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Executive functioning in children with epilepsy: Genes matter

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ABSTRACT

Pediatric epilepsy has emerged as a chronic medical disease with a characteristic behavioral and cognitive phenotype, which includes compromised executive functioning (EF) and attention-related deficits. However, considerable interindividual variability exists; children often display very different or even opposite outcomes, and some children are more likely than others to develop neurocognitive problems in the face of similar individual and disease-related problems. The factors responsible for this interindividual variability are still largely unknown, but we do know that some genetic factors render the developing brain more susceptible to damage or traumatic experiences than others. Dopamine availability has a neuromodulatory function in the prefrontal cortex (PFC) and especially affects EF. Dopamine availability relates to polymorphisms in the gene encoding catechol-*O*-methyltransferase (COMT Val158Met), which in turn is affected by the methylation state of its promoter. Allelic variation of the methylenetetrahydrofolate reductase (MTHFR C677T) gene, alters methylation and may influence the methylation state of the COMT promoter. Given this, we tested the hypothesis that these polymorphisms interact in children with epilepsy, and that variability in allelic expression is associated with variability in cognitive phenotype. Executive function was tested directly and indirectly (parent-rated) in 42 children between 5 and 12 years of age. The MTHFR T allele carriers performed worse than MTHFR homozygous CC carriers on indirect EF, and a significant decline was observed when T allele carriers had at least one met allele of the COMT gene, especially on Working Memory. Direct EF was significantly compromised in COMT Val/Val carriers where reduced dopamine availability seems to confer a higher risk in a test that requests a high degree of executive attention and planning. This finding suggests that in children with epilepsy, genes that influence methylation and dopamine availability affect PFC-related EF. Therefore, we should consider genetic vulnerability as a polygenic risk, which might predispose for a particular phenotype and include specific genetic signatures as part of each patient's behavioral and cognitive profile from the moment that we start to take care of the child.

1. Introduction

When a child is diagnosed with epilepsy, treating the disease should not be the only goal. While we have been able to treat children with epilepsy frequently resulting in satisfactory seizure control, we have not been so good at understanding the cause of both short- and long-term morbidity or disability developed by some of these patients [1–4]. The nature of the disability is often cognitive or behavioral [5], which significantly affects the course of development as well as their quality of life and constitutes a major burden of disease [6,7]. Given this, it is critical to better understand why some children are more likely than others to develop neurocognitive and behavioral problems in the face of similar patient-specific profiles (e.g., age, gender, treatment, pathology, and social context), and to comprehend what drives the variability in short- and long-term cognitive and behavioral disability as well as what research approach would help us to understand the underlying processes. Children and adolescents with diagnosed epilepsy, even the more benign types, often display compromised executive functioning (EF), which are primarily mediated by the prefrontal cortex (PFC) [8–10]. Moreover, the effects of the disease and its treatment on physical and mental health depend on the brain areas that are developing, reorganizing, or declining [11]. At the moment, we do not know what factors are responsible for the intersubject variability and timing of outcome, but we do know that some genetic factors may render the developing brain more susceptible to damage or traumatic experiences than others.

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A significant proportion of children and adolescents, develop clinically meaningful deficits in attention, information processing speed, and executive functions, such as, working memory (WM) [12–15]. Especially, attention and WM play a fundamental transversal function in various domains of executive function and are particularly associated with the availability and activity of dopamine [16,17]. Consequently, polymorphisms of genes capable of altering dopamine availability [18–20] may influence EF, particularly, in tasks where activation of the attention network is critical for an efficient performance.

Two common, functional polymorphisms, catechol-*O*-methyltransferase (COMT) Val158Met [21,22] and methylenetetrahydrofolate reductase (MTHFR) C677T [23,24], have been associated independently or together [25,26] with cognitive functioning [27] and EF [28,29] and/or epilepsy [30,31]. Catechol-*O*-methyltransferase encodes the protein that catabolizes prefrontal dopamine [32,33] and has a functional polymorphism, COMT Val158Met (rs4680) [34], that results in a valine to methionine mutation at gene position 158 (Val158Met) [35]. This polymorphism alters the efficacy of COMT in degrading dopamine, particularly in the PFC and dorsolateral PFC (DLPFC) interfering with the correct formation of cortical structures [36] and connectivity [37] during development while influencing executive processes such as WM and executive control [38,39]. More specifically, the Met variant causes a markedly decrease in enzyme activity, which in turn results in enhanced dopamine availability in the synapse of the PFC [40], and improved performance in prefrontal functioning [35,41]. The Val variant of COMT, characterized by higher activity and higher thermostability [42] resulting in lower dopamine levels in the PFC, has been associated with poorer executive function and anomalous frontal–parietal connectivity during WM [43–45].

Furthermore, dopamine is catabolized by COMT in a transmethylation reaction that relies on methyl-moieties produced through MTHFR from folate. Methylenetetrahydrofolate reductase has a fundamental role in the metabolism of folate, which catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [46]. The latter provides a methyl group for the remethylation of homocysteine back to methionine. This methyl group may then influence cellular methylation, which in turn results in the modification of other genes. Consequently, a common MTHFR polymorphism, characterized by a 677C to T mutation, leads to decreased enzyme activity and elevated plasma homocysteine levels especially in those individuals with a low folate status [47]. Each copy of the 677T variant conveys a 35% reduction in MTHFR activity, affecting downstream biochemical processes, such as, deoxyribonucleic acid (DNA) methylation and homocysteine metabolism [46]. Consequently, T allele carriers likely display diminished promoter methylation [48], increased COMT expression, and reduced dopamine signaling [18,49,50].

Since it is now widely accepted that cognitive phenotypes emerge in part as the consequence of the genetic background against which they occur, we tested the hypothesis that specific allelic variants of the gene encoding for COMT affects EF and that COMT promotor activity is influenced by the allelic expression of the MTHFR gene. More precisely, we tested whether a genotype that results in enhanced availability of dopamine, such as in COMT Met-allele carriers, is protective with respect to EF deficits in children with epilepsy. First, we examined the influence of dopamine bioavailability, operationalized by means of the 677T polymorphisms of the MTHFR gene and Val158Met polymorphism of the COMT gene, on domains of daily EF as assessed by parents. Second, we studied possible differences of MTHFR and COMT allelic expression in relation to EF performance on the Children's Color Trail Test (CCTT) (which has been used in children with epilepsy to test EF) and a Pegboard Test to control for psychomotor speed. We hypothesized that Met carriers would perform better on WM tasks that require substantial attention and visual–motor integration as a result of enhanced dopamine availability. Moreover, we explored whether COMT polymorphic variants interact with MTHFR allelic expression, known to alter the methylation state of the COMT promotor. If so, children with epilepsy that are Met carriers, homozygous for the C allele of MTHFR, which results in normal methylation [46,51], would display better performance on EF, possibly as a result of enhanced dopamine dependent signaling while it would be plausible that the T and Val alleles act synergistically to impair executive function [38].

