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PSYCHIATRY AND PRECLINICAL PSYCHIATRIC STUDIES - ORIGINAL ARTICLE

Refining patterns of *MEF2C* effects in white matter microstructure and psychiatric features

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Abstract

Several GWAS reported Myocyte Enhancer Factor 2 C (*MEF2C)* gene associations with white matter microstructure and psychiatric disorders, and *MEF2C* involvement in pathways related to neuronal development suggests a common biological factor underlying these phenotypes. We aim to refine the *MEF2C* effects in the brain relying on an integrated analysis of white matter and psychiatric phenotypes in an extensively characterized sample. This study included 870 Brazilian adults (47% from an attention-deficit/hyperactivity disorder outpatient clinic) assessed through standardized psychiatric interviews, 139 of which underwent a magnetic resonance imaging scan. We evaluated variants in the *MEF2C* region using two approaches: 1) a *gene-wide analysis*, which uses the sum of polymorphism effects, and 2) *SNP analyses*, restricted to the independent variants within the gene. The outcomes included psychiatric phenotypes and fractional anisotropy for brain images. Results: The gene-wide analyses pointed to a nominal association between *MEF2C* and the Temporal Portion of the Superior Longitudinal Fasciculus (SLFTEMP). The SNP analysis identified four independent variants significantly associated with SLFTEMP and one (rs4218438) with Substance Use Disorder. Our findings showing specific associations of *MEF2C* variants with temporal−frontal circuitry components may help to elucidate how the *MEF2C* gene underlies a broad range of psychiatric phenotypes since these regions are relevant to executive and cognitive functions.

Keywords *MEF2C* · Fractional anisotropy · Neuroimaging · MRI · ADHD

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Introduction

The white matter (WM) comprises the connective segment of the brain, relating cortical and subcortical regions (Le Bihan et al. 2001). Microstructural characteristics of WM can be inferred by Diffusion Tensor Imaging (DTI) data through water diffusivity properties in different neural tissues (Basser 1995). One of the most common DTI measures is Fractional Anisotropy (FA), a global value regarding the water direction in WM fibers. FA varies from zero (fully isotropic) to one (fully anisotropic), allowing inferences on WM orientation and integrity (Mori et al. 2007). FA measures are altered in a series of psychiatric disorders, such as Generalized Anxiety Disorder (GAD) (Wang et al. 2016), Major Depression Disorder (MDD) (Barbu et al. 2019; Bergamino et al. 2016), and Attention-Deficit/Hyperactivity Disorder (ADHD) (Mufford et al. 2017).

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WM has an average SNP heritability of 47.6% across all tracts (Zhao et al. 2021). The Myocyte Enhancer Factor 2C (*MEF2C)* gene, located in the 5q14.3 region, is among the GWAS hits reported for WM integrity (Zhao et al. 2021). *MEF2C* has also been associated through GWAS with several psychiatric and cognitive genetically correlated outcomes (P. H. Lee et al. 2019), including ADHD (Demontis et al. 2019a), Schizophrenia (SCZ) (Huo et al. 2019), MDD (Howard et al. 2019), Educational Attainment (Okbay et al. 2016), and with substance use behaviors (i.e., drinks per week and being ever smoker) (Karlsson Linnér et al. 2019). Microdeletions and point mutations in the *MEF2C* gene region are suggested as the cause of a severe neurodevelopmental disorder (*MEF2C* haploinsufficiency syndrome) characterized by intellectual disability with hypotonia, stereotypic hand movements, and impaired language (Le Meur et al. 2010). The most frequent cerebral alterations in the affected patients involve WM measures and myelination delay (Rocha et al. 2016).

MEF2C is part of a gene family of enhancers, the MEF2s, and part of a large transcription factors family, the MADS-BOX genes. MEF2s have a broad role in development and tissue differentiation, acting in muscular and neural crest cells, endothelium, chondrocytes, neurons, and lymphocyte development (Potthoff and Olson 2007). Moreover, *MEF2C* interacts with a wide variety of development-related proteins, and 16% of these interactions are with proteins relevant to neuronal development (Dong et al. 2017).

