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Opposite molecular signatures of depression in men and women

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Abstract

Background—Major depressive disorder (MDD) affects women approximately twice as often as men. Women are three times as likely to have atypical depression, with hypersomnia and weight gain. This suggests that the molecular mechanisms of MDD may differ by sex.

Methods—To test this hypothesis, we performed a large-scale gene expression meta-analysis across three corticolimbic brain regions, the dorsolateral prefrontal cortex, subgenual anterior cingulate cortex, and basolateral amygdala (N=26 men, 24 women with MDD and sex-matched

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controls). Results were further analyzed using a threshold-free approach, gene ontology, and cell type-specific analyses. A separate dataset was used for independent validation [N=13 MDD subjects/sex; 22 controls (13 males, 9 females)].

Results—Of the 706 genes differentially expressed in men with MDD and 882 genes differentially expressed in women with MDD, only 21 were changed in the same direction in both sexes. Notably, 52 genes displayed expression changes in opposite directions between men and women with MDD. Similar results were obtained using a threshold-free approach, where the overall transcriptional profile of MDD was opposite in men and women. Gene ontology indicated that men with MDD had decreases in synapse-related genes, whereas women with MDD exhibited transcriptional increases in this pathway. Cell type-specific analysis indicated that men with MDD exhibited increases in oligodendrocyte- and microglia-related genes, while women with MDD had decreases in markers of these cell types.

Conclusions—The brain transcriptional profile of MDD differs greatly by sex, with multiple transcriptional changes in opposite directions between men and women with MDD.

Keywords

Sex difference; Depression; Mood disorders; Genetics; Meta-analysis; Corticolimbic

Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide (1), but its impact differs substantially between sexes. Women are twice as likely to be diagnosed with a single MDD episode, and four times more likely to be diagnosed with recurrent MDD (e.g., (2–7). Women with MDD also report greater illness severity, more symptoms (3, 8–10), and different symptomatology than men. For instance, women are three times more likely to have atypical depression, characterized by hypersomnia and weight gain (11–15). Comorbidity of MDD with other disorders also differs between sexes. For instance, women are more likely to have comorbid anxiety disorders, whereas men are more likely to have comorbid substance use disorders (e.g., (16–19)). Some studies suggest that women have more positive treatment outcomes with selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (20, 21), whereas men seem to respond better to tricyclic antidepressants.

Research suggests dysfunction of the corticolimbic network of mood regulation in MDD. We consider three network nodes, the dorsolateral prefrontal cortex (DLPFC; Brodmann area 9 (BA9)), subgenual anterior cingulate cortex (ACC; BA25), and amygdala (AMY). Structural and functional neuroimaging implicates these regions in MDD [e.g. (22–30)]. Since some studies were performed in only women (24, 31), it is unclear whether results are generalizable to both sexes. Additionally, studies that included both sexes often lacked statistical power to stratify by sex. The idea that these brain regions are differentially affected in men and women with MDD is supported by sex differences in activation during normal emotional states. fMRI studies of non-depressed subjects suggest differential regional during emotion-related tasks, with women having more AMY activation and men more cortical activation [e.g., (32–34)].

Postmortem brain studies report reduced density and number of glial cells in MDD in the DLPFC (35), ACC (36, 37), and AMY (38, 39). Additionally, there is reduced neuron size in DLPFC (36) and ACC (37) in MDD. However, these analyses were not stratified by sex. Gene expression studies on tissue homogenate from postmortem brains have identified sex differences in MDD. In the ACC, we reported brain derived neurotrophic factor (BDNF)/TrkB expression changes with greater effect in men compared to women with MDD (40). We also reported a more robust reduction in the GABA neuron marker, somatostatin, in the DLPFC, ACC, and AMY of women compared to men with MDD (41). The AMY of women with MDD exhibited a GABA-/BDNF-related dysfunction not seen in men with MDD (41–43). We also found sex differences in cholinergic signaling changes in the AMY of MDD subjects (44). Sex differences in glutamate-related genes were reported in the DLPFC of MDD subjects, with increased expression of glutamate-related genes in women with MDD and decreases in these same genes in men with MDD (45). Finally, Labonte et al recently reported sex-specific transcriptional signatures of depression (46).