Understanding genes that regulate dopamine in the frontal cortex, such as COMT and MTHFR [52], may help to unravel the complex neurobiological processes that underlie cognitive dysfunction [18,53], which are especially important with respect to the daily functioning of children with chronic or severe illness, such as, epilepsy.

Often, abnormal phenotypic outcomes develop from modifications in basic processes early in development (such as pruning, synaptogenesis, density of neurons, neuro/glia ratio) that are highly flexible, plastic, and open to extensive environmental influences at the level of gene expression, brain cognition, and behavior. Cognitive function, or dysfunction, then, is the emergent outcome of a dynamic system of progressively changing function and structure that continuously interact with each other and with the environment against a background of interindividual variability in the expression of different susceptibility genes. Thus, including genetic markers in the comprehensive testing of children with epilepsy may help to more objectively assess risk for cognitive problems because such markers may represent and sustain a more precise prognosis. At the same time, they may help us to detect neurodevelopmental effects of antiepileptic drugs (AEDs) [54–57] and problems linked to disease progression in a timely manner, which will allow us to evaluate the burden of disease over time of each patient with respect to individual risk factors in relevant domains specific to the context of the patients and their family. Defining risk factors to help plan early interventions in favor of children most vulnerable for the development of cognitive problems may reduce cognitive problems and enhance mental health while growing up.

2. Methods

2.1. Participants

Participants in this study were enrolled in a prospective longitudinal study. Here, we report on the first two time point. The sample consisted of 42 patients between 5 and 12 years of age of which 36 patients were eligible for this study. Six patients received an epilepsy diagnosis of unknown etiology [30,31,58] and recruited from the pediatric neurology out-patient clinic of the University Hospital "Azienda Ospedaliero-Universitario Policlinico di Modena". Exclusion criteria were brain lesions or genetic and metabolic disorders that could justify the onset of epilepsy.

Epilepsy classification was made by a neurologic work-up that included an electroencephalogram (EEG) (according to International League Against Epilepsy [ILAE] [58,59]) and, based on the most pertinent clinical characteristics, patients were divided in 3 groups: 1 Benign Epilepsy with Centrotemporal Spikes (BECTS); 2 Temporal Epilepsy; and 3 Epileptic Dysphasia [30]. The definition of Epileptic Dysphasia is not included in the ILAE classification; in this study, the term refers to patients characterized with EEG abnormalities and anomalies in the language domain, such as, verbal dyspraxia. Because of additional difficulties and the focus of the data presented here, three patients were excluded from the analysis, as well as one patient with a diagnosis of West Syndrome and one patient with a diagnosis of frontal lobe epilepsy. Also, participants were excluded based on comorbid conditions known to affect cognition, for example autism spectrum disorders. Likewise, participants were not excluded based on intellectual functioning alone, and were included if they were capable of adequate comprehension and participation in the neuropsychological tasks administered. The type of treatment was divided in 4 groups based on the active substance: 1 carbamazepine and oxcarbazepine, 2 valproate, 3 levetiracetam, and 4 other therapy. Demographic information (Table 1) was collected via a semistructured interview by a qualified therapist and

Table 1
demographic and clinical characteristics of the sample (N = 42).

General characteristics of the sample	%	N
Gender		
Boys	69	29
Girls	31	13
Ethnicity		
Caucasian	85.71	36
Other	14.29	6
Age of patients		
<10 years and 6 months	61.9	18
≥10 years and 6 months	38.1	24
Diagnosis		
BECTS	52.3	21
Temporal	28.57	12
Epileptic Dysphasia	11.9	5
Other	7.1	3
Onset		
<6 years	59.5	25
Control of seizures		
No	33.33	14
On treatment		
Yes	87.9	37
Type of treatment		
Oxacarbamazepine/Carbamazepine	65.9	27
Valproate	9.8	4
Levetiracetam	9.8	4
Other	2.4	1

included age of onset (Mean = 5 years; standard deviation (SD) = 2.89), presence of comorbidities, caregivers age (mother Mean = 41.2 years; SD = 4.44; father Mean = 44.1 years; SD = 5.25), degree of education (mother: 40.4% high school diploma; father: 45.23% middle school), primary language and ethnicity, close relatives with chronic or severe diseases (40.4% relatives with chronic diseases and 30.9% relatives with mental illness), the course of pregnancy (97.6% good course), and type of delivery (28.6% cesarean section). The Ethics Committee of Modena approved the protocol and informed consent and age-appropriate assent (from 11 years on) was obtained from all young participants and their parents.

2.2. Assessment of executive function

Executive function was tested by *direct* and *indirect assessment* [5,60]. Direct EF was assessed with the Children's Color Trail Test (CCTT). Psychomotor speed was tested using an adaptation of the Pegboard Test. Indirect assessment of EF was evaluated using the Behavior Rating Inventory of Executive Function parent version (BRIEF and BRIEF-P). The CCTT is a standardized paper-and-pencil assessment of executive function and measures alternating and sustained visual attention, sequencing, psychomotor speed, cognitive flexibility, planning, and inhibition–disinhibition [61,62]. The CCTT consists of two parts, Part 1 and Part 2. In Part 1, the child is asked to connect numbers from 1 to 15, alternating the even numbers in a yellow circle with uneven numbers in pink circles. Part 2 is based on the same principle, but now the patient must pay attention to alternate even and odd numbers correctly because the same number appears in two colors (yellow and pink); CCTT Part 1 (CCTT-1) specifically tests sustained visual attention, psychomotor speed, and simple sequencing; Part 2 (CCTT-2) requires more focus, vigilance, working memory, shift, and inhibition skills. The variables measured in the test include completion time in seconds, color or number sequence errors, prompts, and near-misses. Age norm-referenced scores include standard scores for each trial completion time; higher scores indicate faster and better performance. We report on the time the patients needed to complete each test as well as how time and errors translated in the preservation or damage of the executive domains assessed by this test. In order to test whether the CCTT was not merely related to visual–motor coordination and psychomotor speed, children were tested on the speed with which they completed a Pegboard Test. The experimenter first established which hand was the dominant one. Dominance was assessed using the Edinburgh Handedness Test, adapted for children [63]. Our Pegboard Test evaluated motor speed, visual–motor coordination, and single-hand dexterity. We used an adaptation of Annett's Peg Moving Task [64–66]: patients were asked to use one hand to put 10 pegs in one row of 10 holes: the Pegboard task measures the time taken by subjects to move a row of 10 pegs from one hole to the other. The score for each hand is the average of four completed trials [67,68]. We then calculated a measure of relative hand skill (PegQ score = (L − R)/((L + R)/2)) for each child, which was calculated as the difference between the mean time for the left hand (L) and the right hand (R), divided by the mean time for the left and right hand combined [69]; the PegQ score range is from −1 to 1, where 1 means definitely right-handed and − 1 means definitely left-handed [70,71]. Indirect executive function was tested by the parent-report version of the BRIEF. The BRIEF is a norm-referenced rating inventory that assesses EF in relation to the performance of everyday tasks, across multiple domains based on a child's or adolescent's (from 6 to 18 years old) observable behaviors and abilities [72]. The BRIEF includes three higher order index scores of executive functions, as well as several lower order functions. The higher order domains include the Global Executive Composite score (GEC); the Behavioral Regulation Index (BRI), which is the superordinate scale encompassing the subscales Inhibition, Shift, and Emotional Control; as well as the Metacognition Index (MI), which includes the subscales, Initiate, Working Memory, Plan and Organize, Organization of Materials, and Self-Monitoring. The BRIEF has excellent psychometric properties [72,73] and has proven to be sensitive to executive function deficits in children and adolescents with epilepsy in several studies [5,74–77]. Age norm-referenced T scores (M = 50; SD = 10) are computed, whereby higher T scores indicate more difficulties, and T score ≥ 65 indicates clinical significant problems [72].

