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Journal

Molecular Psychiatry, 26(9)

ISSN

1359-4184

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Publication Date

2021-09-01

DOI

10.1038/s41380-020-0752-2

Peer reviewed

HHS Public Access

Author manuscript

Mol Psychiatry. Author manuscript; available in PMC 2023 February 12.

Published in final edited form as:

Mol Psychiatry. 2021 September ; 26(9): 5171–5179. doi:10.1038/s41380-020-0752-2.

Transcriptomic signatures of treatment response to the combination of escitalopram and memantine or placebo in late-life depression

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Abstract

Drugs that target glutamate neuronal transmission, such as memantine, offer a novel approach to the treatment of late-life depression, which is frequently comorbid with cognitive impairment. The results of our recently published double-blind, randomized, placebo-controlled trial of escitalopram or escitalopram/memantine in late-life depression with subjective memory complaints (NCT01902004) indicated no differences between treatments in depression remission, but additional benefits in cognition at 12-month follow-up with combination treatment. To identify pathways and biological functions uniquely induced by combination treatment that may explain cognitive improvements, we generated transcriptional profiles of remission compared with non-remission from whole blood samples. Remitters to escitalopram compared with escitalopram/memantine combination treatment display unique patterns of gene expression at baseline and 6 months after treatment initiation. Functional enrichment analysis demonstrates that escitalopram-based remission associates to functions related to cellular proliferation, apoptosis, and inflammatory response. Escitalopram/memantine-based remission, however, is characterized by processes related to cellular clearance, metabolism, and cytoskeletal dynamics. Both treatments modulate inflammatory responses, albeit via different effector pathways. Additional research is needed to understand the implications of these results in explaining the observed superior effects of combination treatment on cognition observed with prolonged treatment.

Introduction

Depression frequently occurs in the community-dwelling elderly, with significantly increased morbidity and mortality [1, 2]. Despite progress in antidepressant therapies, depressed older adults often develop a chronic disease course characterized by frequent relapse and shorter intervals of recovery [3, 4]. Up to 55% of depressed elderly

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Compliance with ethical standards

Conflict of interest HL received research support from Allergan/Forest Laboratories. All other authors report no financial relationships with commercial interests.

Supplementary information The online version of this article (https://doi.org/10.1038/s41380-020-0752-2) contains supplementary material, which is available to authorized users.

simultaneously suffer from mild cognitive impairment (MCI), which tends to persist despite treatment and may accelerate conversion to dementia [5, 6]. Subjects with subjective memory complaints, even without evidence of impairment on formal testing, express Alzheimer's disease (AD) biomarkers and a heightened risk for developing MCI [7].

Neuroinflammation and excitotoxicity are theorized to underlie the pathophysiology of both depression and neurodegeneration [8]. Antidepressants coupled with drugs that target glutamate transmission and excitotoxicity, therefore, offer a promising novel "mood plus cognitive enhancer" neuroprotective approach to treatment. Memantine, an NMDA antagonist, inhibits calcium influx and excitotoxicity while preserving the physiological activation of the receptor. We recently conducted a randomized, double-blind, placebo-controlled trial of escitalopram combined with placebo (ESC/PBO) or memantine (ESC/MEM) in depressed elderly with subjective memory complaints. No differences were observed in the depression remission rate at 6 or 12 months following initiation of treatment [9]. However, compared with ESC/PBO, ESC/MEM treatment produced improvements in delayed recall and executive functioning at 12-month follow-up. The results were consistent with data from a prior open trial [10].

The full range of neuroprotective effects of memantine remains underexplored in humans. In animal models of AD neurodegeneration, in addition to dampening excitotoxicity, memantine reduces Aβ plaque deposition, tau hyperphosphorylation, neurofibrillary tangle formation, and reactive oxygen species generation. Increased proliferation of hippocampal neural progenitor cells and synaptic plasticity are also described [11, 12]. Enrichment of MAPK, IL-6, and insulin signaling pathways are observed following treatment, which reflects the drug's overlapping functions in stress response, neuronal plasticity, and memory formation [13]. Cholinergic stimulation of the mouse hippocampus and basal forebrain with attenuation of microglial activation are associated with improved attention and memory [14, 15].

Regenerative and remodeling capabilities of memantine are described in acute brain injury models. Memantine improves poststroke injury by inhibiting astrogliosis and promoting vascular integrity [16, 17]. The deleterious effects of whole-brain radiotherapy, which include diffuse devascularization, impaired synaptic plasticity, increased permeability of the blood–brain barrier, and impaired cognition, are attenuated with memantine treatment [18–20]. Antiproliferative and antineoplastic benefits have been observed in tumor cells through the enhancement of autophagy and clearance of damaged cells [21, 22]. Stabilization of endothelial cell integrity, inhibition of microglia activation, and increased survival and differentiation of dopaminergic neurons are also reported [23, 24].

