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Original Article

Response to fluoxetine in children and adolescents: a weighted gene co-expression network analysis of peripheral blood

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Abstract: The inconclusive and non-replicated results of pharmacogenetic studies of antidepressant response could be related to the lack of acknowledgement of its mechanism of action. In this scenario, gene expression studies provide and interesting framework to reveal new candidate genes for pharmacogenetic studies or peripheral biomarkers of fluoxetine response. We propose a system biology approach to analyse changes in gene expression induced by eight weeks of treatment with fluoxetine in peripheral blood. 21 naïve child and adolescents participated in the present study. Our analysis include the identification of gene co-expression modules, using Weighted Gene Co-expression Network Analysis (WGCNA), followed by protein-protein interaction (PPi) network construction coupled with functional annotation. Our results revealed two modules of co-expression genes related to fluoxetine treatment. The constructed networks from these modules were enriched for biological processes related to cellular and metabolic processes, cell communication, immune system processes, cell death, response to stimulus and neurogenesis. Some of these processes, such as immune system, replicated previous findings in the literature, whereas, neurogenesis, a mechanism proposed to be involved in fluoxetine response, had been identified for first time using peripheral tissues. In conclusion, our study identifies several biological processes in relation to fluoxetine treatment in peripheral blood, offer new candidate genes for pharmacogenetic studies and valuable markers for peripheral moderator biomarkers discovery.

Keywords: Selective serotonin reuptake inhibitors, pharmacogenomics, convergent functional genomics, gene expression, neurogenesis, children

Introduction

Between 40 and 50% of patients taking antidepressants relapse or do not respond to treatment [1]. Common genetic polymorphisms explain 42% of this variability in antidepressant response [2]. With some exceptions that include CYP2D6 and CYP2C19, included in guidelines form the Clinical Pharmacogenetics Implementation Consortium (CPIC) [3], pharmacogenetic research has so far failed to identify specific associations through either candidate gene approaches or genome-wide association studies (GWAs) [4].

Although some studies have detected significant associations with antidepressant treatment outcomes, very few of these results have been replicated in independent studies. The lack of replicated candidate gene studies has been attributed to a poor understanding of the biological mechanisms underlying treatment response, phenotypic variability and several limitations of pharmacogenetic studies: differences between studies in terms of design, statistical power, type and dosage of antidepressant and outcome assessment. Pharmacogenetic GWAs have provided tentative hits, but most associations have been inconclusive and

not replicated [5]. Unfortunately, despite the use of large cohorts from multicentric studies and consortia these studies are underpowered and have not revealed reliable predictors of treatment outcomes [2, 6, 7].

In this scenario, genome-wide gene expression studies may reveal the effects of both genetic background and environmental/epigenetic factors, thereby providing an interesting insight into this complex phenotype [8]. These studies make it possible to identify differentially expressed genes associated with antidepressant response that could be used as biomarkers of the phenotype. Numerous studies using this approach have been published, most of which have focused on analyzing the identification of predictor biomarkers by comparing gene expression between groups of responders and non-responders [9-12]. A few studies have also searched for moderator biomarkers by analyzing gene expression changes before and after antidepressant treatment [10, 11, 13-15]. Genome-wide expression analysis not only allows independent and isolated gene analysis to be carried out, but can also be used to explore the biological processes involved in the antidepressant response phenotype. In this regard, an interesting strategy for complex phenotypes involving numerous genes of small effect is the identification of gene co-expression networks (sets of genes that display correlated expressions). Only two studies have proposed this approach in the field of antidepressant response [14, 15].

In the present study, we propose a systems biology analytical approach, based on the identification of gene co-expression modules followed by protein-protein interaction (PPi) network construction and functional annotation analysis, of changes in gene expression induced by eight weeks of fluoxetine treatment in peripheral blood of drug-naïve child and adolescence, to identify biological processes related to fluoxetine treatment.

Materials and methods

Subjects

Twenty-one children and adolescents receiving fluoxetine treatment for the first time participated in the present study. Patients were diagnosed with major depressive disorder (MDD), obsessive compulsive disorder (OCD) or generalized anxiety disorder (GAD) according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [16]. The study was carried out at the Child and Adolescent Psychiatry and Psychology Department of the Institute of Neuroscience at Hospital Clínic, Barcelona. Exclusion criteria were comorbidity of the principal disorder with other psychiatric disorders, Tourette syndrome, autism, somatic disorders and neurological diseases, an intelligence quotient of <70 and non-white ethnicity. All procedures were approved by the hospital's ethics committee. Written informed consent was obtained from all parents and verbal informed consent was given by all subjects following an explanation of the procedures involved.

Information on illness severity was obtained during the initial phase of the study through the assessment of several scales: the Global Assessment of Functioning (GAF) scale [17]; the Children's Global Assessment Scale (CGAS) [18]; the Clinical Global Impression Severity scale (CGI-S) [19]; the Children's Depression Inventory (CDI) [20]; the Obsessive Compulsory Inventory, children's version (OCI-CV) [21]: and the Screen for Child Anxiety-Related Emotional Disorders (SCARED), children's version and parents' version [22]. To assess clinical improvement, these same scales were administered after eight weeks of fluoxetine treatment.

Expression study

RNA isolation and microarray hybridization

For each patient, two blood samples were collected in PAX gene Blood RNA Tubes (Qiagen, Valencia, CA, USA), one prior to the start of fluoxetine treatment and the second after eight weeks of continuous fluoxetine treatment. Plasma concentrations of fluoxetine (FLX) and its metabolite, norfluoxetine (NORFLX), were determined after eight weeks of fluoxetine treatment using a high-performance liquid chromatography method described previously [23]. Patients with concentrations of the active moiety (FLUOX+NORFLUOX) outside the therapeutic range (120-500 ng/mL) [24] were discarded.

