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Predisposition to treatment response in major depressive episode: A peripheral blood gene coexpression network analysis

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ABSTRACT

Antidepressant efficacy is insufficient, unpredictable and poorly understood in major depressive episode (MDE). Gene expression studies allow for the identification of significantly dysregulated genes but can limit the exploration of biological pathways. In the present study, we proposed a gene coexpression analysis to investigate biological pathways associated with treatment response predisposition and their regulation by microRNAs (miRNAs) in peripheral blood samples of MDE and healthy control subjects. We used a discovery cohort that included 34 MDE patients that were given 12-week treatment with citalopram and 33 healthy controls. Two replication cohorts with similar design were also analyzed. Expression-based gene network was built to define clusters of highly correlated sets of genes, called modules. Association between each module's first principal component of the expression data and clinical improvement was tested in the three cohorts. We conducted gene ontology analysis and miRNA prediction based on the module gene list. Nine of the 59 modules from the gene coexpression network were associated with clinical improvement. The association was partially replicated in other cohorts. Gene ontology analysis demonstrated that 4 modules were associated with cytokine production, acute inflammatory response or IL-8 functions. Finally, we found 414 miRNAs that may regulate one or several modules associated with clinical improvement. By contrast, only 12 miRNAs were predicted to specifically regulate modules unrelated to clinical improvement. Our gene coexpression analysis underlines the importance of inflammation-related pathways and the involvement of a large miRNA program as biological processes predisposing associated with antidepressant response.

1. Introduction

Major Depressive Disorder (MDD) is among the most common health problems worldwide, affecting between 5% and 15% of the general population (Kessler et al., 2003). Antidepressants are the most common treatment for MDD, yet roughly one-third of patients experience inadequate response to treatment after several attempts (Rush et al., 2006). This affects patient care, and social and economic outcomes of MDD.

The variability of response to antidepressant is a complex phenomenon that includes a large combination of environmental, genetic and epigenetic factors. Biomarkers have been suggested for treatment response prediction (Thase, 2014) but none of them have been validated. Moreover, the lack of a predictive tool is associated with a scarcity of knowledge of the biological mechanisms underlying treatment response in depression as well as its variability. Although substantial genetic contribution of common variants to treatment response phenotype has been demonstrated (Tansey et al., 2013), GWAS studies failed to achieve statistical significance

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and results need to be replicated (Biernacka et al., 2015). Genome-wide gene expression studies, by revealing the effects of both genetic background and environmental/epigenetic factors, provide an interesting insight into antidepressant response predisposition. These studies rely preferentially on peripheral tissues such as blood, whereas *post-mortem* brain tissues do not allow to correlate precisely clinical state or treatment response with biological measures (Menke, 2013). Gene expression studies allow to identify differentially expressed (DE) genes associated with antidepressant response (Lin and Tsai, 2016), which could provide interesting candidate predictive biomarkers. However, this method only identifies a few of the most significant DE genes, while psychiatric phenotypes are known to be associated with numerous genes that individually confer small and incremental risk to the phenotype (Xu et al., 2016). Moreover, the DE genes approach does not take into account the correlation between genes while co-expressed genes are frequent and tend to be functionally related (Gaiteri et al., 2010). Alternatively, gene coexpression network studies could bridge the gap between individual genes, emergent global properties of transcriptome profiles, and complex traits (Langfelder and Horvath, 2007; Zhu et al., 2012). This method is based on gene coexpression patterns and defined clusters of highly correlated sets of genes, called modules. This method can also be extended to the identification of common biological regulators of genes within a particular module, such as microRNAs (miRNAs) (Gaiteri et al., 2014; Xu et al., 2016). Indeed, each of these small single-stranded, non-coding small RNA molecules, can simultaneously modulate several genes associated with the same biological pathway. Moreover, the same mRNA could be regulated by numerous miRNAs, suggesting a convergent action of miRNAs (Barca-Mayo and De Pietri Tonelli, 2014). Several miRNAs have been individually implicated in antidepressant response or stress resiliency in animal as well as in human studies (Dias et al., 2014; Issler et al., 2014; Launay et al., 2011; Lopez et al., 2014). It is also possible that numerous miRNAs may be involved in orchestrating the antidepressant response, as described for complex dynamic systems regulation (Barca-Mayo and De Pietri Tonelli, 2014). In this study, we aimed to explore gene coexpression network associated with antidepressant response using blood tissue from subjects with MDD. We hypothesize that some of the co-expressed gene network modules may be related to predisposition to antidepressant response. These co-expressed genes modules could provide critical insight into inter-individual variability of treatment response by identifying biological pathways associated with antidepressant response predisposition. Finally, to identify potential common regulator of gene modules, we performed exploratory *in silico* analyses of potential miRNAs associated with these modules, as putative regulators of such biological pathways.