The BRIEF-P is the preschool version of the BRIEF and is used to evaluate executive dysfunction in preschool-age children (from 2 to 5 years old). It is composed of a Global Executive Composite score (GEC), that is an overarching summary score of all scales; the Inhibitory Self-Control Index (ISCI), which includes the subscales: Inhibit and Emotional Control; the Flexibility Index (FI), composed of the subscales Shift and Emotional Control; Emergent Metacognition Index (EMI), which includes the subscales Working Memory and Plan/Organize. Age norm-referenced T scores (Mean = 50; SD = 10) are computed, whereby higher T scores indicate more difficulties, and T score ≥ 65 indicates high clinical significance [77].

2.3. Genotyping

Samples of DNA were obtained from each subject by buccal swab and extracted for analysis of the following polymorphisms: COMT val158met and MTHFR C677T. Noninvasive DNA collection methods were preferred for these young patients to increase study participation and compliance; DNA was isolated from buccal cells using the High Pure PCR Template Preparation Kit (Roche Applied Science, Germany); DNA analysis was performed by PCR-restriction fragment length polymorphism assays. The polymerase chain reactions (PCRs) were carried out in SimpliAMP thermal cycler (Thermo Scientific, USA) in a reaction volume of 50 μl containing 100 ng of genomic DNA, 300 nM of each primer, 200 nM of each dNTP, and 1.25 units GoTaq® DNA polymerase (Promega).

The COMT Val158Met polymorphism was determined by the following PCR primers: Forward 5′-TCGTGGACGCCGTGATTCAGG-3′ and Reverse 5′-AGGTCTGACAACGGGTCAGGC-3′ [78], used to amplify a 217-bp fragment. The PCR products of COMT contained the polymorphic site recognized by *NlaIII* restriction enzyme (New England Biolabs). Homozygotes for COMT158Val generated fragments of 136 and 81 bp, heterozygotes produced 136-, 96-, 81-, and 40-bp fragments and

homozygotes for COMT158Met generated 96-, 81-, and 40-bp fragments. Genotypes were analyzed after electrophoretic run on 2% agarose gel with DNA stain (Atlas).

Genotyping of the MTHFR C677T polymorphism was performed using the forward primer: 5′ GAAGGTGCAAGATCAGAG 3′ and the reverse primer: 5′ CTCAAAGAAAAGCTGCGTGATG 3′ [79]. The PCR products of 232 bp were digested with the *HinfI* restriction enzyme (Sibenzyme) and separated on a 2% agarose gel and stained with DNA stain (Atlas). The 677C allele produced two fragments: one of 199 bp and one of 33 bp, whereas the 677T allele was cut into three fragments of 165, 34, and 33 bp.

2.4. Statistical analysis

Descriptive statistics (mean, SD, and percentages) were reported for demographic and clinical data (Table 1). Independent sample *t*-tests or a one-way analysis of variance (ANOVA) were run to determine the effects of patient characteristics (age at diagnosis, age at assessment, gender, treatment, diagnosis) on direct and indirect EF. There were no outliers, and gender was distributed normally within each group (Shapiro–Wilk test $p > 0.05$). Diagnosis and treatment were tested by one-way ANOVA. Normal distribution was established by the Shapiro–Wilk test ($p > 0.05$ for assumption of normalcy). Also, homogeneity of variances within each group was established by Levene's Test for Equal Variance ($p > 0.05$ for assumption of equal variance), and when violated, the Welch correction for unequal variances was applied. Primary outcome measures were parent-rated and direct EF, as measured by the parent-rated BRIEF (and BRIEF-P) and the CCTT, respectively. For the BRIEF, raw scores for each individual domain, as well as domain-relevant second order indices, were converted into T scores adjusted for age and gender against representative normative data provided by the BRIEF manual. Parents were urged to complete every item on the BRIEF: missing responses were dealt with following the instructions provided by the manual. We excluded BRIEF-P scales not in common with BRIEF from the analysis (N = 3). The same method was applied for scoring of the CCTT. Comparison of disability on indirect EF and EF performance between children with epilepsy and the normative sample of typically developing children was conducted using one-sample *t*-test. Generalized linear modeling was used to analyze the association between COMT polymorphisms, COMT homozygous Val carriers vs COMT Met allele carriers, and MTHFR677 homozygous C carriers vs MTHFR 667T carrier with respect to direct and indirect EF in children with epilepsy. Differences for COMT and MTHFR allelic expression were tested for all indirect and direct EFs using a univariate general linear model (GLM), with age at assessment as a covariate as these variables proved to be associated with direct and indirect EF outcomes. Because the present study should be considered exploratory in nature, statistical significance was set at $p < 0.05$. All analyses were conducted using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Polymorphism distribution (Table 2) did not differ with respect to frequencies reported in other studies, in that, the majority of subjects was heterozygous [28,80].

3.1. Overall impairment of direct and indirect executive function in children and adolescents with epilepsy as compared to normative data

The proportion of patients that performed in the normal range varied with respect to the domains of EF. With respect to indirect executive function assessed by the parents, patients did worse overall as compared to norm-referenced children reported provided by the manual [72] (hereafter called typical developing [TD] children).

