

ORIGINAL ARTICLE

Association of *BDNF*, *HTR2A*, *TPH1*, *SLC6A4*, and *COMT* polymorphisms with tDCS and escitalopram efficacy: ancillary analysis of a double-blind, placebo-controlled trial

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Objective: We investigated whether single nucleotide polymorphisms (SNPs) associated with neuroplasticity and activity of monoamine neurotransmitters, such as the brain-derived neurotrophic factor (*BDNF*, rs6265), the serotonin transporter (*SLC6A4*, rs25531), the tryptophan hydroxylase 1 (*TPH1*, rs1800532), the 5-hydroxytryptamine receptor 2A (*HTR2A*, rs6311, rs6313, rs7997012), and the catechol-O-methyltransferase (*COMT*, rs4680) genes, are associated with efficacy of transcranial direct current stimulation (tDCS) in major depression.

Methods: Data from the Escitalopram vs. Electrical Current Therapy for Treating Depression Clinical Study (ELECT-TDCS) were used. Participants were antidepressant-free at baseline and presented with an acute, moderate-to-severe unipolar depressive episode. They were randomized to receive escitalopram/tDCS-sham (n=75), tDCS/placebo-pill (n=75), or placebo-pill/sham-tDCS (n=45). General linear models assessed the interaction between treatment group and allele-wise carriers. Additional analyses were performed for each group and each genotype separately.

Results: Pairwise group comparisons (tDCS vs. placebo, tDCS vs. escitalopram, and escitalopram vs. placebo) did not identify alleles associated with depression improvement. In addition, exploratory analyses also did not identify any SNP unequivocally associated with improvement of depression in any treatment group.

Conclusion: Larger, combined datasets are necessary to identify candidate genes for tDCS response.

Keywords: Major depressive disorder; non-invasive brain stimulation; single-nucleotide polymorphism; selective serotonin reuptake inhibitors; randomized clinical trial

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation intervention that consists of the application of low-intensity electric currents over the scalp to modify brain activity and excitability according to the parameters selected for stimulation.¹⁻³ For major depressive disorder (MDD), tDCS is applied over the dorsolateral prefrontal cortex (DLPFC), a key hub of the frontoparietal network, which regulates several cognitive functions and is hypoactive in depression.⁴ Although the exact antidepressant mechanisms of tDCS remain unclear, it is

speculated that, by stimulating the DLPFC, tDCS would increase the activity of the frontoparietal network, consequently leading to an improvement of depressive symptoms.⁵

Clinically, tDCS excels in safety and tolerability^{6,7} and can even be used at home.⁸ Nonetheless, tDCS has produced mixed outcomes in terms of antidepressant efficacy.⁹⁻¹² This may reflect a heterogeneous likelihood of response across participants. Therefore, identifying predictors of response may provide useful insights into tDCS, such as clarifying its mechanisms of action, predicting treatment outcomes, and designing better-tailored

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Submitted Jul 22 2019, accepted Aug 20 2019, Epub Nov 11 2019.

How to cite this article: Brunoni AR, Carracedo A, Amigo OM, Pellicer AL, Talib L, Carvalho AF, et al. Association of *BDNF*, *HTR2A*, *TPH1*, *SLC6A4*, and *COMT* polymorphisms with tDCS and escitalopram efficacy: ancillary analysis of a double-blind, placebo-controlled trial. Braz J Psychiatry. 2020;42:128-135. <http://dx.doi.org/10.1590/1516-4446-2019-0620>

trials within the framework of precision psychiatry.^{2,13,14} In this context, the investigation of genomic variants, such as common single nucleotide polymorphisms (SNPs), has been considered a promising avenue to tailor antidepressant strategies.¹⁵⁻¹⁷ Furthermore, compared to other biological markers such as magnetic resonance imaging (MRI) and electroencephalography (EEG), SNPs are relatively more available and affordable to collect and analyse.¹⁸

In the present study, we investigated whether specific SNPs involved in MDD pathophysiology were associated with tDCS response. According to our study protocol,¹⁹ the following genes and SNPs associated with treatment response in MDD, neuroplasticity, and serotonin metabolism were investigated:

- a) brain-derived neurotrophic factor (*BDNF*, rs6265, chromosome 11p13): the *BDNF* gene was chosen because the factor it encodes plays a key role in synaptic plasticity, depression and antidepressant response.²⁰ The most frequent *BDNF* genetic variation is the 196G/A (rs6265) SNP, which causes a change from Val to Met at the 5'-pro protein site. This polymorphism disrupts cellular trafficking, processing, and *BDNF* secretion,²¹ and impairs synaptic transmission and cortical plasticity.²² Although we did not observe an influence of rs6265 on tDCS response in an earlier study,²³ our analyses might have been underpowered.
- b) solute carrier family 6 member 4 (*SLC6A4*, rs25531, chromosome 17q11.1-q12): *SLC6A4* codifies the presynaptic serotonin reuptake transporter (SERT) and is characterized by a functional 44-bp insertion/deletion polymorphism (5HTTLPR) in its promoter region.²⁴ This SNP was chosen because it has been consistently associated with selective serotonin reuptake inhibitor (SSRI) antidepressant response²⁵;
- c) tryptophan hydroxylase 1 (*TPH1*, rs1800532, chromosome 11p15.3-p14) polymorphism: the TPH enzyme metabolizes L-tryptophan to 5-HTP, which is then metabolized to serotonin by the enzyme 5-HTP decarboxylase. TPH regulates the activity of this metabolic pathway and, therefore, the availability of serotonin. Although the TPH1 isoform is less expressed in the brain than TPH2, the *TPH1* gene has been particularly associated with antidepressant effects¹⁷;
- d) 5-hydroxytryptamine receptor 2A (*HTR2A*, rs6311, rs6313, rs7997012, chromosome 13q14-q21) genes: this G protein-coupled receptor triggers long-term, intracellular effects when activated and therefore plays a critical role in the serotonergic system.²⁵ This SNP has been associated with depressive behaviors (including attempted suicide)²⁶ and antidepressant response.²⁷

Finally, we conducted a post-hoc investigation of the impact of the catechol-O-methyltransferase (*COMT*) Val66met polymorphism (rs4680), as later studies investigated the role of this SNP on the effects of tDCS²⁸ and antidepressant agents.²⁹ Biologically, *COMT* enzymes degrade catecholamines, such as dopamine and (nor) epinephrine, and are associated with cognitive functions related to prefrontal cortex activity³⁰ (Table 1).

Methods

Trial design

This is an ancillary study of the Escitalopram versus Electrical Current Therapy for Treating Depression Clinical Study (ELECT-TDCS), a non-inferiority, placebo-controlled trial in which the efficacy of tDCS, escitalopram, and placebo were evaluated.^{19,31} The ELECT-TDCS trial (clinicaltrials.gov number NCT01894815)^{19,31} took place from 2013 to 2016 at the Universidade de São Paulo, the capital of the state of São Paulo, Brazil. Participants were randomized to receive escitalopram-pill/sham-tDCS (henceforth, escitalopram), placebo-pill/active-tDCS (tDCS), or placebo-pill/sham-tDCS (placebo) in a 3:3:2 ratio.

ELECT-TDCS was designed to demonstrate noninferiority of escitalopram vs. tDCS. Specifically, noninferiority would be proven if the improvement in the tDCS vs. placebo groups was at least 50% of the improvement achieved in the escitalopram vs. placebo groups at the primary endpoint (week 10) on our primary outcome scale, the Hamilton Depression Rating Scale, 17 items (HDRS-17). Briefly, the original study showed a mean (standard deviation [SD]) depression improvement in HDRS-17 of 11.3 (6.5) for escitalopram, 9 (7.1) for tDCS, and 5.8 (7.9) for placebo. The main findings showed that tDCS was not noninferior to escitalopram, as the lower boundary of the confidence interval for the difference in the decrease of tDCS vs. escitalopram, a difference of -2.3 (95% confidence interval [95%CI] -4.3 to -0.4), was lower than the noninferiority margin of -2.75. Secondary analyses showed that escitalopram and tDCS were both superior to placebo, and confirmed that escitalopram was superior to tDCS. Moreover, moderator analyses did not identify any clinical or demographic predictor associated with treatment response for any intervention group.

In ELECT-TDCS, we additionally investigated several biological markers that could be associated with clinical depression outcomes, such as plasma biomarkers and motor cortical excitability.^{31,32} Here, we report data from the participants who finished the study and had at least one blood sample collected at baseline for genotyping.