The results of our clinical trial suggested that ESC/MEM treatment induces cognitive improvements that occur subsequent to the onset of depression remission. Here we performed comparative transcriptional profiling of ESC/PBO and ESC/MEM remitters compared with non-remitters to explore pathways unique to ESC/MEM remission that may explain its additional cognitive benefits as well as identify candidate markers of response.

Materials and methods

Subject selection and biospecimen collection

Whole blood samples were collected at baseline and 6-month follow-up from 64 participants undergoing a randomized, double-blinded trial of ESC/PBO compared with ESC/MEM for the treatment of late-life depression (clinicaltrials.gov, NCT01902004). All participants provided informed consent and protocol approved by the UCLA institutional review board. Details regarding participant recruitment, inclusion and exclusion criteria, randomization, and intervention protocol may be found in the clinical trial report and supplementary methods [9]. Supplementary Table S1 and Figure S1 outline baseline and 6-month remission features for participants in each intervention.

RNA isolation and microarray processing

RNA samples were transcribed to double-stranded cRNA using MessageAmp Premier RNA Amplification Kit (Life Technologies) with an oligo(dT) primer following the manufacturer's instructions. Following fragmentation, 11 μg of biotin-labeled cRNA was hybridized for 16 h at 45 °C on Affymetrix Human Genome U133 Plus 2.0 Arrays (Affymetrix). GeneChips were washed and stained in the Affymetrix Fluidics Station 450 (Affymetrix), then scanned with the Affymetrix GeneChip Scanner 3000 G7 (Affymetrix).

Bioinformatics analysis

Detailed analysis information is available in the supplemental methods. Microarray quality control and gene expression analyses were completed using R (version 3.6.2) and Bioconductor libraries (version 3.10) [25]. A total of five arrays failed quality control and were removed from subsequent analysis. Background adjustment, quantile normalization, and probe summarization were achieved by robust multiarray averaging using *oligo* (version 1.50.0) [26]. Log2 fold changes and standard errors between conditions were estimated by fitting a linear model for each feature with empirical Bayes smoothing using *limma* (version 3.42.0) with batch, sex, and age as co-variates [27]. The false discovery rate (FDR) was corrected using the Benjamini–Hochberg procedure [28]. Transcriptional profiles were compared using an FDR adjusted p value < 0.05 as the threshold for differential expression as well as by rank-rank hypergeometric overlap analysis [29]. Gene set enrichment analysis (GSEA) was conducted using *fgsea* (version 1.12.0) [30]. GSEA results were visualized using the EnrichmentMap (version 3.2.1) module for Cytoscape (version 3.7.1) [31, 32].

Results

ESC/PBO and ESC/MEM remission profiles differ pretreatment and at 6-month follow-up

Baseline and 6-month follow-up transcriptional profiles were generated from whole blood samples for ESC/PBO (n = 30) and ESC/MEM (n = 34) participants using Affymetrix Human Genome U133 Plus 2.0 microarray data. ESC/PBO and ESC/MEM remitters compared with non-remitters did not differ significantly in demographics, depression history, cognitive status, or baseline depression (Supplementary Table S1 and Fig. S1). At the 6-month follow-up, 15 participants (50%) in the ESC/PBO group compared with 23 subjects (67.6%) in the ESC/MEM group met the criteria for depression remission.

The difference in remission rate between the two treatments was not significant ($\chi^2(1) = 2.1$; $p = 0.2$). Three transcriptional profiles were generated for each intervention: (1) remitters compared with non-remitters at baseline, (2) 6-month follow-up compared with baseline in remitters, and (3) 6-month follow-up compared with baseline in non-remitters (Supplementary Fig. S2A, SB). For exploratory analysis, we set an FDR adjusted p value < 0.05 as cutoff of differential gene expression. A total of 827 genes were significantly altered in at least one of the three remission profiles for ESC/PBO treatment and 1352 genes for ESC/MEM treatment (Supplementary Tables S2, S3). Agglomerative hierarchical clustering of differentially expressed genes within each profile is shown in Fig. 1a. Supplementary Fig. 2C–E shows a selection of top-scoring genes unique to remission in each condition as well as genes common to remission in both interventions.