Total RNA was isolated with the PAXgene Blood RNA Kit and purified using RNeasy MinElute

Cleanup Kit (both from Qiagen, Valencia, CA, USA). The quantity and quality of RNA was determined with a spectrophotometer (ND10-00, NanoDrop, Wilmington, OF, USA) and a Bioanalyzer (Agilent Bioanalyzer, Agilent, Santa Clara, CA, USA). A total of 1 μg of purified RNA from each of the samples was submitted to Kompetenzzentrum für Fluoreszente Bioanalytik Microarray Technology (KFB, BioPark Regensburg GmbH, Regensburg, Germany) for labelling and hybridization to Human Gene 2.1 ST Array (Affymetrix, Santa Clara, CA, USA), in accordance with the manufacturer's protocols.

Microarray data analysis

Full details of the extraction, labelling and hybridization protocols, raw array data (.cel files) and the pre-processed data matrix are available in Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128387).

The microarray data pre-processing analysis was performed using the Babelomics 5.0 suite (http://www.babelomics.org/) [25]. The data were standardized using robust multichip analysis. Multiple probes mapping to the same gene were merged using the average as the summary of the hybridization values. The sample size was not predetermined using a formal power analysis; instead it was determined based on previous estimations to identify greater than two-fold changes in gene expression levels at P=0.01 [26]. No data points were excluded as outliers.

Weighted correlation network analysis (WGCNA) procedure

Co-expression modules were identified using the WGCNA R software package (https://cran.r-project.org/web/packages/WGCNA/index.html) [27]. Co-expression analysis commences with the construction of a matrix of pairwise correlations between all pairs of genes across all selected samples. Next, the matrix is raised to a soft-thresholding power (β=8 in this study) to obtain an adjacency matrix. In order to identify modules of co-expressed genes, we constructed the topological overlap-based dissimilarity, which was then used as the input for average linkage hierarchical clustering. This step results in a clustering tree (dendrogram) whose branches are identified for cutting, depending on their shape, using the dynamic tree-cutting algorithm. The above steps were performed using the automatic network construction and module detection function (blockwiseModules in WGCNA), with the following major parameters: minModuleSize of 30; reassign Threshold of 0; and merge CutHeight of 0.25. The modules were then tested for their associations with fluoxetine treatment by correlating module eigengenes (MEs, the first principal component of each module) with treatment status (pre- vs. post-treatment). Modules with significant ($P<0.05$) correlation were selected for further analysis. For each significant module, the correlation between the gene significance (GS, the absolute value of the Pearson correlation between each gene expression and treatment status) and its module membership (MM, the correlation between gene expression and the module eigengene at baseline) was calculated.

PPi network construction and evaluation

The SNOW program [28], implemented in the Babelomics 5.0 suite, was used to create PPi networks. If a module exceeded 500 genes (the maximum number allowed by SNOW to construct a network), to ensure higher connectivity, we selected the 500 top hub genes according to gene significance (more likely to be associated to fluoxetine treatment) and module membership (higher connectivity) values. The minimum connected network (MCN), defined as the shortest network that connects all the interacting nodes within a gene list, was obtained. Briefly, we used the curated interactome (validated by at least two independent methods) and allowed the inclusion of extra nodes that connected two or more nodes in the list. Network parameters for each gene were computed: connection degree (which accounts for the number of direct interaction partners a particular node has), clustering coefficient (which accounts for the connectivity of a given node and also for the connectivity of the neighbourhood to which this node is connected), and betweenness centrality (which is related to the existence of hubs connecting different parts of the network). Moreover, the results of the global topological values were compared with the same values of networks with the same size but made up of randomly chosen genes, and a significance value was obtained.

Gene set enrichment analysis and visualization

The MCN constructed was uploaded into Cytoscape 3.5.0 (http://www.cytoscape.org) [29]. We then used ClueGO v2.3.3 [30], a Cytoscape plug-in, to perform a gene set enrichment analysis (GSEA), as described previously [31]. Briefly, we selected the unstructured terms of biological processes from Gene Ontology (GO) (http://geneontology.org/). Genes involved in each MCN were mapped to their enriched term based on the two-sided hypergeometric test, with the Bonferroni-corrected *P*-value. ClueGO created a functional module network in which the different GO terms were clustered according to the association strength between terms calculated using chance-corrected kappa statistics.

Quantitative real-time polymerase chain reaction (Q-RT-PCR)

A Q-RT-PCR was used to verify the microarray results for two selected genes (*NAP1L2* and *ANXA1*) on a 7500 Real-Time PCR System (Applied Biosystems, Warrington, UK). *GADPH* and *ACTB* were used as endogenous controls. First, a reverse transcriptase-PCR was conducted using the "High-capacity cDNA Reverse Transcriptase" kit of Applied Biosystems, following manufacturer's protocol. After this, the real-time RT-PCR was completed using TaqMan Gene Expression Master Mix and a TaqMan Gene Expression Assays for selected genes, following also the Applied Biosystems protocol. The genes analyzed in this study were examined for their relative expression by means of the $\Delta\Delta C_T$ method. The 32 samples analyzed by means of the microarray were validated, and each assay was performed in duplicate.

