UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Medicina
Programa de Pós-graduação em Medicina Molecular

Nathália Gualberto Souza e Silva

A GENETIC PROFILE OF REFRACTORY INDIVIDUALS WITH MAJOR DEPRESSIVE DISORDER AND THEIR RESPONSIVENESS TO TRANSCRANIAL MAGNETIC STIMULATION

PERFIL GENÉTICO DE INDIVÍDUOS REFRATÁRIOS COM TRANSTORNO DEPRESSIVO MAIOR E RESPOSTA À ESTIMULAÇÃO TRANSCRANIANA MAGNÉTICA

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Dissertação apresentada ao Programa de Pós-graduação em Medicina Molecular da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Mestre em Medicina Molecular.

Orientadora: Professora Débora Marques de Miranda (MD-PhD)

Coorientador: Professor Marco Aurélio Romano Silva (MD-PhD)

Belo Horizonte

2020

Souza, Nathália Gualberto.
SO729p Perfil Genético de Indivíduos Refratários com Transtorno Depressivo Maior e Resposta à Estimulação Magnética Transcraniana [manuscrito]. / Nathália Gualberto Souza. - - Belo Horizonte: 2020.
66f.: il.
Orientador (a): Débora Marques de Miranda.
Coorientador (a): Marco Aurélio Romano-Silva.
Área de concentração: Medicina Molecular.
Dissertação (mestrado): Universidade Federal de Minas Gerais, Faculdade de Medicina.

1. Transtorno Depressivo Maior. 2. Estimulação Magnética Transcraniana. 3. Estudo de Associação Genômica Ampla. 4. Transtorno Depressivo Resistente a Tratamento. 5. Perfil Genético. 6. Dissertação Acadêmica. I. Miranda, Débora Marques de. II. Romano-Silva, Marco Aurélio. III. Universidade Federal de Minas Gerais, Faculdade de Medicina. IV. Título.

NLM: QU 500

Bibliotecário responsável: Fabian Rodrigo dos Santos CRB-6/2697

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA MOLECULAR

FOLHA DE APROVAÇÃO

A GENETIC PROFILE OF REFRACTORY INDIVIDUALS WITH MAJOR DEPRESSIVE DISORDER AND THEIR RESPONSIVENESS TO TRANSCRANIAL MAGNETIC STIMULATION

NATHÁLIA GUALBERTO SOUZA

Dissertação submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em MEDICINA MOLECULAR, como requisito para obtenção do grau de Mestre em MEDICINA MOLECULAR, área de concentração MEDICINA MOLECULAR.

Aprovada em 28 de fevereiro de 2020, pela banca constituída pelos membros:

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Belo Horizonte, 28 de fevereiro de 2020.

RESUMO

A depressão é caracterizada pela alteração de humor, particularmente por tristeza ou irritabilidade, acompanhada de várias alterações psicofisiológicas, como distúrbios no sono e apetite, pensamentos suicidas e diminuição da velocidade e da ação. A incidência de depressão ao longo da vida é de duas a três vezes maior nas mulheres do que nos homens. Segundo a Organização Mundial da Saúde (OMS), a depressão é o principal motivo de incapacidade no mundo, afetando cerca de 300 milhões de pessoas. Embora a depressão possa ser efetivamente tratada, na maioria dos pacientes, por medicação ou por alguma forma de psicoterapia baseada em evidências, apenas 30% respondem a intervenções padrão e alcançam remissão. A estimulação magnética transcraniana repetitiva (EMTr) tem sido proposta como uma possível alternativa ao tratamento de pacientes com depressão resistente ao tratamento (TRD). Embora o EMTr seja um tratamento eficaz para muitos pacientes com TRD, nem sempre é eficaz. As taxas de remissão de EMTr em pacientes com TRD estão entre 30% e 40%. A capacidade de resposta à farmacoterapia e à terapia com EMTr pode ser influenciada por fatores genéticos. O objetivo do presente estudo é caracterizar o perfil genético de indivíduos refratários com TDM e sua responsividade à EMTr. Realizamos um estudo de associação genômica com 48 participantes e 593.260 variantes e identificamos 53 associações significativas de SNPs. A análise de enriquecimento por conjunto de genes mostrou que genes significativamente associados são carregados nas vias de regulação da plasticidade sináptica. Entre os genes encontrados estavam os genes APP, GRID2 e SPPL2A. Esses genes já foram descritos na literatura associados ao distúrbio neuropsiquiátrico, incluindo transtorno depressivo maior e doença de Alzheimer. Baseado nestes resultados, sugerimos que os genes identificados podem influenciar a responsividade a ETMr por meio da interação de vias gênicas. A partir de nosso conhecimento, este é o primeiro estudo de perfil genético de resposta a ETMr usando abordagem de estudo de associação genômica ampla. Portanto, mais estudos se fazem necessário para esclarecer o mecanismo molecular por qual estes genes podem afetar a resposta a estimulação transcraniana magnética.

Palavras-chave: Transtorno Depressivo Maior, Estimulação Transcraniana Magnética, Estudo de Associação Genômica Ampla, Depressão Resistente ao Tratamento.

ABSTRACT

Depression is characterized to change of mood, particularly sadness or irritability, accompanied by several psychophysiological disturbances such in sleep and appetite, suicidal thoughts, and slowing of speed and action. The lifetime incidence of depression is two to threefold higher in women than in men. According to the World Health Organization (WHO), depression is the main reason of disability worldwide, affecting around 300 million people.

Depression can be effectively treated in the majority of patients by medication or/and evidence-based psychotherapy, but only 30% respond to standard interventions and achieve remission. Repetitive transcranial magnetic stimulation (rTMS) has been proposed as a possible alternative to treat the individuals with treatment-resistant depression (TRD). rTMS seems an effective treatment for many TRD patients, but it is not always effective. Remission rates of rTMS in TRD patients are between 30% and 40%. The responsiveness to pharmacotherapy and rTMS therapy may be influenced by genetic factors.

The aim of the present study is to characterize the genetic profile of refractory individuals with MDD and their responsiveness to rTMS. We conducted a genomic association study with 48 participants and 593,260 variants and identified 53 significant SNPs associations. Gene-set enrichment analysis showed that significantly associated genes loaded onto synaptic plasticity regulation pathways. Among the genes founded were APP, GRID2 and SPPL2A genes. These genes were already described in the literature associated with neuropsychiatry disorders, including major depressive disorder and Alzheimer Disease. From the findings, we suggests that the genes found may influence the response to rTMS. Although, the responsiveness to rTMS may be associated to several pathways and not just to the influence of a single gene. Therefore, further studies are necessary to investigate the molecular mechanism by which these genes affect response to rTMS treatment.

KEYWORDS: Major Depressive Disorder, Transcranial Magnetic Stimulation, Genome Wide Association, Treatment-resistant Depression.

