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A genetic profile of refractory individuals with major depressive disorder and their responsiveness to transcranial magnetic stimulation

Dear Editor

Effective in treatment-resistant depression (TRD), repetitive transcranial magnetic stimulation (rTMS) presents a remission rate between 30% and 40%. Although genetics could be one potential source of inter-individual variability in rTMS responsiveness, few studies have sought to identify possible genetic basis of rTMS response [1,2]. Therefore, we used an extreme-phenotype design in which we compared genome-wide allelic variation between rigorously defined rTMS responders and non-responders.

A total of 99 TRD patients provided informed consent and this study was approved by the Human Research and Ethics Committee of the Alfred Hospital. All were submitted to a rTMS protocol and completed at least 18 sessions of 10 Hz at left dorsolateral prefrontal cortex (DLPFC). Clinical outcomes (responder or non-responder) were determined based on scores on the Montgomery Asberg Depression Rating Scale (MADRS).

We used the extreme-phenotype design. Patients were separated into two groups, responders - patients with at least 60% reduction on the MADRS scale (n = 29) and non-responders - patients with 10% or less reduction on the MADRS scale (n = 19). We used this criteria considering that extreme scoring patients (<10 >60) may present more representative genetic results, improving power to detect phenotype-genotype associations [3,4].

Genotyping was performed using the Infinium PsychArray-24 BeadChip (Illumina, Inc., San Diego, CA, USA) and analyzed with PLINK 1.9. We performed quality control to remove individuals or markers with high error rates. Data quality control parameters were: call rate (GENO) > 90%, maximum individual missingness rate (MIND) < 10%, minor allele frequency (MAF) > 5% and Hardy-Weinberg equilibrium (HWE) *p*-value > 10−6. We performed standard association analysis to compare allele frequency in both groups with a 95% CI. After identifying the significant SNPs and genes a functional enrichment analysis using STRING and Cytoscape databases was performed.

Of the initial 593,260 SNPs in 48 individuals, 958 variants were removed due to missing genotype data, 310,522 SNPs were removed due to minor allele threshold and 4 people were removed due to missing genotype data. This left 281,780 SNPs and 44 subjects for the association study. In order to estimate the effective number of significant SNPs, we applied the False Discovery Rate (FDR) correction considering sample and SNPs per chromosome. A new *p* value was then determined for each chromosome (Supplementary Table 01). Analysis revealed 53 significantly SNPs associations mapped to coding genomic regions: 2 SNPs associated with treatment response mapped to coding genomic regions and 18 associated with non-responsiveness (Table 1).

Protein-protein interaction network analysis (STRING database) showed no pathway association among the identified genes. Genes and pathway interaction networks were obtained after enrichment analysis using ClueGo (Cytoscape), and flagged a synaptic plasticity regulation pathway containing the genes *APP* (Amyloid Beta Precursor Protein), *SPPL2A* (Signal Peptide Peptidase-like 2a), *EXOSC7* (Exosome Component 7), *GRID2* (Glutamate Ionotropic Receptor Delta Type Subunit 2), *ADGRB3* (Adhesion G Protein-Coupled Receptor B3), *COL9A3* (Collagen Type IX Proteoglycan), *LY9* (Lymphocyte Antigen 9), *FOXN3* (Forkhead Box N3).

The *SPPL2A* gene was associated with positive response. This gene encodes an aspartic intramembrane protease important in the development and function of antigen presenting cells such as B-lymphocytes and dendritic cells. *SPPL2A* is an enzyme related to presenilins [5], which are linked to generation of beta-amyloid (Aβ) peptide, that deposits in the brain in the Alzheimer disease (AD).

The following genes are associated with low response. *EXOSC7* is a gene encoding RNA exosome component 7. The RNA exosome complex participate in the processing of stable RNA species [6]. Mutational changes in genes encoding RNA exosome subunits may trigger inherited tissue-specific diseases, for example, it was found differentially expressed in Grade II and III glioma [7].

GRID2 is a member of the family of ionotropic glutamate receptors. The *GRID2* gene is selectively expressed in Purkinje cells, playing a key role in synaptogenesis, synaptic plasticity and motor coordination. SNPs in glutamate-related genes have been associated with antipsychotic response [8].

ADGRB3 also known as *BAI3* is a gene that encodes a brain-specific angiogenesis inhibitor and is a member of the secretin receptor family. This gene plays a key role in axon guidance, myelination and synapse formation. ADGRB3 SNPs have been associated with schizophrenia, bipolar disorder and drug addiction [10].