Statistical analysis

Statistical analyses were performed in SPSS version 17 (SPSS inc, Chicago, Ill). Normal distributions of the data were confirmed using Shapiro-Wilk test, and equality of the variance between groups was assessed by means of Levene's test. For comparing two groups, a two-tailed Student's t test or U-Mann Whitney was used. Significance was set at $P<0.05$.

Results

Table 1 shows the demographic and clinical characteristics of the twenty-one study participants. In order to obtain a more homogeneous sample, we selected sixteen female samples with a diagnosis of MDD that were not taking antipsychotics and whose RNA samples had enough quality for microarray hybridization. No significant differences in clinical characteristics were detected between the whole sample and the 16 samples selected. None of the patients showed active moiety plasma levels outside the therapeutic range.

Figure 1 shows the analysis pipeline followed in the present study. Firstly, 46 modules of co-expressed genes were obtained in the WGCNA (Figure S1). Three modules were found to significantly correlate with fluoxetine treatment: black module (1081 genes) (r^2=0.396, P=0.02), light cyan module (383 genes) (r^2=0.389, P=0.03) and medium purple3 module (60 genes) (r^2=-0.391, P=0.03, respectively) (Figure S2). Black and light cyan modules were selected for further analysis, as the genes included in these modules showed a significant correlation between gene significance (GS) and module membership (MM) (black module r^2=0.36, P=2×10^{-34}; light cyan module r^2=0.48, P=1.83×10^{-23}; and medium purple3 r^2=0.2, P=0.13) (Figure S3). Lack of GS-MM correlation could indicate that only a submodule relates to the trait or suggests considering the association more tentative, needing further validation or evidence.

Secondly, genes included in the black and light cyan modules were used to construct a PPi network for each module. The MCN obtained from the black module contained 443 proteins, 193 (43.6%) of which came from the black module and 250 (56.4%) of which were added externally. The nodes of the network obtained showed more connections (connectivity degree *p*-value $<1\times10^{-3}$), greater connectivity (clustering coefficient *p*-value $<1\times10^{-3}$) and more hub nodes (betweenness centrality *p*-value $=4\times10^{-4}$) compared to random expectations. The MCN from the light cyan module also presented significant values (betweenness P<0.001; connectivity P<0.001; and clustering coefficient <0.001) and contained 329 genes, 138 (41.94%) of which came from the light cyan module and 191 (58.06%) that were added externally.

Finally, GSEA was performed with MCNs constructed with the black module and light cyan genes. MCNs from the black module were

Table 1. Demographic and clinical data of the study population

	Recruited sample	Microarray Sample	Statistical Test	*p*-value
Patients, N	21	16		
Gender, male, N (%)	2 (10.5%)	0 (0%)	_	_
Age, years (mean ± SD)	14.9 ± 1.5	14.9 ± 1.5	U=165.5	0.952
Diagnosis, N (%)			_	_
MDD	19 (90.5%)	16 (100%)	_	_
OCD	1 (4.8%)	0 (0%)	_	_
GAD	1 (4.8%)	0 (0%)	_	_
Comedication, N (%)				
Antipsychotics	3 (14.29%)	0 (0%)	_	_
Benzodiazepines	5 (23.8%)	5 (31.25%)	_	_
Baseline, score (mean ± SD)				
GAF/CGAS	44.8 ± 9.2	45.38 ± 8.14	U=162.0	0.865
CGI-S	4.5 ± 0.8	4.38 ± 0.62	U=162.5	0.881
CDI	27.1 ± 11	29.2 ± 8.6	t=-0.613	0.272
OCI-CV	15.0 ± 8.2	15.75 ± 7.9	t=-0.295	0.384
SCARED	34.2 ± 14.8	38 ± 12.7	t=-0.823	0.208
8 weeks, score (mean ± SD)				
GAF/CGAS	55.7 ± 12.6	56.6 ± 13.6	U=141.5	0.741
CGI-S	3.7 ± 0.9	3.6 ± 11.8	U=144.5	0.818
CDI	23.6 ± 13.2	24.7 ± 11.8	t=-0.794	0.219
OCI-CV	16.3 ± 9.8	16.8 ± 10.2	t=-0.146	0.442
SCARED	34.1 ± 15.7	35.8 ± 15.3	t=-0.324	0.756
Fluoxetine 8 weeks, ng/mL (mean ± SD)	123.68 ± 71.3	121.81 ± 71.92	t=0.078	0.469
Norfluoxetine 8 weeks, ng/mL (mean ± SD)	154.63 ± 74.02	159.88 ± 73.32	t=-0.207	0.418
FLU+NORFLU 8 weeks, ng/mL (mean ± SD)	278.31 ± 113.997	281.69 ± 110.62	t=-0.086	0.466

enriched with 336 GO biological process terms merged in a network that included 51 clusters (**Figure 2A**) (Table S1). These clusters were involved in cellular processes (12 clusters, 23.53%), metabolic processes (10 clusters, 19.60%), cell communication (8 clusters, 15.68%), immune system processes (6 clusters, 11.76%), protein localization and transport (6 clusters, 11.76%), cell death (6 clusters, 11.76%), response to stimulus (5 clusters, 9.80%) and neurogenesis (2 clusters, 3.92%). MCN from light cyan was enriched with a network of 192 terms in 32 clusters (**Figure 2B**) (Table S2). Regarding the light cyan module, the clusters related mainly to immune system processes (8 clusters, 25% of the genes), metabolic processes (7 clusters, 21.87%), response to stimulus (6 clusters, 18.75%), cellular metabolic processes (5 clusters, 15.62%), cell communication (5 clusters, 15.62%), neurogenesis (4 clusters, 12.5%), protein localization and transport (2 clusters, 6.25%) and cell death (2 clusters, 6.25%). These biological GO processes were similar across the two networks, since they had 117 in common (35% black and 41% light cyan). These 117 processes belonged to 33 clusters in the black module (64.7%) and 26 clusters in the light cyan module (81.25%). Out of these 117 processes, most were involved in the same functions as clusters of individual networks: cellular metabolic processes (29.91%), metabolic processes (16.24%), cell communication (11.96%), localization (11.11%), immune system processes (7.69%), cell death (5.98%), neurogenesis (5.13%) and response to stimulus (4%). We compared the lists of the two modules and analysed the over-representation of GO biological processes, but no significant processes were observed.