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LIST OF ACRONYMS AND INITIALS

5-HTTLPR – Serotonin Transporter-linked Promoter Region

Aβ - Amyloid-beta peptide

AD – Alzheimer's disease

ADGRB3 – Adhesion G Protein-coupled receptor B3

APP – Amyloid Beta Precursor Protein

BAI3 – Brain-Specific Angiogenesis Inhibitor 3

BDNF – Brain-Derived Neurotrophic Factor

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CD22 – B-cell Receptor CD22

CD6 – B-cell Recptor CD6

CD7 – B-cell Receptor CD7

CNPq- Conselho Nacional de Desenvolvimento Científco e Tecnológico

COL9A3 – Structural Constituent of the Extracellular Matrix

COMT – Catechol-O-Methyltransferase

CREB – cAMP Response Element-binding

DLPFC – Dorsolateral Prefrontal Córtex

DMS IV – Diagnostic and Statistical Manual of Mental Disorders 4th Edition

DNA – Deoxyribonucleic acid

EGR – Early growth Response

ELMO1 – Engulfment and Cell Motility 1

ETMr – Estimulação Transcraniana Magnética repetitiva

EXOSC2 – Exosomal Component 2

EXOSC3 – Exosomal Component 3

EXOSC7 – Exosomal Component 7

FDA - Food and Drug Administration

FDR – False Discovery Rate

FOXN3 – Forkhead box N3

GENO – Missing Genotype Rates

GR – Glucocorticoid Receptor

GRID2 – Glutamatergic Ionotropic Receptor

GWAS - Genome-wide Association Study

HWE – Hardy-Weinberg Equilibrium

Hz - Hertz

I-CLiPs – Intramembrane cleaving Proteases

IL-1 – Interleucin 1

IL-6 – Interleucin 6

LY9 - Lymphocyte Antigen 9

MADRS – Montgomery Asberg Depression Rating Scale

MAF – Minor Allele Frequency

MAOI – Monoamine Oxidase Inhibitor

MDD - Major Depressive Disorder

MIND – Maximum Individual Missing Rate

MINI – Mini-International Neuropsychiatric Interview

NASSA – Noradrenergic and Specific Serotonergic antidepressant

NHMRC – National Health and Medical Research Council

OR – Odds Ratio

PCH1b – Pontocerebellar Hypoplasia type 1

PPI – Protein-protein Interaction

PRS – Polygenic Risk Score

PS1 – Presenilins 1

PS2 – Presenilins 2

RIMA – Reversible Inhibitor of Monoamine Oxidase-a

RNA – Ribonucleic acid

rTMS - Repetitive Transcranial Magnetic Stimulation

SCA – Spinocerebellar Ataxia

SLAM – Signalling Lymphocytic activation Molecule

SLC6A1 – Solute Carrier Family 6 Member 1

SNP – Single Nucleotide Polymorphism

SNRI - Serotonin-norepinephrine Reuptake Inhibitors

SNV – Single Nucleotide Variant

SPPL2A – Signaling GPCR Transmembrane Protein

SSRI - Selective Serotonin Reuptake Inhibitors

STRING – Search Tool for the Retrieval of Interacting Genes

TCA – Tricyclic Antidepressant

TDM – Transtorno Depressivo Maior

TF – Transcription Factors

TMS - Transcranial Magnetic Stimulation

TNF-α - Tumor Necrosis Factor-α

TRD - Treatment-resistant Depression

WHO - World Health Organization

SUMMARY

1. INTRODUCTION

Major depressive disorder (MDD) is one of the most prevalent and debilitating psychiatric disorders characterized by impairments in cognition, emotional, memory, motivation and behavior (Dean & Keshavan, 2017). According to World Health Organization (WHO) (2017) (World Health Organization, 2017), about 350 million people are affected by a depressive disorder. Major depressive disorder is associated with functional impairment and medical comorbidities, which leads to increased mortality and poor quality of life (Yan et al., 2019). Life expectancy of people with depression is on average 15 years less than the general population (Jia, Zack, Thompson, Crosby, & Gottesman, 2015). The Global Burden of Disease, Injuries, and Risk Factors Study (Vos et al., 2017), revealed that MDD was the fifth leading cause of years lived with disability in 2016. These factors put MDD in a scenario among the higher burden diseases (Liu Quingquing et al., 2019). Previous studies have shown that psychiatric disorders represent a substantial economic burden for patients, caregivers, healthcare providers and society (Cloutier, Greene, Guerin, Touya, & Wu, 2018).

Treatment options for depression are vast. Multiple modalities of treatment are effective for depression, including antidepressant medications, psychotherapies and various brain stimulation techniques (Akil et al., 2018). The most common treatment used for MDD are the second-generation antidepressants (e.g. selective serotonin reuptake inhibitors (SSRI) or serotonin-norepinephrine reuptake inhibitors (SNRI)), considered as first-line treatment (Gartlehner et al., 2016, 2011). However, more than 50% of patients with MDD do not reach full remission with a first treatment; of those, 30%-40% also do not present any kind of response (Mrazek, Hornberger, Altar, & Degtiar, 2014; Rush, 2007). Therefore, for non-responders, therapeutic strategies include switching or combining antidepressants and improving antidepressant treatment with non-pharmacological therapy (Moret, 2005). The matching between the patient to the optimal treatment generally requires multiple trials of different treatments, although that more treatments tried without success, the less likely a successful outcome (Liston et al., 2014).

A significant percentage of all MDD patients exhibits resistance to all available standard treatments. Treatment-resistant depression (TRD) is used to describe patients who do not respond to antidepressant therapy after two adequate trials (duration of at least six weeks and use of adequate dosages) (Mrazek et al., 2014). Resistance to treatment can emerge in patients previously responsive to treatment or as a progressive, deteriorating illness course over time (Thase & Schwartz, 2015). According to Akil and cols. (Akil et al., 2018), the resistance can manifest as the presence of residual depressive symptoms following treatment as well as loss of effectiveness with ongoing treatment. An alternative treatment to pharmacoresistant patients are the neuromodulation strategies. In contrast to pharmacotherapies that exert their efficacy at the molecular level, neuromodulation techniques target entire neuronal circuits (Kraus, Kadriu, Lanzenberger, Zarate, & Kasper, 2019).

Transcranial magnetic stimulation (TMS) is a non-invasive procedure that has been approved by the Food and Drug Administration (FDA) for the treatment of major depressive disorder in adults who have failed to receive satisfactory improvement with antidepressant medications (Janicak & Dokucu, 2015). TMS involves passing an electrical current through a coil placed against the scalp. The rapidly changing electrical current creates a time-varying magnetic field, which passes unimpeded through the scalp and skull and induces an electrical field in the cortex (Levkovitz et al., 2015) (Figure 01). If these currents are of a suitable size, duration, and localization, they will depolarize neural tissue and generate an action potential, which then propagates by the body’s normal nerve conduction mechanisms (Barker & Shields, 2017).

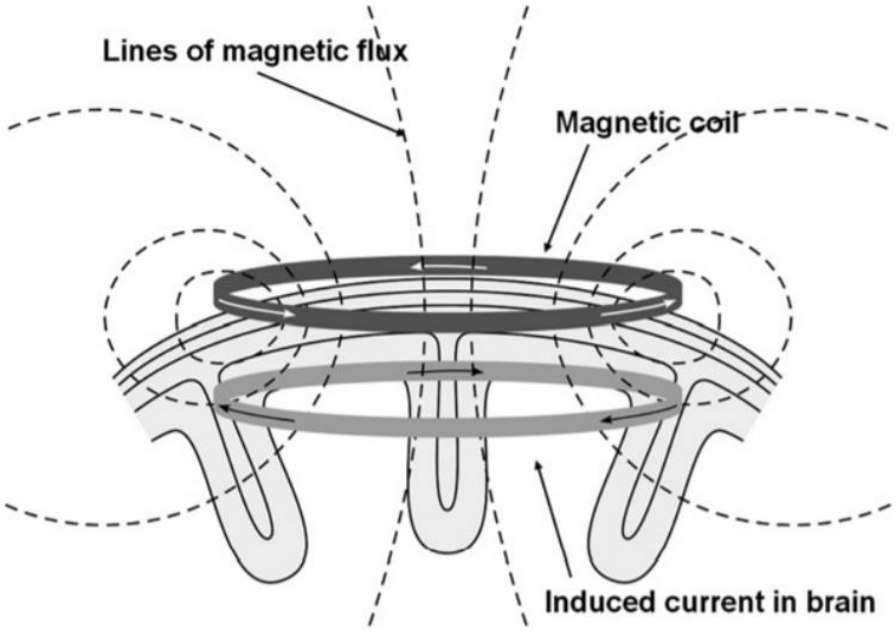

FIGURE 1. Illustration of direction of current flows in a magnetic coil and the induced current in the brain. Adapted from Hallet, 2007.