Subjects

Participants with unipolar depression aged 18 to 75 years were included. Diagnoses were established by certified psychiatrists using the DSM-5 criteria, according to the Mini-International Neuropsychiatric Interview (MINI).³³ Only those participants who scored ≥ 17 points on the HDRS-17 and had low suicidal risk per the MINI were included.

The exclusion criteria were bipolar disorder, substance abuse/dependence, any history of psychotic disorder, current suicidal ideation, personality disorders, neurological diseases, and severe or unstable clinical conditions. In fact, only anxiety disorders were allowed as a comorbidity. Patients who had any contraindications to tDCS or escitalopram, previous non-response to escitalopram, pregnancy, breastfeeding, or use of escitalopram in the

current major depressive episode were not included. Participants either were not using antidepressants or were required to undergo an antidepressant drug wash-out of at least five drug half-lives. Benzodiazepines were allowed at a maximum dose of 20 mg/day of diazepam or equivalent. Drug doses remained constant during the study.

Procedures

Clinical assessments were performed by board-certified psychiatrists and psychologists at specified time points according to the original protocol.¹⁹

For brain stimulation, we used tDCS-CT devices (Soterix Medical, New York, USA). The anode and cathode electrodes were inserted in saline-soaked, 5 × 5 cm² sponges and then positioned using specific headgear to target the left and right dorsolateral prefrontal cortices.³⁴ The protocol consisted of 22 sessions lasting 30 minutes each (15 sessions in the first 3 weeks, from Monday to Friday, and then weekly until the endpoint) with 2 mA intensity. For sham tDCS, the same protocol was used but the current was turned off automatically after 30 seconds, according to the configuration of the device. Trained nurses blinded to group assignment administered the tDCS regimen.

For drug treatment, escitalopram (or placebo) was given at 10 mg/day in the first 3 weeks and 20 mg/day thereafter until week 10. Placebo pills had the same taste, shape, and color of the *verum* pills. Both escitalopram and placebo pills were placed in drug bottles that contained only a numeric code prepared by a third person not involved in the trial.

Blood samples were collected by venipuncture, between 2:00 and 4:00 p.m., into ethylenediamine tetraacetic acid (EDTA)-containing tubes. Within 24 hours, samples were transferred to the Genetic Laboratory of the Instituto do Coração, Hospital das Clínicas, São Paulo, Brazil, where DNA samples were extracted and further stored at -80 °C. After the trial was complete, we performed quality checks (DNA concentration and volume) of the samples at the Laboratório de Neurociências, Instituto de Psiquiatria, of the same hospital complex. The samples were genotyped at the National Genotyping Center (CEGEN) in Santiago de Compostela, Spain (www.usc.es/cegen/).

The SNPs were analyzed using the MassARRAY SNP genotyping system (Agena Bioscience, San Diego, USA), in accordance with manufacturer instructions, at CEGEN.

Briefly, the primers for amplification and extension were designed using Extend Primer Assay Design software v4. Sequenom iPLEX GOLD chemistry was used for locus-specific amplification, followed by a single-base primer extension reaction, which generated products of different masses that were quantitatively analyzed using MALDI-TOF mass spectrometry. The resulting data were analyzed using TyperAnalyzer software version 4, followed by manual inspection of the spectra by trained personnel.³⁵ All assays were performed in 384-well plates, including negative controls and a trio of Coriell samples (Na10860, Na10861, and Na11984) for quality control.

Statistical analysis

We used Stata 14 (StataCorp, College Station, Texas, USA) for all analyses. Results are described for the per-protocol sample. For descriptive data, clinical and demographic variables were compared across groups using one-way analysis of variance (ANOVA), chi-squared tests, or Fisher's exact tests, when appropriate. Hardy-Weinberg equilibrium (HWE) was verified using the Full Bayesian Significance Test (FBST), an intuitive Bayesian approach that does not assign probabilities to zero-measure tests when testing sharp hypotheses.³⁶ Analyses were considered significant at a threshold level of 0.05. To enhance statistical power, SNP analyses were performed allele-wise. We compared the minor allele frequency (MAF) allele vs. homozygotes for the major allele. MAF information was obtained on the National Institutes of Health (NIH) Single Nucleotide Polymorphism Database (dbSNP)³⁷ (Table 1).