Altogether, these several independent above-mentioned GWAS pointing *MEF2C* associations with white matter microstructure and psychiatric features, together with its critical role in neurodevelopment, pose this gene as an important player for neuroimaging genomics in psychiatry. The mounting GWAS results demand increasing efforts to refine phenotypes architecture considering fundamental genetic and neurobiological data (Sullivan and Geschwind 2019). Therefore, this study aimed to narrow down the *MEF2C* association patterns in an integrated analysis of white matter and psychiatric phenotypes in an extensively characterized sample of subjects with ADHD and healthy controls.

Methods and materials

Sample

Out of the 870 (mean age: 31.3 years; males: 50.3%) adult volunteers studied, 407 were recruited in the ADHD Outpatient Program, adult division (ProDAH-A) from Hospital de Clínicas de Porto Alegre (HCPA), and 463 are healthy volunteers recruited in the same institution. The diagnosis of ADHD followed DSM-IV criteria (American Psychiatric Association, 1996) from 2001 to 2012 and DSM-5 criteria (APA 2013) from 2013 onwards. ADHD diagnosis was performed using the Portuguese version of the Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS-E(Mercadante et al. 1995); adapted for adults (Grevet et al. 2005). Other psychiatric comorbidities were evaluated through the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al. 1998), from 2001 to 2012, an adapted version of SCID from 2012 to 2015, and the SCID-5(First et al. 2015) from 2015 onwards. The exclusion criteria were evidence of clinically significant neurological disease (e.g., delirium, dementia, epilepsy, head trauma) and intelligence quotient (IQ) ≤ 70. The remaining sample comprised 463 subjects (mean age: 29.37 years; males: 47.9%) with negative screening for ADHD, assessed by the 6-item Adult ADHD Self-Rated Scale Screener (ASRS) (Kessler 2005). In addition, psychiatric disorders were evaluated through the screening module of SCID-IV (First et al. 1998) from 2001 to 2015 and SCID-5(First et al. 2015) after 2015, covering anxiety, mood, psychosis, substance use, and eating disorders. All participants signed an informed consent form and the protocol approved by the hospital ethical committee (IRB 0000921).

Genotyping

DNA was extracted from peripheral blood from all subjects using *salting out* method (Lahiri and Nurnberger 1991). The variants were genotyped through the Illumina Infinium PsychArray-24 v1.1. Pre-imputation quality control at individual and SNP levels and principal component analyses for ancestry genetic outlier detection was performed through the *Rapid Imputation and COmputational PIpeLIne* (Ricopili). Phasing of genotype data and imputation were performed using SHAPEIT2 and IMPUTE2 algorithms, respectively, considering as reference the European ancestry panels of the 1000 Genomes Project Phase 1 version 3 (v3) (April 2012) from the genome build hg19.

Magnetic Resonance Imaging (MRI) scan

As part of a longitudinal cohort evaluated at ProDAH-A (Guimarães-da-Silva et al. 2018; Karam et al. 2015, 2017), a subsample including subjects with ($n = 85$, mean age: 34.3 years, males 43.5%) and without ADHD (n = 54, mean age: 29.3 years, males: 61.1%) was reassessed (Grevet et al. 2005) and scanned in a 3 T Siemens Spectra MRI scanner with a 16-channel head coil. The diffusion-weighted imaging acquisition protocol applied a single-shot echo planar imaging sequence (62 contiguous axial slices, TE = 110 ms, TR = 11000 ms, voxel size = 2 × 2x2mm, slice thickness = 2.0 mm, FOV = 240 mm, one b0 image and 64 diffusion-weighted images with gradient directions b = 1400 s/mm^2). An adapted protocol with reduced acquisition time (with differences in the number of diffusion-weighted images = 32, TE = 106 ms, voxel size = 2.4 × 2.4x2.4 mm,

and slice thickness = 2.4 mm) was applied for the restless or claustrophobic individuals ($n = 10$ cases and 15 controls).