Two genes (*NAP1L2* and *ANXA1*) were selected for further validation using quantitative RT-PCR evaluation. These genes were chosen based on the following criteria: 1) each of them belonged to a different GO category (Neurogenesis and Immune system); 2) their expression was

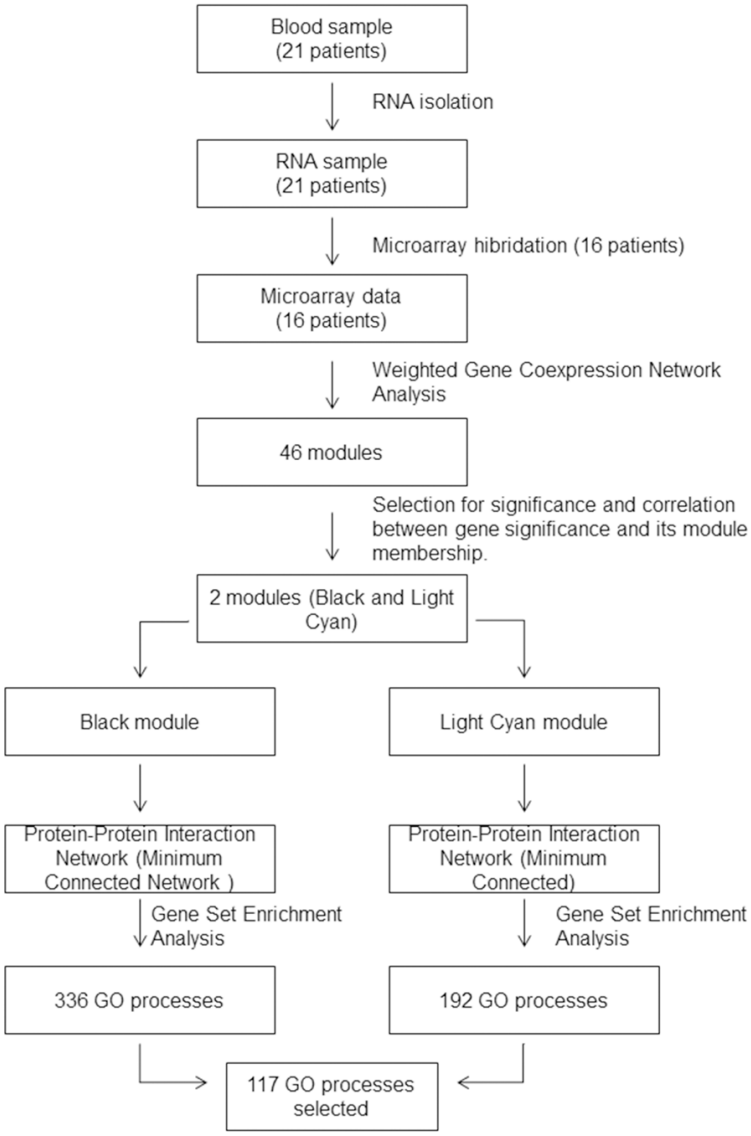

Figure 1. Analysis workflow followed in the present study. Briefly, 21 child and adolescences were recruited, and blood samples were collected before treatment initiation and after eight weeks of fluoxetine treatment. 16 patients participated in the gene expression study. WGCNA identified 46 modules of co-expressed genes, two significantly associated with fluoxetine treatment. PPi were constructed from the significant modules, and GSEA was performed to provide functional interpretation.

altered significantly after fluoxetine treatment (*P<0.05*); and 3) each of them had central role in PPi network. As can be seen in **Figure 3**, the genes analyzed where clearly validated, as they exhibited an identical pattern of expression, without significant differences between both methodologies according to ANOVA test.

Discussion

We propose a systems biology analytical approach, based on the identification of gene co-expression modules followed by protein-protein interaction network construction and functional annotation analysis, of changes in gene expression induced by eight weeks of treatment with fluoxetine in peripheral blood of drug-naïve children and adolescents. The main objective of the present study was to identify key biological processes involved in fluoxetine response. These processes could be a possible source of peripheral biomarkers of fluoxetine treatment or candidate genes for pharmacogenetic studies of the fluoxetine response. Our findings replicate previous results that support the role of the inflammatory system in the antidepressant response. And, for the first time in the literature, we identified processes related to neurogenesis in the peripheral blood of children and adolescents as possible biomarkers of antidepressant treatment.