COL9A3 is a gene that encodes one of the three alpha chains of the type IX collagen. Some of the brain collagen proteins are expressed by neurons, suggesting their involvement in brain architecture development [11]. Collagen type IV is known to inhibit glial differentiation in cortical cell cultures and to be enhanced in the frontal and temporal cortex of patients with Alzheimer's disease [12].

Lymphocyte antigen 9 (LY9) belongs to the signaling lymphocytic activation molecule (SLAM) family of immunomodulatory receptors. The activation of upstream gene regulatory pathways that modulate gene expression in immune cells may be linked to MDD.

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Table 1
SNPs founded. Description of significant SNPs ($p < 0.05$). A1, lower frequency allele. A2, highest frequency allele. MAF, minor allele frequency. SNV, single nucleotide variant. OR (0dds ratio) > 1 related to treatment response and OR < 1 associated to non.

Chr	SNP	Position	Gene	Chi square	Odds Ratio	P value	A1	A2	MAF
19	rs960995	57039169	*ZNF471*	13.49	0.18	0.0002397	G	A	0.4886
7	rs17164813	11616500	*THSD7A*	16.54	0.1373	0.000047516	A	C	0.3068
15	rs8035452	51040798	*SPPL2A*	14.53	7.25	0.0001376	G	A	0.3977
18	rs595562	18449508	snv variation near genes *LINC01541* and *LOC107985179*	15.50	0.1603	0.000082719	G	A	0.3714
1	rs4648426	3773089	snv variation near genes *DFFB* and *CEP104*	14.43	0.1489	0.0001455	G	A	0.2727
3	rs12487861	160535721	*PPM1L*	18.38	16	0.000018101	A	C	0.3295
11	rs198475	61526071	*MYRF*	14.43	0.1489	0.0001455	A	G	0.2727
1	rs560681	160786670	*LY9*	14.98	0.09502	0.0001088	G	A	0.1818
1	rs11265485	160764759	*LY9*	16.98	0.08403	0.000037682	G	A	0.1932
9	rs1934115	23103266	*LOC107987055*	14.49	0.04528	0.000141	C	A	0.125
18	rs872994	73171838	*LOC107985177*	13.919	0.1769	0.0001909	A	C	0.375
22	rs5995416	37719004	*LOC105373024*	12.643	5.61	0.0003769	A	G	0.4432
19	rs2189698	57014071	*LOC105372471*	13.52	0.1839	0.0002364	C	A	0.4318
18	rs4243296	73219777	*LOC105372202*	13.92	0.1769	0.0002	G	A	0.375
6	rs6899975	138275769	*LINC02528*	14.38	0.1729	0.0001494	A	G	0.3977
17	rs1014129	49517224	*LINC02073*	17.10	0.1407	0.000035449	A	G	0.3523
13	rs626904	39984946	*LHFPL6*	14.90	0.1674	0.0001131	A	G	0.4205
2	rs17673232	144860827	*GTDC1*	19.07	0.07451	0.000012614	A	G	0.2045
4	rs11942069	94494455	*GRID2*	14.43	0.1489	0.0001455	A	G	0.2727
14	rs447347	89992265	*FOXN3*	14.79	0.1654	0.0001205	A	G	0.4773
13	rs2271926	39979675	*EXOSC7*	14.90	0.1674	0.0001131	G	A	0.4205
19	rs4646515	15658569	*CYP4F3*	9.122	0.2503	0.002525	G	C	0.3636
X	rs2273081	4594630	*COL9A3*	12.17	0.1571	0.0004864	C	A	0.3429
22	rs229526	47236880	*C1QTNF6*	16.40	0.1324	0.00005141	G	A	0.3409
X	rs2980075	152794075	*ATP2B3*	11.62	0.1282	0.0006537	C	A	0.2286
21	rs373521	27257660	*APP*	12.5	0.1963	0.0004067	A	C	0.3864
6	rs1283468	70038147	*ADGRB3*	15.56	0.07051	0.00007978	A	G	0.1591
5	rs11956034	178754468	*ADAMTS2*	14.98	0.09502	0.0001088	A	G	0.1818
3	rs501118	95116949	–	15.66	8.7	0.000075922	A	G	0.375
21	rs9981074	33165958	–	13.54	6.29	0.0002341	A	G	0.4205
X	rs17317597	116660237	–	8.202	12.55	0.004185	G	A	0.2143
18	rs4347699	51183679	–	15.74	10.33	0.000072533	A	G	0.3409
X	rs12559502	128048545	–	6.893	0.2462	0.008651	G	A	0.3
X	rs17333434	27133302	–	8.072	0.2027	0.004495	G	A	0.2571
21	rs2829964	27242396	–	12.823	0.1914	0.0003425	A	G	0.4659
21	rs2142419	19928069	–	12.908	0.1778	0.0003272	A	G	0.3068
18	rs8082822	73209334	–	13.92	0.1769	0.0001909	A	G	0.375
X	rs6640653	4579981	–	9.714	0.1645	0.001829	A	C	0.2429
10	rs2068888	94839642	–	15.50	0.1603	0.0000827	G	A	0.4432
X	rs1343974	4594630	–	12.17	0.1571	0.0004864	C	A	0.3429
13	rs9548721	39846266	–	14.569	0.1546	0.0001351	G	A	0.2955
12	rs7135989	48655268	–	16.40	0.1324	0.000005141	A	G	0.2841
X	rs5916687	4596138	–	11.62	0.1282	0.0006537	A	G	0.2286
21	rs2829950	27223152	–	19.23	0.124	0.000011565	C	A	0.3636
X	rs5980684	69300000	–	13.57	0.1222	0.0002298	A	G	0.2714
6	rs1074349	22838984	–	18.63	0.1217	0.000015902	G	A	0.3182
22	rs134913	27413509	–	18.41	0.1111	0.000017857	G	A	0.2727
X	rs12390729	4597922	–	15.68	0.1053	0.00000055659	A	G	0.2857
13	rs944868	39843411	–	18.47	0.102	0.000017268	C	A	0.25
10	rs10787147	111079538	–	14.6	9.595	0.0001327	G	A	0.3295
14	rs2094718	99434288	–	13.5	8.908	0.000238	C	A	0.3182
X	rs1144863	144582143	–	12.83	7.986	0.0003411	A	G	0.4143
X	rs5915786	4642016	–	9.775	5.353	0.001769	G	A	0.4571