Repetitive TMS (rTMS) applied repeated TMS pulses at set frequencies or burst of stimulation to induce changes in cortical excitability. The alterations according to the applied frequency have generally been observed as a decrease in cortical excitability with low-frequency stimulation (≤ 1 Hz) and an increase in cortical excitability with high-frequency stimulation (≥ 5 Hz) (Allen, Kluger, & Buard, 2016; Liu, Sheng, Li, & Zhang, 2017; O'Reardon et al., 2007). From therapeutic and rehabilitative perspectives, the main interest of rTMS resides in the persistence of clinical improvement beyond the time of stimulation (Lefaucheur et al., 2014). Previous studies have demonstrated that when rTMS is given in daily sessions for 1-6 weeks the duration of such after-effects increases and may persist minutes to hours or even days after the end of an rTMS session (Kelly et al., 2017). Acute rTMS benefits patients with pharmacoresistant major depressive disorder and shows a sustained durability of effect across 12 months of follow-up (Dunner et al., 2014). However, the response to treatment is variable, with response

rates reported between 45% and 60% and remission rates between 30% (Kelly et al., 2017). The reasoning behind TMS responsiveness is yet to be fully understood. An overall hypothesis could be based on variation of genetic factors, such as single nucleotide polymorphisms (SNPs).

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people and are appropriate candidates for molecular marker development due to their abundance within the genome, helping scientists to locate genes associated with disease (Miranda, Romano-Silva, & De Marco, 2008). When SNPs occur within a gene or in a regulatory region, they may play a more direct role in disease by affecting the gene's function. SNP genotyping is a technique used for genome-wide association studies (GWAS) which identify significantly associated variants with a trait or disease (Howard et al., 2018). GWAS evaluate common variation in the genome, usually variants with frequencies greater than 5% and typically requires a genome-wide significance threshold of 5×10^{-8} (Flint & Kendler, 2014). Analysis of the genotype–phenotype associations makes it possible to establish a link between the allelic variant in some particular region of the genome with the trait studied.

An important feature of GWASs is that the identified variants indicate genomic regions, without a direct link to the underlying biological mechanisms. Thus, the identified variant may not be a cause to the phenotype of interest, but to other phenotypes strongly associated with the phenotype of interest, including comorbid conditions and intermediate characteristics in the biological disorder pathway that lead to the final outcome. Additional bioinformatics analyses may explore whether genes within GWAS loci are preferentially expressed in certain tissues or enriched in certain networks and pathways, and whether these genes are targets for existing (e.g., psychiatric) medications (Ormel, Hartman, & Snieder, 2019).

Several studies of the genetic architecture of depression indicate that it is polygenic, meaning that the contribution of genetic factors is attributable to small effects of hundreds or thousands of genetic variants spread across the gene (Peterson et al., 2017; Patrick F. Sullivan, Daly, & O'Donovan, 2012). Family and twin studies have

provided strong evidence about the contribution of genetic factors to the risk of depression (Shadrina, Bondarenko, & Slominsky, 2018). A meta-analysis conducted by Sullivan and cols. (2000) (P. F. Sullivan, Neale, & Kendler, 2000) shows that the heritability rate for depression is 37% (95% IC: 31% - 42%), and data from family studies show a two to threefold increase in the risk of depression in first-degree offspring of patients with depression.

Many genetics studies have sought to identify loci that are significantly associated with MDD, however a lot of GWASs have had a notable difficullties in identifying individual associated loci. This failure to identify clearly the genetic associations indicate that the depession is a polygenic psychiatric disorder. A recent genome-wide association meta-analysis conducted by Wray and cols. (2018) based in 135,458 cases and 344,901 controls identified 44 independent and significant loci. Of these 44 loci, 30 are new and 14 were significant in a prior study of MDD and depressive symptoms. Between the significant SNPs founded showing genome-wide significant association or suggestive significant association with psychiatric disorder were the genes WFDC11 (protease inhibitors) and HTR1B (regulation of the serotonin system). The CONVERGE (China, Oxford and Virginia Commonwealth University Experimental Research on Genetic Epidemiology) consortium studied 5,303 Han Chinese women with recurrent MDD and 5,337 controls and identified two loci, near the SIRT1 gene and in a intron of the LHPP gene (Cai et al., 2015).

2. OBJECTIVES

2.1. Overall Objective

The present study aims to characterize the genetic profile of refractory individuals with MDD and their responsiveness to TMS under two distinct protocols.

2.2. Specific objective

2.2.1. To find out which SNPs are related to responsiveness to TMS.
2.2.2. To identify which genes are related to the SNPs found.
2.2.3. To identify genes pathways.

3. SUBMITTED ARTICLE

A Genetic Profile of Refractory Individuals with Major Depressive Disorder and Their Responsiveness to Transcranial Magnetic Stimulation

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ABSTRACT

Background: Major depressive disorder (MDD) is a debilitating illness characterized by the persistence of negative thoughts and emotions. Although antidepressant medications are effective, less than half of patients achieve complete remission despite multiple treatment trials. Repetitive transcranial magnetic stimulation (rTMS) has proven effective in the treatment of depression, especially for patients resistant to antidepressant medications. Remission rates when using rTMS for treatment-resistant depression (TRD) patients are between 30% and 40%. The responsiveness to pharmacotherapy and rTMS therapy may be influenced by genetic factors.

Objective: Here we aim to characterize the genetic profile of refractory individuals with MDD and their rTMS responsiveness.

Methods: We used an extreme-phenotype design (rTMS responders vs. non-responders) and conducted a genome wide association study on 48 participants and 593,260 SNPs.

Results: We identified 53 significant SNP associations. Gene-set enrichment analysis showed that significantly associated genes loaded onto synaptic plasticity regulation pathways. Among the genes found differentially expressed in rTMS responders compared to non-responders were *APP, GRID2* and *SPPL2A* genes.

Conclusions: Based on these findings, we suggest that the identified genes may influence of rTMS responsiveness. Furthermore, the rTMS responsiveness may be associated with several pathways and not just to the influence of a single gene. To the best of our knowledge, this is the first report on the genetic profile of rTMS response using a GWAS approach. Nevertheless, further studies are necessary to enlight the molecular mechanism by which these genes affect response to rTMS treatment.

KEYWORDS: Major Depressive Disorder, Transcranial Magnetic Stimulation, Genome Wide Association Study, Treatment-resistant Depression

Introduction

Major depressive disorder (MDD) is characterized by the persistence of negative thoughts and emotions that disturb mood, cognition, motivation and behavior [1]. According to the World Health Organization (WHO) [2] depression is the main reason of disability worldwide, affecting around 300 million people. Depression can occur at any stage of life, from childhood until old age, with a twofold higher incidence in women compared to men [6]. Several treatment options are available for depression, such as pharmacological and non-pharmacological therapy, psychotherapy and brain stimulation techniques. However, less than half of patients with MDD achieve complete remission after a first line treatment with antidepressants [7].