As previously mentioned, some genome-wide gene expression studies of SSRI have used WGCNA [8, 14]. However, our study presents some differences with respect to those studies. Firstly, our study was performed in a sample of children and adolescents. This represents a homogeneous sample because the onset of the illness was in childhood. Age at onset is an important trait in mental illnesses, and sometimes even defines subgroups of patients with different clinical traits and outcomes [32-35]. Moreover, our patients were debuting or in the initial phases of the illness, and confounders relating to disease progression or chronicity were avoided. Lastly, they were naive patients, and therefore there were no confounders related to previous drug treatment. Studies by Hodgson et al. (2016) [14] and Belzeaux et al. (2016) [15] used larger samples, but these were more heterogeneous in terms of age of onset. We also

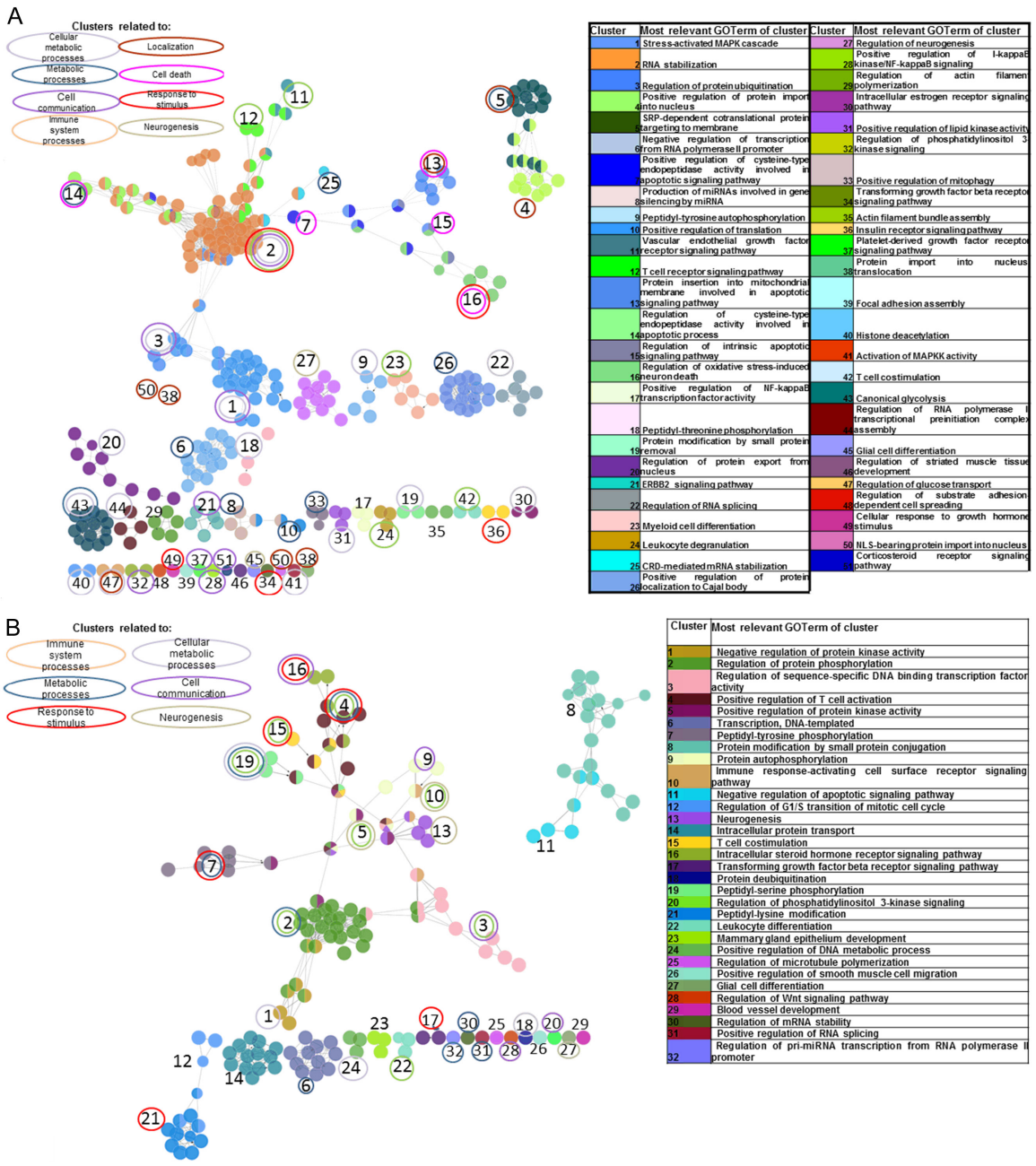

Figure 2. Functional network of Gene Ontology (GO) biological processes obtained from each PPI network, according to ClueGO: black module (A) and light cyan module (B). Each node represents a GO biological process. The node size represents the enriched *p*-value corrected with the Benjamini-Hochberg method. Edge between nodes based on their kappa score level. Nodes were grouped in clusters represented by different colors. Clusters were related to common processes represented by circles. Legends listed all clusters and grouped related clusters according to common biological process.

analysed fluoxetine and non-fluoxetine plasma levels to ensure that they were within therapeutic levels and that the results were not biased due to a lack of adherence in some patients. Secondly, our study focused on fluoxetine. Belzeaux's study [15] analyzed the effect of citalopram, and Hodgson's study [14] examined the effects of the SSRI escitalopram and the tricyclic antidepressant nortriptyline. Another aspect to consider is that, in our study and the study by Hodgson et al. (2016) [14] the follow-up period was eight weeks of treatment, where-