Especially, WM and plan and organize were compromised. Almost 60% of our patients displayed moderate to severe WM difficulties while only 40% displayed no difficulties related to WM. More importantly, 20% of the children that had more difficulties had scores in the clinical range, ≥65. Furthermore, a rather large percentage around 30%–40% performed in the borderline range. The impairment level of the child regarding the capacity to plan and organize a thought or action was similar to the level of impairment observed for WM; only 50% of the patients had no impairment while 38% displayed moderate impairment, and 18% scored in the clinical range. Also, the overall pattern of impairment in the various other domains of daily EF showed significantly more deficits as represented by elevated impairment on Metacognition and Global Executive Composite indexes (62% and 56% respectively), with 14% performing in the clinical range of the MI. This suggests that adequate surveillance should be implemented in order to timely detect worsening of EF over time.

Table 2
MTHFR C677T and COMT Val158MeT allelic distribution in boys and girls with epilepsy.

Genotype	%	N
COMT Val158Met		
Val/Val	31.7	13
Val/Met	53.7	22
Met/Met	14.6	6
MTHFR C677T		
CC	39	16
CT	56.1	23
TT	4.9	2

Distribution of the participants according to COMT and MTHFR genotype (percent and number). DNA could not be obtained from one patient. Val, Valine; Met, Methionine.

3.2. Difference between male and female patients with respect to indirect executive functioning

Gender significantly affected various subscales of executive function observed by the BRIEF in our patients when compared to normative data in TD children. Males performed significantly worse on WM ($F(1,33) = 5.417$, $p < 0.05$, $\eta^2 = 0.141$), when planning and organizing a thought or action (PlanOrg) ($F(1,33) = 5.801$, $p < 0.05$, $\eta^2 = 0.150$), and when monitoring an action ($F(1,33) = 5.955$, $p < 0.05$, $\eta^2 = 0.170$) (Table 3). On the composite scores males did worse than females with respect to the MI, which includes emotional control ($F(1,33) = 5.421$, $p < 0.05$, $\eta^2 = 0.141$) (Table 3). Thus, gender significantly affects some of the executive parameters but not others, with boys displaying enhanced impairment (Table 3). The type of epilepsy, nor the type of treatment affected domains of daily EF.

Table 3
BRIEF Index and Subscales Scores for boys and girls with epilepsy.

Scales	Boys (N = 26)			Girls (N = 10)			
	M	*SD*	% Clin.	*M*	*SD*	% Clin.	Sign.
Inhibit	48.09	9.34	<5%	45.22	8.32	<5%	ns
Shift	54.35	12.25	>5%	48.78	12.07	<5%	ns
Emotional control	50.26	10.27	>5%	46.11	11.97	<5%	ns
Initiate	51.47	11.48	<5%	46.32	12.35	<5%	ns
Working Memory	58.04*	10.17	>15%	49.0	9.35	<5%	$p < 0.05$
Plan/Organize	55.47*	12.74	>15%	45.22	6.90	<5%	$p < 0.05$
Org. Mat.	52.52	8.69	<5%	49.22	8.22	<5%	ns
Monitor	52.61	11.48	>5%	43.22	7.46	<5%	$p < 0.05$
BRI	50.34	10.85	<5%	46.00	11.74	<5%	ns
MI	55.12*	11.16	>10%	46.11	8.55	0»	$p < 0.05$
GEC	53.43	11.06	<5%	45.89	9.64	<5%	ns

Effects of gender on executive function reported by the BRIEF (parent version): Behavioral Rating Inventory of Executive Function; M: mean; SD: standard deviation; % Clin.: (proportion) of patients in the clinical significant range (T-score ≥ 65); Plan/O.: plan/organize; Org. Mat.: organization of materials; BRI: Behavioral Regulation Index MI: Metacognition Index; GEC: Global Executive Composite; ns: not significant.

* Level of significance (Sign.) set at $p = 0.05$ vs male counterparts.

3.3. Effect of patient and disease-related characteristics on direct executive function and psychomotor speed

Time-related T scores were significantly lower on the CCTT-1 when compared to a sample of TD children $t = 4.450$ $p < 0.001$ (TD Mean = 43.00 SD = 9.95 and patients Mean = 31.20 SD = 12.12), but no difference was observed on the CCTT-2. Patients made significantly more number sequence errors than estimated based on the standard number sequence errors on the CCTT-1 (28.6%) while almost no number sequence errors was made on the CCTT-2 (5.7%). Furthermore, patients made more color-related errors on the CCTT-2 than expected (25.7%) (Table 4).

3.4. Difference between male and female patients with respect to direct executive functioning and psychomotor speed

The CCTT-1 required markedly more effort in boys (Mean = 62.3 s. SD = 12.64) than in girls (Mean = 47.4 s. SD = 17.68). Similarly, boys needed significantly more time to complete the CCTT-2 than girls (Mean = 129.5 s. SD = 20.45 as compared to Mean = 78.6 s; SD = 19.9) ($p < 0.05$ OR of 2.4). Neither the type of epilepsy, nor the type of treatment affected color trail performance.

When testing for psychomotor speed and coordination, we first assessed handedness. According to the Edinburgh Handedness Test [63], 80% of the sample was predominantly right-handed, 8.1% left-handed, and 11.9% did not display a clear preference. With respect to psychomotor speed, that is, performance on the Pegboard Test, time to complete the task with the dominant hand differed significantly between boys and girls. Males were slower than girls (boys Mean = 12.11 s; SD = 3.56 and girls Mean = 9.58 s; SD = 0.98; $p < 0.01$, $\eta^2 = 0.11$, OR 3.5). Completion of the Pegboard Test with the nondominant hand also took significantly more time in males than in females (boys Mean = 11.79 s; SD = 2.74 and girls Mean = 9.59 s; SD = 1.25; $p < 0.01$, $\eta^2 = 0.14$) (Table 4). Thus, in our patients, gender significantly affected the patient's psychomotor speed, with males displaying enhanced impairment. Interestingly, although 80% of the patients expressed the right hand (left brain) as being the preferred one, the PegQ score indicated an underlying construct that showed a preference for the left hand (right brain). The PegQ score for the total sample was -013 ± 0.067. However, girls approached 0 (PegQ girls = -0.0009 ± 0.069) while in boys the underlying tendency to display preference for the left hand was much more pronounced, (PegQ boys = -0.016 ± 0.067) (Table 4).

Table 4
Performance on the CCTT and Pegboard Test.