The motion and eddy currents were preprocessed, and the correction was performed using FMRIB Software Library (FSL) tools (https://fsl.fmrib.ox.ac.uk/fsl/; Woolrich et al., 2009), with posterior visual quality control. We generated a whole-brain FA map per each individual through *drift*. All their maps were registered, and skeleton created using tract-based spatial statisticsIit (TBSS). Mean FA values within the TBSS skeleton were extracted for the whole brain and 11 tracts (anterior thalamic radiation–ATR, corticospinal tract–CST, dorsal cingulate gyrus–CING, ventral cingulate gyrus–HIPPCING, forceps minor, forceps major, inferior fronto-occipital fasciculus–IFOF, inferior longitudinal fasciculus–ILF, superior longitudinal fasciculus–SLF, uncinate fasciculus–UF, temporal part of SLF–SLFTEMP) extracted according to the John Hopkins University white matter tractography atlas(Mori et al. 2007; Wakana et al. 2007) (https://identifiers.org/neurovault.image:1403).

Statistical analysis

Gene-wide analysis

We extracted the *MEF2C* gene region plus a window of 10kb upstream and 10kb downstream (GRCh37 genomic positions 5:88,004,058 to 5:88,209,922), comprising 97 variants using the *-make-set* command in PLINK v1.9(Chang et al. 2015). We tested these variants for association with FA of the eleven WM tracts, plus the average whole brain in a gene-wide analysis using the *--set-based* command, under the additive model, with 10,000 permutations for the calculation of the empirical p-value ($p<0.05$), adjusted for sex, age, ten first principal components, head motion, and ADHD diagnosis. This analysis besides the association of the whole gene calculated as the mean of a single SNP statistic, it also retrieves the independent variants presenting the lowest p-values on the outcome. To verify if ADHD diagnosis or dimensional severity scores impacted the associations of FA measures of the whole brain and the eleven tracts mentioned above, we used linear regression models, adjusted for sex, age, and head motion in SPSS v18 (SPSS Inc.).

SNP analysis

Hereafter, we tested the effects of the independent variants associated with WM (retrieved in the *gene-wide* analysis) on psychiatric phenotypes previously associated with *MEF2C* (i.e. (BD (Ruderfer et al. 2014), GAD(A. Watanabe et al. 2018), MDD (Howard et al. 2019) and SUD (Karlsson Linnér et al. 2019) within the subjects with ADHD sample ($n = 407$), for which a reasonable sample size for comorbidities was present, using the additive genetic model within the *–assoc* command in PLINK 1.9 software(Chang et al. 2015). This analysis was adjusted for sex, age, and the first ten principal components. Covariates were included according to their association ($p \leq 0.2$) with predictor and outcome (Maldonado and Greenland 1993), or its clinical relevance. False discovery rate (FDR) was applied to correct for multiple tests.

In silico analysis

We also evaluated the *MEF2C* independent variants through in silico analyses to explore possible regulatory functions. We used the following tools: GWASatlas (https://atlas.ctglab.nl/)(K. Watanabe et al. 2019) to assess associations with neurological and psychiatric features, Variant Effect Prediction (VEP), to predict functional effects of the variants (Yates et al. 2016); HaploReg v4.1 (Ward and Kellis 2012), to examine noncoding genome annotations of disease-associated loci by GWAS (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php)

RegulomeDB (Boyle et al. 2012), to evaluate the SNPs with known and predicted regulatory elements in the intergenic regions of the genome This *in silico* analysis also comprised the variants in high LD ($R^2>0.8$) with the three independent variants previously associated in the gene-wide analyses, based on the LD matrix tool (Machiela and Chanock 2015) and the CEU reference panel (Gao et al. 2012).