Here, we use large-scale gene expression studies, meta-analysis across corticolimbic brain regions, and meta-regression for sex to examine the brain molecular pathology in MDD. Given the sex differences in MDD incidence, symptomatology, and neuroimaging, we hypothesize that the molecular signature of MDD is distinct in men and women.

Materials and Methods

Detailed methods are available in the supplements.

Human subjects and microarray studies

Brain samples were obtained during autopsies conducted at the Allegheny County Medical Examiner's Office (Pittsburgh, USA) after next-of-kin consent using procedures approved by University of Pittsburgh's Institutional Review Board and Committee for Oversight of Research Involving the Dead. Consensus DSM-IV diagnoses were made by an independent committee of experienced clinical research scientists using information from clinical records, toxicology results, and a standardized psychological autopsy. Unaffected comparison subjects were assessed with identical procedures.

50 MDD subjects (26 men, 24 women) and 50 sex-matched unaffected comparison subjects were included. We combined 8 microarray datasets from three brain regions, with half the studies performed in men, half in women (43, 47, 48). Four studies were in ACC (2/sex), two in DLPFC (1/sex), and two in AMY (1/sex). Tables S1 and S2 contain details on subjects and areas investigated. Group means for age, postmortem interval (PMI), RNA integrity number (RIN), and brain pH were nearly identical and not statistically different.

For replication, we used recently published publically available RNA-seq data (BA11, BA25) generated using brains from a different brain bank (GEO GSE102556; (46)). The effect of MDD was analyzed separately in men and women. We then assessed overlap in DE genes identified in men and women and the percent of overlapping genes that were changed in opposite directions in men and women with MDD.

Meta-analysis of gene expression in MDD

Datasets and meta-analysis methods and results were described previously (49–51). Briefly, we adopted linear models to account for potential confounding covariates and applied a meta-analysis pipeline to combine studies for identification of MDD-associated genes. A random effects model (REM) was used to detect changes in gene expression by combining effects across studies. We adopted REM separately for the 4 female and 4 male studies. We used $q<0.05$ as the cutoff for differential expression (DE). We then combined all eight studies by REM and used meta-regression to probe for genes that were changed differently in men and women with MDD ($q<0.05$).

Overlap of gene expression profiles in MDD in men and women

Rank-rank hypergeometric overlap test (RRHO)—We used RRHO (52) to compare MDD DE genes between men and women. RRHO is a threshold-free algorithm that identifies trends of overlap between two ranked lists of DE genes. The genes are ranked by the −log10 of DE p-value multiplied by the effect size direction. Up, down, and unchanged genes are at the bottom, top, and middle of the list, respectively. A one-sided p-value for the overlap of gene lists from two datasets is calculated according to the hypergeometric distribution.

Spearman's Correlation—We used Spearman's rank-order correlation as a complementary threshold-free method. We compared ranked effect sizes between men and women for all genes.

Gene ontology enrichment analysis

The area under the receiver operating curve (AUROC) statistic was used to measure enrichment of Gene Ontology (GO) groups in a specific gene ranking. This value is equal to the probability that a gene in a GO group will rank higher than a gene not in the group. DE results were ranked from the most significant gene in the negative direction to the most significant gene in the positive direction (signed −log(pvalues)). Mann-Whitney *U* test p-values were calculated. GO groups with 10–200 genes were used (5081 groups).

Cell type-specific analysis

From single-cell transcriptome analysis of healthy human adult cortex, we obtained six lists of the top 21 most enriched genes in transcriptomic-determined cell types (astrocytes, neurons, oligodendrocytes, oligodendrocyte precursors, microglia, and endothelial cells; Table S3 in (53)). The number of genes tested varies because not all 21 genes were assayed in our meta-analysis. We calculated AUROC statistics and Mann-Whitney U p-values for each cell-type list with Bonferroni correction.

Results

Divergent molecular signatures of MDD in men and women

We used large-scale gene expression meta-analysis to probe for sex differences in the brains of men and women with MDD (See strategy in Figure S1). We first performed the meta-

analysis in each sex separately. There were 706 DE transcripts (252 upregulated, 454 downregulated) in men and 882 DE transcripts (524 upregulated, 358 downregulated) in women. When comparing DE genes in men and women with MDD, 633 of 706 transcripts were found in men only and 809 of 882 transcripts in women only. Interestingly, only 73 genes were DE in both MDD men and women, and 52 of these 73 genes were changed in opposite directions between sexes. Therefore, only 21 DE genes were affected in the same direction in men and women with MDD. Results are summarized in Figure 1A. Results are not driven by differences in sex chromosomes, as only 2.5% of genes identified in men and 3.2% of genes identified in women are found on sex chromosomes.