The degree of overlap between ESC/PBO and ESC/MEM remission and non-remission profiles is minimal. The classical p value threshold approach to defining differential expression enforces an arbitrary cutoff that may underestimate subtle concordance or discordance between transcriptional profiles. As such, we evaluated differences in ESC/PBO and ESC/MEM remission profiles using a threshold-free rank-rank hypergeometric overlap (RRHO) algorithmic approach. In the RRHO analysis, each gene list is ranked by −log10(p value) and effect size direction. The algorithm compares the rank lists and calculates the significance of overlap across the entire continuous significance gradient of tested genes. Analysis results are visualized as a heatmap, in which intensity corresponds to the −log(p value) from the hypergeometric distribution. A signal in Cartesian quadrants II and IV (upper left, lower right) indicates significant discordant gene expression (red = lower p value. A signal in quadrants I and III (upper right, lower left) indicates significant concordant gene expression. ESC/PBO and ESC/MEM remitters compared with non-remitters at baseline show a higher degree of discordant than concordant gene expression (Fig. 1b). ESC/PBO and ESC/MEM remitters continue to display discordant gene expression post-treatment, although concordant expression is increased (Fig. 1c). Metric scatterplots additionally demonstrate low correlation between the tested transcriptional profiles (Supplementary Fig. S3).

Unique signaling pathways enriched in ESC/PBO and ESC/MEM remission

Similar to RRHO analysis, GSEA enables assessment of the enrichment of differential signal in predefined gene sets without employing a cutoff for differential expression. We performed GSEA of the three remission profiles for each treatment to assess for biologically meaningful differences between ESC/PBO and ESC/MEM remission profiles. An FDR adjusted p value of 0.05 was used to define significant enrichment. The top enrichment results for hallmark and canonical pathway gene sets are shown in Fig. 2 for ESC/PBO (A) and ESC/MEM (B) remitters compared with non-remitters. Supplementary Tables S4, S5 include the full comparative enrichment results. A gene set was defined as uniquely enriched in ESC/PBO remission if significantly enriched (FDR adjusted p value 0.05) in ESC/PBO remitters not significantly enriched in ESC/PBO non-remitters or ESC/MEM remitters (FDR adjusted p value > 0.1). Similarly, ESC/MEM unique gene sets are significantly enriched in ESC/MEM remitters but not ESC/MEM non-remitters or ESC/PBO remitters. The more positive the value of normalized enrichment score (NES),

the more enriched the tested gene set is in upregulated genes for that profile; conversely, negative NES corresponds to enrichment in the downregulated genes. Upregulated gene sets for ESC/PBO remitters included interferon signaling and inflammatory response, cell division, and apoptosis. Downregulated gene sets in ESC/PBO remitters included long term potentiation and serotonin receptors with downregulated genes, an internal validation of ongoing SSRI treatment. Upregulated gene sets in ESC/MEM remitters included proteasomal degradation, autophagy (including longevity associated FOXO transcription factors), and NF-kB signaling. Downregulated gene sets in ESC/MEM remitters included PI3K/AKT signaling, cytoskeletal dynamics (specifically microtubule organization), cell adhesion, and insulin signaling.

The “leading edge” of a GSEA gene set is the subset of genes that most impact the enrichment score. Genes that appear in the leading edge of multiple gene sets represent genes of potentially high biological significance. Frequency analysis of the leading edges of all significantly enriched remission gene sets revealed that bone morphogenetic protein 4 (BMP4), a member of transforming growth factor-beta superfamily, is the most frequent leading-edge gene, followed by apoptosis adapter molecule, FADD, in ESC/PBO remitters (Fig. 3a). The leading edges of gene sets enriched by ESC/MEM remitters demonstrate an array of proteasome proteins common among enriched gene sets, as well as dopamine receptor D2 and apolipoprotein E (Fig. 3b). The degree of leading-edge overlap among gene sets was also assessed by correlogram. Branching morphogenesis and chromatin maintenance-related gene sets show the highest overlap in ESC/PBO remission (Fig. 3c). ESC/MEM remission gene sets have the highest overlap among sets related to proteasome degradation, stem cell differentiation, apoptosis, and inflammation signaling gene sets (Fig. 3d).

Biological functions differentially regulated in ESC/PBO and ESC/MEM remission

To synthesize GSEA results into more interpretable biological functions, significantly enriched gene sets were clustered and visualized as an enrichment network for each intervention. The enrichment algorithm organizes gene sets based on the degree of overlap among the signature genes of each set. Clusters were annotated using a word-frequency algorithm. The ESC/PBO remission network is characterized by functions related to branching morphogenesis, cellular proliferation, cell death, and inflammation (Fig. 4a). Among the most highly interconnected subnetwork is the one related to inflammatory response (Fig. 4b). The ESC/MEM remission network includes functions related to cellular clearance, inflammation, metabolism, and cytoskeletal dynamics (Fig. 4c). The most connected ESC/MEM remission subnetwork demonstrates the significant degree of overlap between these processes (Fig. 4d).