Treatment-resistant depression (TRD) refers to the occurrence of an inadequate response after antidepressant treatment among patients that suffer from unipolar depressive disorder [8]. The most traditional concept of TRD is based on the non-responsiveness to at least two protocols of antidepressant therapy [9]. Among the patients who receive adequate treatment for MDD, only 30% respond to treatment and achieve remission. Of the remaining 70%, approximately 20% of the patients present an improvement in depressive symptoms, although not achieving remission, while 50% do not present any kind of response [10]. Due to the low efficacy of antidepressants after two attempts of treatment without success, new alternative therapies have been developed. The use of neurostimulation strategies are potential candidates acting as alternative or complementary therapies for neuropsychiatric disorders.

Repetitive transcranial magnetic stimulation (rTMS) has been demonstrated to be an effective depression treatment [11]. In rTMS, electromagnetic induction is used to induce focal currents in superficial brain regions and modulate cortical function [12]. Previous studies have demonstrated that low-frequency stimulation of rTMS ($\leq$ 1 Hz) leads to cortical activity inhibition, whereas high-frequency stimulation ($\geq$ 5 Hz) increases cortical excitability [13]. Although rTMS is an effective treatment for many patients with TRD, it is not always effective with remission rates ranging from 30% to 40% and response rate between 45% and 60% [14]. The factors contributing to rTMS responsiveness remain unclear. Although one potential source of inter-individual variability in responsiveness to rTMS could be genetics, few studies have sought to identify a genetic basis of rTMS response [15].

The genetic basis of depression is well established through twin and family studies with heritability estimates ranging from 30% to 40%, and SNP-based heritability estimates ranging from 9% to 29% [16,17]. Risk of MDD is highly polygenic and involves many genes with small effects. This coupled with the clinical heterogeneity of MDD requires very high numbers of patients to find significant associations [18]. Genome-wide association study (GWAS) is a powerful tool for investigating the genetic risk factors of complex human disease, providing information about variants that may be associated with a trait [19,20]. GWAS has been used to map genetic loci, associated with MDD [21,22]. A recent GWAS conducted by Wray et al (16,823 MDD cases and 25,632 controls) identified 44 risk variants and significant loci associated to MDD [17]. Only hypothesis driven SNP genotyping approaches have been so far applied in studies with rTMS response, mainly focusing on BDNF [23–26] or serotonergic genes such as 5-HTTLPR [27].

In face of such knowledge, we hypothesized that inter-individual differences in genetics may influence the responsiveness of rTMS in patients with treatment resistant MDD. To explore this hypothesis we used an extreme-phenotype design in which we compared allelic variation genome-wide between rigorously defined rTMS responders and non-responders.

Materials and Methods

Participants

The study was approved by the Human Research and Ethics Committee of the Alfred Hospital. All patients had a DSM IV diagnosis of major depressive disorder applied by an experienced psychiatrist and confirmed by the Mini-International Neuropsychiatric Interview (M.I.N.I) [28]. Patients who received rTMS treatment whilst participating in one of two clinical trials [29,30] were recruited for genetic analysis. All patients received high frequency left sided 10Hz rTMS, either in a standard daily format or in a more accelerated treatment protocol (Table 01). Patients were asked to donate saliva for DNA samples. The DNA extraction was conducted using a standard protocol as recommended by the supplier Oragene® (Kit Oragene-DNA | OG-600 prepIT-L2P). The resulting purified gDNA is ideal for microarray analysis.

A total of 99 (100%) individuals consented and provided samples for analysis. Clinical outcomes (response or non-response) were determined based on scores on the Montgomery Asberg Depression Rating Scale (MADRS). We compared MADRS scores from baseline to the end of acute treatment. We included individuals for analysis who were either clear responders to rTMS (a greater than 60% reduction on the MADRS scale – N = 29 (29.29%) or clear non-responders (below a 10% reduction on the MADRS scale – N = 19 (19.19%)). The remaining 51 (51.51%) subjects who demonstrated a reduction of between 11-59% were excluded from the analysis. These criteria were applied considering that extreme scoring patients (<10 >60) may present more representative genetic results to the allocated groups. Recently association studies have been sampling the extremes as a strategy for achieving good statistical power under sample size limitations. This strategy is based on the assumption that extreme phenotype sampling may increase power to detect causal variants [31–33].

TMS Treatment

Treatment followed the same conditions described by Fitzgerald *et al* [29,30]. In one study all patients received a 3 week course of 10 Hz stimulation applied to the left DLPFC with an extension of this course up to 6 weeks in total or switching to low frequency right sided rTMS or bilateral rTMS if not meeting partial response criteria at 3 weeks [29]. Patients in the second study received one of two treatment conditions – accelerated rTMS and standard rTMS. In the accelerated treatment, in week 1, patients were provided 3 sessions per day over 3 days. In week 2, patients were provided 3 sessions per day over 2 days and in week 3, 3 sessions in a single day were provided. In a standard treatment, 20 daily sessions provided 5 days per week over 4 weeks [30]. Both treatments provided trains of 10 Hz rTMS to the left dorsolateral prefrontal cortex (DLPFC).

Genotyping

Genotyping was performed using the Infinium PsychArray-24 BeadChip (Illumina, Inc., San Diego, CA, USA) and automated workflow according to the manufacturer's instructions. Raw data were analyzed using PLINK 1.9 [34].

Data quality control

Due to large number of marker loci tested in GWAS a rate of error or bias can be harmful. Therefore, to remove false-positive or false-negative associations, steps of quality control was performed to remove individuals or markers with high error rates. Data quality control was performed using PLINK 1.9 [34]. SNP inclusion required: call rate (GENO) > 90%, maximum individual missingness rate (MIND) > 10%, minor allele frequency (MAF) < 5% and Hardy-Weinberg Equilibrium (HWE) p-value > 10^{-6}.

Association analysis

For the analysis of association between the phenotype and the response to rTMS therapy we used the resources available in PLINK 1.9 [34]. We performed standard association analysis to compare allele frequency in both groups (responders and non-responders) with a 95% confidence interval through the following commands (--assoc), (--ci 0.95) (--adjust).

Pathways Analysis

The protein-protein interaction (PPI) network analysis of the identified hub genes was constructed using the Search Tool for the Retrieval of Interacting Genes (STRING) database (database of known and predicted protein-protein interactions) [35]. The resulting PPI network was then visualized using Cytoscape [36] software (software platform for visualizing molecular interaction networks and biological pathways that integrating these networks with annotations, gene expression profiles) (ClueGO plug-in) for the functional enrichment analysis.

Results

Since the observed clinical responses between both trials were similar, data analysis and results were presented in conjunction (Table 02). Quality control analysis on the raw genotypic data was conducted in 48 individuals and 593,260 SNPs. After application of data quality control criteria, 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 submitted the results to the False Discovery Rate (FDR) correction

considering sample and SNP size per chromosome [37,38]. A new *p* value was then determined for each chromosome (Table 03). GWAS analysis using PLINK 1.9, revealed 53 significantly SNP associations, 11 of which were related to treatment response and 42 associated with non-responsiveness to treatment. Of the 53 associated SNPs, 25 were mapped to non-coding genomic regions. The remaining 28 SNPs mapped to protein coding genes; 9 SNPs mapped to described pathways (Table 04).

Protein-protein interaction network (PPI) analysis performed through the STRING database, presented no pathway association between the identified genes (Table 05). In an attempt to explore an interaction network analysis between the selected genes, we included common pharmacological target genes for depression treatment in the analysis. Among the pharmacological target genes were *BDNF, COMT, SLC6A1*, however, no significant protein-protein interaction network was identified (Figure 01).