Results

Clinical and neuroimaging samples are characterized in Table 1. The workflow of the study is summarized in Fig. 1.

Considering that FA measures of the whole brain and the eleven white matter tracts evaluated were not associated with ADHD status or severity (Supplementary Tables 1 and 2), we performed *gene-wide* analyses between *MEF2C* and the white matter tracts pooling the whole neuroimaging sample (n=139), controlling for ADHD status. The results pointed to a nominal association between *MEF2C* and FA measures of the SLFTEMP (Table 2). In the *SNP analysis*, thirty-six variants were associated with this tract, four of which are independent: chr5:88083991, rs244756, rs4518438, and rs10075941 (Table 3). These associations are detailed in Supplementary Table 3. In the *SNP analysis* assessing ADHD comorbidities (n = 407), we found that SUD was associated with one of the tested polymorphisms (rs4518438) (Table 3).

The results of our in silico analysis are compiled in Table 4. We found 41 variants in strong LD with the independent variants associated with SLFTEMP (n =3),

Table 1 Sample characteristics

Neuroimaging subsample	Subjects with ADHD ($n=85$)		Healthy volunteers ($n=54$)		p
	n	%	n	%	
Gender (male)	37	43.5	33	61.1	0.15
Age (years)[a]	46.6	9.7	38.2	8.9	0.09
Lifetime comorbidities					
Generalized anxiety disorder	63	74.1	7	13	< 0.01
Bipolar disorder	31	36.5	–		< 0.01
Major depressive disorder	37	43.5	22	40.7	0.7
Substance use disorder	29	34.1	5	9.2	< 0.01
Total sample	Subjects with ADHD ($n=407$)		Healthy volunteers ($n=463$)		p
	n	%	N	%	
Gender (male)	216	53	222	47.9	0.1
Age (years)[a]	33.6	10.8	29.4	8.7	< 0.01
Comorbidities lifetime					
Generalized anxiety disorder	154	37.8	63	13.6	< 0.01
Bipolar disorder	94	23.1	16	3.4	< 0.01
Major depressive disorder	166	40.8	141	30.4	< 0.01
Substance use disorder	98	24	22	4.7	< 0.01