Discussion

The results of our previous clinical trial demonstrated that while ESC/PBO and ESC/MEM are equally effective in inducing remission of depressive symptoms by 6-month follow-up, ESC/MEM remission is additionally associated with later cognitive improvement [9]. Neuropharmacological response is a delicate balance of competing processes, including

proliferation of developing neurons versus selective apoptosis of unviable neurons, branching versus pruning, synaptogenesis versus synaptic pruning, long-term synaptic potentiation versus long-term depression, and pluripotency versus differentiation [33, 34]. In murine models, memantine enhances poststroke recovery through tissue remodeling and enhancement of neural plasticity through reduced astrogliosis and increased capillary formation. These benefits, however, are only observed with prolonged treatment [16, 35]. The majority of controlled clinical trials of memantine treatment, alone or in combination with other drugs, are under 12 months in duration. Neurodegenerative processes are inherently progressive in nature with molecular changes evident prior to measurable cognitive impairment [36]. Molecular changes induced by neuropharmacology are reasonably assumed to be similarly progressive with molecular changes preceding detection on clinical scales.

Adult hippocampal neurogenesis is a process negatively impacted by stress-induced hypothalamic–pituitary–adrenal axis activation and inflammation, processes reversed with antidepressant treatment [37, 38]. Examinations of peripheral blood collected following escitalopram treatment indicate induction of pathways related to neuroplasticity and stress response [39]. Depression remission is additionally associated to differential regulation of immune response and neurotrophin signaling pathways [40]. Consistent with these data, in the current study, ESC/PBO remitters enriched pathways related to cell division, apoptosis, and inflammation. The most frequent gene present in the leading edge of the enriched gene sets was BMP4, a factor with well-characterized functions in neurogenesis, including regulation of the balance between proliferative and quiescent cells of neural stem cells in the adult hippocampus [41]. Outside of the brain, BMP signaling is critical in regulation of bone resorption and turnover. Similarly, the enrichment of pathways related to apoptosis observed here is supported in other studies, although whether this constitutes an adverse effect, benefit, or both, remains to be determined [42]. Preliminary data from our OPTIMUM (Optimizing Outcomes of Treatment-Resistant Depression in Older Adults) study indicates that upwards of 50% of patients on antidepressants have concurrent osteopenia (data not shown). Serotonin receptors are present in all major bone cell types [43]. The current data provides molecular evidence of antidepressant use as a risk factor in bone health. The most interconnected upregulated network in ESC/PBO remission contained processes related to B and T cell activation and differentiation. SSRIs possess both immunosuppressive and immunostimulatory effects [44]. Depletion of regulatory T cells and reduced activation of B cells are observed in depressed patients compared with healthy controls with upregulation of production noted with SSRI treatment [45, 46].

By contrast, ESC/MEM remission is characterized by significant enrichment of functions related to the ubiquitin proteasomal system (UPS) and autophagy suggestive of enhanced cellular clearance. In addition, remitters significantly enrich pathways related to cytoskeletal dynamics, specifically, microtubule dynamics, as expected given memantine’s known function in reversing abnormal tau tubulin hyperphosphorylation [47]. The UPS controls numerous cellular processes, including cell cycle, DNA integrity, and apoptosis. Inhibition of proteasomal function is a well-studied animal model of neurodegeneration. The most upregulated and interconnected network identified in ESC/MEM remission intersects immune response, cellular clearance, stem cell maintenance and differentiation, cell survival,

and angiogenesis along with NF-κB, Notch4, and Hedgehog signaling, all of which possess a multiplicity of known functions in these pathways [48–50]. A role for memantine in monitoring of genomic stability and clearance of unstable cells through enhancement of autophagy has been previously described [21, 51–53]. Remitters to ESC/MEM treatment also show enrichment of insulin and insulin-like growth factor signaling pathways as well as lipid metabolism. Dysregulated insulin signaling is a known risk factor for neurodegeneration, including increased rates of increase of amyloid production, tau phosphorylation, mitochondrial dysfunction, and oxidative stress [54]. In murine models, memantine treatment has been shown to improve pancreatic β-cell function and the metabolic effects of a high-fat diet [55, 56].

Preclinical data indicates reduction in neuronal senescence following memantine treatment [57]. Senescence-accelerated mice show improved learning and reduced hippocampal CA1 neurofibrilliary tangles and amyloid precursor protein in response to memantine [58]. Senescence-associated secretory phenotype (SASP) is the metabolically active state of cells during cell cycle arrest that results in active secretion of numerous signaling factors and proteases [59]. SASP's role in neurodegeneration and association to late-life depression have been previously described [60, 61]. SASP is also theorized to be neuroprotective in certain contexts by increasing the clearance of damaged senescent cells and promoting cellular regeneration [62]. Transient senescent cells exist in development and wound healing [63]. Senescence-associated inflammation may also increase epigenetic plasticity, termed "transflammation," resulting in nuclear reprogramming and increased cellular longevity [64]. Wnt and Notch signaling, enriched in ESC/MEM remission, have reported functions in mediating the balance between neural progenitor cell proliferation and differentiation in the adult hippocampus in response to SASP induction [65, 66]. Regulation of SASP-related pathways may explain memantine's dual function in cellular clearance and regeneration with improvement in cognition over time.