CCTT and Pegboard scores				
Test	Characteristic	Statistic/measure	Mean (N)	SD, percentage
CCTT-1	Time T score	Mean (SD)	31.0 (36)*	±12.12
	Number sequence errors	>161	25/35	77.7%*
		≤16	10/35	22.3%*
CCTT-2	Time T score	Mean (SD)	37.31 (36)*	±11.58
	Color sequence errors	>16	26/35	74.3%*
		≤16	9/35	25.7%*
	Number sequence errors	>16	33/35	94.3%
		≤16	2/35	5.7%
Pegboard Test	Dominant hand males	Time in sec (SD)	12.11	±3.56
	Dominant hand females		9.58	±0.98
	Nondominant hand males	Time in sec (SD)	11.79	±2.74
	Nondominant hand females		9.59	±1.25
	PegQ	(L − R)/((L + R)/2)	−0.013	±0.067
	PegQ males	(L − R)/((L + R)/2)	−0.0.016	±0.067
	PegQ females	(L − R)/((L + R)/2)	−0.0009	±0.069

CCTT = Children's Color Trails Test. Statistical tests are based on a two-sided one-sample test of a binomial proportion with a null hypothesis of 16% (CCTT manual). Lower levels mean worse performance. Patients committed more number sequence errors on the CCTT-1, while on the CCTT-2 no difference was observed with respect to number sequence errors. However, on the CCTT-2, the number of color sequence errors was higher in patients when compared to a normative sample of TD children.

* Indicates statistical significance at the 0.05 level with respect to a normative population of typically developing children.

3.5. Association of MTHFR and COMT polymorphisms with parent reported executive function

The targeted genetic variants were selected a priori based on the literature and/or hypothesized associations with executive function and attention. Because the intervariability on neurocognitive impairment in children with epilepsy, and especially EF, may potentially be mediated by genes regulating neurotransmitter availability and by folate depletion and related metabolic processes, we included SNPs of genes known to alter folate concentrations (MTHFR) as well as dopamine availability (COMT).

Overall, when controlled for age, allelic expression of the MTHFR gene influenced EF in everyday functioning, $F(8,22) = 2.573$, $p < 0.05$, Wilks $\Lambda = 0.517$, partial $\eta^2 = 0.483$. Patients with at least one T allele of the MTHFR gene displayed significant difficulties especially with respect to WM $F(1,29) = 5.921$ $p < 0.05$, partial $\eta^2 = 0.170$. Plan and Organize and Shift as well as MI were marginally compromised and displayed a medium effect size ($\eta^2 = 0.056$, 0.06 and 0.048 respectively). Allelic variation of the COMT gene was not predictive of EF overall nor did COMT allelic expression differentially affected various subscales of the BRIEF. However, a general trend was observed where heterozygosity for COMT predicted worse performance on the BRIEF. Thus, only variation in the allelic expression of the MTHFR but not COMT moderated WM-related to everyday tasks as reported by the parents.

3.5.1. Impairment on direct EF and allelic expression of MTHFR C677T and COMT Val158Met

The CCTT is especially useful for measuring attention and mental processing speed in children suffering from neurological disorders, such as, seizures and trauma (closed head injuries). In general, twice as many MTHFR T carriers scored in the moderate to extremely impaired range when compared to CC carriers on the CCTT-1 (47.4% vs 23.1%), while the percentage that scored in the normal range did not differ (38.5% vs 31.6%) (Fig. 2). Performance on the CCTT-2 displayed a similar pattern in impairment. Moreover, 50% of our patients showed moderate to severe problems on the CCTT-2 with more than 10% displaying extreme impairment. With respect to COMT, Met carriers displayed significantly milder to moderate problems but markedly less severe impairment on the CCTT-1. Also, Met carriers showed significantly less extreme impairment on the CCTT-2 than Val/Val carriers (4.8% vs 18.2%), while overall Met carriers had more moderate to severe problems (36.8% vs 23.1%). Together, MTHFR T and COMT Val/Val carriers manifest more difficulties on EF performance.

Also, we did not observe a significant main effect of MTHFR allelic variation on time to complete the CCTT while COMT influenced CCTT performance ($F(2,29) = 3.88$ $p < 0.05$, Wilks $\Lambda = 0.789$, $\eta^2 = 0.211$). Given the significance of the overall test, univariate main effects were examined. The COMT allelic variation significantly influences time to complete the CCTT-1 ($F(1,30) = 8.016$, $p < 0.008$, $\eta^2 = 0.210$) but not the CCTT-2 ($F(1,30) = 3.963$, $p = 0.056$, $\eta^2 = 0.117$). Moreover, a significant interaction was observed between MTHFR and COMT allelic expression on overall color trail performance ($F(2,29) = 5.210$, $p < 0.025$, Wilks $\Lambda = 0.736$, $\eta^2 = 0.264$, and power 0.789). A significant interaction between MTHFR and COMT alleles was observed both with respect to the CCTT-1 ($F(1,30) = 10.67$, $p < 0.01$, $\eta^2 = 0.264$) as well as the

CCTT-2 ($F(1,30) = 5.164$, $p < 0.05$, $\eta^2 = 0.147$). Against a MTHFR CC homozygous background, COMT Met carriers performed better than homozygous Val/Val carriers (29.7 vs 103.5 s). However, performance on the CCTT-1 task of COMT Val/Val and Met carriers took more time against a background of at least one MRHFR T allele, but no difference was observed among carriers of different COMT alleles (47.7 vs 50.4 s). A similar pattern was observed for the CCTT-2. Here, COMT Val/Val and MTHFR CC allele homozygous patients, needed almost threefold the time to complete the task than MTHFR homozygous CC, COMT Met allele carriers (134 vs 54 s). Again, the time to complete the task was higher in the presence of at least one MTHFR T allele; against this background, Val/Val and Met carriers spend the same amount of time to complete the task (86 vs 94 s). This suggests that carrying the Met allele is protective in a task that requires psychomotor speed, sustained visual attention, sequencing, mental flexibility, and WM. Furthermore, in MTHFR T allele carriers, COMT allelic variation is not predictive of performance on this type of task.

Motor speed as defined by the time needed to complete the Pegboard Test differed according to both MTHFR allelic expression and the dominant hand ($F(1,33) = 4.836$, $p < 0.05$, $\eta^2 = 0.128$); T allele carriers were faster than the CC homozygous patients. The MTHFR allelic variation did not affect performance speed of the nondominant hand. The COMT allelic variation had no effect on motor speed of either hand.

