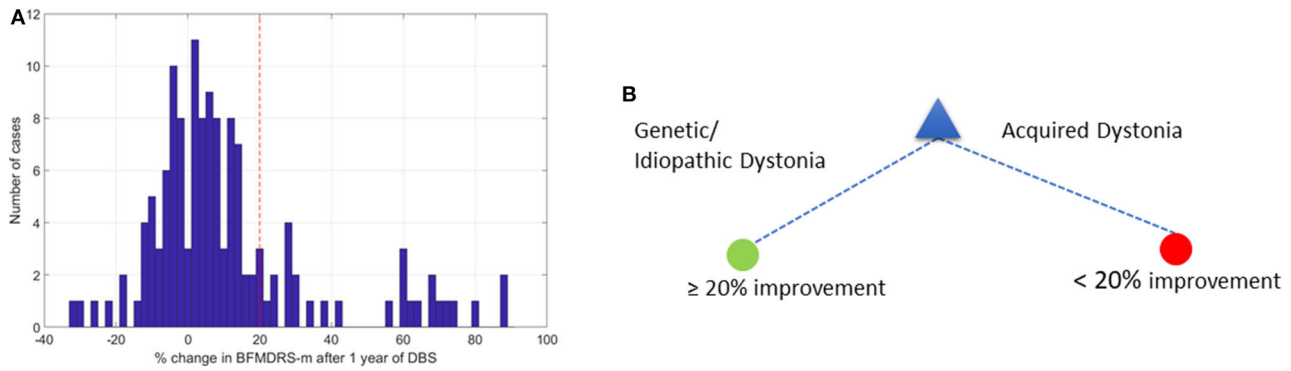
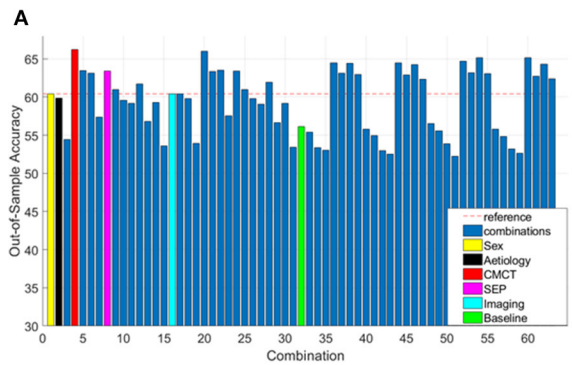


FIGURE 3 | Change in BFMDRS-m after 1 year and decision tree with 20% cutoff. **(A)** Distribution of % change in BFMDRS-m across all patients, with 20% improvement marked by the red vertical line. **(B)** Decision tree based on cut-off of ≥20% improvement in BFMDRS-m, suggesting that DBS prognosis is favorable when patients have genetic or idiopathic dystonia, and less favorable in cases with acquired dystonia.



B

Index	Baseline BFMDRS-m	Imaging	SEP	CMCT	Etiology	Sex	Mean Accuracy
4	0	0	0	1	0	0	66.21
20	0	1	0	1	0	0	66.02
54	1	1	0	1	1	0	65.16
60	1	1	0	1	0	0	65.14
52	1	1	0	1	0	0	64.74
36	1	0	0	1	0	0	64.47
44	1	0	1	1	0	0	64.46
38	1	0	0	1	1	0	64.43
62	1	1	0	1	1	0	64.33
46	1	0	1	1	1	0	64.28

FIGURE 4 | Decision tree performance and winning tree with cut-off ≥0% improvement in BFMDRS-m considering all possible combinations. **(A)** Out-of-sample accuracy of decision trees investigating all possible combinations (63) for patients with acquired dystonia, reference accuracy corresponding to the percentage of majority class, and decision trees based on single feature highlighted, suggesting that CMCT is the most informative feature. **(B)** The top 10 trees, based on accuracy, with the index number associated with those in **(A)**. Each possible combination in the table is binary-coded (1 indicating inclusion of the feature, and 0 indicating the feature was not included in that combination) and the associated accuracy is reported (minimum leaf size of 10, i.e., the minimum number in end nodes, 100 iterations, 10 fold cross validation).

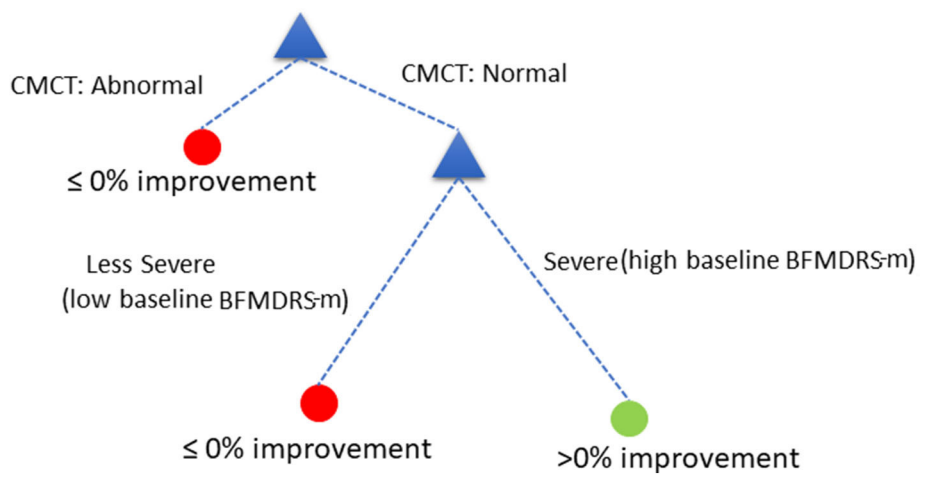


FIGURE 5 | Decision tree based on cut-off of ≥0% improvement in BFMDRS-m, that uses CMCT and baseline severity.

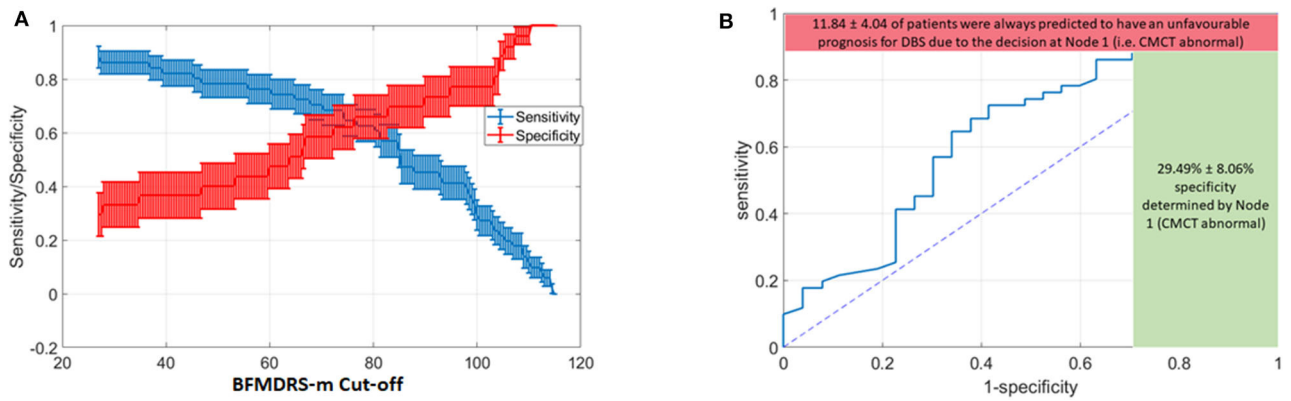


FIGURE 6 | Variation in Decision tree performance with different cut-offs for baseline severity and ROC curve for baseline severity cut-off at point of transection of specificity and sensitivity curves **(A)** Sensitivity and Specificity for different thresholds on BFMDRS-m at Node 2, after applying the decision tree based on CMCT and BFMDRS-m on patients using Monte Carlo simulation. **(B)** Corresponding ROC curve which also shows the upper bound on sensitivity (88.16%), and lower bound on specificity (29.49%) determined by the decision at Node 1 (i.e., CMCT abnormal).

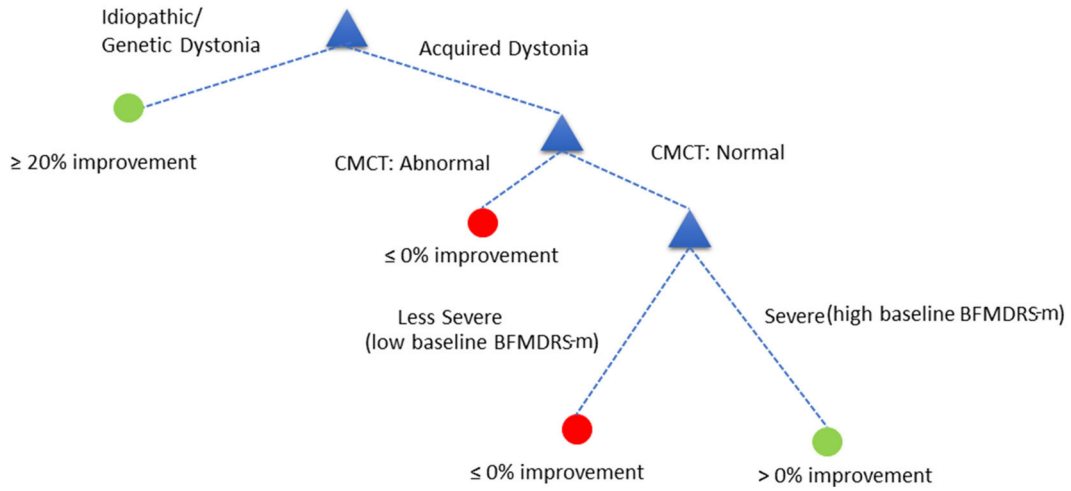
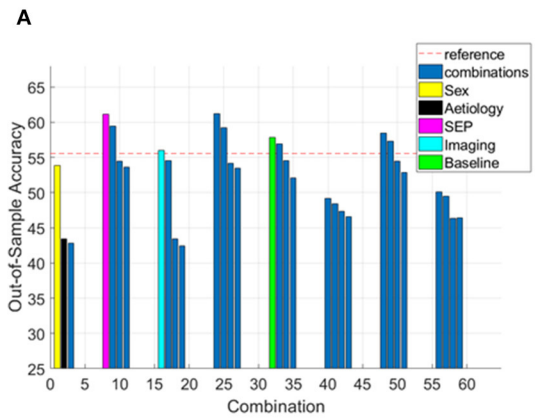


FIGURE 7 | Overall decision tree to determine if DBS will have favorable prognosis.

shows the resulting sensitivity and specificity plotted with error bars corresponding to one standard error and **Figure 6B** shows the associated ROC curve. From the figure, it is obvious that there is a trade-off between sensitivity and specificity. Some working examples taking specific baseline severity cut-offs are as follows:

(a) If one sets the cut-off baseline severity BMFDRS-m score at 80, then patients with a baseline score of >80 would be predicted by the DT to have favorable prognosis from DBS while those with baseline score of <80 would be predicted to have less favorable prognosis. The **sensitivity** at this cut-off is 0.62, which means that 62% of those with a positive outcome would be correctly identified by this DT, while 38% of those who could have had a positive outcome with DBS would be predicted wrongly to have a poor outcome. The **specificity** at this cut-off is 0.64 which means that 64% of those with a poor outcome would be correctly identified by the DT as having a poor prognosis (true negatives), whereas 36% of children predicted to have a good outcome by the DT would actually have a poor outcome (false positives).