Limitations to the current study include the small cohort size and use of whole blood. A previous study of human blood and CNS tissues demonstrated an intermediate level of correlation with ~50% shared expression between blood and brain for a subset of schizophrenia genes [67]. Association between gene expression levels in peripheral blood and theorized function in the brain must, therefore, be approached with caution, particularly in regard to activation and repression of predicted factors and pathways, which may reflect feedback of upstream or earlier processes. Finally, a single posttreatment time point does not permit determination of the sequence or direction of regulatory events, which may differ across time and tissues.

Conclusion

ESC/PBO and ESC/MEM remitters display transcriptional differences both pre- and post-treatment compared with non-remitters. Such differences may serve as future biomarkers for prediction of positive response to combination treatment. Memantine is theorized to complement escitalopram's underlying mood benefits while enacting a beneficial neuroprotective program of cellular clearance, metabolic protection, and regeneration leading to enhanced synaptic transmission and improved cognition. The benefits of

combination treatment are likely better appreciated with longer treatment, although the current study offers evidence of early molecular changes. The interplay between the deleterious and neuroprotective effects of SASP suggested by the ESC/MEM remission profile offers an exciting experimental framework to increase our understanding of the interplay between depression, cognition, and medical comorbidity in the elderly. Future work will examine genomic and epigenomic differences in remission between interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was funded by the NIH grants R01MH097892; AT009198 to HL. This work was further supported by NIH grants MH097892, AT009198, and the National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR001881. AG was partly funded by a research fellowship from the American Psychiatric Association Foundation. The authors thank the Semel Institute Biostatistics Core (SIStat) for database management and support.

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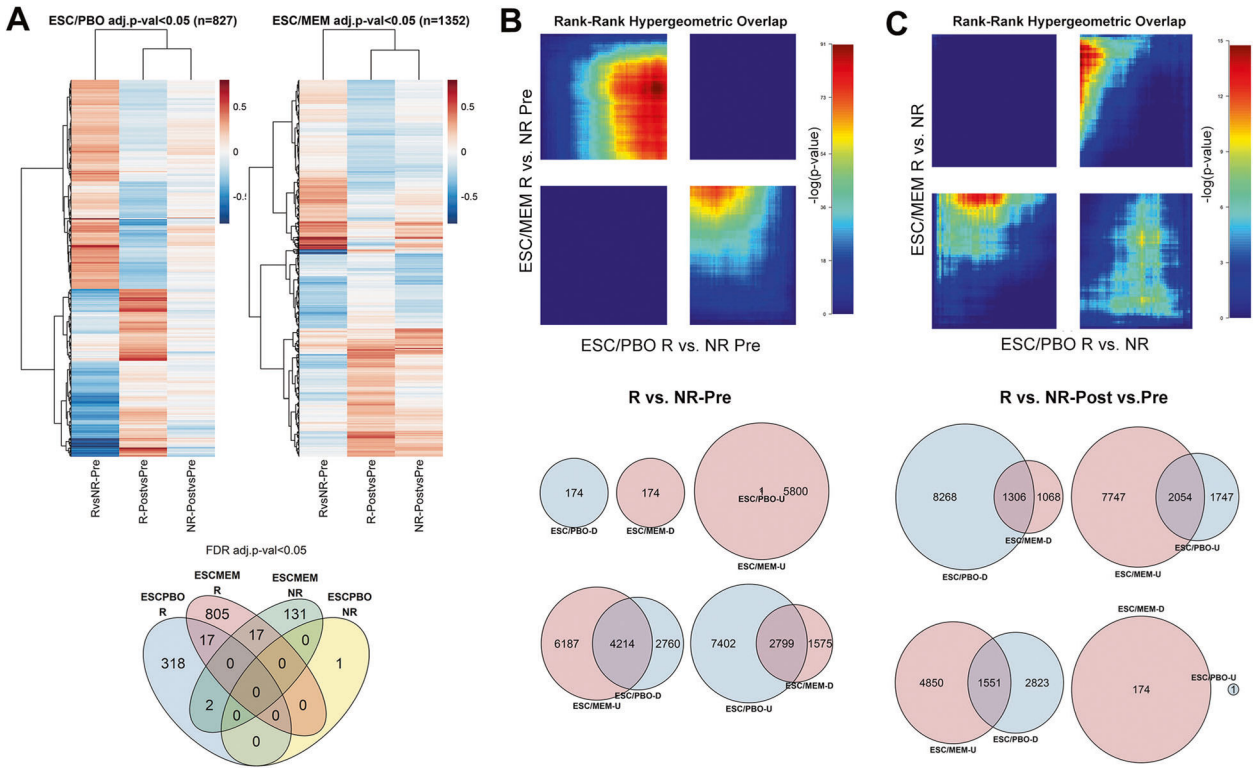