Next, we performed meta-regression on all studies to directly test for expression differences between men and women with MDD. This approach is more stringent than assessing the overlap of DE gene lists identified in men and women separately and is better powered since it includes all eight studies. We identified 1027 genes that were significantly differentially altered in men and women with MDD (Figure 1A). A comparison of the meta-regression gene list (1027 genes) indicates that these genes are changed in opposite directions in men and women with MDD (Figure 2A). Of these meta-regression genes, 198 and 338 were significant in men or women only, respectively. 52 of the 1027 meta-regression genes were significant for both men and women with MDD, but in opposite directions. These same 52 genes were identified in the previous men/women separate analysis (Table S3; Figure 2B). The remaining genes with meta-regression main effect (439) did not reach significance ($q<0.05$) in either sex. Notably, less than 1% of the meta-regression genes were sexually dimorphic in control subjects, indicating that the differential effects in MDD are not driven by baseline sex differences (Table S4).

Although most of our male and female MDD studies were performed in separate experiments, one ACC study was performed at the same time in men and women; we directly compared individual gene expression results in this study. We selected three meta-regression genes that were significantly changed in opposite directions in males and females with MDD (*ARPP21*, *P2RY12*, *MTHFR*). We selected an additional 5 genes identified by meta-regression that were significant in only one sex (*CACNA1I*, *ARHGEF3*, *SLCO1A2*, *GABRD*, *CAMK2B*). We confirmed significant interactions of sex and diagnosis for 7/8 genes (Figure 3A–H). We also confirmed main effects of diagnosis on expression of *NOL3*, *NUB1*, and *PSMA3*; these genes were changed in the same direction in men and women with MDD (Figure 3I–K). To confirm that these changes were consistent across brain regions, we performed an independent qPCR experiment in the AMY for *ARPP21*, *P2RY12,* and *MTHFR*, and found significant interactions of sex and diagnosis for *ARPP21* and *P2RY12* (Figure S3). In the AMY, there was a sex difference in *MTHFR*, but no interaction of sex and diagnosis, suggesting that the meta-regression result is driven primarily by the ACC and DLPFC for *MTHFR*.

We confirmed this opposite direction effect in male and female MDD using a separate RNA-seq dataset generated using subjects from a different brain bank (46). We again found very little overlap in DE genes in men and women with MDD (~8%). Notably, ~55% of these overlapping genes changed in opposite directions in men and women with MDD (Figure 1B–C; Tables S5–S6).

Opposite transcriptional profiles in men and women with MDD

We used a threshold-free approach to validate our divergent gene expression findings in men and women with MDD. Typical DE studies use somewhat arbitrary DE and effect size thresholds to identify relevant genes, which might miss small but reproducible changes. To complement this approach, we used RRHO as an exploratory, threshold-free method to assess patterns of overlap between two DE datasets. For each of the two datasets, RRHO ranks the entire gene list by DE p-value and effect size direction, with one dataset represented on the X-axis and one on the Y-axis. We first performed RRHO using results from the cross-brain region meta-analysis. We compared the rank ordered gene list generated in depressed men compared to controls (X-axis Figure 4) to the rank ordered gene list generated in depressed women compared to controls (Y-axis Figure 4). Figure 4A indicates interpretation of RRHO plots. Consistent with the lack of overlap in DE genes reported above, there was no statistically significant overlap in genes that were upregulated in both men and women with MDD or downregulated in both sexes (Figure 4B). However, there was a statistically significant overlap in genes affected in opposite directions in men and women with MDD (Figure 4B; Figure S4A). We confirmed this result using Spearman correlation. There was a significant negative correlation in effect sizes for genes in the male-specific dataset to the effect sizes of genes in the female-specific dataset ($\rho=-0.130$; slope=−0.127; $p=4.39\times10^{-41}$), indicating that genes were changed in opposite directions in men and women with MDD.