Genes and pathway interaction networks were obtained after enrichment analysis using ClueGo, a Cytoscape plug-in. The pathways involved were: synaptic plasticity regulation pathway, containing - *APP* (precursor beta amyloid protein), *SPPL2A* (signaling GPCR - transmembrane proteins), *GRID2* (glutamatergic ionotropic receptor), *ADGRB3* brain-specific angiogenesis inhibitor), *COL9A3* (structural constituent of the extracellular matrix) genes (Figure 02).

Discussion

The results of this study reveal a number of SNPs that may be associated with the response to rTMS in treatment-resistant patients with major depressive disorder. Among the findings are genetic polymorphisms that have already been mapped to genes associated with the innate and adaptive autoimmune response, genes involved in the pathophysiology of Alzheimer's disease (AD), regulation of synaptogenesis and dendritic spine formation [39–41]. Although these genes point to a contribution to the overall phenotype, it is important to elucidate the effects of each gene on disease development. It is worth mentioning that the sum of multiple genetic and environmental factors leads to different clinical presentations and therapeutic responses in each patient.

In our study, the repetitive TMS (rTMS) protocol to the left DLPFC was performed in predict and accelerated modalities. It was possible to observe that the treatment model adopted did not interfere in the individuals' responsiveness to rTMS (Table 02). Therefore, for the genetic analysis patients from both protocols (predict and accelerated) were grouped.

Although we were not able to characterize a complete protein-protein interaction network with the SNP carrying genes, we were able to further describe their individual functionality and possible association to our disease and treatment in focus.

In the synaptic plasticity regulation pathway the significant genes found were related to the signaling of transmembrane proteins (*SPPL2A*), precursor of the beta amyloid protein (*APP*), exosomal component (*EXOSC7*), glutamatergic ionotropic receptor (*GRID2*), brain-specific angiogenesis inhibitor (*ADGRB3*), structural constituent of the extracellular matrix (*COL9A3*), lymphocyte antigen 9 (*LY9*) and forkhead box N3 (*FOXN3*) (Table 05).

Positive Response Associated Genetic Variants

Signal peptide peptidase-like 2a (*SPPL2A*) is a gene that encodes an aspartic intramembrane protease that plays an important role in the development and function of antigen presenting cells such as B-lymphocytes and dendritic cells. Regulated intramembrane proteolysis (RIP) is a process that controls communication between cells and the extracellular environment mediated by a family of proteases, the intramembrane cleaving proteases (I-CLiPs) [42]. The founding members of this I-CliPs family are the presenilins (PS1 and PS2), the catalytically active subunit of the ɣ-secretase complex [43]. *SPPL2A* has been described as an enzyme related to presenilins [42]. The prominent class of ɣ-secretase is an aspartyl I-CLiPs involved in the generation of the beta-amyloid (Aβ) peptide from the amyloid precursor protein (*APP*). In AD patients it is possible to find the presence of amyloid plaques in neural tissue, and it is believed that the accumulation of these polypeptides is involved in the development of the disease [44]. Previous studies have shown that depression is one of the most frequent comorbid psychiatric disorders in Alzheimer's disease and up to 50% of patients with AD will suffer from depression at some stage during the progression of dementia [45]. A study of Zhu *et al* [46] showed that similar environmental risk factors have been implicated in different neuropsychiatric diseases

(including major depressive disorder and Alzheimer's disease), indicating the existence of common epigenetic mechanisms underlying the pathogenesis shared by different illnesses.

Negative Response Associated Genetic Variants

EXOSC7 is a gene encoding RNA exosome – exosome component 7. The RNA exosome is a ribonuclease complex composed of both structural and catalytic subunits that participate in the processing of stable RNA species [47]. Mutational changes in genes encoding RNA exosome subunits may trigger inherited tissue-specific diseases [48]. A study conducted by Di Donato *et al* [49] showed that mutations in *EXOSC2* have been linked to a novel syndrome characterized by early onset retinitis pigmentosa, progressive sensorineural hearing loss, hypothyroidism, premature aging and mild intellectual disability. Other studies reveal that mutations in *EXOSC3* have been linked to pontocerebellar hypoplasia type 1 (PCH1b), an autosomal-recessive, neurodegenerative disease characterized by significant atrophy of the pons and cerebellum, Purkinje cell abnormalities, and degeneration of spinal motor neurons [50].

Glutamate ionotropic receptor delta type subunit 2 (*GRID2*) is a gene member of the family of ionotropic glutamate receptors which are the predominant excitatory neurotransmitter receptors in the mammalian brain. Single nucleotide polymorphisms (SNPs) in glutamate-related genes have been associated with antipsychotic response or treatment resistance. A GWAS conducted by Stevenson *et al* [51] identified two SNPs in the *GRID2* gene (rs9307122 and rs1875705) that were associated with reduced response to antipsychotic treatment according to the Brief Psychiatric Rating Scale change score. The results found by Stevenson *et al* [51] support the hypothesis that genetic variation in glutamate system genes may impact the clinical trajectory of the patients treated with antipsychotic medications, and that these may represent a broader involvement of neurodevelopmental pathways. Furthermore, the *GRID2* gene is selectively expressed in Purkinje cells in the cerebellum where they play a key role in synaptogenesis, synaptic plasticity and motor coordination. For that matter, different mutations in *GRID2* have been shown to cause cerebellar ataxia in human [52]. In a study conducted by Schwenkreis *et al* [53] proved the existence of abnormal motor cortex activation by TMS in some types of genetically defined spinocerebellar ataxia (SCA), whereas other genetic subgroups show normal responses.

Adhesion G protein-coupled receptor B3 (*ADGRB3*) also known as *BAI3* is a gene that encodes a brain-specific angiogenesis inhibitor and is thought to be a member of the secretin receptor family. This gene play a key role in the regulation of several aspects of the central nervous system, such as axon guidance, myelination and synapse formation and function [54]. *ADGRB3* SNPs has already been associated with schizophrenia, bipolar disorder and drug addiction. In a family-based study conducted by Scuderi *et* al [41] shows a correlation between a disrupting intragenic duplication involving several exons of the *ADGRB3* and intellectual disability, cerebellar atrophy and behavioral disorder. The BAI proteins are highly expressed in the brain and have been identified at postsynaptic densities in the forebrain and cerebellum. The involvement of these proteins in the development of functional neuronal networks is related to their structural characteristics. The morphology and complexity of dendritic arborization allow functional differences of neurons, and deficits in neuronal morphogenesis correlate with psychiatric disorders. Lanoue *et al* [55] presented evidence both *in vivo* and in vitro for a signaling pathway regulating the morphogenesis of dendrites involving *BAI3*. The authors suggest that an interaction between BAI3 and the ELMO1 protein (important regulator of RAC1 RhoGTPase) is involved in this signaling.

COL9A3 is a gene that encodes one of the three alpha chains of the type IX collagen. Mutations in this gene are associated with multiple epiphyseal dysplasia type 3. Some of the brain collagen proteins are expressed by neurons, suggesting their involvement in growth regulation and axonal orientation, synaptogenesis, cell adhesion, and brain architecture development [56]. Collagen biosynthesis in the brain can be abnormal in many hereditary diseases. Much of the brain pathology associated with collagen are related to neurodevelopment. 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 [57,58].