Fig. 1. ESC/PBO and ESC/MEM remission profiles differ pre- and post-treatment.

a Agglomerative hierarchical clustering and Venn diagram of differentially expressed genes in at least one of the tested remission profiles at an FDR adjusted *p* value < 0.05. Red: positive log2 fold change. Blue: negative log2 fold change. **b** Rank-rank hypergeometric overlap (RRHO) heatmap and Venn diagram of concordant and discordant genes in ESC/PBO and ESC/MEM remitter compared with non-remitter profiles at baseline. Overlapping genes that change significantly in the same direction are located in the bottom left and upper right quadrants. Overlapping genes that change significantly in different directions between conditions are located in the upper left and bottom right quadrants. **c** RRHO analysis of ESC/PBO and ESC/MEM remitter compared with non-remitter profiles at 6-month follow-up (controlled for pretreatment expression). ESC/PBO escitalopram/placebo, ESC/MEM escitalopram/memantine, R remitter, NR non-remitter, Pre baseline, Post 6-month follow-up, D downregulated, U upregulated.

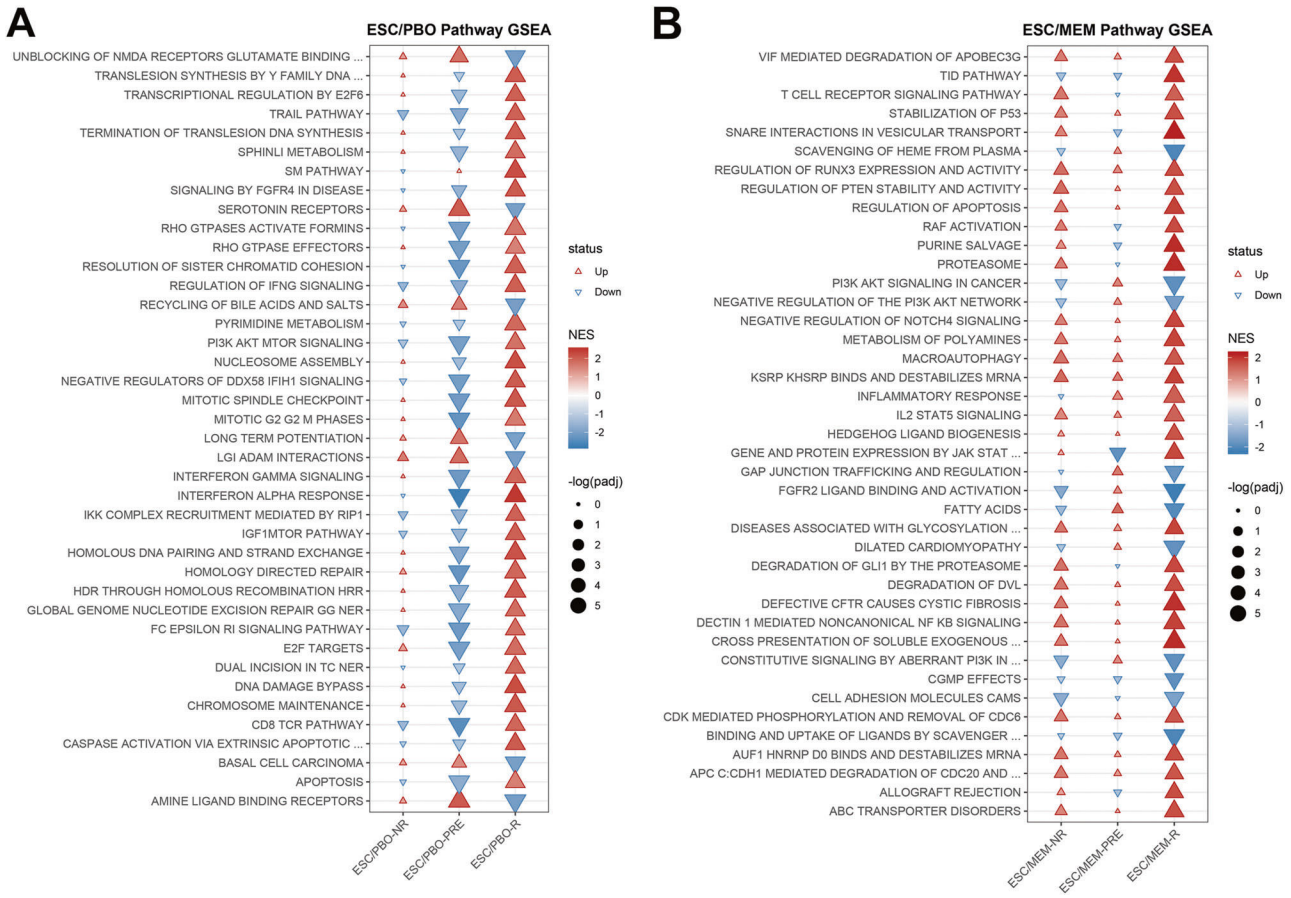

Fig. 2. Signaling pathways uniquely enriched by ESC/PBO and ESC/MEM remitters compared with non-remitters.