The Peg Q score differed according to allelic expression both of the MTHFR gene ($F(1,33) = 6.562$, $p < 0.05$, $\eta^2 = 0.166$, and power 0.701), as well as the COMT gene ($F(1,33) = 8.525$, $p < 0.01$, $\eta^2 = 0.205$, and power 0.809). Homozygous CC patients preferred the left hand over the right hand, while T carriers did not demonstrate a clear hand preference when considering the PegQ score. Furthermore, Val/Val carriers had a PegQ score of 0.025, indicating a right-hand preference while Met carriers had a PegQ score of −0.03, which indicates a left-hand preference.

4. Discussion

We found that a subset of patients with pediatric epilepsy had compromised EF, especially those related to WM. Forty percent of our patients (Fig. 1) were at risk for decreased performance on tasks that asked for intact mental flexibility and executive attention. Up to 10% of our young patients displayed substantially reduced performance across various subscales. Subscales at risk for deficits were WM and Plan and Organize where more than 20% of children were reported to display considerable problems (Fig. 1). This suggests that young patients with epilepsy have difficulties in multiple facets of daily EFs despite otherwise adequate cognitive abilities [13]. Parents and caregivers are an important source of information on EF [81], and their assessment of EF and difficulties related to attention in children with epilepsy should be included in treatment plans aimed at reducing problems at home and at school [9,82–84]. Interestingly, many of these problems appeared to be associated with specific genetic profiles related to dopamine availability.

A deficit in EF is particularly debilitating during childhood and adolescence because they may undermine the individual's capacity to adapt to the surrounding environment, even with general intelligence within the norm [82]. In our study with a limited number of patients, we analyzed the relationship between polymorphisms in a folate-related gene and effects on direct and indirect executive function. We examined alleles of the methylenetetrahydrofolate reductase (MTHFR) gene, which cause enzyme activity to vary, altering the availability of the methyl donor and thus changing the efficiency of methylation. We hypothesized that alleles of the MTHFR gene would influence behavior and executive function [85] and found that patients heterozygous for the MTHFR gene displayed difficulties in every day executive function especially with respect to WM. More importantly, from the direct testing of the child using the CCTT, which requires sustained attention, visual–spatial working memory, sequencing, and cognitive flexibility, emerged an important interaction between the allelic variations of the COMT and MTHFR genes. This suggests that genetic vulnerability in our patients should be expressed as polygenic risk that we might want to consider as part of behavioral and cognitive risk assessment.

In line with our hypothesis, children homozygous for the C allele of MTHFR resulting in standard (unchanged) methylation did not interfere with COMT Val158Met promoter activation, which in turn led to normal gene expression (Fig. 3). Given this, in Val/Val carriers dopamine breakdown is enhanced, which leads to less dopamine availability in the synapse and reduced efficiency on the CCTT. Particularly, patients with this

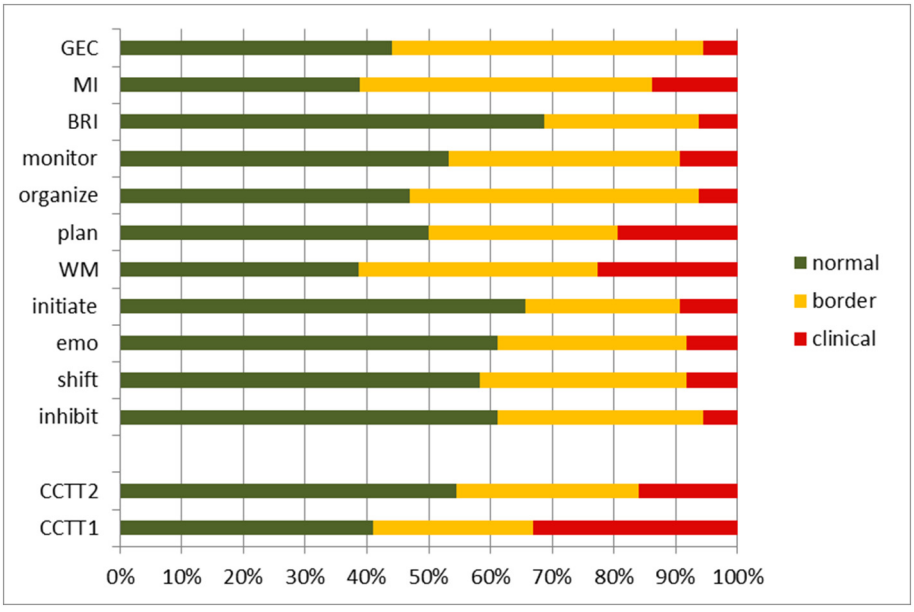

Fig. 1. Impairment on direct and indirect EF in children with epilepsy. BRIEF parent version: Behavioral Rating Inventory of Executive Function; emo: emotional control; WM: working memory; plan: plan/organize; organize: organization of materials; BRI: Behavioral Regulation Index; MI: Metacognition Index; GEC: global executive composite; CCTT: Child Color Trail Test 1 and 2. For the BRIEF: Green: no impairment T score < 50; Orange: light to medium impairment (T score > 50 and ≤ 65); Red: (T score > 65), for the CCTT impairment defined according to manual; N = 36.