We also performed RRHO and Spearman correlations separately for each brain region. There was no statistically significant overlap in genes upregulated in both men and women with MDD or downregulated in both sexes for any brain region (Figure 4C, D, E). Instead, we observed in the DLPFC and ACC a statistically significant overlap in genes affected in opposite directions in men and women with MDD (Figure 4C and 3D; Figure S4B and S4C). We confirmed this negative correlation in effect size direction using Spearman correlation in the DLPFC ($\rho=-0.204$; slope=−0.197; $p=2.20\times10^{-100}$) and ACC ($\rho=-0.224$; slope=−0.149; $p=4.94\times10^{-122}$). In the AMY, there was no statistically significant overlap in genes changed in opposite directions in men and women with MDD (Figure 4E). Importantly, RRHO analysis in the replication cohort confirmed these opposite transcriptional profile in male and female depression (Figure S5).

Pathway analysis of molecular signatures of MDD in men and women

In men, the DE genes were enriched for synapse-related pathways, inner mitochondrial membrane protein complex, and G-protein coupled amine receptor activity (Table 1; top three pathways are synapse-related, with overlapping genes (Figure S6)). Results indicated that genes in these pathways were downregulated in men with MDD.

In women, the DE genes were enriched for pathways related to antigens and mitochondrial function (Table 2; top four pathways are antigen-related, with overlapping genes (Figure S7)). Genes in these top pathways were downregulated in women with MDD.

Given that some pathways might still be enriched in both men and women with MDD, but to varying degrees, we examined the top pathways identified in each sex in the opposite sex. In

other words, a pathway might be enriched in both sexes, but might only be a top pathway in one sex. Interestingly, all five pathways identified in men were also enriched in women (Table 1). However, while genes in 4 of the 5 top male pathways were downregulated in men with MDD these same pathways had genes that were upregulated in women with MDD. When we examined the top female identified pathways in men, most were not enriched in men with MDD (Table 2).

We next performed GO pathway analysis using genes identified by meta-regression. These genes enriched for regulation of synapse-related pathways, antigen-related pathways, and MHC protein complex (Table 3; overlap of genes in the top pathways in Figure S8).

Cell type enrichment analysis

We next asked whether sex-specific DE genes were enriched for markers of particular cell types. The goal is to identify candidate cell populations that are likely disrupted in MDD. Results are summarized in Table 4. Genes specifically expressed in oligodendrocytes and microglia were upregulated in men with MDD, but downregulated in women with MDD. Genes specifically expressed in astrocytes were upregulated in men with MDD, but unchanged in women with MDD. Neuronal genes were downregulated in men with MDD, but unchanged in women with MDD. We confirmed these cell-type specific results using a single cell dataset generated in mouse cortex (Table S7). Together, this cell type enrichment analysis suggests that oligodendrocytes and microglia are oppositely affected in men and women with MDD.

We previously reported reduced expression of oligodendrocyte-specific genes in the AMY of men with MDD (42). Thus, we were surprised that when all three corticolimbic brain regions were combined, there was an increase in expression of oligodendrocyte-specific genes in men with MDD. A closer look at the cell type-specific findings for each brain region in fact confirms our previous AMY finding. While oligodendrocyte-specific genes increase in expression in the DLPFC and ACC of men with MDD, these same genes are decreased in the AMY (Figure S9A). Interestingly, the oligodendrocyte-specific genes were upregulated in the AMY, but downregulated in DLPFC and ACC in women with MDD. Together, the cortical patterns for oligodendrocyte-specific genes drives the cross-brain region findings. A closer look at microglia- and neuronal-specific genes showed consistent findings across all three brain regions (Figure S9B, S9C).

Discussion

We report almost no overlap in transcriptional changes across corticolimbic brain regions in men and women with MDD, but instead opposite transcriptional changes. Our results suggest that men with MDD have decreases, but women with MDD have increases in synapse-related genes. Immune-related reductions characterized female MDD. Cell type-specific analysis suggests increases in oligodendrocyte- and microglia-specific genes in men with MDD, but decreases in markers of these cell types in women with MDD. Together, these findings point towards distinct, and even opposite molecular changes in MDD in men and women.