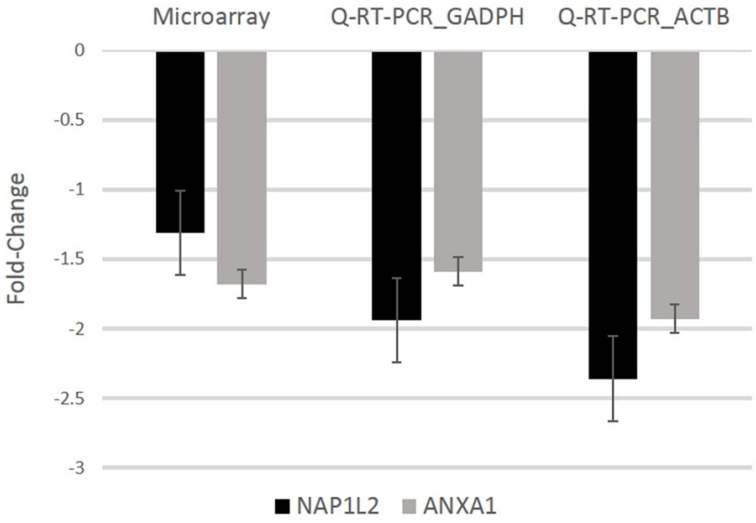

Figure 3. Results of the quantitative real-time PCR (using *GADPH* or *ATCB* as endogenous controls) and microarray analysis for validation of selected genes (*NAP1L2* and *ANXA1*). Each value is the mean ± SD of 32 values; duplicate measurements of 16 biological replicates for each condition (pre- and post-treatment). The Y-axis shows the fold-change (treated vs. untreated) from both Q-RT-PCR and microarray.

as in the study by Belzeaux et al. (2016) [15], it was 12 weeks. Finally, although the three studies used WGCNA, the functional genomic analysis of the significant modules in each study was slightly different. Moreover, Hodgson et al. (2016) [14] focused on molecular knowledge, and used molecular functions and cellular component categories of GO instead of biological processes. By contrast, Belzeaux et al. (2016) [15] focused on GO categories of biological processes and regulation by mi-RNAs. We performed an analysis of GO biological processes, not directly in the genes belonging to significant modules, but in the MCNs created from these significant modules.

The study by Belzeaux et al. (2016) [15] identified 59 modules, nine of which were associated with the citalopram response. Interestingly, four of these significant modules were related to the immune system. In our study, the two significant modules showed enrichment of biological processes related to the immune system, thus supporting the hypothesis that the inflammatory state plays a role in the antidepressant response [36]. Immune system is highly implied in mental illness [37]: in mood and anxiety disorders [38], bipolar disorder [39], obsessive compulsive disorder [40], autism spectrum disorders [41], even in psychosis spectrum disorders [42, 43] and in alzheimer and dementia [44-46]. Specifically, inflammatory response are involved in the neuroprogression of MDD. Moreover, these inflammatory mediators have been investigated as putative biomarkers and therapeutic targets for MDD [47, 48]. Concerning SSRI response, the main SSRI target, the serotonin transporter, is regulated by proinflammatory cytokines [49, 50]. Studies in both, cell cultures [51] and animal models [52-54], showed modulation of inflammatory mediators and immune responses by antidepressants. In humans, many studies have demonstrated that immune alterations related with MDD, such as levels of some interleukines, cytokines or interferons (i.e. IL-6, TNF-α, and IL-1β), may return to normal in MDD patients after treatment with SSRIs and with other antidepressants [48, 55].

Hodgson et al. (2016) [14] identified 10 modules of co-expression genes, one of which was significantly correlated with fluoxetine treatment. This module was enriched with five GO molecular categories linked to Mrna-UTR binding and with the cellular component of the cortical cytoskeleton [14]. Cytoskeletal reorganization is an important event during neurogenesis, a process identified in our significant modules. During development, neural progenitor cells begin a series of morphological changes to adapt their form, migrate to their destination and create synapses [56, 57]. Neurogenesis is a mechanism that has previously been related to mental illness [58-60], including MDD [61], OCD [62] and anxiety [63]. Several studies have demonstrated that neurogenesis processes are involved in the SSRI response using both cell cultures [64, 65] and animal models [66-68]. In humans, it has been demonstrated that the hippocampal volume is decreased in patients with MDD when compared to controls [69, 70]. Patients treated with antidepressants have shown an increased hippocampal volume, and this increase correlates with a better clinical outcome [71, 72].

Limitations

It is important to treat the results of this study with caution, because it presents some unavoidable limitations. First, blood was used as a proxy for the key tissue of interest in antidepressant research, i.e. the brain. Studies that explore the degree of gene co-expression in blood and brain in humans suggest that there is a moderate correlation [73-76]. Nevertheless, other sample types, such as post-mortem brain tissue, prevent biological measurements from being taken before and after treatment [77]. Second, no placebo or control group was used. Both of these groups would have allowed us distinguish the response to antidepressants, the response to placebo, and the spontaneous improvement of symptoms [78]. Third, the biological validity of gene co-expression modules was not fully explored. Co-expressed genes are supposed to be correlated because of common biological functions and master regulators, not random ones [79]. Four, the sample size did not allow us to stratify the sample by different psychiatric illnesses, thereby making it impossible to capture differences. Moreover, it meant that we had to indicate that these results were exploratory.

Conclusions

Expression changes detected in peripheral blood after treatment with fluoxetine in a sample of naive children and adolescents were found to be related to several biological processes. The processes related to immune system replicated previous findings in the literature using similar approaches. We identified neurogenesis for the first time by measuring expression changes in peripheral blood. This makes sense from a biological point of view, as this is a mechanism proposed to be involved in fluoxetine response. Our results identifying several biological processes in relation to fluoxetine treatment in peripheral blood, offering new candidate genes for pharmacogenetic studies and valuable markers for peripheral moderator biomarkers discovery.