To investigate whether the selected SNPs were predictors of depression improvement, we used generalized linear models (GLMs), implemented via the *glm* command in Stata. The dependent variable was the difference in HDRS-17 depression scores from baseline to endpoint. The independent variables were the assigned group intervention, the gene alleles, and the interaction thereof. We used different models comparing tDCS vs. placebo, tDCS vs. escitalopram, and escitalopram vs. placebo. Additionally, we ran separate models to assess the influence of alleles on each group and genotype-wise analyses investigating the influence of each of the three genotypes independently. Finally, we performed additional exploratory analyses, adding baseline depression scores (continuous variable) and self-declared ethnicity (white vs. non-white) as independent variables in our models.

Table 1 Single nucleotide polymorphisms (SNPs) investigated in this study

Gene name	Gene	SNP	MAF allele
Brain-derived neurotrophic factor	<i>BDNF</i>	rs6265	T (Met)
Solute carrier family 6 member 4	<i>SLC6A4</i>	rs25531	Short (s) form
Tryptophan hydroxylase 1	<i>TPH1</i>	rs1800532	T
5-Hydroxytryptamine receptor 2A	<i>HTR2A</i>	rs6311	T
		rs6313	A
		rs7997012	A
Catechol-O-methyltransferase	<i>COMT</i>	rs4680	A (Met)

For each SNP, analyses were performed to compare carriers of the minor allele frequency (MAF) allele vs. homozygotes for the major allele.

Ethics statement

Participants provided written informed consent and the trial was approved by the local and national ethics committees.

Results

Overview

We report data from 195 participants (202 completed the study; two participants refused DNA collection, three samples were not collected due to technical reasons, and two DNA samples could not be identified). There were 75, 45, and 75 participants in the escitalopram, placebo, and tDCS groups, respectively.

We used Bayesian approaches to examine HWE based on the Bayesian asymptotic e-value. All the SNPs followed the population genotype proportions, except for rs6311, whose e-value was 0.04 (Table 2).

There were no differences regarding main clinical and depression characteristics, including self-reported ethnicity and allele distribution (Table 3).

Depression scores according to group and SNPs are summarized in Table 4.

Influence of SNPs on tDCS vs. placebo

We found no interaction between the SNPs and tDCS vs. placebo groups for allele-wise analyses of any of the investigated genes: *BDNF* ($p = 0.80$), *TPH1* ($p = 0.64$), *COMT* ($p = 0.58$), *SLC6A4* ($p = 0.13$), or the rs6311

Table 2 Bayesian analysis of Hardy-Weinberg equilibrium (HWE)

Gene name	Gene	SNP	Bayesian asymptotic e-value
Brain-derived neurotrophic factor	<i>BDNF</i>	rs6265	0.71
Solute carrier family 6 member 4	<i>SLC6A4</i>	rs25531	0.07
Tryptophan hydroxylase 1	<i>TPH1</i>	rs1800532	0.17
5-Hydroxytryptamine receptor 2A	<i>HTR2A</i>	rs6311	0.04
		rs6313	0.07
		rs7997012	0.15
Catechol-O-methyltransferase	<i>COMT</i>	rs4680	0.33

Full Bayesian test for examining HWE.