N is the number of subjects and % the percentage considering the whole sample

[a]n is the mean years and % the standard deviation

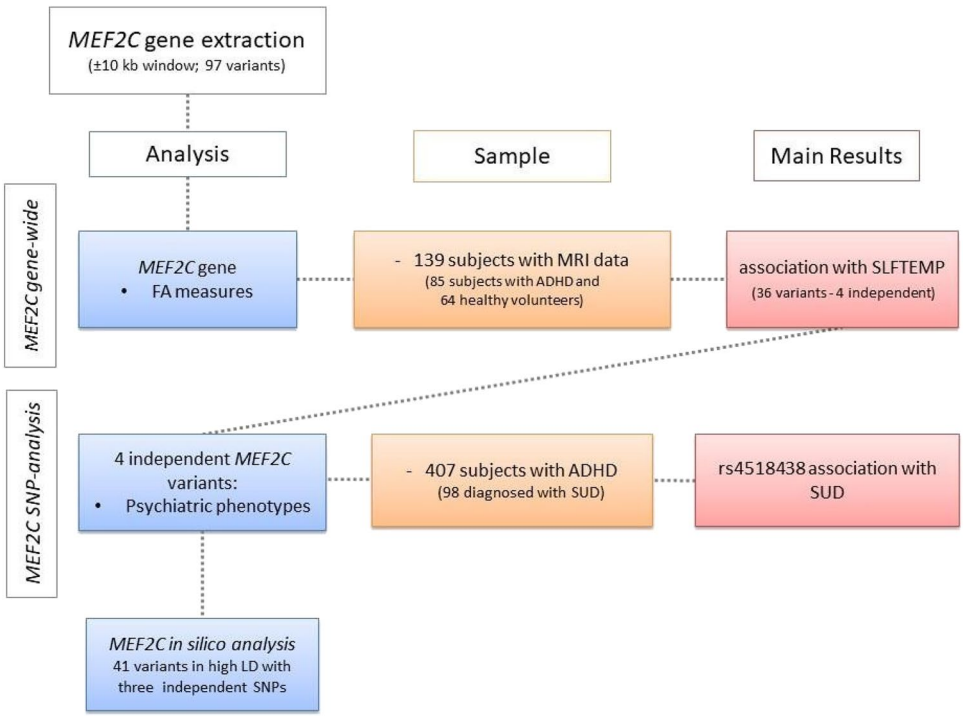

Fig. 1 Analyses flow for MEF2C gene and outcome measures. The gene-wide analysis evaluated FA measures in the subsample of subjects with MRI data resulting in an association of 36 MEF2C variants with SLFTEMP, four of which are independent. These independent variants were further explored in a SNP analysis including ADHD comorbidities evaluated in our sample of subjects with ADHD, resulting in one association with substance use disorder (SUD). In addition, three of the four independent variants and 41 additional variants in high LD were further investigated regarding their regulatory potential in an in silico analysis

excluding the chr5:88083991 deletion, because of missing data in the search platforms. After that, we selected only those variants with a RegulomeDB Score superior to 0.6 (meaning a high probability to be a regulatory variant), totalizing 11 variants. All included variants are suggested to have a functional role in several tissues' differentiation,

Table 2 Gene-wide effects of *MEF2C* (total of 97 variants) on Attention-Deficit/Hyperactivity Disorder status and on Fractional Anisotropy measures of the 11 White Matter tracts and the average measure of the whole brain

	N_{SNP}	N_{SIG}	P_{-emp}	P_{-FDR}
Inferior fronto-occipital fasciculus	97	–	1	1
Inferior longitudinal fasciculus	97	3	0.3163	0.4744
Superior longitudinal fasciculus	97	41	0.1444	0.3466
Uncinate fasciculus	97	24	0.0596	0.3466
Temporal portion of SLF	97	36	**0.0271**	0.32520
Anterior thalamic radiation	97	–	1	1
Corticospinal tract	97	–	1	1
Dorsal cingulate gyrus	97	30	0.1286	0.3466
Ventral cingulate gyrus	97	–	1	1
Forceps major	97	35	0.1739	0.3478
Forceps minor	97	16	0.2562	0.4392
Average fractional anisotropy*	97	19	0.0972	0.3466

N_{SNP} are the number of SNPs in the *MEF2C* gene region. N_{SIG} is the number of significantly associated SNPs with the outcome. P-emp stands for the P-empirical value after 10,000 permutations. P_{-FDR} stands for the p association value after FDR correction FA analysis adjusted for: ADHD diagnosis, sex, age, head motion, and the ten first principal components. analysis adjusted for: sex, age, headmotion, average fractional anisotropy and the ten first principal components*Average FA was only adjusted for: sex, age, headmotion and the ten first principal components. Abbreviations: SFL: Superior Longitudinal Fasciculus

development, and function, besides being associated with neuropsychiatric and cognitive domains by GWAS. The rs4518438, which was also associated with SUD, has chromatin enhancer marks in different brain tissues, including temporal and frontal regions, in addition to changing motifs of the Pou2f2 transcription factor.