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Disclosure of conflict of interest

None.

Abbreviations

CPIC, Clinical Pharmacogenetics Implementation Consortium; CDI, Children's Depression Inventory; CGAS, Children's Global Assessment Scale; CGI-S, Clinical Global Impression Severity scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; FLX, Fluoxetine; GAD, Generalized Anxiety Disorder; GAF, Global Assessment of Functioning; GSEA, Gene Set Enrichment Analysis; GWAs, Genome-Wide Association Studies; MCN, Minimum Connected Network; MDD, Major Depressive Disorder; NORFLX, Norfluoxetine; OCD, Obsessive Compulsive Disorder; PPi, Protein-Protein interaction; SCARED, Screen for Child Anxiety-Related Emotional Disorders; SNPs, Single Nucleotide Polymorphisms; WGCNA, Weighted Correlation Network Analysis.

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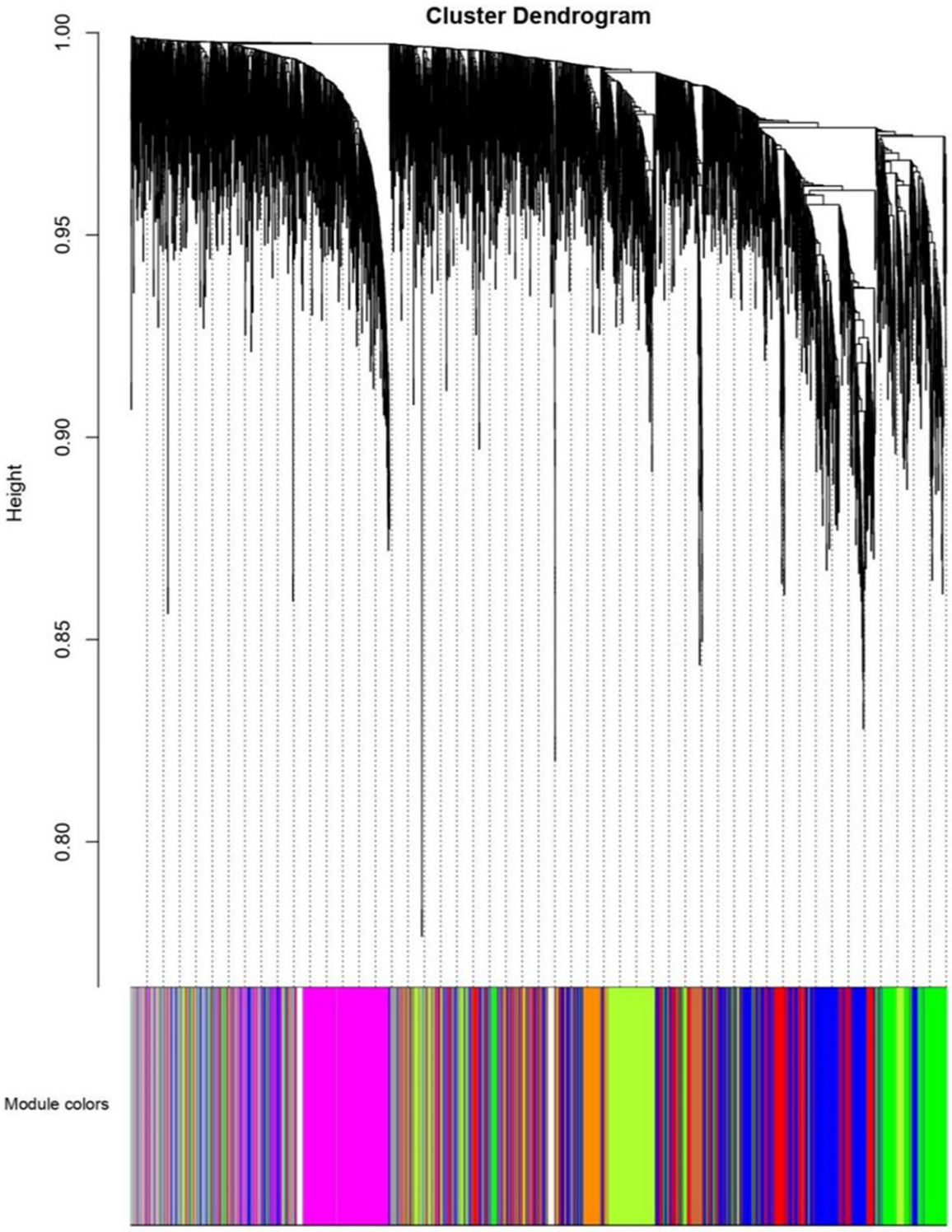

Figure S1. Gene co-expression networks obtained in the WGCNA. Branches in the hierarchical clustering dendrograms correspond to modules. Modules of co-expressed genes were assigned colours corresponding to the branches.