(b) If one sets the cut-off baseline severity BMFDRS-m score at 100 then patients with a baseline score of >100 would be predicted by the DT to have favorable prognosis from DBS while those with baseline score of <100 would be predicted to have less favorable prognosis. The specificity at this cut-off is 0.78, which means that 78% of those predicted to have a positive outcome would do so and there would be fewer (22%) false positives. However, the sensitivity at this cut-off is 0.28, which means that only 28% of those with a positive outcome would be correctly identified by this DT and 72% of those who could have benefitted would be predicted wrongly to have a poor prognosis.



B

Index	Baseline BFMDRS-m	Imaging	SEP	Etiology	Sex	Mean Accuracy
8	0	0	1	0	0	61.24
24	0	1	1	0	0	61.15
9	0	0	1	0	1	59.49

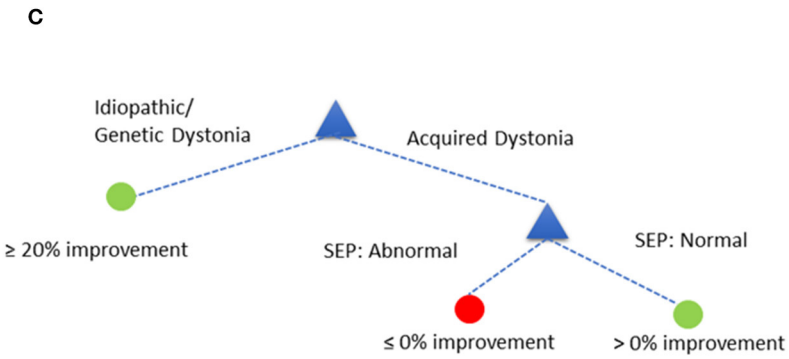


FIGURE 8 | Decision tree performance and winning tree with cut-off ≥0% improvement in BFMDRS-m considering all possible combinations except CMCT. **(A)** Out-of-sample accuracy of decision trees investigating all possible combinations (31) for patients with acquired dystonia, reference accuracy (55.56%) corresponding to the percentage of majority class (based on the 54 patients with SEP data), and decision trees based on single feature highlighted. **(B)** The top three trees, based on accuracy, with the index number associated with those in **(A)**. Each possible combination in the table is binary-coded (1 indicating inclusion of the feature, and 0 indicating the feature was not included in that combination) and the associated accuracy is reported. **(C)** Overall decision tree found for cases with acquired dystonia when CMCT is presumed unavailable.

(c) If one sets the cut-off baseline severity BMFDRS-m score at 60 then patients with a baseline score of >60 would be predicted by the DT to have favorable prognosis from DBS while those with baseline score of <60 would be predicted to have less favorable prognosis. The sensitivity at this cut-off is around 0.75, which means that 75% of those with a positive outcome would be correctly identified by this DT, with fewer (25%) false negatives (those who *could* have had a positive outcome with DBS but were predicted wrongly to have a poor outcome). The trade-off is that the specificity at this cut-off is around 0.45, which means that only 45% of those predicted to have a positive outcome would do so and there would be more (55%) false positives.

Increasing the cut-off of BFMDRS-m makes it less likely that a patient will get a positive prognosis leading to increased specificity at the cost of decreased sensitivity. Thus, if the patient, family and clinician together feel that they would rather not miss the possibility of a positive surgical outcome, they could opt for a BFMDRS-m cut-off to the left, and the corresponding sensitivity and specificity of the outcome prediction considered. Conversely, if the patient, family and clinician together take a more risk adverse view and want to be more certain of the predicted outcome then the BFMDRS-m cut-off might be taken to be to the right, and the corresponding sensitivity and specificity considered in coming to a final decision.

In summary, the range of sensitivity/specificity in the figure will vary according to threshold selected for BFMDRS-m at the second node of the decision tree. However, the decision at node 1 (whether CMCT is normal or abnormal) results in the red and green bounds shown in **Figure 6B**. On average, there were 11.84% of patients who, despite favorable DBS outcome, had an abnormal CMCT. If this decision tree was to be used for clinical decision making, then 11.84% of cases who could have benefited from DBS would instead be deemed to have a poor DBS prognosis. As sensitivity captures the proportion of patients with favorable DBS prognosis who are correctly identified, missing these 11.84% cases results in the upper bound on sensitivity shown in red. However, at the same time, 29.49% of patients with unfavorable DBS outcomes are correctly identified at node 1. As specificity captures the accuracy of identifying the proportion of people with unfavorable DBS outcomes, there were

29.49% of such patients with an abnormal CMCT (shown by the green bound on specificity) and hence would be correctly deemed to have an unfavorable DBS prognosis (based on these criteria).

Figure 7 proposes the overall decision tree that combines the analysis of the whole population (when using a threshold of ≥20% change in BFMDRS-m as improvement) and the analysis carried out on cases with acquired dystonia (when using a threshold >0% change in BFMDRS-m as improvement).

Since TMS is not available in all centers and, as we report above, is sometimes not performed for clinical reasons, or cannot be completed, we also looked at the data from the viewpoint of CMCT not being done. There were 54 patients with acquired dystonia who had SEP performed. We analyzed this cohort of 54 patients using a similar approach as before, testing all possible combinations (but without CMCT this time). **Figure 8A** shows the overall accuracy after investigating all 31 possible combinations of clinical variables (sex, etiology, baseline severity, cranial MRI, and SEP). Compared with **Figure 4**, fewer DTs are seen to exceed the majority class classifier. However, several trees did lead to improved accuracy and the top 3 best performing combinations are listed in **Figure 8B**. These results suggest that SEP is an important factor for predicting DBS prognosis for a child with acquired dystonia in cases where CMCT is not present. However, no other feature (cranial MRI, sex, etiology, and baseline severity) provides any further predictive value in this analysis. **Figure 8C** shows the resulting decision tree in cases where CMCT is not available.

Furthermore, given that inter-rater reliability for scoring BFMDRS-m is not 100%, we also investigated the impact of choosing different thresholds of the BFMDRS-m to define positive and negative surgical outcomes in acquired dystonia. **Table 2** summarizes the number of cases with positive and negative outcomes, and features that appeared in the best performing models (i.e., those that appeared in >5 models out of the top 10 models). While the results vary due to the limited number of cases, CMCT appears as one of the best features for all possible thresholds of BFMDRS-m that were investigated.

Lastly, given the limitations of using BFMDRS-m in a pediatric population (8), especially in acquired dystonia and dyskinetic cerebral palsy (13, 27, 28), we also investigated the use of COPM to separate positive DBS outcomes from negative outcomes. The number of patients who had COPM data was less than in the original group of patients with post-operative results (96 vs. 133). A change in COPM score of ≥2 is considered clinically significant (13), so in our preliminary analysis, we used this threshold to separate positive from negative DBS outcomes. However, using these data, we were not able to find any decision tree that performed better than the majority class classifier.

TABLE 2 | Variation of number of positive/negative cases in acquired dystonia, and the top features (those that appear in >5 top 10 models) as the threshold on BFMDRS-m to define improvement/no improvement is varied.

Threshold on % change in BFMDRS-m	Improved (%)	Not Improved (%)	Best features (i.e., appeared >5 models out of the top 10 models)
0	58 (60.4)	38 (39.6)	CMCT, Baseline Severity
1	58 (60.4)	38 (39.6)	CMCT, Baseline Severity
2	52 (54.2)	44 (45.8)	CMCT, Etiology
3	48 (50.0)	48 (50.0)	CMCT
4	45 (46.9)	51 (53.1)	CMCT, Baseline Severity
5	41 (42.7)	55 (57.3)	CMCT, Sex
>5	Performance degradation (not enough models that perform better than majority class classifier)		

DISCUSSION

We investigated the prognostic value of six key pre-surgical clinical features (sex, etiology, baseline severity, cranial MRI, CMCT, and SEP) in a cohort of 133 children progressing to DBS for dystonia. Concurring with previous reports, the clearest distinction in outcome was between those children with genetic or idiopathic dystonias, compared with acquired dystonias. Focusing on the 96 children with acquired dystonia, we found that CMCT is the feature with greatest value in predicting improvement after surgery, and where this test is not available or technically unsatisfactory, then SEPs offer an alternative source of prognostic information. Based on these findings we suggest a data-driven, clinically useful tool as an illustration of how ML techniques could support the decision whether or not to proceed to DBS in young people with dystonia (**Figures 7**, **8**). The decision support tool is the product of decision tree analysis, and the sensitivity and specificity of the predictions underpinning the tool are specified. In addition, we suggest a novel method whereby decisions can accommodate case-specific preferences. Such ML-based decision support tools should be revised as necessary as more data become available, and we emphasize that our primary goal here is to use the present cohort to illustrate how ML could support clinical decision making, rather than producing a definitive final decision tree.

Standard Feature-Outcome Associations

Standard feature-outcome associations in our cohort (reported in details in **Supplementary Material**) confirmed and extended previously published data showing that patients with abnormal CMCT and/or abnormal SEP show less reduction in dystonia (measured using BFMDRS-m) with pallidal DBS than those without such abnormalities (6). These conclusions are derived from contrasts of the outcome between groups with and without a given feature, or from correlations between features and outcome. However, feature-outcome associations do not provide a measure of the sensitivity and specificity of any derived prediction, nor, in their simplest form, do they lend themselves to the consideration of combinations of features.

Decision Tree Analysis

We were particularly interested in using this cohort to explore how decision trees can provide clinically useful decision support tools able to inform surgical decisions while at the same time

providing estimates of the sensitivity and specificity of the underlying outcome predictions. In effect, these estimates allow the clinician to weigh the significance of each step in the decision tree. The first clear "decision point" in our decision tree analysis is whether the patient has an idiopathic or genetic dystonia vs. an acquired dystonia, as DBS for the former categories is likely to have a favorable outcome. This decision point is consistent with both the literature (see Introduction) and our own feature-outcome association analyses. The second "decision point" suggests that the CMCTs and baseline BFMDRS-m can be used in combination to help decide on whether DBS may have a favorable outcome in patients with acquired dystonia. In this analysis, a normal CMCT and more severe BFMDRS-m suggest that DBS is, on balance, likely to have a positive outcome in acquired dystonia.