Discussion

This is the first study to simultaneously address associations relating *MEF2C*, WM microstructure, and psychiatric phenotypes, and our findings are in line with the previous suggestions that *MEF2C* effects on psychiatric phenotypes might involve alterations in the WM microstructure. Our results are conspicuously supported by *MEF2C* genome-wide associations with DTI measures (Zhao et al. 2021) as well as previous findings relating this gene to substance use behaviors (Karlsson Linnér et al. 2019). The *MEF2C* variants associated with SLFTEMP and SUD are also implicated in WM integrity in other related tracts by GWAS. Our in silico analysis predicted that most of these variants have a role in neuronal function and developmental pathways, by disrupting binding sites to important transcript factors

Table 3 SNP-analysis results for the four independent *MEF2C* variants associated to the temporal portion of the Superior Longitudinal Fasciculus (SLFTEMP) Fractional Anisotropy measures ($n = 139$) and ADHD comorbidities ($n = 407$)

	SLFTEMP			GAD			MDD			BD			SUD		
	Beta	CI 95%	p_{-FDR}	OR	CI 95%	p_{-FDR}	OR	CI 95%	p_{-FDR}	OR	CI 95%	p_{-FDR}	OR	CI 95%	p_{-FDR}
chr5_88083991_D	0.015	(− 0.03– 0.001)	0.1075	0.95	(0.63 1.46)	0.8480	0.83	(0.55 1.27)	0.3400	1.42	(0.88 2.28)	0.2820	0.58	(0.34 0.98)	0.1075
rs244756_T	0.019	(− 0.03– 0.009)	**0.0200**	0.90	(0.65 1.25)	0.6074	0.67	(0.48 0.93)	0.0680	1.23	(0.85 1.80)	0.3800	0.62	(0.41 0.91)	0.0680
rs4518438_C	0.011	(0.001 0.02)	0.1075	1.09	(0.80 1.49)	0.6074	1.13	(0.84 1.54)	0.5493	0.74	(0.52 1.07)	0.2422	1.72	(1.18 2.50)	**0.0266**
rs10075941_T	0.018	(− 0.03– 0.006)	**0.0266**	0.85	(0.57 1.28)	0.5600	0.78	(0.51 1.16)	0.3400	0.87	(0.54 1.40)	0.6074	0.73	(0.45 1.18)	0.3400

Beta (CI 95%) stands for Beta of linear regression with a confidence interval of 95%, SLFTEMP SNP analysis adjusted for: ADHD diagnosis, sex, age, headmotion, and the ten first principal components. OR (CI 95%) stands for odds ratio with a confidence interval of 95% P_{-FDR} stands for the *p* value after the FDR correction *GAD* Generalized Anxiety Disorder, *MDD* Major Depression Disorder *BP* Bipolar Disease, *SUD* Substance Use Disorder, Comorbidities SNP analysis adjusted for: sex, age, and ten first principal components

Table 4 SNP analysis with SLFTEMP and in silico analyses of the four independent variants and their SNP LD pairs (r2 > 0.8) with RegulomeDB score > 0.6

Variants*	Location (hg19)	LD	A1	SLFTEMP		HaploReg				Regulome DB		GTEx–eQTL	GWAS atlas
				Beta	*P*	H3K4me1*	H3K4me3*	H3K27ac*	Motifs changed	Motifs	Score		PheWas
rs244756	5:88,098,064	Index	T	− 0.01882	0.00034						0.18		EA; SFOF; IFOF
rs6872000	5:88,070,416	1.0[1]	T	− 0.01672	0.00154					GATA2, GATA3, TCF7, TCF7L1	0.62		EA; SFOF; IFOF
rs10942537	5:88,077,448	1.0[1]	G	− 0.01682	0.00146					SRF	0.94		EA; SFOF; IFOF
rs770189	5:88,088,439	1.0[1]	G	− 0.01682	0.00146					BRCA1, USF2	0.78		EA; SFOF; IFOF
rs244755	5:88,095,785	1.0[1]	C	− 0.01682	0.00146					OBOX6	0.78		EA; SFOF; IFOF
rs700594	5:88,101,142	1.0[1]	G	− 0.01682	0.00146						0.60		EA; SFOF; IFOF
rs700593	5:88,101,150	1.0[1]	T	− 0.01678	0.00129						0.60		EA; IFOF
rs4518438	5:88,157,552	Index	C	0.01103	0.03551	Hippocampus; Cingulate Gyrus; Angular Gyrus; Dorsolateral PFC; Fetal Brain	Dorsolateral PFC	Cingulate Gyrus; Inferior Temporal Lobe; Angular Gyrus; Dorsolateral PFC	Pou2f2		0.18	Thyroid	Verbal-numeric reasoning;Intelligence; EA; Cognitive Performance
rs10075941	5:88,206,889	Index	T	− 0.01837	0.00451				NRSF,ZBRK1		0.18		SCZ; EA
rs10044342	5:88,178,683	0.91[3]	C	− 0.01733	0.00826						0.60		SCZ; EA
rs80043958	5:88,179,575	0.95[3]	G	− 0.01682	0.00941					SP4	0.63		SZC; EA