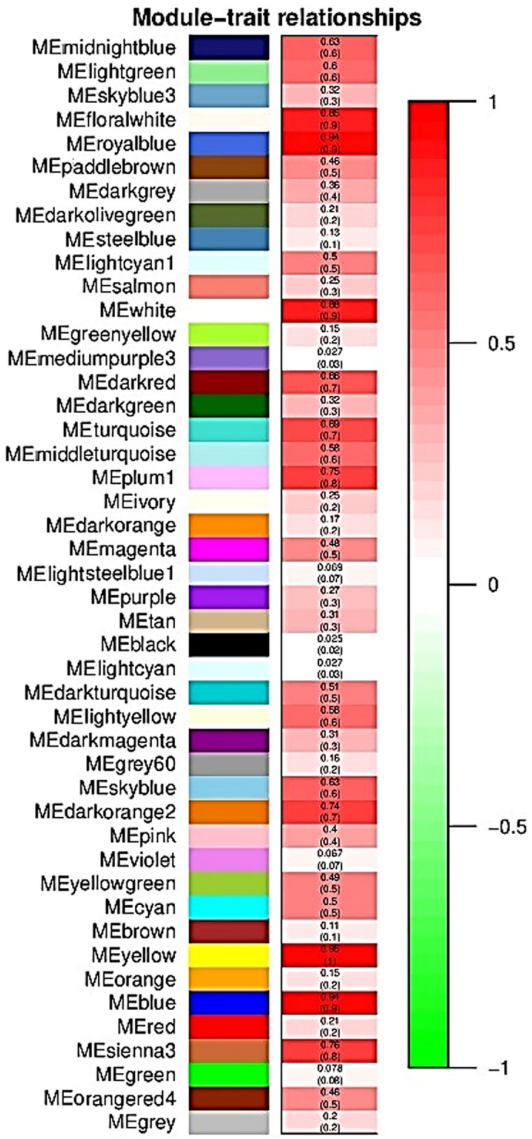

Figure S2. Correlation and significance of module eigengenes (MEs, the first principal component of each module) identified in the WGCNA with treatment status (pre- vs. post-treatment).

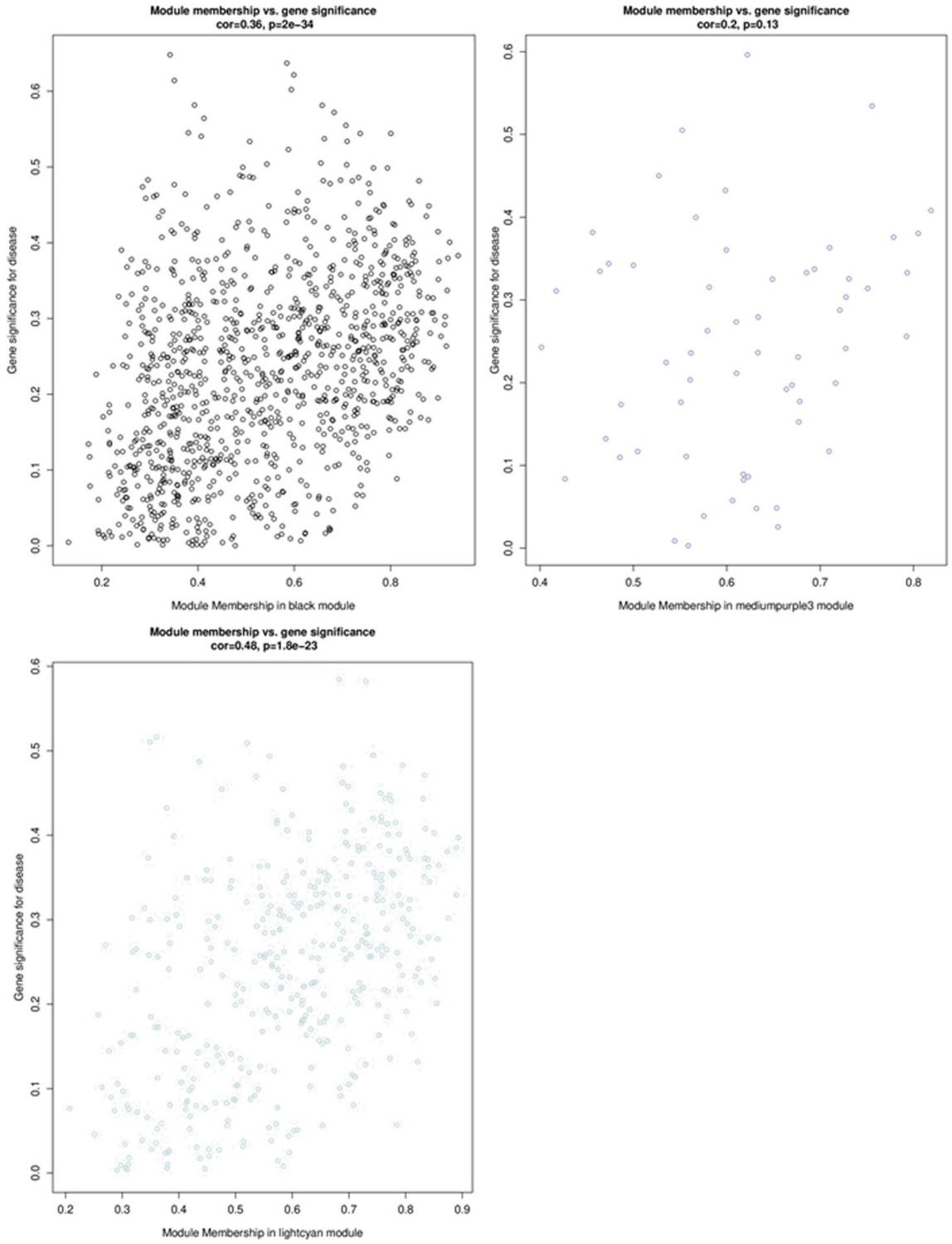

Figure S3. Representation of correlation between the gene significance for treatment status and its membership in the module. Only significant WGCNA modules are shown.