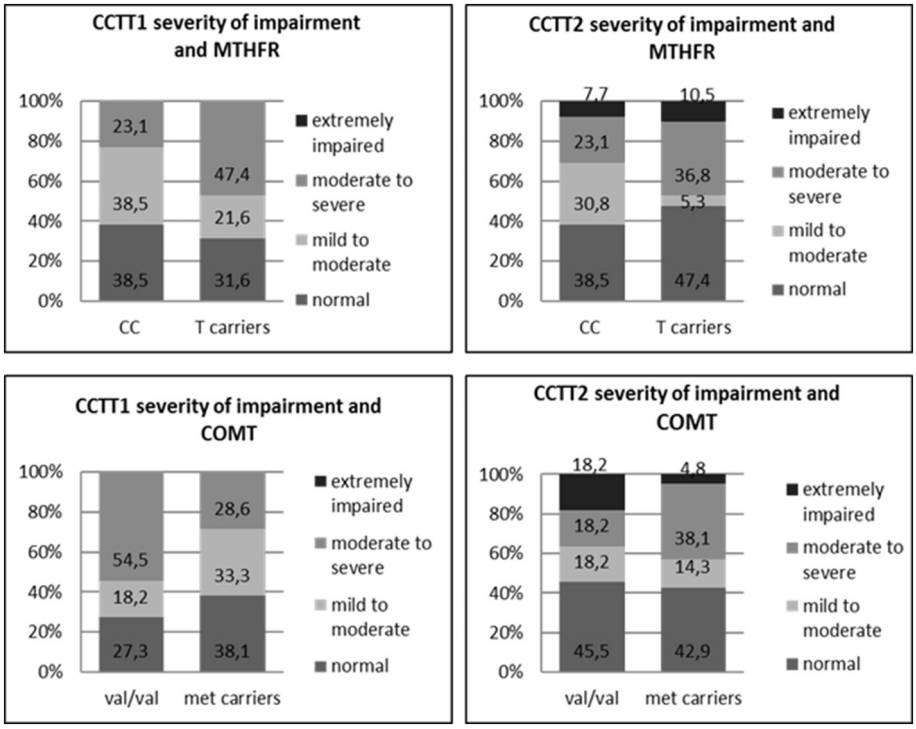

Fig. 2. Percentage, genotype and severity of impairment on the CCTT-1 and 2 CCTT: Child Color Trail Test; COMT Val158Met: Catechol-O-Methyltransferase valine/methionine mutation at position 158; MTHFR C677T: Methylenetetrahydrofolate reductase C/T mutation at position 677; Val: Valine; Met: methionine; Upper part: association of the MTHFR genotype with level of impairment on the CCTT Part 1 (left) and the CCTT Part 2 (right). Lower part: association of the COMT genotype with level of impairment on the CCTT Part 1 (left) and the CCTT Part 2 (right).

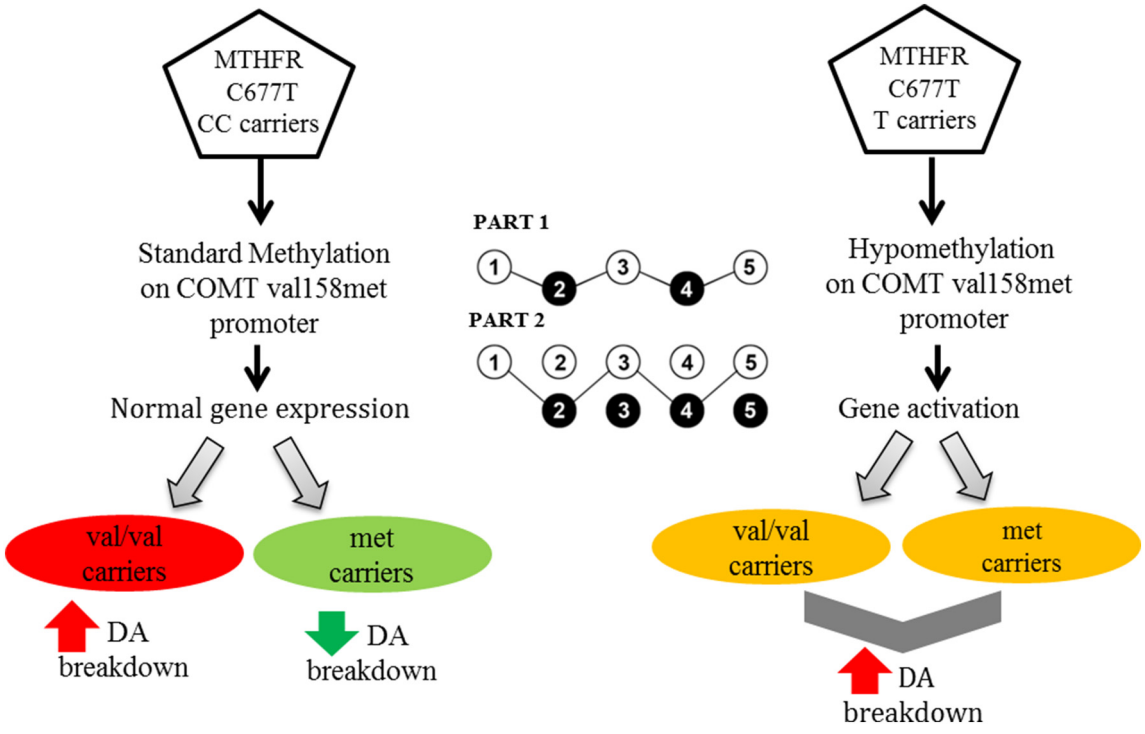

Fig. 3. Representation of the hypothesized interaction between MTHFR and COMT on dopamine (DA) availability. When DA breakdown is increased, time of CCTT execution increased and patients had poorer performance. C carriers have normal MTHFR enzyme activity, thus, the COMT gene is regularly activated and DA breakdown depends only on the COMT polymorphism expressed. In presence of at least one Met allele, the activity of the COMT enzyme is reduced and there is more DA circulating in PFC and DPFC cortex. As a result, Met carriers present faster and better performance on the CCTT Part 1 and 2. Val homozygous patients have worse performance on the CCTT Part 1 and 2, which might be related to elevated DA degradation. The presence of at least one T allele causes an impairment in all folate pathways that result in the hypomethylation of the COMT promoter gene and COMT gene transcription activation. More presence of COMT, then, results in an increase in the degradation of dopamine which influence both COMT SNP's, where both Val/Val and Met carriers display and intermediate phenotype with medium impaired performance (COMT Val158Met: Catechol-O-Methyltransferase valine/methionine mutation at position 158; MTHFR C677T: Methylenetetrahydrofolate reductase C/T mutation at position 677; Val: Valine; Met: methionine; DA: dopamine).

genetic signature were three times slower than MTHFR CC homozygous Met carriers. In these patients, dopamine breakdown was hypothesized to be reduced, thus, providing more dopamine in the synapse in neurons in the PFC leading to enhanced performance on the CCTT [38,52]. On the contrary, the T allele of MTHFR likely leads to hypomethylation of the COMT Val158Met promoter. This then drives the activation in both Val/Val as well as Met carriers of the COMT Val158Met gene; time necessary to perform the CCTT was higher for both. In fact, dopamine breakdown is presumably higher, which results in less dopamine. Consequently, execution of the CCTT took longer in MTHFR Val/Val than CC Met carriers, while no difference was observed between these two genotypes in the presence of at least one MTHFR T allele.

Especially with respect to direct executive function with an important emphasis on visual spatial attention, our data suggest a protective role for the COMT Val158Met allelic variant both in overall impairment and in CCTT performance. This is in accordance with the findings reported by Dumontheil et al. [28] that showed that the Met allele has a protective role. Especially with respect to PFC-related tasks for which adequate dopamine-related neurotransmission is fundamental [86].