H3K4me1; H3K4me3, H3K27ac: Enhancer histone marks in this cell types, Motifs: The SNPs evaluated binds to the referred transcripts, RegulomeDB score: is ranging from 0 to 1, with 1 being most likely to be a regulatory variant, PheWas: *EA* Educational Attainment, *SFOF* Superior Fronto-Occipital Fasciculus, *IFOF* Inferior Fronto-Occipital Fasciculus, *SCZ* Schizophrenia, all included studies have a *p* association value < 5.10^{-4} [1] LD score with rs244756, [3]LD score with rs10075941

(TFs) involved in embryogenesis, cell development, and differentiation.

SLF is a bidirectional association fiber tract connecting the parietal, occipital, and temporal lobes with frontal cortices (Schmahmann et al. 2007). Its temporal portion SLFTEMP is directly involved in processes related to human cognition, such as attention, language, memory, and emotions (Kamali et al. 2014), and therefore it is a relevant structure to executive and cognitive functions. Besides that, SLFTEMP partially overlaps with SFOF (superior fronto-occipital fasciculus), which has been associated with *MEF2C* variants through GWAS (Zhao et al. 2021). SLFTEMP has been implicated in psychiatric phenotypes also associated with *MEF2C,* such as MDD (van Velzen et al. 2020), SUD (Hampton et al. 2019), Bipolar Disorder (Ching et al. 2020), and SCZ (Kelly et al. 2018).

Among the four independent variants found associated with SLFTEMP in the *gene-wide* analysis, the rs4518438 was also associated with risk for SUD. This SNP has promoter and enhancer features in epigenetic marks present in brain tissues, some of which co-localized with SLFTEMP, supporting its role in this structure. This SNP has been also associated with intelligence (Savage et al. 2018), cognitive performance (Savage et al. 2018), and educational attainment (J. J. Lee et al. 2018), and has eQTL data in thyroid tissue (Westra et al. 2013), suggesting a broad influence in neuroendocrine and neuropsychiatric domains.

The *MEF2C* gene has binding sites to different proteins, DNA dimerization, and chromatin associated elements (Shalizi and Bonni 2005). The in silico analysis of the associated SNPs showed enriched TFs related to glial cells (GATA3), Wnt (TCF7), and MAPK (SRF) signaling pathways, and the same variants were also associated with FA measures of the SFOF and IFOF tracts by the previous GWAS (Zhao et al. 2021; 2019), which are closely related to SLFTEMP. The Wnt and MAPK signaling pathways are hypothesized to be related to ADHD (Ohki et al. 2020), and multimorbid psychiatric disorders (Boyle et al. 2012), respectively. Accordingly, Zhao et al. 2021 observed that FA measures are more related to glial cells than neurons. *MEF2C* effects in the microglia are demonstrated both by animal studies showing that the Mef2c protein is related to resilience in pro-inflammatory stimuli in microglia (Deczkowska et al. 2017) and by human studies associating *MEF2C* haploinsufficiency syndrome with deficits in white matter integrity and myelination (Rocha et al. 2016; Lesch 2019). Taken together our in silico results and the literature, we suggest that the association between *MEF2C* variants, WM microstructure, and its possible effect in psychiatric disorders might be related to these molecular pathways.