However, CMCT is not always available. TMS is not performed in some centers and, even when available it is sometimes contra-indicated, for example in the presence of a cochlear implant. Furthermore, despite best efforts, it may be attempted but without successful data acquisition as a small number of patients do not tolerate the stimulus, especially children with high thresholds. Our ML analyses indicate that where CMCT is not available, SEP alone is still helpful in improving accuracy of outcome prediction, and this forms the basis for an alternative decision tree where SEP status provides the basis for the second decision point. The feature-outcome association analyses previously performed by McClelland et al. (6) and extended here were also able to identify CMCT and SEPs as having predictive value, helping to again validate the DT approach. However, they were not able to assess the predictive value of combined features.

The specific etiology of acquired cases, baseline severity (alone), the results of cranial MRI, and gender were of no additional value at this stage, in deciding whether surgery might be worthwhile, and did not improve upon the majority class classifier when considered in isolation (Note this does not diminish the role of imaging. Cranial MRI already plays an important role in the classification of dystonia as acquired or not, by demonstrating whether structural or degenerative changes are present). BFMDRS-m severity only improved the accuracy with which outcome could be predicted in acquired dystonia when considered in combination with CMCTs, but none of the other non-electrophysiological attributes afforded additional predictive value in combination. Baseline severity did not improve the accuracy of prediction in the smaller analysis performed to reflect the situation where CMCT was not available (**Figure 8**). The reason for this is not clear, but could be a reflection of the smaller numbers in this analysis.

Previous literature reporting the association between disease severity and DBS outcomes shows conflicting results (7, 31, 32). Moro et al. (31) in a meta-analysis of 523 isolated inherited or idiopathic cases undergoing DBS surgery reported a multivariate meta-regression of absolute BFMDRS-m scores indicating that higher BFMDRS motor and disability scores before surgery, together with younger age at time of surgery, were the main factors associated with significantly better DBS outcomes at the latest follow-up. In contrast, the study by Badhiwala et al. (7) reported that in 125 patients with acquired dystonia (derived from a systematic review and individual patient data meta-analysis), a higher disease severity was associated with poor DBS outcomes. It is important to highlight the key differences between our study and the work by Badhiwala et al. (7) that may explain this difference. Firstly, their work derived latent variables (each such variable is a combination of several features with different weights, estimated in a data-driven manner) and did not assess the contribution of individual features. Secondly, it is possible that the difference in proportion of children with neurodegenerative conditions between our study (<10%) and their study (∼40%) may explain this discrepancy (patients with neurodegenerative conditions often have higher baseline severity scores, but will inevitably worsen with time despite an initial response to DBS). Lastly, the latent variable that Badhiwala et al. (7) found to suggest that higher baseline severity leads to poorer DBS outcomes was not only dependent on baseline severity, but several other features (such as age at onset, age at surgery, duration and proportion of life with dystonia). In our case, however, the DT we derived suggests that only in patients with normal CMCT are children with low baseline severity likely to have less favorable DBS outcomes.

Some previous reports (7, 8, 31) have looked at the potential role of age at onset of dystonia or proportion of life lived with dystonia as potential predictive factors for DBS outcome. Age at onset and proportion of life lived with dystonia are inextricably linked with etiology (e.g., those with dystonic cerebral palsy, the largest sub-group of acquired dystonia, have onset in the perinatal period and have therefore spent virtually the whole of their lives with dystonia). These parameters were therefore not chosen for the present analysis because of this potential confound, especially since our work focused on patients with childhood-acquired dystonia.

Unlike the basic feature-outcome associations, the DT analysis requires a threshold to be set for outcome, above which the DT considers a good outcome/favorable prognosis and below which the DT considers a poor outcome/less favorable prognosis. Choosing a threshold is not straightforward, particularly using the BFMDRS-m, with its inherent limitations in this population. A level of 20% improvement in BFMDRS-m has been used in several previous studies, but clinically important changes are still observed in many young people with acquired dystonia, even where this level is not achieved (13, 27, 28, 30). Day to day fluctuations in performance on this scale within a given individual are noted and inter-rater reliability is a further important consideration. Within our own service, inter-rater reliability is ∼6–10% (Gimeno unpublished observations). A cut-off point will therefore always, in reality, reflect a range of values and this caveat should be kept in mind when interpreting the findings. The threshold of 0 chosen here has the benefit of showing negative changes (worsening of BFMDRS-m) as a poor outcome and positive changes (any improvement in BFMDRS-m) as a good outcome, which is visually easy to conceptualize. Adjusting the cut-off threshold between 0 and 5% in the current analysis produced comparable results in terms of which factors improved the accuracy above the majority class classifier. Above this level there were insufficient cases

classified as having a positive outcome for the ML algorithm to work effectively.

An alternative outcome measure, the COPM, was also assessed in the current study. Patients with abnormal SEPs show a trend towards less benefit in terms of functional goal achievement (COPM) compared with those with normal SEPs (see **Supplementary Material**). Applying the current DT methods using a threshold of $\geq$2 point improvement in COPM score did not identify any parameter that performed better than the majority class classifier. The reasons for this are uncertain but could include the smaller number of cases for whom COPM data was available, or the higher proportion of cases that improve with this threshold i.e., there may be insufficient numbers of cases in the "no improvement" group for the ML algorithm to work effectively. The findings could also reflect the nature of the goal-setting process, which takes into account all the clinical assessments in setting realistic and achievable goals. Further objective, blind-rated outcome measures are therefore needed for future work.

Another consideration when assessing outcomes is the potential impact of GPi DBS on non-motor functions. We have evaluated cognitive abilities before and after DBS across the etiological spectrum in childhood dystonia and found overall no adverse consequences of GPi DBS in genetic (33) or acquired dystonias (including dystonic cerebral palsy) (34) or neurodegeneration with brain iron accumulation (NBIA) (35). Recent work also demonstrates the benefit of DBS in reducing pain in children with dystonia and dystonic cerebral palsy (36). Further work is currently ongoing to assess non-motor functioning pre- and post-DBS in more detail.

Study Strengths

Previous work has shown a significant correlation between the outcome of neurophysiological tests, CMCT and SEP, and change in BFMDRS-m (and for SEP, the change in COPM scores) following DBS (6). However, it was not obvious from that study how much value there might be in basing a decision about DBS surgery on CMCT and SEP results (37). This analysis extends the previous work by using Machine Learning techniques to incorporate these features into a clinical decision support tool, where the recommendations can be assessed through the sensitivity and specificity of the predictions underscoring each step. In addition, a larger sample of patients was available for the current analysis. The study shows how the DT methodology can be used to assess combinations of features (e.g., baseline severity, CMCT, etc.) and convert them into a practical tool that can provide a guide to prognosis for DBS. As these results are based on existing data they therefore represent an evidence-based approach to clinical decision making. This work also quantifies the extent of error to be expected if the proposed decision tree is used, in terms of sensitivity and specificity. It therefore identifies which features should be included in clinical decision making and provides a methodological framework to systematically explore contributions from multiple features. An evidence-based DT and sensitivity/specificity curve might also potentially assist clinical teams when counseling patients and families about expected benefit from DBS. It can therefore help with managing expectations and makes steps toward providing a personalized prognosis, sensitivity and specificity. Indeed, we demonstrate how case-specific preferences, such as the strong wish not to miss the possibility of a positive outcome, can be accommodated within this framework. Lastly, the algorithm developed is highly interpretable and accessible to health care professionals.

Study Limitations

Firstly, our decision trees are based on numbers of patients that are relatively small for typical machine learning applications that have previously been shown to work well in healthcare with large datasets (38). Nevertheless, the DT method was able to pick out the features that were most predictive of outcome and produce a tree that makes clinical sense, and which is concordant with other analyses. Secondly, the data are all from a single center and we were only able to do an internal validation with k-fold cross validation technique. It is thus possible that the results presented in this work may be subject to overfitting and may not generalize well. Third, this study was a retrospective study and the clinical decision to proceed or not to DBS may have already been influenced by theoretical assumptions relating to neurophysiological results (6) leading to a possible circularity. A further factor is that the analysis here is based on 1-year outcome data. Improvements following DBS continue beyond 1 year and even up to 5 years, so future work is needed to assess whether the DTs developed here will still help in predicting these later improvements. Lastly, the outcomes in this study were based primarily on changes in the BFMDRS-m. This scale has significant limitations for childhood-onset dystonia and acquired dystonia and is not sensitive to some changes which are still meaningful for young people and their families (13, 27). We are working towards obtaining data with other objective outcome scales, but numbers are not yet sufficient to apply a ML method to these data. Preliminary data (Gimeno and McClelland, unpublished observations) indicate that patients with abnormal neurophysiological tests show less benefit from DBS as measured using other such scales, but this requires confirmation by further analysis with larger subject numbers.

CONCLUSION

This study is the first exploration of how a ML-based approach could be used to predict potential benefit from DBS in children with dystonia and to aid clinical decision making and counseling of families about expected outcome. Although ML methods generally excel in very large datasets, the Decision Tree methodology provides a data-driven, highly interpretable, example decision support tool even in our modestly sized cohort. The key finding is that neurophysiological parameters can help to predict the outcome of DBS. We encourage other centers to introduce neurophysiological measures to their assessment pathways and to collect data to facilitate future multi-center evaluation of these potential predictive markers and the testing of the illustrative decision support tools presented here. Future work will also consider additional outcome measures and thereby broaden DT-based decision support tools.

DATA AVAILABILITY STATEMENT

The code of our analysis is available at https://github.com/syedahmar/ChildrenDystoniaDBS/tree/master. The data analyzed in this study is subject to the following licenses/restrictions: De-identified data will be made available to interested researchers upon scientific protocol evaluation committee approval and subject to study governance structure and patient consent form, following a time restriction. Requests for data access can be submitted to Verity M. McClelland. Note the data management plan approved by the funders states the following: The research team will have exclusive use of the data for the project duration and 3 years afterwards to allow for publications to be achieved. After this period, the majority of data would be made available for data sharing via the King's College London data repository. Requests to access these datasets should be directed to verity.mcclelland@kcl.ac.uk.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by London-Harrow National Research Ethics Committee, London, UK (17/LO/0439). 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

PB with VM conceived the initial idea. SS led and undertook the development of the machine learning methodology and data analysis. VM curated and analyzed the neurophysiological data. SS wrote the initial draft of the paper and all co-authors contributed to and approved the final version of the manuscript. J-PL created and leads the Evelina London Children's Hospital Complex Motor Disorders Service for deep brain stimulation neuromodulation, initiated measuring the Central Motor Conduction Time (CMCT) and Somatosensory Evoked Potentials (SEP) as an extension to the clinical phenotype of children with dystonia and other movement disorders.