Gene set enrichment analysis of **a** ESC/PBO and **b** ESC/MEM pretreatment, remitter, and non-remitter transcriptional profiles. Analyzed gene sets include hallmark and canonical pathways. Red: positive normalized enrichment score, indicating activation post-treatment. Blue: negative normalized enrichment score, indicating repression post-treatment. Triangle symbol size is proportional to −log(FDR adjusted *p* value). ESC/PBO escitalopram/placebo, ESC/MEM escitalopram/memantine, R remitter, NR non-remitter, Pre baseline, Post 6-month follow-up.

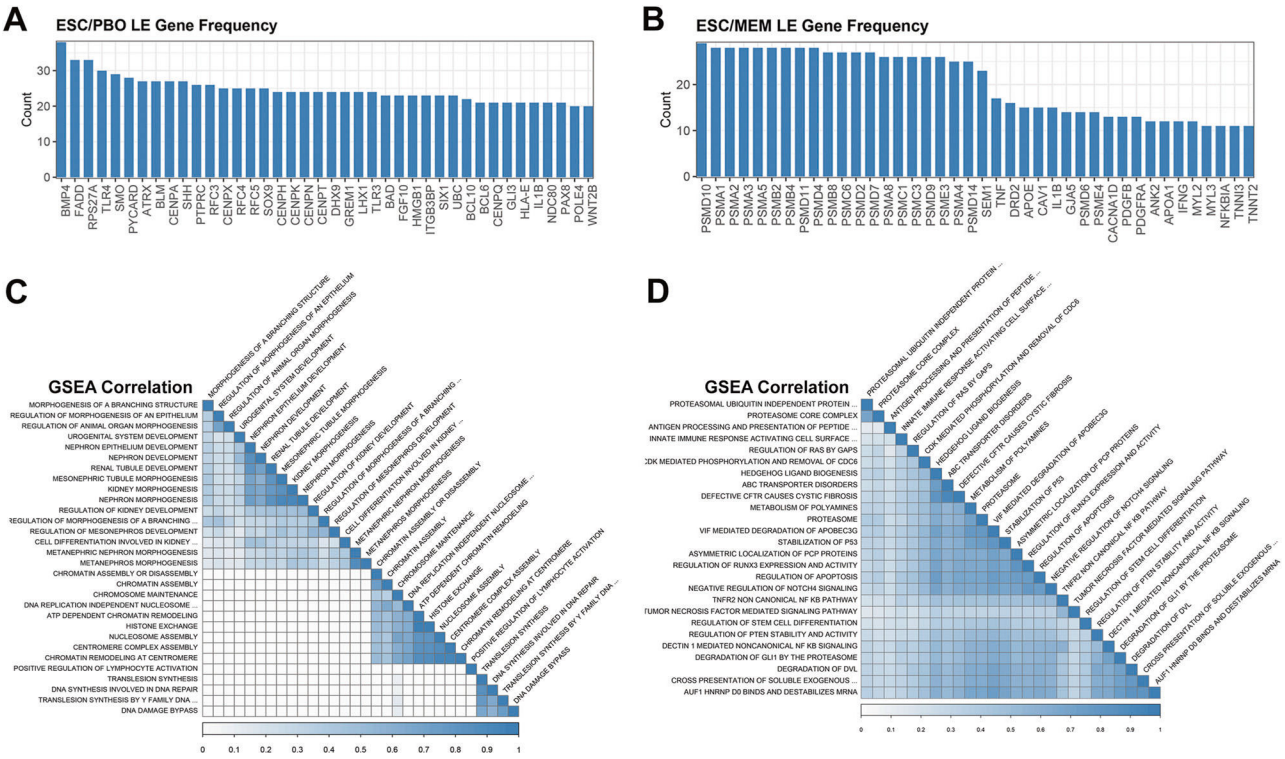

Fig. 3. Leading edge analysis of ESC/PBO and ESC/MEM enriched gene sets.

Frequency count of genes that appears in the leading edge of significantly enriched hallmark pathway, canonical pathway, and gene ontological gene sets in **a** ESC/PBO and **b** ESC/MEM remission (FDR adjusted *p* value < 0.05). The Jaccard similarity index was calculated between the leading edges of all significantly enriched gene sets. The most highly correlated gene sets are shown by correlogram for **c** ESC/PBO and **d** ESC/MEM remission where intensity of hue signifies a higher Jaccard similarity index.