2. Material and methods

2.1. Design setting and population

We included in our analysis three prospective cohorts of MDD patients based on a similar design and recruited in Pittsburgh (PA, USA), Montréal (Quebec, Canada) and Marseille (France) as previously described (Belzeaux et al., 2012; Guilloux et al., 2015; Mamdani et al., 2011).

The discovery cohort was provided by the Pittsburgh study (Guilloux et al., 2015). The MDD group included 34 patients suffering from mild to severe MDE according to DSM-IV SCID interview at baseline, without comorbid substance use disorders, any other major psychiatric diagnosis (i.e. schizophrenia or bipolar disorder), or any unstable medical condition. Patients were unmedicated at baseline and then received citalopram, a widely used selective serotoninergic recapture inhibitor antidepressant. All patients were evaluated for clinical improvement with Hamilton Depressive Rating Scale 17 items (HDRS-17) at baseline and 12 weeks later. Control group included age and sex-matched subjects without any psychiatric disorders according to structured interview (SCID), and any unstable medical condition.

Replication cohorts were provided by Montreal and Marseille studies. They also included a MDD group with identical inclusion criteria. In the Montreal study, patients were unmedicated at baseline and then also treated with citalopram (Mamdani et al., 2011). In the Marseille study, patients were already receiving antidepressant treatment at baseline and were maintained on their treatment during the study (Belzeaux et al., 2012). For both replication cohorts, patients were evaluated for clinical improvement with HDRS-17 at baseline and 8 weeks later. There was no control group in the Montreal study (Table 1). A clinical improvement ratio was calculated for each patient, based on the difference of HDRS scores between inclusion and follow-up visit weighted by the initial HDRS score.

The investigation was carried out in accordance to the last version of Declaration of Helsinki. The appropriate local committee approved the 3 studies design and written informed consent was obtained after a complete description of the study to the subjects.

2.2. Blood mRNA extraction and microarray assay

At baseline, whole blood was collected using PAXgene blood RNA tube for Pittsburgh and Montréal studies and peripheral blood mononuclear cells (PBMCs) were obtained using Ficoll density centrifugation, for Marseille study. The preparation of samples and microarray assays has been previously described (Belzeaux et al., 2012; Guilloux et al., 2015; Mamdani et al., 2011). Total mRNAs were used for gene expression using three different microarray platforms: Illumina HT 12-v4.0 (Pittsburgh study), Affymetrix GeneChip Human Genome U133 Plus 2.0 array (McGill study) and Agilent SurePrint G3 humans GE 8*60 K (Marseille study). Technical reliability of the 3 micro-array data set has been previously validated by independent qPCR measurement for most significant deregulated genes between responders and non-responders (Belzeaux et al., 2012; Guilloux et al., 2015; Mamdani et al., 2011).

As microarray data were generated using different platforms, which contain different gene sets, we first matched genes across the three studies. In each study, for those multiple probesets targeted to a single gene, we selected the one with largest interquartile range (IQR). After this filtering procedure, a unique gene set for each study was kept. A universal gene set was derived by taking the intersection of unique gene sets from the three studies ($n = 12{,}602$ genes).

2.3. Weighted gene coexpression network analysis

Gene clusters/modules were identified using weighted gene coexpression network analysis (WGCNA) package in R (Langfelder and Horvath, 2008; Zhang and Horvath, 2005). The coexpression network was constructed by weighted adjacency matrix, which contained pairwise correlation between two genes and was derived from unsigned correlation matrix with power equal to six for soft-thresholding. Similarity measure was then defined based on topological matrix, which reflected the interconnectedness between two genes. Modules were identified based on hierarchical clustering method, with 1 - topological matrix as dissimilarity matrix. The minimum module size was set to 20 and the threshold for merging module was set to 0.15 as default. Each module was then assigned a unique color. First, the coexpression network was built in the healthy control group in the discovery cohort. To assess the