Table 3 Clinical, demographic, and allele distribution of the sample

	Placebo (n=45)	Escitalopram (n=75)	tDCS (n=75)	p-value
Clinical characteristics				
Female	31 (68.9)	52 (70.3)	49 (66.2)	0.86
Age, mean (SD)	41.8 (13.1)	41.5 (12.5)	45.2 (11.8)	0.15
Years of schooling	15.7 (3.45)	14.5 (4.1)	15.7 (5.2)	0.24
White ethnicity (self-declared)	10 (22.2)	19 (25.3)	25 (33.3)	0.35
Body mass index, mean (SD)	27.2 (6)	26.3 (5)	25.7 (4.8)	0.31
Depression				
Baseline HDRS-17, mean (SD)	22.5 (4.1)	21.8 (3.6)	21.6 (4.1)	0.44
Endpoint HDRS-17, mean (SD)	16.5 (8.3)	10.3 (5.9)	13.1 (6.6)	< 0.001
Response rate	11 (24.4)	38 (50.7)	30 (40)	0.02
Treatment-resistant depression	15 (33.3)	19 (25.3)	26 (34.7)	0.42
SNPs				
<i>BDNF</i> , Met-carriers	17 (37.8)	27 (36)	24 (32)	0.78
<i>BDNF</i> , Val/Val	28 (62.2)	48 (64)	51 (68)	
<i>SLC6A4</i> , short allele carriers	31 (68.9)	45 (60)	53 (70.7)	0.35
<i>SLC6A4</i> , long/long	14 (31.1)	30 (40)	22 (29.3)	
<i>TPH1</i> , T-carriers	32 (71.1)	47 (62.7)	42 (56)	0.25
<i>TPH1</i> , C/C	13 (28.9)	28 (37.3)	33 (44)	
<i>COMT</i> , G-carriers	14 (31.1)	24 (33.3)	58 (77.3)	0.32
<i>COMT</i> , A/A	31 (68.9)	50 (66.7)	17 (22.7)	
<i>HTR2A</i> (rs6311), T-carriers	28 (62.2)	57 (76)	46 (61.3)	0.11
<i>HTR2A</i> (rs6311), C/C	17 (37.8)	18 (24)	29 (38.7)	
<i>HTR2A</i> (rs6313), A-carriers	28 (62.2)	58 (77.3)	46 (61.3)	0.07
<i>HTR2A</i> (rs6313), G/G	17 (37.8)	17 (22.7)	29 (38.7)	
<i>HTR2A</i> (rs7997012), A-carriers	26 (57.8)	35 (46.7)	37 (49.3)	0.49
<i>HTR2A</i> (rs7997012), G/G	19 (42.2)	40 (53.3)	38 (50.7)	

Data presented as n (%), unless otherwise specified.

BDNF = brain-derived neurotrophic factor; *COMT* = catechol-o-methyltransferase; HDRS = Hamilton Depression Rating Scale; SD = standard deviation; *SLC6A4* = solute carrier family 6, member 4; tDCS = transcranial direct current stimulation; *TPH1* = tryptophan hydroxylase 1.

Table 4 Depression scores at baseline (week 0), endpoint (week 10), and score difference (baseline minus endpoint) according to group and investigated single nucleotide polymorphisms (SNPs)

	Placebo	Escitalopram	tDCS
BDNF			
Met			
Baseline	21.2 (3.7)	21.3 (4)	23 (3.6)
Endpoint	14.8 (6.3)	9.8 (6.4)	13.7 (4.9)
Difference	6.3 (6)	11.5 (7.8)	9.3 (4.9)
Val/Val			
Baseline	23.3 (4.2)	22 (3.4)	20.9 (4.2)
Endpoint	17.5 (9.3)	10.5 (5.6)	12.8 (7.2)
Difference	5.9 (8.7)	11.5 (5.8)	8.1 (7.6)
SLC6A4			
Short			
Baseline	22.5 (4.1)	21.7 (3.9)	21.7 (4.3)
Endpoint	17.5 (7.6)	10.7 (6.1)	12.9 (6.4)
Difference	5 (6.9)	11 (6.9)	8.8 (7)
Long/Long			
Baseline	22.6 (4.4)	21.9 (3.2)	21.2 (3.6)
Endpoint	14.3 (9.6)	9.7 (5.5)	13.6 (7.1)
Difference	8.3 (9.1)	12.2 (6)	7.7 (6.7)
TPH1			
T			
Baseline	22.2 (4)	21.8 (3.7)	21.9 (3.9)
Endpoint	16.3 (8.4)	11.4 (5.9)	13 (5.7)
Difference	5.9 (7.7)	10.3 (6.7)	8.9 (6.1)
G/G			
Baseline	23.2 (4.6)	21.8 (3.4)	21.1 (4.6)
Endpoint	16.8 (8.3)	8.4 (5.4)	13.2 (7.6)
Difference	6.4 (8)	13.5 (5.9)	8 (7.9)
COMT			
G			
Baseline	21.9 (3.3)	21.6 (3.6)	21.4 (3.8)
Endpoint	16.4 (7)	11.4 (4.8)	12.2 (7)
Difference	5.5 (6)	10.1 (5.2)	9.2 (6.6)
A/A			
Baseline	22.8 (4.5)	21.9 (3.6)	21.6 (4.2)
Endpoint	16.5 (8.9)	9.7 (6.3)	13.4 (6.5)
Difference	6.3 (8.5)	12.2 (7.1)	8.3 (7)
HTR2A (rs6311)			
T			
Baseline	23 (4.2)	21.9 (3.5)	21.9 (3.3)
Endpoint	17.4 (8.6)	10.6 (5.7)	13.4 (5.8)
Difference	5.6 (8.4)	11.2 (6.6)	8.6 (6.4)
C/C			
Baseline	21.7 (4)	21.5 (4.2)	21 (5.3)
Endpoint	15 (7.9)	9.2 (6.4)	12.6 (7.7)
Difference	6.7 (6.7)	12.3 (6.5)	8.4 (7.7)
HTR2A (rs6313)			
A			
Baseline	23 (4.2)	21.8 (3.5)	22 (3.3)
Endpoint	17.4 (8.6)	10.5 (5.7)	13.4 (5.8)
Difference	5.6 (8.4)	11.2 (6.5)	8.5 (6.4)
G/G			
Baseline	21.7 (4)	21.9 (4)	21 (5.2)
Endpoint	15 (7.9)	9.5 (6.5)	12.6 (7.7)
Difference	6.7 (6.7)	12.4 (6.7)	8.4 (7.7)
HTR2A (rs7997012)			
A			
Baseline	22.2 (3.9)	21.9 (3.9)	21.7 (4.7)
Endpoint	15.8 (8.6)	9.6 (6.6)	13.6 (6.8)
Difference	6.4 (8.7)	12.2 (7.4)	8 (7.8)
G/G			
Baseline	23 (4.5)	21.7 (3.4)	21.5 (3.5)
Endpoint	17.3 (8)	10.8 (5.2)	12.5 (6.3)
Difference	5.6 (6.4)	10.8 (5.7)	8.9 (6)