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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.2020.00825/full#supplementary-material

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METHODS
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Brain Age Prediction of Children Using Routine Brain MR Images via Deep Learning

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Predicting brain age of children accurately and quantitatively can give help in brain development analysis and brain disease diagnosis. Traditional methods to estimate brain age based on 3D magnetic resonance (MR), T1 weighted imaging (T1WI), and diffusion tensor imaging (DTI) need complex preprocessing and extra scanning time, decreasing clinical practice, especially in children. This research aims at proposing an end-to-end AI system based on deep learning to predict the brain age based on routine brain MR imaging. We spent over 5 years enrolling 220 stacked 2D routine clinical brain MR T1-weighted images of healthy children aged 0 to 5 years old and randomly divided those images into training data including 176 subjects and test data including 44 subjects. Data augmentation technology, which includes scaling, image rotation, translation, and gamma correction, was employed to extend the training data. A 10-layer 3D convolutional neural network (CNN) was designed for predicting the brain age of children and it achieved reliable and accurate results on test data with a mean absolute deviation (MAE) of 67.6 days, a root mean squared error (RMSE) of 96.1 days, a mean relative error (MRE) of 8.2%, a correlation coefficient (R) of 0.985, and a coefficient of determination (R^2) of 0.971. Specially, the performance on predicting the age of children under 2 years old with a MAE of 28.9 days, a RMSE of 37.0 days, a MRE of 7.8%, a R of 0.983, and a R^2 of 0.967 is much better than that over 2 with a MAE of 110.0 days, a RMSE of 133.5 days, a MRE of 8.2%, a R of 0.883, and a R^2 of 0.780.

Keywords: magnetic resonance imaging, deep learning, brain age, convolutional neural network, artificial intelligence

INTRODUCTION

The brain development of children undergoes a rapid and complex process, especially in the first 2 years after birth (1, 2). The early brain development follows the law of myelination from caudal to rostral, posterior to anterior regions, central to peripheral locations, which is closely related to the development of sensory, motor, and cognitive ability (3). Delayed brain development can lead to

intellectual disability, language disorder, activity limitation, and other manifestations in children, which seriously affect their quality of life. Therefore, accurate and quantitative evaluation of brain development, early identification, and intervention treatment is particularly important for children with brain development analysis and brain disease diagnosis.

At present, brain magnetic resonance (MR) imaging is a reliable method to evaluate brain development (brain age) due to its non-invasive, high soft tissue resolution and multi-parameter imaging advantages. Recently, the main ways of MR image to evaluate brain development are as follows: morphometry [including measurement of brain volume (4–6), cortical thickness (7), surface area (7, 8), etc.], white matter diffusion (9, 10), functional connectivity (11–14). However, there are some drawbacks within these studies: the need of some special sequences with long scanning time, complex data post-processing, and group-level comparison results without quantitative analysis to individuals, which limit their wide use in clinical situations.

With the development of deep learning, more and more sophisticated deep neural networks have been proposed to analysis massive image, voice, or video data. Of these, convolutional neural network (CNN) of deep learning has achieved great success with superior performance beyond human experts in many computer vision and speech recognition tasks since it was put forward (15–20). In the field of medical image analysis, CNN-based method has been also proposed for disease diagnosis and lesion detection with high performance in accuracy, such as the classification and detection of lung nodules (21, 22), the recognition of melanoma (23), the detection of cerebral microbleeds (24–26), as well as the classification of Alzheimer's disease (27, 28). In addition, brain age prediction based CNN model has been proved to be a reliable and heritale biomarker of brain aging and can be used to indicate the risk of brain degenerative diseases (29, 30), whereas it has not been reported in young children up to now. Furthermore, unlike traditional machine learning approaches that implement feature extraction, feature reduction, and classification separately, CNN combines them as an end-to-end system, from raw images to the corresponding target values, avoiding complicated image preprocessing and manual design of appropriate features. The excellent performance and transferability lead us to believe that CNN-based method should be the most promising resolution for most clinical applications, including brain age prediction of children.

In this paper, we collected 220 routine brain MR images of healthy children for investigating the brain age of children based on deep learning. Data augmentation was utilized to extend the training data for avoiding the potential over-fitting and enhancing generalizability of the model. With delicate design of structure and careful setting of hyper parameters, we proposed a 3D deep neural network and achieved high performance. We analyzed the prediction results of different age groups in detail and compared them with those of other two state-of-the-art methods. The factors in the proposed model that may affect the prediction results were investigated comprehensively. Furthermore, we compared the proposed 3D CNN with the corresponding 2D CNN that has a similar structure in predicting brain age of children with 3D MR image data.

METHODS

Dataset Acquisition

Ethical approval for the research was obtained from the ethics committee of Children's Hospital of Nanjing Medical University. This is a retrospective study, and informed written consent was thus waived. The dataset consists of T1-weighted images of 220 healthy children aged 0 to 5 years old. The data were all acquired using a 1.5T Siemens Avanto Scanner, but scanning parameters of newborns ($\leq$1 month) are different from older children due to variation in water content of brain tissue. Scans of newborns were imaged using a T1-weighted spin-echo sequence (repetition time [TR] = 4,490 ms, echo time [TE] = 7.5 ms, flip angle [FA] = 150°, 18 slices, slice thickness = 4.5 mm, FOV = 180 × 180 mm, voxel dimensions = 1.0 × 0.7 × 4.5 mm). Scans of older children (>1 month) were also imaged using the T1-weighted spin-echo sequence (TR = 3,850 ms, TE = 7.3 ms, FA = 150°, 22 slices, slice thickness = 5.0 mm, FOV = 220 × 220 mm, voxel dimensions = 1.4 × 1.0 × 5.0 mm). Those whose brain MR image quality was good enough to diagnose and reports were diagnosed as normal by two experienced radiologists, and whose history, clinical data, and phone call following-up can't show the existence of neurological disease were enrolled into our dataset. Premature infants, subjects who were diagnosed with congenital diseases (congenital heart diseases, Down's syndrome, etc.), neurodevelopmental or mental disorders (neurodevelopmental delay, autism, etc.), and other serious illnesses (hypoxic-ischemic encephalopathy, cerebral hemorrhage, septicopyemia, etc.) affecting brains were excluded from our dataset. Furthermore, we used downsampling method to convert the stacked 2D brain MR images of newborns and older children to the same size of 128 × 116 × 12.

Data Augmentation Technology

Basically, data augmentation methods are extensively used to train a deep neural network having huge parameters for improving prediction accuracy that had been validated in the "Results" section. In our experiments, four commonly used methods of data augmentation were employed to enhance the training dataset. They are listed as: (a) scaling, (b) image rotation, (c) translation, and (d) gamma correction. We scaled images with scaling factor of 0.85 to 1.15 with step of 0.03 for generating 10 new images. Image rotation was used to generate 10 new images with rotation angle of −15 to 15 degrees increased by 3 degrees. We translated images with factor of −0.1 to 0.1 with step of 0.02 diagonally for generating 10 new images. Gamma correction with gamma value of 0.85 to 1.15 increased by 0.03 was employed to generate 10 new images. At last, we augmented the training dataset by 41 times using data augmentation methods.

Noted: For one routine brain MR image with size of 128 × 116 × 12 in this paper, we split the volume into 12 slices, then used the same transformation method to process every slice, and finally stacked those slices into a 3D image (see **Figure 1**). We achieved the transformation of the whole 3D image by this way.

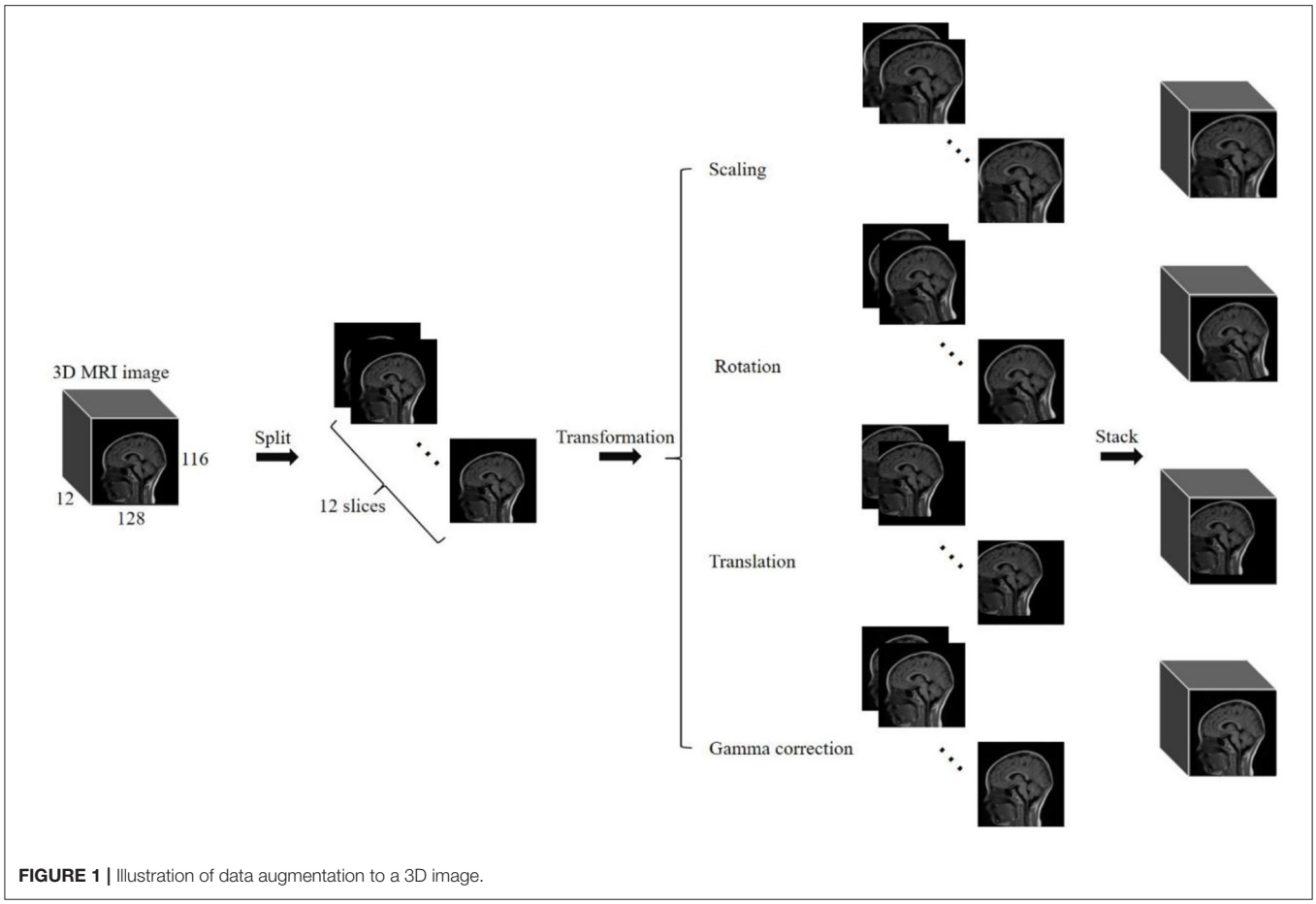

FIGURE 1 | Illustration of data augmentation to a 3D image.