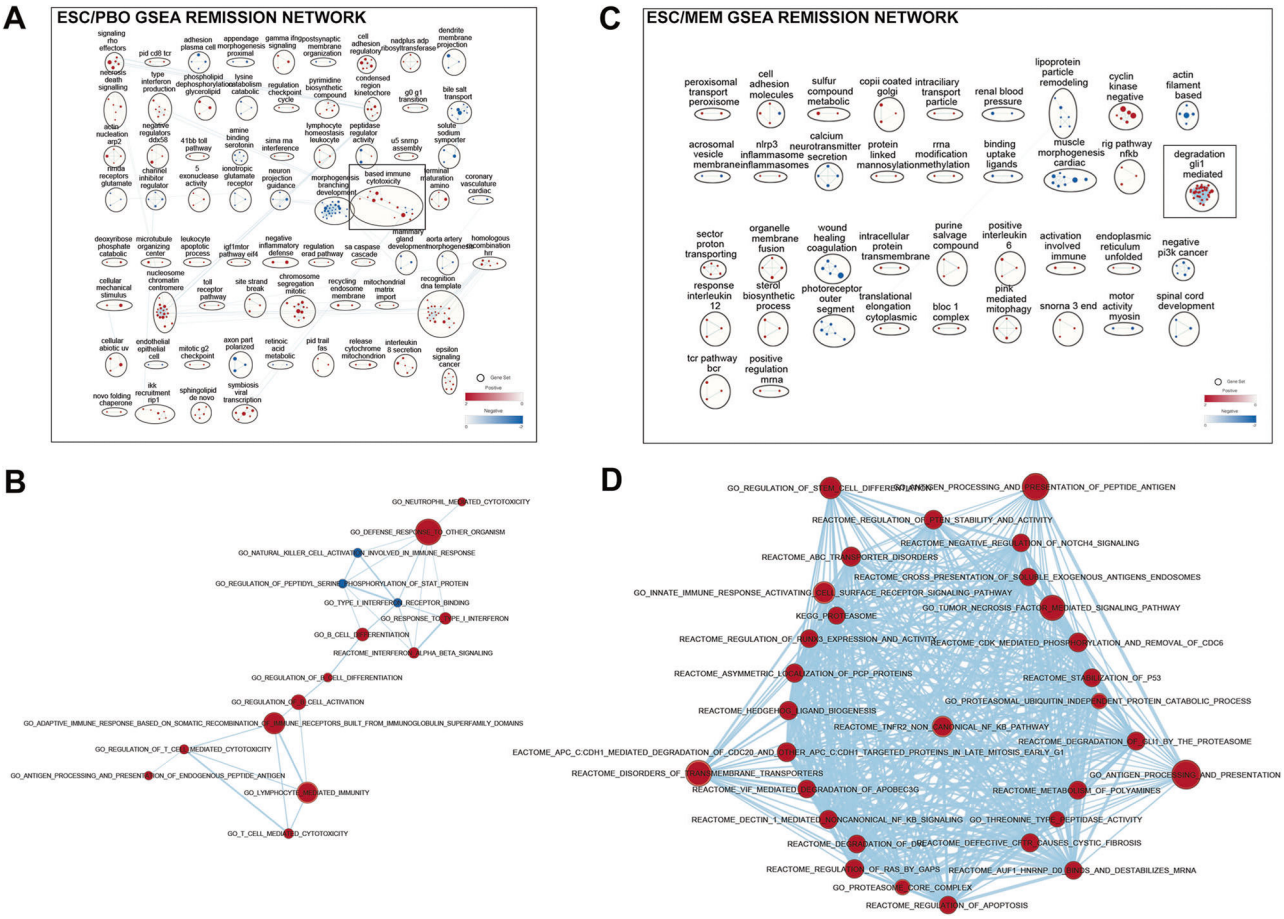

Fig. 4. Network analysis of enriched gene sets in ESC/PBO and ESC/MEM remitters compared with non-remitters.

Clustering of gene set enrichment analysis results of **a** ESC/PBO and **b** ESC/MEM remitter profiles. Tested gene sets included hallmark pathways, canonical pathways, and gene ontology gene sets. Only gene sets with an FDR adjusted *p* value < 0.05 were included. Nodes represent gene sets and lines demonstrate their connectivity based the degree or overlap between set genes. Highly related terms are enclosed in a circle with a label automatically summarized from the word-frequency of the contained terms. **c** Immune response subnetwork in ESC/PBO remission. **d** Proteasomal degradation subnetwork in ESC/MEM remission. Red: positive normalized enrichment score, indicating activation posttreatment. Blue: negative normalized enrichment score, indicating repression post-treatment.