Data presented as mean (standard deviation) scores on the Hamilton Depression Rating Scale, 17-items (HDRS-17). BDNF = brain-derived neurotrophic factor; COMT = catechol-o-methyltransferase; SLC6A4 = solute carrier family 6, member 4; tDCS = transcranial direct current stimulation; TPH1 = tryptophan hydroxylase 1.

($p = 0.68$), rs6313 ($p = 0.68$), and rs7997012 ($p = 0.56$) SNPs of *HTR2A*.

Influence of SNPs on escitalopram vs. placebo

We found no interaction between the SNPs and escitalopram vs. placebo groups for allele-wise analyses of any of the investigated genes: *BDNF* ($p = 0.85$), *TPH1* ($p = 0.33$), *COMT* ($p = 0.65$), *SLC6A4* ($p = 0.44$), or the rs6311 ($p = 0.98$), rs6313 ($p = 0.98$), and rs7997012 ($p = 0.81$) SNPs of *HTR2A*.

Influence of SNPs on tDCS vs. escitalopram

We found no interaction between the SNPs and tDCS vs. escitalopram group for allele-wise analyses of any of the investigated genes: *BDNF* ($p = 0.60$), *TPH1* ($p = 0.06$), *COMT* ($p = 0.23$), *SLC6A4* ($p = 0.32$), or the rs6311 ($p = 0.62$), rs6313 ($p = 0.62$), and rs7997012 ($p = 0.31$) SNPs of *HTR2A*.

Influence of SNPs on each group separately

There was no influence of the MAF alleles of the *BDNF* ($p = 0.84$), *TPH1* ($p = 0.87$), *COMT* ($p = 0.75$), *SLC6A4* ($p = 0.17$), and *HTR2A* SNPs rs6311 ($p = 0.66$), rs6313 ($p = 0.66$), and rs7997012 ($p = 0.76$) on depression improvement in patients assigned to placebo.

Likewise, for tDCS, there was no influence of the MAF alleles of the *BDNF* ($p = 0.48$), *TPH1* ($p = 0.55$), *COMT* ($p = 0.63$), *SLC6A4* ($p = 0.51$), or *HTR2A* SNPs rs6311 ($p = 0.95$), rs6313 ($p = 0.95$), and rs7997012 ($p = 0.59$) on depression improvement.