Proposed 3D CNN Architecture

A 3D CNN was proposed to predict the brain age of children using brain MR images with size of 128 × 116 × 12. The 3D image was input to the model and then a single scalar denoting the brain age was output. The proposed 3D CNN model, shown in **Figure 2**, contains 7 3D convolution layers, 4 3D max pooling layers, and 3 fully connected layers. All convolution layers are followed by 3D batch-normalization (31) and ReLU activation function (32), while the first two fully connected layers are followed by ReLU activation function. All convolution layers have the same kernel size of 3 × 3 × 3, stride size of 1 × 1 × 1, and padding size of 1 × 1 × 1, which means the feature map size is the same as that of the input. The kernel size in the first, second, third, and fourth max pooling layer are 4 × 4 × 1, 3 × 3 × 1, 3 × 3 × 3, and 3 × 3 × 3, respectively, and the stride size is equal to the kernel size in all max pooling layers.

The mean absolute error (MSE) was used as the loss function. The reliable and commonly used stochastic gradient descent with momentum of 0.9 (SGDM) was employed as the optimization method. The mini-batch and epoch were set to 64 and 40, respectively. The initial learning rate was set to 0.0000008 and decreased by 10% every epoch. The weights were initialized randomly.

Note: 10 runs were implemented for accounting for the stochastic properties of CNN, and the average value of the 10 runs is regarded as the final result.

2D CNN Architecture

We designed a 2D CNN model, shown in **Figure 3**, according to the structure of the proposed 3D CNN, so both have similar hierarchical structures. The 3D image with size of 128 × 116 × 12 was split into 12 slices, and those slices were then input to the 2D CNN model, and finally the age was given. Same as the proposed 3D CNN, all convolution layers in 2D CNN are followed by batch normalization and ReLU, and the first two fully connected layers are followed by ReLU. The kernel size, stride size, and padding size in convolution layers are 3 × 3, 1 × 1, and 1 × 1, respectively. The kernel size is equal to stride size in all max pooling layers, and they are 4 × 4, 3 × 3, 3 × 3, and 3 × 3, respectively, from the first to the last max pooling layer. In terms of hyperparameters, the loss function, optimizer, mini-batch, epoch, learning rate, and weights initialization were set the same as the proposed 3D CNN.

Software Availability and PC Configuration

All data augmentation methods were implemented using imgaug (https://github.com/aleju/imgaug). All experiments of deep learning were carried out on PyTorch (https://pytorch.org/). The running environment of the programs: i9-9900k CPU, NVIDIA GeForce RTX 2080 Ti GPU, and 16.0 GB RAM.

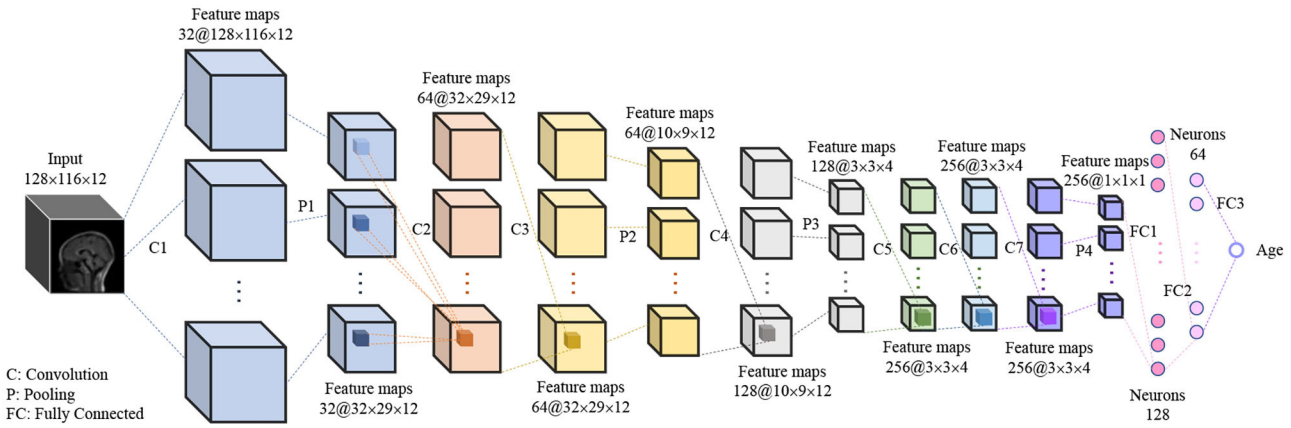

FIGURE 2 | The hierarchical architecture of proposed 3D CNN. The "32" and "128 × 116 × 12" in "32@128 × 116 × 12" denote the number and size of feature maps.

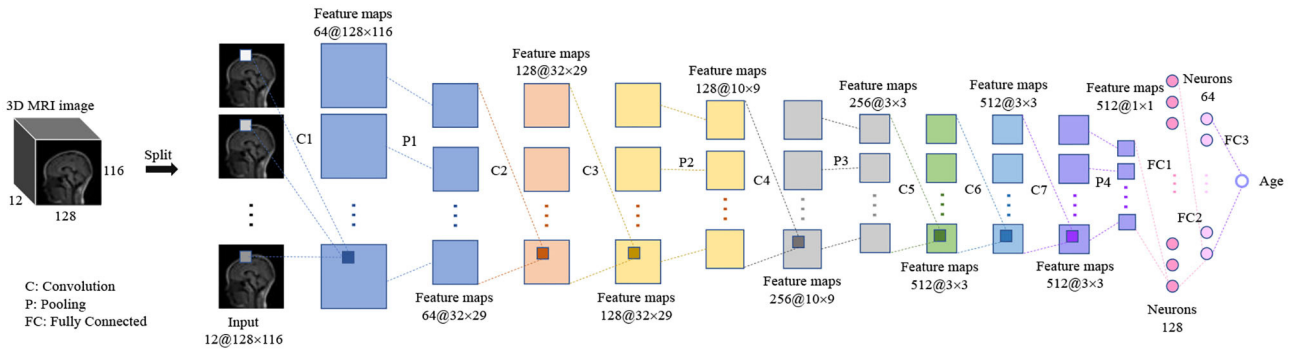

FIGURE 3 | The hierarchical architecture of the 2D CNN.

RESULTS

Dataset Characteristics

To develop an AI system for predicting the brain age of children using routine clinical brain MR images, we enrolled 220 subjects aged 0 to 5 years old (**Figure 4** shows the distribution of participant ages with 100-day intervals) and scanned them to achieve the brain MR images. The hold-out method was employed to divide the 220-image dataset into two parts randomly, and one part containing 176 images (80%) was regarded as training dataset and the other part containing 44 images (20%) as test dataset. The reason for abandoning the validation dataset is that the whole dataset only contains 220 subjects. **Table 1** gives the demographic information of the training and test datasets. Since the amount of the training dataset is slightly small to train a deep neural network, data augmentation was implemented for generating new "fake" images. At last, the training dataset containing 7,216 images and the test dataset containing 44 images were obtained.

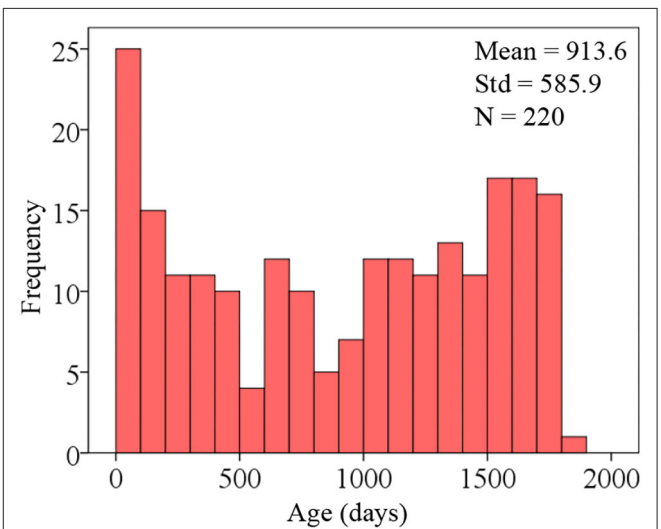

FIGURE 4 | Distribution of participant ages.

Performance of the Proposed Model

With grid search and trial-and-error methods, we optimized a 3D CNN model for predicting the children's age more accurately and reliablly. The detailed information of the proposed model can be found in **Figure 2**. The model was trained by the data-augmented dataset including 7,216 images and evaluated on the test dataset including 44 images. In our experiments, the learnable weights

TABLE 1 | Subjects demographic (Std denotes standard deviation).