An alternative explanation for faster performance in children with the Met allele is that their psychomotor performance is altered by dopamine availability not only in the PFC but also in the striatum and other parts of the limbic system where dopaminergic receptors are more abundantly distributed. T carriers did not demonstrate a clear hand preference, and speed did not differ according to the hand used, which supports the (intermediate) speed on the CCTT. Interestingly, we found a discrepancy between hand preference and PegQ scores. T carriers performed faster than CC homozygotes only for the dominant hand. However, CC patients who indicated right-hand preference, in fact had a negative PegQ score suggesting a preference for the left hand. This, in turn, suggests that psychomotor speed is likely affected by nonverbal foci that primarily affect the right side of the brain. In children with temporal lobe epilepsy, seizures are often associated with intractable focal epilepsy involving the temporal lobe, the hippocampus, and areas such as subcortical and cerebellar areas. Moreover, in these children, hemispheric lateralization of their temporal lobe epilepsy may correlate with hemisphere related function deficits. Thus, most likely connections and networks are affected more so than one specific area. This also sustains the claim that functions and domains are not fixed in time and place and may become more specialized over time.

The results of this study extend our current knowledge to include specific genetic profiles in comprehensive testing as factors underlying intervariability and encourage us the use signs together with symptoms from the moment that we start to take care of the child with epilepsy. Furthermore, our data expand the literature suggesting a role for specific genotype profiles across various domains of executive function [87]. Clinicians as well as researchers could use this information to identify patients at risk for EF and provide recommendations with respect to necessary treatment modalities and especially, when to best introduce them.

4.1. Significance and limits of the study

First of all, we studied three heterogenetic and relatively small groups of patients with different clinical and EEG characteristics, which lowers the overall power of the results, especially when we correlated the effects of different genes. Notwithstanding the small number and the heterogeneity, the findings of this exploratory study seem to be rather robust and suggest that variability in the MTHFR and COMT genotype may be associated with or affect particular domains of EF, which underscores the notion that different domains of EF function need a different degree of dopamine availability and system integrity. Additional studies are necessary to address this issue. Future studies should also address the fact that COMT genetic variation alone does not seem to be predictive of parent-rated executive function and other processes that influence dopamine availability should therefore be considered. Also, the interaction between the MTHFR and COMT gene does not necessarily reflect a strict interdependency but may result from the cumulative effect of each individual gene on the function and connective architecture to, from and within the prefrontal cortex.

Second, we analyzed our data against normative data of typically developing children and adolescents and did not include a control group of children that we tested. However, this is the study reports on the first time point while the complete protocol envisions a longitudinal study of five years we strongly believe that what is important is the change over time of the individual patient. That's why our patients will be followed in a truly longitudinal way with an explicit neurodevelopmental focus. Each chronically ill child (and its family) develops its own idiosyncratic manner of coping, which change according to the developmental needs of the patient. Consequently, we aimed to detect what factors are predictive of the strengths and difficulties of each single patient. With respect to the group of patients included in this study so far, we did not observe an effect of treatment on EF, which is in contrast with the general notion that AED affect cognitive function. Most patients in our sample were in monotherapy with adequate seizure control, which may the reason why we did not find an effect of treatment on executive function also in light of recent findings reporting that polytherapy is deleterious to cognitive function [88].

Finally, other confounding factors related to this chronic disease may have altered the results of this study; however, medication, diagnosis, age of onset, and some psychosocial parameters did not substantially differ among the genotypes. We only had two MTHFR TT homozygous patients, and for them, a warning was issued not to use valproate acid as a treatment option, because of altered methylation and the resultant toxicity with respect to this drug.

5. Conclusions

The MTHFR gene stands out as the most significant gene in our sample because of a) its role in treatment-toxicity, b) its general role in methylation, and c) its interaction with the COMT gene where the presence of the T allele may cause hypomethylation of the COMT promoter, which increases COMT expression and synaptic dopamine breakdown.

Cognitive and behavioral comorbidities often emerge in children and adolescents with epilepsy [89–92]. These problems frequently represent an important factor in increasing the burden of disease and reducing quality of life [6,7], together with other patient and disease-related risk factors, such as, age and gender, the use of polytherapy, and the resistance to therapy. The presence or development of uneven cognitive and behavioral profiles over time [93] within the same patient and among patients with similar age of onset, gender, diagnosis, risk stratification, treatment, and psychosocial circumstances, cannot ignore the developmental history of the brain and organism of the pediatric patient in order to perform adequate, efficient follow-up. More importantly, cognitive and behavioral problems are frequently underdiagnosed, and consequently, they are not considered in the comprehensive care of the patient. Therefore, we should enhance the awareness with respect to these problems and implement regular and recurrent screening.

Executive functioning is importantly related with Health-Related Quality of Life (HRQoF) and the present and future burden of disease, as they relate to the impact of a disease and its treatment on the physical, cognitive, behavioral, emotional, and psychosocial functioning [15, 94]. Recently, it has been advocated that the care of children with newly diagnosed epilepsy should include treatments focused on all these different aspects, as soon as possible, after treatment has started with the scope to reduce EF problems and lessen the burden of disease by improving HRQoL [95,96]. Because EF changes substantially over time, and thus, differentially affects the trajectories of specific domains

of functioning, a better understanding of the factors that influence problematic functioning of some patients could help identify patients at risk and suggest critical periods when EF is most vulnerable. Identifying genetic factors as predictors linked to the development of EF deficits over the course of epilepsy could help in recognizing children at risk for EF deficits and in what domains.

Early more diffuse structural brain organization develops into more specific and segregated modules over time [97] with enhanced specialization in specific domains of function, especially in executive function and emotional control. Cognition, executive function, and emotional control are multidimensional psychological constructs driven by functional networks and domains that mature and improve substantially during development as structural networks emerge and become more separated. In children, with diagnosed epilepsy, disease as well as treatment often interferes with the maturation of the structural architecture function. As brain maturation is a highly dynamic and unfolding process, disease-related structural and functional toxicity needs to be monitored [98,99].

Domain-specific deficits in EF are contingent upon development, and their trajectory and significance of optimal functioning change over time and with respect to external and internal constraints [100, 101]. Domain specificity or lack thereof in children and adolescents with epilepsy is the emergent outcome of the dynamic interaction between the illness and its treatment on neurodevelopmental processes and the patients' sensitivity to context. This then warrants a domain-relevant context sensitive approach where specific neurocognitive endophenotypes together with allelic expression of EF-related genes would represent useful biomarkers and offer an additional tool to help predict early who is more susceptible to what particular cognitive problems and late effects, when, and what intervention would be most age-appropriate for the neurodevelopmental stage of the patient [100,101]. Such a multidisciplinary risk assessment incorporating neurodevelopment and genetic predisposition will help to capture possible problems in time and permit us to intervene preventively and organize their care and follow-up by providing developmentally appropriate interventions as early as possible.

Conflict of Interest

We report no conflict of interest.

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