In a study by Mellon et al. [13], the authors found that among the main negatively regulated transcripts in MDD patients are the cell surface antigens of leukocytes (CD6, CD7, CD22 and LY9). Differential expression of these transcripts may be associated with possible changes in the distribution of leukocyte subset in MDD patients.

FOXN3 is a protein coding a member of the forkhead/winged helix transcription factor family. Recent GWAS analysis found significant correlation between suicidality and the FOXN3 gene [14,15].

In this study, we set out to test whether polymorphic profiles are associated to rTMS treatment outcomes. Our findings suggest that the responsiveness to rTMS might be associated with several pathways and not just under the influence of a single gene. However, the molecular mechanisms by which these genes may influence the response to rTMS treatment are unknown, requiring further investigation.

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Declaration of competing interest

PBF has received equipment for research from Medtronic, MagVenture A/S and Brainsway Ltd. He is on scientific advisory boards for Bionomics Ltd and LivaNova and is a founder of TMS Clinics Australia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.04.019.

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Nathália G. Souza-Silva, Eduardo S. Nicolau
Centro de Tecnologia em Medicina Molecular, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

Kate Hoy[a]
[a] *Epworth Centre for Innovation in Mental Health, Epworth HealthCare and Department of Psychiatry, Central Clinical School, Monash University, Camberwell, Victoria, Australia*

Ziarih Hawi, Mark A. Bellgrove
School of Psychological Sciences and Turner Institute for Brain and Mental Health, Monash University, Melbourne, Australia

Débora M. Miranda
Centro de Tecnologia em Medicina Molecular, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

Departamento de Pediatria, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Marco A. Romano-Silva[*]
Centro de Tecnologia em Medicina Molecular, Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil

Departamento de Saúde Mental, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Paul B. Fitzgerald
Epworth Centre for Innovation in Mental Health, Epworth HealthCare and Department of Psychiatry, Central Clinical School, Monash University, Camberwell, Victoria, Australia

[*] Corresponding author. Alfredo Balena, 190, sala 114, Belo Horizonte, Minas Gerais, CEP 30130-100, Brazil.
E-mail address: romano-silva@ufmg.br (M.A. Romano-Silva).

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