For escitalopram, there was no influence of the MAF alleles of the *BDNF* ($p = 0.98$), *COMT* ($p = 0.19$), *SLC6A4* ($p = 0.45$), or *HTR2A* SNPs rs6311 ($p = 0.53$), rs6313 ($p = 0.54$), and rs7997012 ($p = 0.36$) on depression improvement. For *TPH1*, T-allele carriers experienced less depression improvement than G/G homozygotes, with a significant ($p = 0.038$) difference of 3.2 (95%CI 6.2-0.18) points.

Genotype-wise analysis

For tDCS vs. placebo, we found no significant interactions between intervention and SNPs ($p > 0.1$). Nonetheless, trends were observed for the gene *COMT*, in which the tDCS-placebo difference tended to be larger in the AG vs. AA and GG vs AA genotypes ($p = 0.06$ and $p = 0.09$, respectively). In other words, the A (Met) allele tended to decrease improvement of depression with tDCS compared to placebo, although this effect was not statistically significant.

For tDCS vs. escitalopram, we found no significant interactions between intervention and SNPs ($p > 0.1$). Nonetheless, trends were observed for the gene *TPH1*, in which G/G homozygotes tended to present greater antidepressant response for escitalopram than tDCS ($p = 0.057$), and patients receiving escitalopram tended to present greater response when harboring the G/G rather than T/T genotype ($p = 0.062$). In other words, the

G allele favored larger antidepressant effects of escitalopram vs. tDCS.

For escitalopram vs. placebo, no significant interactions between intervention and SNPs were found (all $p > 0.01$), except for the gene *TPH1*. For this gene, patients presenting the G/G and G/T genotypes presented significantly greater antidepressant response for escitalopram than placebo ($p = 0.002$ and $p = 0.001$, respectively)

Exploratory analyses

Additional analyses introducing baseline depression severity and self-reported ethnicity as covariates in our models did not change our findings – i.e., no SNP was identified as a predictor of response.

Discussion

In the present study, we investigated whether SNPs in genes associated with neuroplasticity (*BDNF*), serotonin (*SLC6A4*, *HTR2A*, *TPH1*), and dopamine and noradrenaline (*COMT*) metabolism were associated with antidepressant response in the ELECT-TDCS trial. Except for the *COMT* polymorphism, which was analyzed after a recent international tDCS trial,⁹ the SNPs were chosen *a priori*. In contrast to our hypotheses, no significant associations between these SNPs with tDCS vs. escitalopram and with tDCS vs. placebo antidepressant improvement were observed. Nonetheless, genotype-wise analyses showed non-statistically significant trends of the *COMT* polymorphism on tDCS vs. placebo and of the *TPH1* polymorphism on tDCS vs. escitalopram and on escitalopram vs. placebo comparisons. These findings are further discussed below.

The *COMT* polymorphism is involved in regulating prefrontal dopamine levels. For instance, A/A (Met/Met) homozygotes have reduced dopamine catabolism³⁸ and greater prefrontal availability of dopamine. Plewnia et al.²⁸ observed that, among healthy subjects, Met/Met homozygotes had poorer performance on executive tests after anodal tDCS over the prefrontal cortex. This finding is in line with the concept of an inverted-U-shaped response for dopamine-related tDCS effects on neuroplasticity.³⁹ However, mixed effects of the *COMT* influence on tDCS effects in cognitive functioning have been reported.^{40,41} In a recent tDCS trial in depression, *COMT* did not predict antidepressant effects of tDCS²⁹; however, no main effects of tDCS were observed in that study.⁹ Here, our results pointed out to a nonsignificant trend of dose-dependent effects of the A (Met) allele and lower tDCS vs. placebo effects. Possibly, higher dopaminergic activity decreased tDCS effects by shifting the U-dose dopamine curve beyond optimal activity. Another possible explanation for our finding is a greater placebo effect associated with higher dopaminergic activity, as observed in studies of Parkinson's disease.⁴²

The *TPH1* gene regulates serotonin availability and, therefore, has been investigated as a predictor of response to antidepressant drugs, particularly SSRIs. Although a meta-analysis suggested that this SNP could modulate antidepressant response,¹⁷ a more recent, updated

meta-analysis including 12 individual studies and the STAR*D trial⁴³ found that the SNP was not associated with antidepressant response, regardless of ethnicity, type of antidepressant drug, or an interaction between these variables.⁴⁴ In our study, the G allele was associated with greater escitalopram effects, in line with previous studies.¹⁷