The lack of difference between FA measures in subjects with and without ADHD reported here is in line with another recent DTI study with a larger sample of 654 subjects, being 258 ADHD cases (Damatac et al. 2020). We also did not observe any alterations in FA measures concerning the ADHD dimensional scores. Previous meta-analysis pointed to inconsistent results relating DTI measures and ADHD (Sáenz et al. 2019), which is not unexpected considering the highly heterogeneous nature of this disorder.

Despite ADHD GWAS findings indicating the *MEF2C* region as a significant hit (Demontis et al. 2019; 2022), we did not find any association between *MEF2C* and the symptoms of ADHD or its diagnosis. These differences can be attributable to the highly heterogeneous presentation of the disorder, with underlying distinct functional deficits that converge in the diagnosis. A GWAS with a substantial representation of adult samples(Rovira et al. 2019), showed differences in the genetic findings of affected adults and children, even with a high level of genetic correlation among both. Interestingly, in this same GWAS, *MEF2C* was not a significant hit. As *MEF2C* was a significant hit in GWAS of other psychiatric disorders presenting high genetic correlation with ADHD, as MDD (Howard et al. 2019), and SCZ (Ripke et al. 2014), some specific association with ADHD could also be ascribed to a shared underlying trait among these disorders. The overall scenario, where our sample is composed of adult patients with high schooling levels and frequent comorbidity (Karam et al. 2015), could possibly explain the nominal (but not significant) association with *MEF2C*.

This study must be seen in light of some limitations. First, as usual in neuroimaging genetics studies, unfortunately, the sample size might be limiting the identification of additional associations. However, we adopted the gene-wide strategy to optimize statistical power, narrowing the analysis to few independent SNPs. Second, we analyzed complex and multifactorial features that are all deeply related to each other, hindering the establishment of causal effects. Finally, the brain parcellation used to identify the WM tracts varies across studies, hindering possible comparisons. Regarding strengths, our study bears the ability to provide an integrated analysis of several phenotypes previously related to *MEF2C*.

The overall pattern of our findings supports the view that *MEF2C* effects on psychiatric phenotypes could involve alterations in the WM microstructure. Further studies are needed to understand better how *MEF2C*, psychiatric disorders, and white matter microstructure are interconnected.

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Author contributions MEAT and RBC designed the study, performed the data analysis, and prepared the first drafts of the manuscript. CEB, MEAT, and RBC collected and processed the neuroimaging data, providing essential contributions to these analyses. ESV, BSS, FAP, and DLR provided substantial contributions to data acquisition, analyses, and interpretation of the results. CAIS and ESV were responsible for the clinical assessment of the patients and helped with the evaluation of the clinical outcomes. RSS contributed to the manuscript preparation and editing. DLR, LAR, and EHG provided critical discussion and insights into the intellectual content of the manuscript. CHDB contributed to the conception and design of the study and participated in all its stages of its preparation. All authors carefully revised and approved the final version of this manuscript.

Data availability Our data is available under request.

Declarations

Conflict of interest The authors declare the following potential conflict of interest: Dr. Grevet was on the speaker's bureau for Novartis and Shire for three years and received travel awards (air tickets and hotel accommodations) for participating in two psychiatric meetings from Shire and Novartis. Luis Augusto Rohde has received grant or research support from, served as a consultant to, and served on the speakers' bureau of Aché, Bial, Medice, Novartis/Sandoz, Pfizer/Upjohn, and Shire/Takeda in the last three years. The ADHD and Juvenile Bipolar Disorder Outpatient Programs chaired by Dr Rohde have received unrestricted educational and research support from the following pharmaceutical companies in the last three years: Novartis/Sandoz and Shire/Takeda. Dr. Rohde has received authorship royalties from Oxford Press and ArtMedAll other authors declare that they have no conflict of interest.

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