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.), 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.

https://doi.org/10.1016/j.brs.2020.04.019

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Recent GWAS analysis found significant changes in the distribution of leukocyte subset in MDD patients. Differentially regulated transcripts in MDD patients are the cell surface antigens of leukocytes (CD6, CD7, CD22 and LY9). FOXN3 is a protein coding a member of the forkhead/winged helix transcription factor family. Their expression is associated to suicidality and the FOXN3 gene [14,15].

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 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.

### 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 (odds ratio) > 1 related to treatment response and OR < 1 associated to non.

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**Funding**

The study was supported by grants from the National Health and Medical Research Council (NHMRC) (1041890) and Alfred Hospital. PBF was supported by a Practitioner Fellowship grant from National Health and Medical Research Council (NHMRC) (1078567). NGSS was supported by a scholarship from CAPES-Brazil. KEH was supported by the NHMRC Fellowship (1135558). MAB was supported by a Senior Research Fellowship (level B) from the NHMRC (1154378). MARS and DMM were supported by Research Fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)-Brazil and a CAPES-PGCI grant (47/2014).

**Declaration of competing interest**
PBF has received equipment for research from Medtronic, Mag-Venture 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.

References


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9 April 2020
Available online 6 May 2020