Lymphocyte antigen 9 (*LY9*) belong to signaling lymphocytic activation molecule (SLAM) family of immunomodulatory receptors. According to previous studies, the activation of upstream gene regulatory pathways that modulate gene expression in immune cells may be linked to MDD [59]. Between the active pathways there are a family of transcription factors (TFs), the glucocorticoid receptor (GR), cAMP response element-binding (CREB), early growth response (EGR) family TFs, and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α),

interleukin (IL)-1β and IL-6 [60–63]. In a study conducted by Mellon *et* al [64] to test the theory on transcriptional control pathways that may be active in MDD, 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 gene member of the forkhead/winged helix transcription factor family. Recent GWASs was conducted to analyze possible gene that are associated with suicide found significant correlation between evidence for suicidality and the gene *FOXN3* [65,66]. However, the way this gene may influence the risk of suicide is not fully elucidated.

Conclusions

In this study, we set out to test whether polymorphic profiles are associated to rTMS treatment outcome. From the findings, we may consider that the responsiveness to rTMS may be associated to several pathways and not just to the influence of a single gene. As already reported in the literature the influence of genes such as *APP, GRID2, SPPL2A* and others on MDD (also described here), suggests that the genes found may influence the response to rTMS. However, the molecular mechanisms by which these genes may influence the response to rTMS treatment are unknown, requiring further investigation.

This study has some limitations that should be noted. The sample size used in this study was smaller than typically employed in genetic association studies and stratified (Australian patients with a diagnosis of major depression disorder refractory to pharmacological treatment). Although traditional GWAS require a vast number of genotyped individuals, this method is expensive and time-consuming. A potential solution for this is extreme phenotypic sampling. Recent studies have compared the results of extreme phenotypic sampling with large-scale samples, and showed that extreme phenotypes are effective [67]. This method allow to identify rare causal SNPs with increased efficiency. Due to heterogeneity of MDD the study with homogenous patient subgroups allows a better understanding about etiological mechanisms and thus the development of patient-specific treatment [68]. In addition, few reports have been found in the literature associating genetic profile and response to rTMS therapy in treatment-resistant

depression patients [69–71]. Further replication is necessary to confirm the present findings and to further uncover the genetic profile of refractory individuals with MDD and their responsiveness to rTMS.

Acknowledgements

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 an 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).

Conflict of 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.

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Tables

Table 01. Characteristics of participants. Abbreviations: SSRI, selective serotonin reuptake inhibitor; MAOI, monoamine oxidase inhibitor; SNRI, serotonin-norepinephrine reuptake inhibitor; TCA, tricyclic antidepressant; RIMA, reversible inhibitor of monoamine oxidase-a; NASSA, noradrenergic and specific serotonergic antidepressant.

Age - Mean (SD)	
Age (years)	47 (13.26)
Range (years)	19-74
Gender (%)	
Male (#)	21 (43,75)
Female (#)	27 (56,25)
Occupational status (%)	
Employed (#)	15 (31,25)
Unemployed (#)	21 (43,75)
Part-time (#)	6 (12,5)
Retired (#)	3 (6,25)
N/A (#)	3 (6,25)
Age onset (%)	
Childhood (#)	5 (10,42)
Adolescence (#)	15 (31,25)
Early adulthood (#)	16 (33,33)
Mid adulthood (#)	12 (25)
Use of antidepressant (%)	
SSRI	8 (16,66)
MAOI	3 (6,25)
SNRI	14 (29,16)
TCA	3 (6,25)
RIMA	1 (2,08)
NaSSA	1 (2,08)
Combination	7 (14,58)
None	9 (18,75)
N/A (#)	2 (4,16)

Table 02. Response rates after treatment with rTMS. Analysis conducted in GraphPad Prism with Chi-square Test and Fisher's exact test to show the difference between groups after treatment with rTMS (*p* 0.5544).

Data analyzed	Accelerated (n)	Predict (n)
Non-responders	13 (43,33%)	6 (33,33%)
Responders	17 (56,66%)	12 (66,66%)
Total	30 (100%)	18 (100%)

Table 03. New *p* value per chromosome after FDR correction.

Chr	Total SNP	New *p*-value (FDR)
1	19934	0.000106
2	20574	0.000090
3	17491	0.0000936
4	16104	0.0001141
5	15623	0.0001225
6	17610	0.0001126
7	14047	0.0001552
9	11283	0.0001893
10	12745	0.0001457
11	12742	0.0001628
12	12093	0.0001572
13	9490	0.000218
14	7895	0.0002183
15	7537	0.0002380
17	7233	0.0002747
18	7408	0.0002840
19	5896	0.0003472
21	3703	0.0004098
22	3687	0.0004761
X	6442	0.0001432

Table 04. 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-responsiveness treatment.

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

Table 05. Description of significant genes.

Gene	Brain Expression	Function
SPPL2A	+	Catalyzes the intramembrane cleavage of a several proteins and may play a role in the regulation of innate and adaptive immunity.
APP	+	Performs physiological functions on the surface of neurons relevant to neurite growth, neuronal adhesion and axogenesis.
EXOSC7	+	Presents exoribonuclease activity and participates in a multitude of cellular RNA processing and degradation events.
GRID2	+	Plays a role in synapse organization between parallel fibers and Purkinje cells.
ADGRB3	+	Plays a role in the regulation of synaptogenesis and dendritic spine formation.
COL9A3	+	Possesses the function of structural component of hyaline cartilage.
LY9	-	Modulates the activation and differentiation of a wide variety of immune cells and are involved in the regulation and interconnection of both innate and adaptive immune response.
FOXN3	+	Acts as a transcriptional repressor and may be involved in DNA damage-inducible cell cycle arrests (checkpoints).

Figures

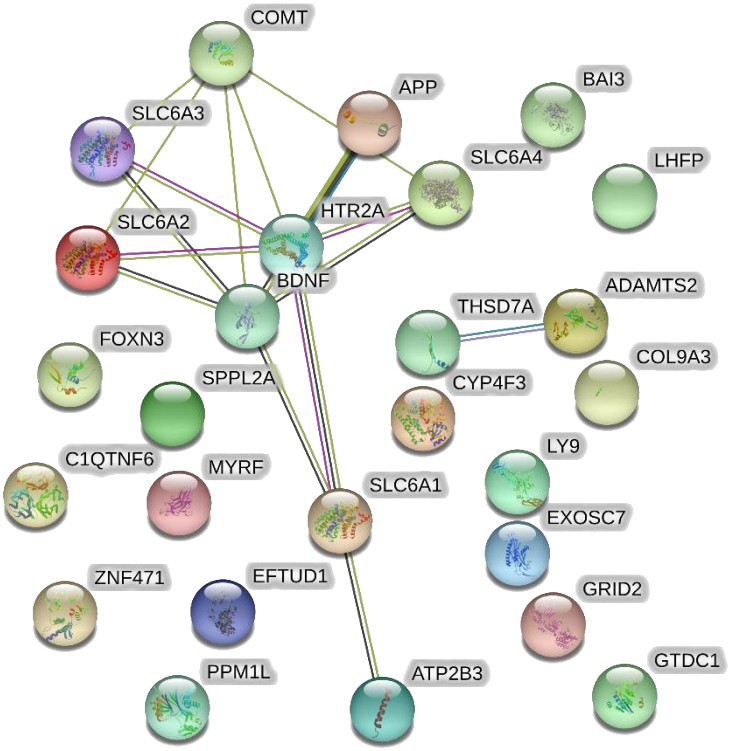

Figure 01. Protein pathway network from STRING. Genes of positive SNPs with pharmacological targets. Colors of edges: black (co-expression), green (textmining), pink (experimentally determined) and blue (from curated databases). Colors of nodes: colored nodes – query proteins and first shell of interactors, white nodes – second shell of interactors. Node content: empty nodes – proteins of unknown 3D structure, filled nodes – some 3D structure is known or predicted.