	Total dataset		Test dataset		Training dataset	
	0–2 years old	**2–5 years old**	**0–2 years old**	**2–5 years old**	**0–2 years old**	**2–5 years old**
Subjects	88	132	23	21	65	111
Age (days)	4–697	731–1820	36–680	749–1687	4–697	731–1820
Mean ± Std	283.6 ± 215.7	1333.5 ± 314.2	244.2 ± 201.0	1267.0 ± 288.4	297.6 ± 220.5	1346.1 ± 318.4

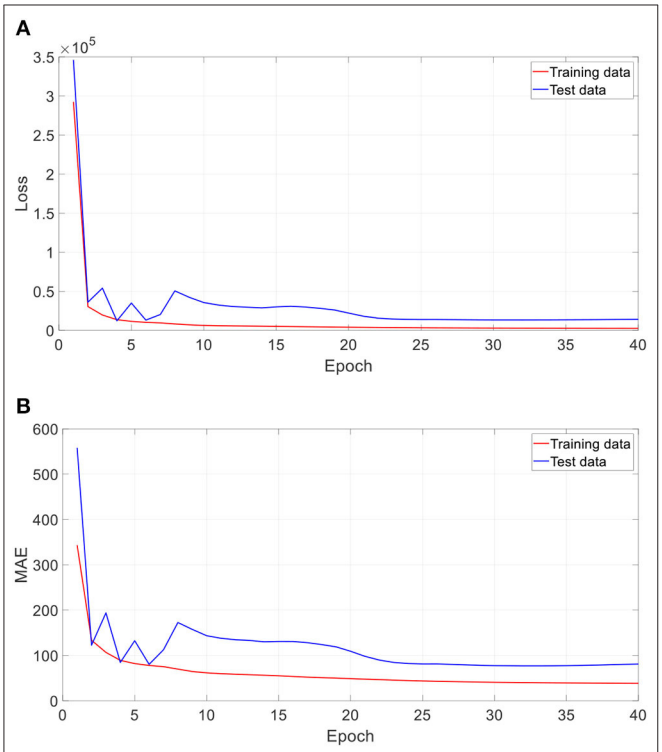

FIGURE 5 | Training performance of one run. **(A)** Loss against training epoch, and **(B)** MAE against training epoch. The loss and MAE are the average of all iterations in one epoch.

of model were initialized randomly, and the random seed was not fixed, causing the randomness of prediction results. Thus, we implemented 10 runs under the same settings of the model for ensuring the reliability of the results.

Figure 5 shows the training performance of one typical run. As **Figure 5** shows, after 40 epochs (iterations through the whole training dataset), both training dataset and test dataset in loss and MAE reached a plateau and were at a minimum, which means that the training process has converged.

Figure 6 shows the average and standard deviation of prediction results of 10 runs under the same setting. It is found that most true data fall within the standard deviation of predicted data, which means that the predictions can fit the true data well. To further quantitatively evaluate the prediction accuracy of the model, MAE, RMSE, MRE, *R*, and R^2 between the average values and the true values were employed (**Table 2**). With a MAE of 67.6 days, a RMSE of 96.1 days, a MRE of 8.2%, a *R* of 0.985, and a R^2 of 0.971, the proposed model was considered to achieve quite high accuracy in predicting the brain age of children aged 0 to 5 years old.

Since the brain development of infants under 2 years old is heterogeneous and particularly rapid, it is necessary to divide the age into two age groups according to 2 years old and evaluate the prediction results of the two groups separately. **Table 2** gives the assessment results. We found that age predictions for children under 2 years old are significantly better than those over two according to all evaluation indicators. We can also observe that most predictions under 2 years old are closer to the true values compared with those over 2 years old in **Figure 6**. Comparing to the 0–2 age group, there is a stronger correlation between predicted and true values in the age group from 0 to 5 years old according to *R*, but there is a bigger MAE. The reason is that the true values in 0–2 age group are smaller as a whole than that in 0–5.

To further assess the reliability of the predicted results, we gave the residual plot (Bland-Altman plot) and performed paired samples *T*-test. The residual plot was employed to show the relationship between mean and difference of the predicted and actual value, which is show in **Figure 7**. The *P*-values of the paired samples *T*-test were 0.5665, 0.9407, and 0.7979 in 0–2, 2–5, and 0–5 age groups, respectively, showing that there are no significant statistical differences between the predicted and the actual values of all age groups. As the **Figure 7A** indicating the 0–2 age group shows, 95.7% (22/23) of the points fall within the 95% limits of agreement, and the mean of difference is 4.6, which is close to 0. Similar to the 0–2 age group, 95.2% (20/21) of the points fall within the 95% limits of agreement, and the mean of difference is −2.3 according to **Figure 7B**. In terms of 0–5 age group, which is shown in **Figure 7C**, 90.9% (40/44) of the points fall within the 95% limits of agreement, and the mean of difference is 3.8 which is quite close to 0.

Impact of Data Augmentation

In this research, the amount of the obtained 220-subject dataset is big enough to draw a conclusion of statistical analysis, but it is still insufficient for training a deep neural network with huge parameters. Many researches have reported that increasing the number of samples in training data can avoid over-fitting, enhance generalizability, and improve the performance on test set (33–37). Therefore, we performed data augmentation on a 176-subject training dataset and extended the dataset to 7,216 in our proposed method.

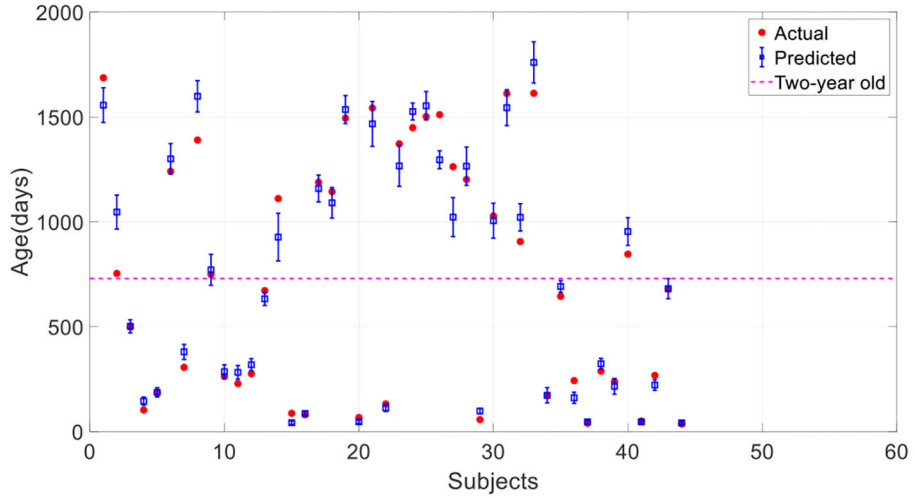

FIGURE 6 | Prediction results of the proposed 3D CNN. The error bar represents the average and standard deviation of the prediction results over 10-run.

TABLE 2 | Performance of the proposed 3D CNN in predicting children aged.

Age group (years old)	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
0–2	28.9	37.0	7.8	0.983	0.967
2–5	110.0	133.5	8.2	0.883	0.780
0–5	67.6	96.1	8.2	0.985	0.971

Here we investigated the impact of data augmentation on predicting children's age using stacked 2D routine clinical brain MR images. **Figure 8** offers the prediction results of our proposed method without data augmentation. All the results are average on 10 runs. Comparing with **Figure 6**, it is found that most of the predicted values deviate from the true values further, and the standard deviation of the predicted values is larger, showing the instability of the model. **Table 3** gives a detailed comparison of our proposed method with and without data augmentation, which further confirms data augmentation can improve the prediction accuracy of the model.

Impact of Network Depth

To test the impact of network depth on performance of predicting children age, different 3D CNN including different convolution layers and fully connected layers were validated. The evaluation results are given in **Table 4**. Ten runs were implemented, and the average values were regarded as the final results. It is found that the proposed 3D CNN structure containing seven convolution layers and three fully connected layers achieved the best performance according to the comprehensive assessment of four indicators. **Figure 9** is utilized to further visualize the performance differences between different 3D CNN.

Impact of Batch Normalization

In the proposed 3D CNN structure, every 3D convolution layer is followed by a 3D batch normalization. We investigated the impact of batch normalization on prediction accuracy in this section. All batch normalization layers were removed, and initial learning rate was set as 0.000000008. All other settings remain the same. **Table 5** gives the comparison result of the proposed approach with and without batch normalization. All the results are averaged on 10 runs. As **Table 5** shows, the 3D CNN without batch normalization achieved a MAE of 132.6 days, a RMSE of 189.9 days, a MRE of 15.0%, a *R* of 0.945, and a R^2 of 0.893, which is obviously worse than the proposed 3D CNN with batch normalization. **Figure 10** gives the training performance of the 3D CNN without batch normalization. As we can see, both training dataset and test dataset in loss and MAE reached the minimum plateau, indicating that the network is fully trained.

Impact of Batch Size and Learning Rate

Except for the structure, hyper parameters also can affect the 3D CNN performance. We compared different prediction results of the proposed 3D CNN trained by different batch size and initial learning rate for understanding the influence of them on the performance. **Table 6** gives the survey results. All results are average on 10 runs. As the **Table 6** shows, the 3D CNN with batch size of 64 and learning rate of 0.0000008 achieved the best prediction results according to all four evaluation indicators.

Comparing With 2D CNN

The input of 2D CNN is a 2D image with three color channels (i.e., RGB) in most natural scenes. With this regard, the simplest way for 2D CNN to deal with 3D input is to replace the color channels with the slices of the volumetric image. We designed a 2D CNN model, shown in **Figure 3**, according to the architecture of the proposed 3D CNN model for predicting the brain age of children using stacked 2D routine clinical brain MR image (gray-level) and investigated the performance differences between the two models. The comparison results are given in **Table 7**.

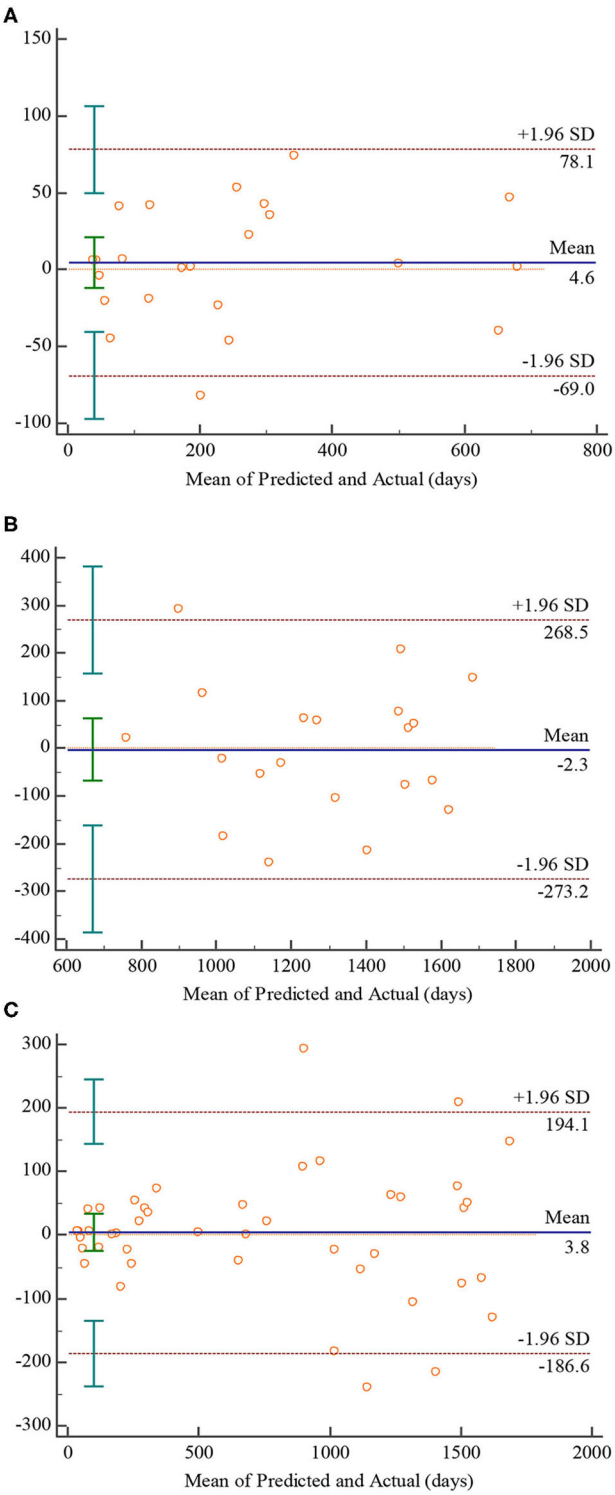

FIGURE 7 | Bland-Altman plots for the proposed 3D CNN. Plot **(A–C)** denote 0–2, 2–5, 0–5 age groups, respectively.