Regarding the *BDNF* gene, the rs6265 polymorphism determines a substitution of Val66 for Met. Although the Met allele is associated with changes in brain anatomy, memory, and behavior in experimental animals,⁴⁵ its role in antidepressant drug response is controversial. Although one meta-analysis demonstrated that Met/Met and Val/Met genotypes were associated with clinical response,¹⁷ another study showed association only with the Val/Met genotype,⁴⁶ whereas another one showed no association.⁴⁷ On the other hand, a study with rTMS⁴⁸ showed an association between Val/Val and depression improvement in 36 patients. Regarding tDCS, the present study and two previous clinical trials showed no association between rs6265 and clinical response to tDCS.^{9,23} Moreover, several studies showed that BDNF plasma levels do not increase after tDCS treatment in depression.^{32,49-51}

Regarding the *SLC6A4* serotonin transporter polymorphism studied herein, the short allele (*s*) is related to lower availability and activity of the serotonin transporter compared to its long form. In fact, subjects with the *s* allele display worse clinical response to SSRIs, probably because the efficacy of SSRIs decreases when the serotonin transporter is not functioning optimally.⁵² In an earlier study, we found a significant antidepressant effect of tDCS vs. placebo for long-arm carriers, although the result was not conclusive, since the SNP effects on tDCS response (not considering placebo effects) were not significant.²³ For rTMS, the LL genotype has been associated with greater antidepressant effects.⁵³

Regarding the *HTR2A* polymorphisms, no association was observed between any of the three investigated SNPs and escitalopram or tDCS response. In a recent meta-analysis, these three polymorphisms were associated with antidepressant response in both allele- and genotype-wise analyses in depression.⁵⁴ Therefore, the lack of observed effects might be explained by our small sample size.

Some limitations of this study must be underscored. First, although ELECT-TDCS used an adequately powered sample for its primary outcome, SNPs analyses were exploratory outcomes and therefore were likely to be underpowered. Noninvasive brain stimulation (NIBS) studies usually recruit smaller sample sizes than pharmacological trials, as treatment is delivered daily, for several weeks, at the study center. To overcome this limitation, clinical trials of tDCS could collect SNP data to be further combined using meta-analytic techniques, as was done recently for clinical and cognitive outcomes.^{55,56} Second, we could only evaluate the role of self-reported ethnicity on tDCS clinical response in our sample, not of ancestry. This is a significant limitation, as Brazilian population has a very mixed ancestry; therefore, self-declared or directly observed physical phenotype correlate poorly with genomic ancestry.⁵⁷ Third, several other SNPs associated with

antidepressant response (e.g., in *HTR1A*, *HTR1B*) were not investigated, as we restricted our analyses to SNPs determined *a priori* in our study protocol and to the COMT polymorphism. Therefore, further studies should explore the impact of additional SNPs on tDCS response. Finally, our sample was composed of individuals who were antidepressant-free at baseline and received a specific tDCS regimen. Therefore, we cannot extrapolate these findings to other tDCS protocols, including designs in which subjects receive tDCS combined with other pharmacotherapeutic or non-pharmacotherapeutic interventions.

In conclusion, this ancillary study of ELECT-TDCS investigated whether a series of SNPs related to neuroplasticity and serotonin metabolism (selected *a priori*) were associated with antidepressant response to tDCS. Post-hoc, we also investigated whether a *COMT* polymorphism was associated with tDCS response. The nonsignificant effects observed in our study are probably explained by our low sample size. To increase statistical power and draw definite conclusions, other trials investigating tDCS in depression should explore the impact of these and other SNPs on antidepressant response.

Acknowledgements

This study was primarily supported by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Programa Jovens Pesquisadores em Centros Emergentes (grant 2012/20911-5) and from Brain and Behavior (Alliance for Research in Schizophrenia and Affective Disorders [NARSAD]), Young Investigator (grant 2013/20493). ARB is a former recipient of a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/Alexander von Humboldt fellowship award for experienced researchers and current recipient of a Returning Fellowship from the Humboldt Foundation, as well as a scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Laboratório de Neurociências receives financial support from the Associação Beneficente Alzira Denise Hertzog da Silva and the CAPES/Institutos Nacionais de Ciências e Tecnologia (INCT) program via the Instituto Nacional de Biomarcadores em Neuropsiquiatria (INBioN).

Disclosure

The authors report no conflicts of interest.

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