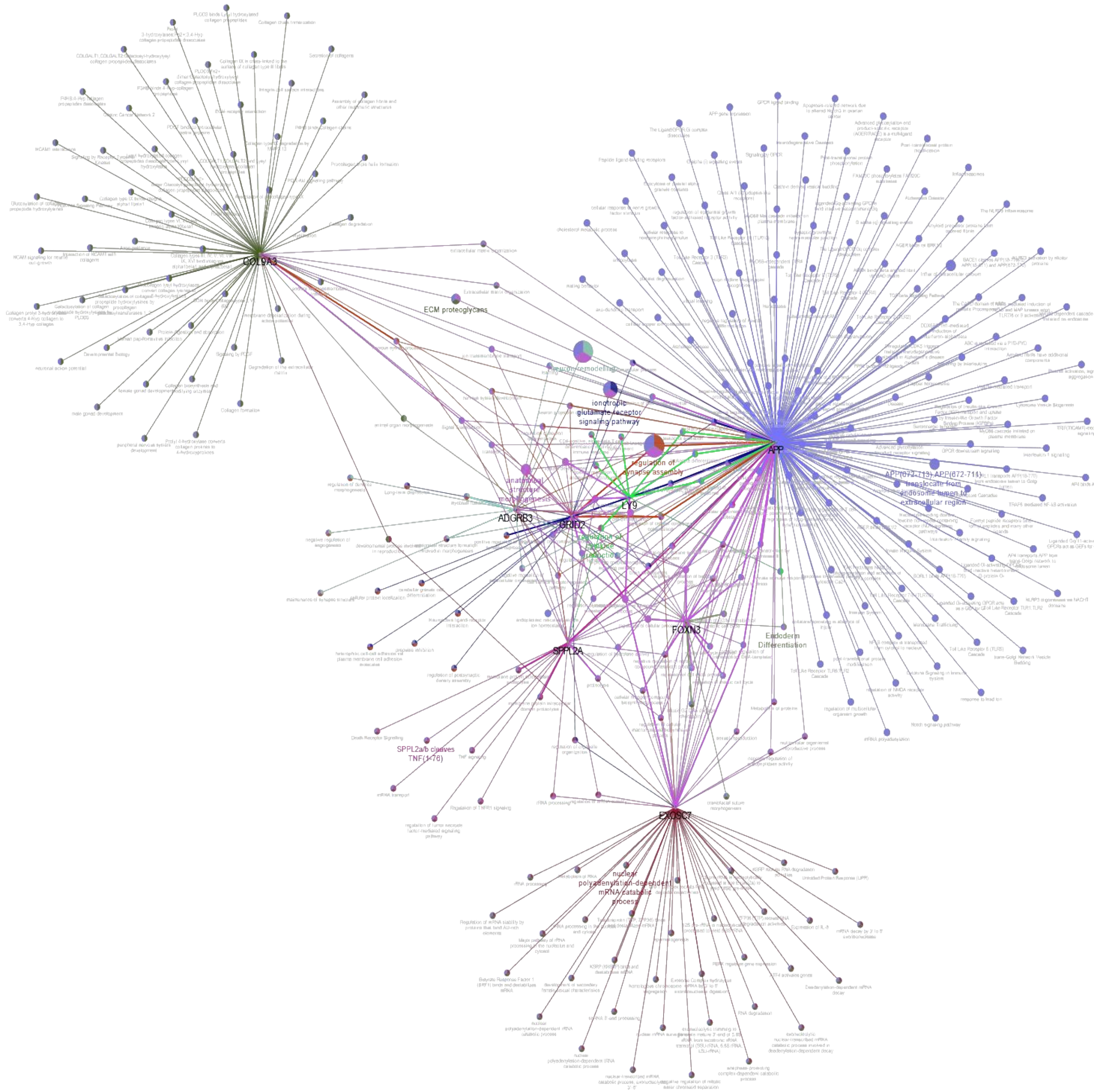

Figure 02. Regulation of synaptic plasticity pathway. In this way, the genes that are in bold and their hexagon-shaped nodules are the genes found after genome analysis through PLINK. Ball-shaped nodules represent the pathways to which these genes participate. The interaction between the genes found results in the regulation pathway of synaptic plasticity. The genes involved in this pathway are: *APP* (amyloid beta protein precursor), *SPPL2A* (GPCR signaling - transmembrane proteins), *EXOSC7* (exosome component), *FOXN3* (forkhead/winged helix transcription factor family), *GRID2* (glutamatergic ionotropic receptor), *LY9* (Self-ligand receptor of the signaling lymphocytic activation molecule (SLAM) family), *ADGRB3* (brain-specific angiogenesis inhibitor), *COL9A3* (extracellular matrix structural constituent).

4. MATERIALS AND METHODS

4.1. POLYGENIC RISK SCORE

The information about the participants of the study and the steps of GWAS approach are described in the article above.

Polygenic risk score (PRS) was performed using PLINK 1.9 (--score; --read-freq). The allelic frequency data with the allelic association data of the 53 significant SNPs were used to perform the polygenic risk (Table 1). We use a logistic regression to assess the association between polygenic risk, depression and responsiveness to rTMS.

TABLE 1. Variants used to scoring.

SNP	A1	OR
rs134913	G	0.333333
rs17333434	G	0.245769
exm-rs5916687	A	0.24152
rs5980684	A	0.223359
exm2273081	C	0.222605
rs1343974	C	0.222605
rs2980075	C	0.222237
rs12559502	G	0.213704
rs4648426	G	0.19938
rs11942069	A	0.19938
rs229526	C	0.198951
rs373521	A	0.194275
rs2829964	A	0.18589
rs6899975	A	0.185237
rs12390729	A	0.185049
rs9548721	G	0.179835
rs6640653	A	0.177548
exm-rs2068888	G	0.176742
rs960995	G	0.172951
rs447347	A	0.170243
rs1014129	A	0.16315
rs198475	A	0.160563
rs2142419	A	0.15646
rs2189698	C	0.149201
rs872994	A	0.143006
rs4243296	G	0.143006
rs8082822	A	0.143006

rs1074349	G	0.141307
rs17164813	A	0.141094
rs595562	G	0.138827
rs2829950	C	0.122303
rs944868	C	0.119689
rs560681	G	0.108799
rs11956034	A	0.108799
rs626904	A	0.100796
exm2271926	G	0.100796
rs11265485	G	0.084996
psy_rs17673232	A	0.064825
rs4646515	G	0.0569564
rs1934115	C	0.0552054
rs7135989	A	0.0533886
rs1283468	A	0.0333333
rs9981074	A	68.841
rs4347699	A	100.604
rs10787147	G	133.305
rs12487861	A	152.481
rs5915786	G	433.774
rs8035452	G	566.598
rs5995416	A	583.614
rs2094718	C	600.544
rs501118	A	708.646
rs17317597	G	817.192
rs1144863	A	972.964

5. RESULTS

5.1. POLYGENIC RISK SCORE

Results concerning the genome association study may be found in the article above, therefore will not be cited here. Moreover, the results presented below refer to the polygenic risk score (Table 2).

TABLE 2. Polygenic risk score results.