All the results are average on 10 runs. We observed that the proposed 3D CNN achieved better performance in terms of all the evaluation indicators.

DISCUSSION

High Reliability and Accuracy of 3D CNN for Brain Age Prediction

It is important to predict the brain age reliably and accurately for brain development analysis and brain disease diagnosis in pediatric patients. Basically, methods for predicting brain age can be divided into two categories: shallow learning algorithms and deep learning algorithms (38). So far, numerous shallow learning algorithms have been developed, such as gaussian processes regression (GPR) (29, 39, 40), support vector regression (SVR) (41, 42), partial least squares (PLS) regression (43), relevance vector regression (RVR) (44), hidden Markov model (HMM) (45), and Bayesian linear discriminant analysis (46). In terms of deep learning algorithms, CNN (29, 47) and back propagation neural network (BPNN) (48) were proposed to predict the brain age with brain MR images.

As the above references report, for achieving fairly good prediction result, all methods except CNN need to accomplish the complicated preprocessing task well including feature selection, dimension reduction, and segmentation of brain MR image into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) tissues. The manual interventions in preprocessing lead to high intra-observer and inter-observer variability, which easily biased the final interpretation. Comparing to the traditional machine learning methods, CNN-based methods are an end-to-end system that uses the raw MR image data as the input and output the age value without manual interventions, showing higher reliability and improving clinical practice (38).

Although there is no error caused by manual intervention in predicting brain age using CNN-based model, there may be systematic bias (49, 50). As reported in (49), CNN-based model will overestimate the younger and underestimate the older, decreasing the reliability of prediction results. To evaluate the reliability of the predicted results in this paper, the Bland-Altman plots characterizing the relationships between the mean and the difference of the predicted and actual value were given, showing in **Figure 7**. According to **Figures 7A,B**, the mean of difference in the 0–2 age group is slightly higher than 0, while that in the 2–5 age group is slightly lower than 0. This observation seems to indicate that the prediction results in this paper confirm the conclusion of (49). However, the means in age group of 0–2 and 2–5 are quite close to 0, and the paired samples *T*-test results revealed there are no significant statistical differences between the predicted and the actual values on both age groups, which means the predicted results of 0–2 and 2–5 age group are in good agreement with the actual age. Similarly, the predicted results of 0–5 age group are also in good agreement with the actual age according to **Figure 7C**. Therefore, the predicted results achieved by the proposed CNN-based model are considered to be reliable overall.

Furthermore, the CNN-based methods can achieve more accurate prediction results compared with traditional machine learning methods. Cole et al. (29) reported the detailed comparison between 3D CNN and GPR method in predicting the brain age using different input data (GM, WM, GM+WM, and raw data). We found that the 3D CNN achieved higher

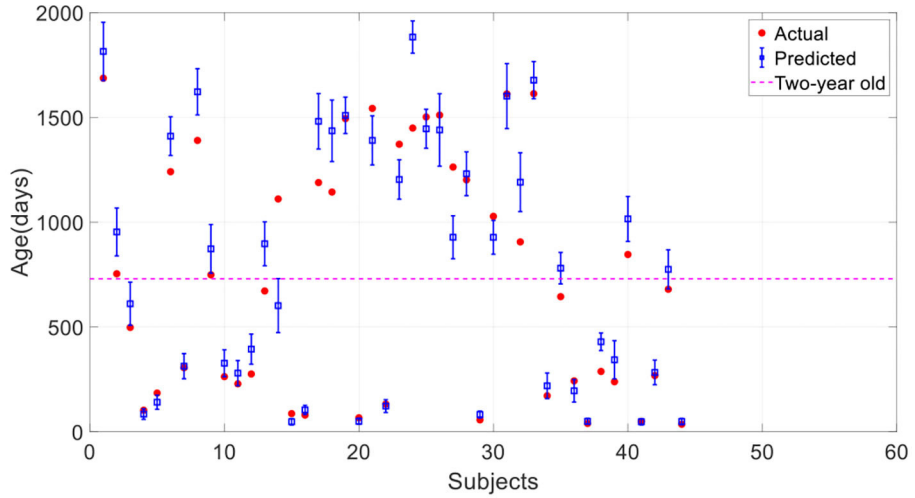

FIGURE 8 | Prediction results of the proposed method without data augmentation. The error bar represents the average and standard deviation of the prediction results over 10-run.

TABLE 3 | Comparison of the proposed method with and without data augmentation.

	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
Without data augmentation	118.3	166.7	13.7	0.963	0.926
With data augmentation	67.6	96.1	8.2	0.985	0.971

TABLE 4 | Performance of different network depths.

No. of convolution layers	No. of fully connected layers	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
6	2	75.9	102.8	9.2	0.983	0.967
6	3	73.8	101.6	9.0	0.984	0.968
6	4	70.1	95.1	8.4	0.985	0.971
7	2	80.4	110.5	10.0	0.981	0.962
7	3	67.6	96.1	8.2	0.985	0.971
7	4	70.3	97.1	8.6	0.985	0.970
8	2	73.4	106.8	9.2	0.982	0.964
8	3	68.7	99.5	8.2	0.984	0.969
8	4	69.5	96.6	8.3	0.985	0.971

performances than the GPR in all kinds of input data in this reference. Especially, the MAE of 4.65 years obtained by 3D CNN are much lower than the MAE of 11.81 years obtained by GPR when the raw data was used as the input.

However, it is not particularly reasonable to compare our results with the above example since the subjects used above are aged 18 to 90 years old. To the best of our knowledge, only two traditional machine learning-based methods for age prediction of young children were investigated currently. Toews et al. (51) firstly developed a feature-based developmental model for predicting infant age using structural brain MR images. They enrolled 92 subjects aged 8–590 days and achieved a MAE of 72 days. Hu et al. (46) proposed a two-stage prediction method named Hierarchical Rough-to-Fine (HRtoF) model for predicting infant age. They enrolled 50 infants aged 14–797 days and achieved a MAE of 32.1 days. Since it is hard to collect the brain images of young children, the data amount reported in (51) and (46) is not large, <100. In our study, we spent over 5 years collecting 220 subjects, which is enough for reaching a convincing conclusion comparing to the above two studies. **Table 8** gives the performance comparison of our proposed method and the above two methods. It is found that the proposed 3D CNN gained the best performance in predicting the brain age of infant aged about 0–2 years old. The prediction accuracy of the 3D CNN for the age of 4–1,820 days is even better than the prediction accuracy of Toews's method (51) for the age of 8–590 days.

In addition to traditional machine learning-based methods, we also compared 3D CNN with 2D CNN in predicting brain age of young children using 3D MR images. The inputted 3D images are stacked 2D brain MR images (slices) and there is a gap between two adjacent slices in the actual location of the brain. Thus, we speculated that the correlation between slices will not be great, and we think that 2D CNN model may also be able to complete the age prediction task well with the inputted 3D images. If the prediction effect of the 2D CNN is the same as that of the 3D CNN, then the 2D CNN will be more recommended in clinical practice, because the 2D model requires much less computation and computer memory. However, as **Table 7** shows, the proposed 3D CNN outperformed the 2D CNN significantly. This result shows that the small correlation between adjacent slices is beneficial to the prediction accuracy of the model, and also shows the 3D CNN employing 3D kernels is a more reliable resolution that can take all full advantage of spatial contextual information of the 3D MR images for more accurate age prediction (16, 29, 52).

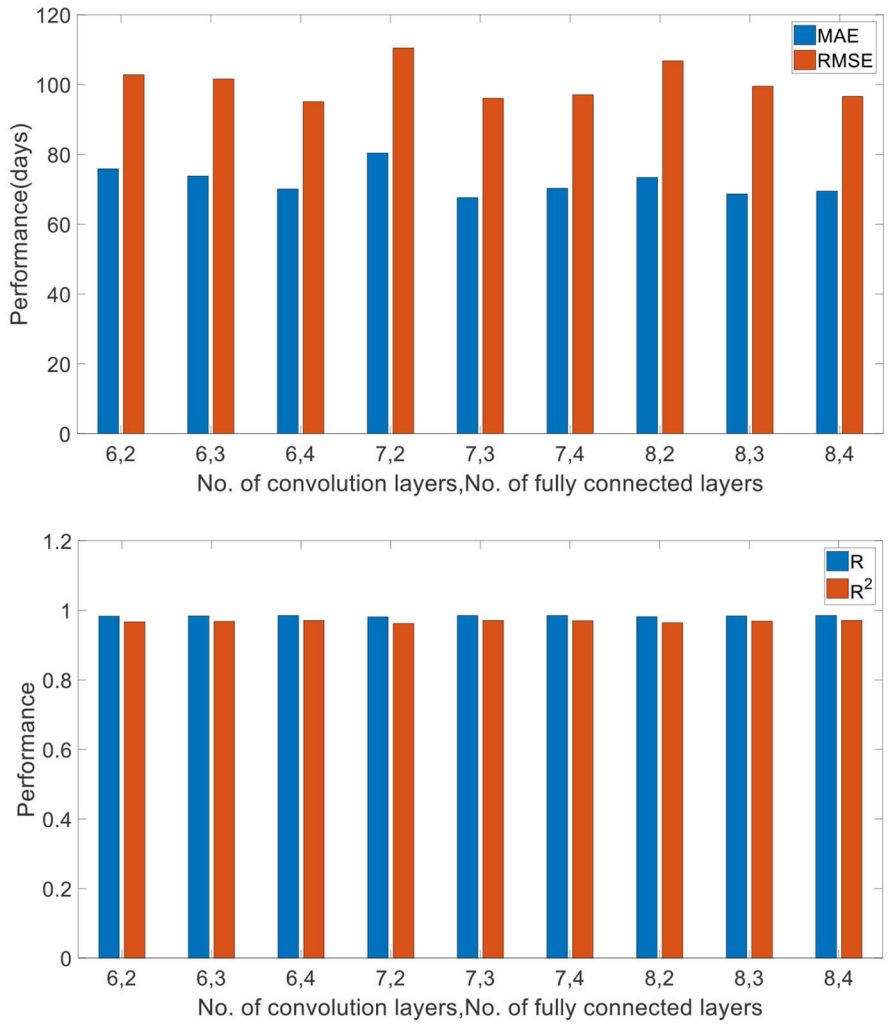

FIGURE 9 | Comparison of different network depths. "6, 2" ("No. of convolution layers, No. of fully connected layers") denotes 6 convolution layers and 2 fully connected layers.