IID	PHENO	SCORE_AVG
Acc_143	1	103.945
Pred_4	1	885.227
Pred_12	1	104.993
Acc_114	1	109.184
Acc_148	1	139.977
Pred_33	1	965.219
Acc_120	1	163.646
Pred_19	1	151.788
Acc_103	1	739.918
Acc_124	1	197.847
Acc_104	1	131.249
Acc_125	1	990.193
Acc_105	1	266.781
Acc_161	1	109.375
Pred_23	1	734.569
Acc_137	1	0.0819187
Acc_165	1	556.363
Pred_1	2	348.349
Pred_24	2	483.326
Acc_108	2	43.651
Pred_26	2	805.507
Acc_110	2	398.081
Acc_144	2	339.807
Pred_6	2	343.453
Pred_27	2	810.177
Acc_112	2	493.482
Acc_146	2	590.323
Pred_8	2	520.638
Pred_29	2	717.694
Acc_113	2	472.715

Acc_147	2	527.197
Pred_30	2	514.552
Pred_17	2	720.176
Acc_150	2	396.954
Acc_122	2	341.726
Acc_153	2	42.724
Acc_154	2	419.462
Pred_20	2	739.814
Acc_157	2	343.361
Acc_127	2	548.293
Pred_22	2	637.158
Acc_106	2	410.687
Acc_134	2	431.067
Acc_107	2	486.496

IID: Identification of subjects – Pred_: standart rTMS protocol, Acc_: accelerated rTMS protocol
PHENO: 1 – Non-responders, 2 – Responders. SCORE_AVG: score of weighted allelic dosage.

Data distribution was non-normal. PRS showed a subgroup-pattern distribution divided in three peaks of distribution, according to histogram analysis. Bases on this distribution we used percentiles scores to create the low, medium and high responsive subgroups used on further analysis.

We used chi-square tests to compare PRS group distribution and the TMS response group. The chi-square test was significant (χ^2=20.43, p<0.001) and showed a large effect size (Φ=0.68). Figure 2 show the distribution (%) of groups divided by phenotype. We found significant differences (after Bonferroni correction) between respondents and non-respondents in Low (79% x 21%) and Medium (0% x 100%) PRS groups, but not in the High (39% x 61%).

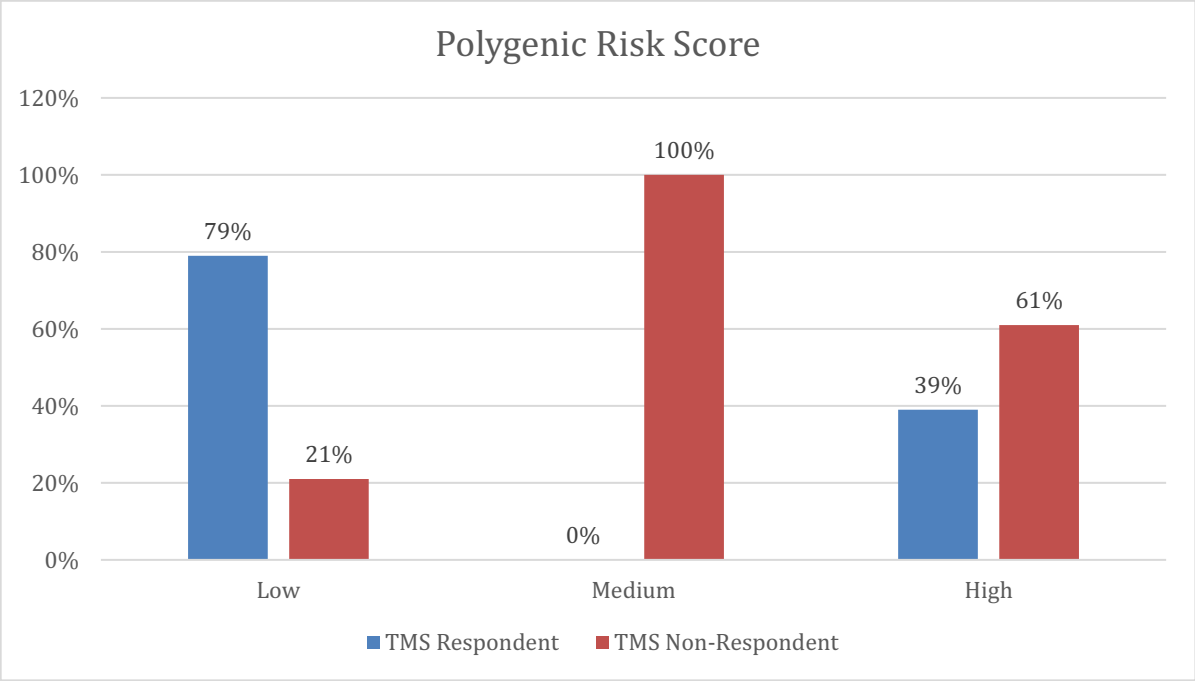

FIGURE 2. Polygenic risk score and distribution of groups.

6. DISCUSSION

In this study, we tried to find out if there is any relationship between genetic polymorphisms and responsiveness to rTMS. In our knowledge, this is the first study that investigates the relationship between genetic variations and responsiveness to rTMS throughout GWAS. In our results, we found polymorphisms that have already been associated with neuropsychiatric disorders, such as Alzheimer's, immune system responses, synaptogenesis regulation and dendritic spine formation. Our findings are in accordance with the literature, since depression is a polygenic trait that is influenced by many different genetic variants (Ormel, Hartman, & Snieder, 2019).

Among the genes found from the significant SNPs, the genes those are interacting on synaptic plasticity regulation pathway demanding special attention to study them since synaptic plasticity represents one of the most fundamental important functions of the brain. Synaptogenesis is regulated by a complex interaction of signalling pathways, and disruption of many of the key pathways have been implicated in the susceptibility to depression (Duman, Aghajanian, Sanacora, & Krystal, 2016; Vose & Stanton, 2016).

Although these genes are already described in the literature as participants in the synaptic plasticity regulation pathway, thus far there is no report on the molecular mechanisms by which these genes interfere in the pathway. Therefore, more studies are needed. The use of polygenic risk score has become widespread in biomedical science, which demonstrated reliable, though modest prediction using straightforward scoring methods (Purcell et al., 2009). Polygenic risk can be characterized in a GWAS by creating a summary score of weighted allelic dosage across all single nucleotide polymorphisms associated with the outcome.

There is growing consensus that complex psychiatric disorders, including major depressive disorder have a significant polygenic risk component, in which genetic liability is conferred by the combined additive effects of large numbers of variants with small effect sizes (Musliner et al., 2019).

In our study, we examined the associations between polygenic risk score, depression and responsiveness to rTMS. We found that the variants might influence the responsiveness to rTMS. Within our sample, we observed an increased effect of polygenic risk score on TMS nonrespondent, with evidence for interaction between MDD

and significant SNPs. In the group of TMS nonresponders, the subjects that have a polygenic score presents higher chance do not respond to TMS treatment.

7. OVERALL CONCLUSION

- We set out to test whether polymorphic profiles are associated to rTMS treatment outcome.
    - We may consider that the responsiveness to rTMS seems associated to several pathways and not just to the influence of a single gene.
    - Although these SNPs/genes point to a contribution to the overall phenotype, it is important to elucidate the single effects of each gene on disease development.
    - Considering the phenotype of depression as a polygenic characteristic, a gradient in the level of depressive symptoms with an increasing number of risk alleles is expected. This hypothesis leads us to think that individuals with a high number of risk alleles would have a worse response to treatment with rTMS.
    - Genes related to pathways evolved to neurodegeneration and plasticity emerged once more in this study.
    - The molecular mechanism by which these genes may influence the response to rTMS treatment requires further investigation.

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