TABLE 5 | Comparison of the proposed method with and without batch normalization.

	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
Without batch normalization	132.6	189.9	15.0	0.945	0.893
With batch normalization	67.6	96.1	8.2	0.985	0.971

Predictions for Children Under 2 Years Old Are Better Than Those Over 2

As **Table 2** shows, the proposed 3D CNN achieved a MAE of 28.9 days, a RMSE of 37.0 days, a MRE of 7.8%, a *R* of 0.983, and a R^2 of 0.967 in predicting brain age of children aged 0–2 years old, while a MAE of 110.0 days, a RMSE of 133.5 days, a MRE of 8.2%, a *R* of 0.883, and a R^2 of 0.780 were obtained in predicting brain age of children aged 2–5 years old. It is found that the predictions for children under two years old are much better than that over two. Actually, this phenomenon is consistent with the understanding of clinical practice—that is, brain development under 2 years old is rapid and heterogeneous, while the brain over 2 years old develops relatively statically (1, 2). Slow development of the brain over 2 years old leads to low distinguishability and high prediction error.

Optimizing Model Parameters Can Improve Prediction Accuracy

Generally, the prediction performance is quite dependent on the structure of CNN and the hyper parameters. Thus, we optimized the 3D CNN structure and the hyper parameters with grid search for achieving the best performance on training set and reported the performance on the test set independently. Recently, some evidence reports that network depth is crucially

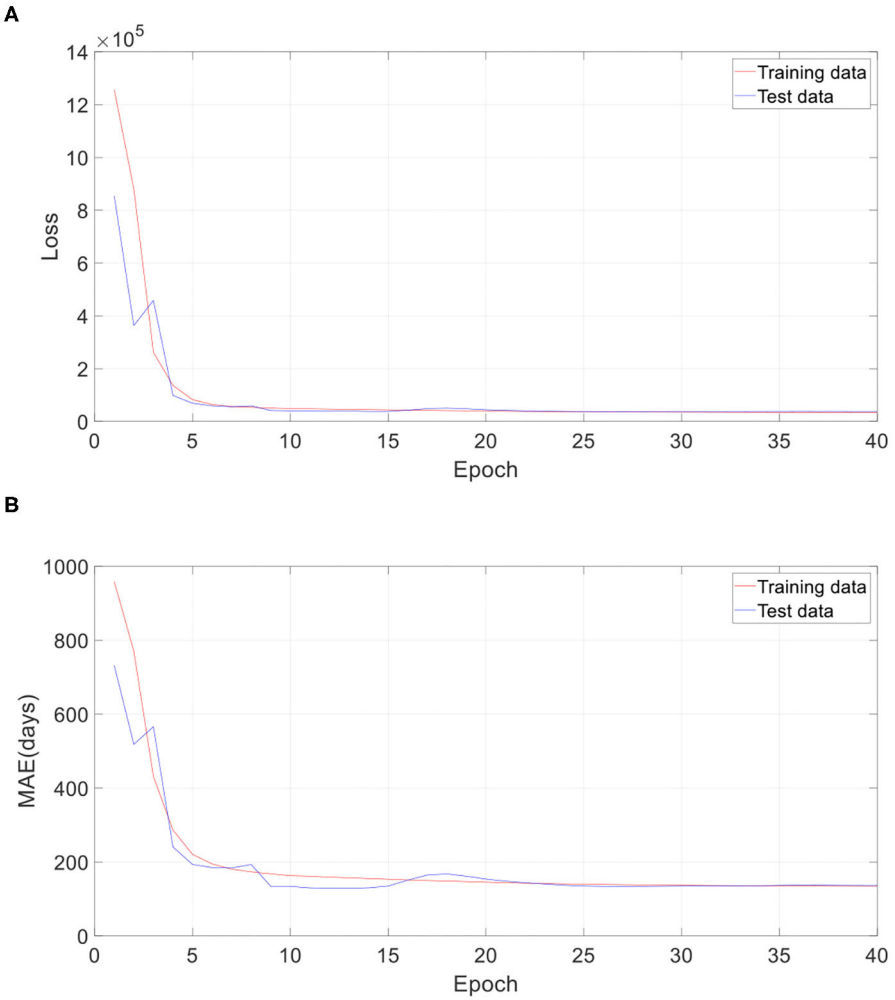

FIGURE 10 | Training performance of one run without batch normalization. **(A)** Loss against training epoch, and **(B)** MAE against training epoch. The loss and MAE are the average of all iterations in one epoch.

important for achieving remarkable prediction results (53, 54). Thus, we investigated the influence of different network depths on the prediction results, showing in **Table 4** and **Figure 9**. As we can see, the best performance was achieved by the 3D CNN containing seven convolution layers and three fully connected layers, not the deepest or shallowest network. Theoretically, the more convolution layers, the higher the extracted feature levels, and the more fully connected layers, the more complex the mapping function that can be fitted. However, too many neuron layers will produce redundant parameters, easily resulting in overfitting. Except for overfitting, a degradation problem may also occur when the deep network starts to converge: with the network depth increasing, accuracy gets saturated (55, 56). Therefore, in order to obtain the best performance, it is necessary to choose a network structure with the appropriate depth.

If the neural network is too deep, the gradient will become very small when it propagates back to the shallow layer, so that the parameters of the shallow layer cannot be updated or the amplitude of the update is very small. This phenomenon called gradient dispersion will lead to the requirement of lower learning rate and careful parameter initialization. Batch normalization was developed to address the above problems (31). In this study, we firstly tried to set the initial learning rate of the 3D CNN without batch normalization the same as that of the proposed 3D CNN. However, it is found that the learning rate is too high, which leads to the failure of training the 3D CNN without batch normalization. With grid search and trial-and-error methods, we set the learning rate of the network to 0.000000008. This observation fully proves that the network without batch normalization requires more careful parameter setting. According to **Figures 5**, **10**, the training loss of the 3D CNN without batch normalization is bigger than that of the proposed 3D CNN, indicating that the former fits the training data worse than the latter. Furthermore, as **Table 5** shows, we observed that batch normalization can greatly improve the prediction performance according to MAE, RMSE, R, and R^2. Thus, batch normalization is strongly recommended for use in

TABLE 6 | Comparison of the proposed 3D CNN trained by different batch size and initial learning rate.

Batch size	Learning rate	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
16	0.0000008	72.3	96.2	8.3	0.985	0.971
32	0.0000008	74.1	97.4	9.2	0.985	0.971
64	0.0000008	67.6	96.1	8.2	0.985	0.971
64	0.0000012	69.9	97.1	8.6	0.985	0.971
64	0.0000004	75.0	104.8	9.3	0.983	0.966

TABLE 7 | Comparison of 2D CNN and our proposed 3D CNN.

	MAE (days)	RMSE (days)	MRE (%)	*R*	R^2
2D CNN	75.1	104.6	9.2	0.982	0.965
3D CNN	67.6	96.1	8.2	0.985	0.971

TABLE 8 | Comparison with state-of-the-art approaches.

Method	Subjects	Age (days)	MAE (days)
Feature-based developmental model (51)	92	8–590	72
HRtoF model (46)	50	14–797	32.1
The proposed 3D CNN	88	4–697	28.9
The proposed 3D CNN	220	4–1,820	67.6

3D CNN for predicting brain age using stacked 2D routine clinical brain MR images.

In terms of hyper parameters, we investigated the influence of batch size and learning rate on the performance of the 3D CNN. Basically, the larger the batch size, the more stable the gradient descent and the more accurate the direction. However, large batch size may cause the model to fall into local minimums and cannot come out because of the little noisiness. Small batch size may cause the data distribution to be too random to converge. Thus, the best batch size should be obtained by experiments for making the model converge to the global minimum as much as possible. In this paper, we set the batch size as 16, 32, and 64 for observing their effects on the predictions, showing in **Table 6**. It is found that the batch size of 64 achieved the best performance. The reason for not increasing the batch size is because the computer does not have enough computing memory, which is also the disadvantage of large size that cannot be ignored. Learning rate controls the convergence speed of model. When the learning rate is set too small, the convergence process becomes very slow and may make the model overfit. When the learning rate is set too large, the gradient may oscillate back and forth around the minimum value, and may not even converge. Thus, it is necessary to select the appropriate learning rate with grid search for achieving the best performance. As **Table 6** shows, the middle-sized learning rate yields the best predictions.

CONCLUSION

In this paper, we developed an end-to-end AI system based on 3D CNN for predicting the brain age of children aged 0 to 5 years old and achieved reliable and high performance with a MAE of 67.6 days, a RMSE of 96.1 days, a MRE of 8.2%, a *R* of 0.985, and a R^2 of 0.971. We found that the predictions for children under 2 years old are much better than those over two, which is also better than two state-of-the-art methods of predicting brain age of infants. The changes in the structure of the model have small effects on the prediction results, as do the changes in learning rate and batch size. The tricks of data augmentation and batch normalization have a significant impact on model performance. The proposed 3D CNN outperformed the 2D CNN having similar structure in prediction results.

In the future, we will collect more subjects for enhancing the performance of the model since CNN is a kind of data-driven method. Furthermore, we will enroll child patients with neurodevelopmental or mental disorders for validating the performance of the model in predicting the biological age of their brains.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material. Code Repository: https://github.com/Captain-Hong/Brain-Age-Prediction-of-Children.

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

ZF and MY collected the data. JH designed the algorithm. JH and Y-DZ preprocessed the data, designed the algorithm, and tested the model. S-HW, MY, and Y-DZ interpreted the results. JH and ZF drafted the work. MY and YZ gave guidance on experiment design. JH, ZF, and S-HW organized the literature. YS and AP substantively revise the manuscript. All authors gave critical comments and approved the submission.

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