Our results are partially consistent with results from a recent publication reporting sex-specific changes in MDD (46). While we also found very little overlap in DE genes in men and women with MDD, our results indicate a high level of transcriptional overlap in genes changed in opposite directions. In fact, we used our statistical methods on the data generated by Labonte et al. (46) and found very similar, but unreported opposite transcriptional results. Brains used in the previous publication were from a different brain bank, supporting the generalizability of our findings. Here, we include results from the AMY, which is not included in Labonte et al. (46). Although consistent with our hypothesis, it is somewhat surprising that these sex-specific molecular changes in MDD were not reported previously. One reason might be because many previous postmortem brain analyses in MDD were performed in mostly (or only) men. Studies that included both sexes mostly did not have sufficient statistical power to stratify by sex, although a few prior reports have hinted at sex differences in MDD (see examples in the Introduction) We believe that our meta-analysis/regression approach gave us the statistical power to investigate larger-scale profiles of molecular changes occurring in the brains of men and women with MDD.

Previous studies reported reduced expression of neuron-specific genes in the AMY of men with MDD and reduced neuronal density in DLPFC (35, 42). Our findings in men with MDD are consistent with those reports. However, we did not find a significant change in neuronal genes in women with MDD. In fact, our results, while not significant after correction for multiple testing, suggest upregulation of neuron-specific genes in women with MDD. Hence future studies should directly compare neuron and synapse density in the brains of men and women with MDD.

Our findings in men with MDD are consistent with previous reports (that included mostly men) showing reduced markers of synapses, increased markers of inflammation, and reduced spine synapses in the DLPFC (54, 55). Specifically, we report reduced expression of genes related to synapse function and increased expression of microglia-specific genes in men with MDD. Our current findings suggest opposite synapse and inflammation-related changes in women with MDD.

Prior studies demonstrated reduced glial cell densities in DLPFC, ACC, and AMY in MDD (35–37, 39). These studies included both men and women, but did not stratify by sex. Thus, it is unclear whether the findings are sex-specific. Here, we report increases in markers of glia in men, but decreases in women with MDD. Making comparisons between density of glia and changes in expression of glia-specific cells might not be appropriate, as reduced glia density does not necessarily translate into reduced expression of glia-specific genes. Future studies will examine the glial deficits in both sexes, with attention to different glia cell types.

The region-specific findings for oligodendrocyte-specific genes are interesting. In men with MDD, we report increases in oligodendrocyte-specific genes in the DLPFC and ACC, but decreases in expression of these same genes in the AMY. Additionally, in women with MDD, these genes showed decreased expression in the DLPFC and ACC, but increased expression in the AMY (Figure 4). Thus, the oligodendrocyte changes in MDD are not only sex-specific, but brain region-specific as well. The cell type-specific findings for microglia-, astrocyte-, and neuronal-specific genes were consistent across brain regions.

The sex differences in MDD that we report might be driven by developmental processes. Developmental exposure to testosterone around the time of birth and through puberty permanently masculinizes the structure of several brain regions (termed organizational effects of hormones). Notably, adolescence is also a sensitive developmental time-period in which there is extensive neuroanatomical, functional, and chemical brain maturation. Events during adolescence that interact with these developmental processes can increase risk for adult psychopathology. We and others have used various rodent models to manipulate gonadal hormone exposure during critical periods of brain development (perinatal through puberty). For instance, we showed that giving newborn female mice a single dose of testosterone partially masculinizes adult mood-related behavior (i.e., these females had lower anxiety-/depressive-like behaviors in adulthood) (56). Differences in gonadal hormone exposure during development might also influence how the brain responds to a challenge (e.g., chronic stress) in adulthood. Sex differences due to developmental processes might be more relevant to the developmental origins of MDD compared to sex differences emerging in adulthood (e.g., reflecting environmental effects) (57). Since our study includes only adults, we are unable to determine whether the observed sex differences emerge during development or in adulthood.

Our cell type specific and pathway analyses suggest divergent changes in the brains of men and women with MDD. It is quite interesting that in both men and women, the neuronal- and microglial-related changes occur in opposite directions. Women with MDD have decreased markers of immune function and microglia, with increased markers of synaptic function and neurons. On the other hand, men with MDD have increased markers of microglia, with decreased markers of synaptic function and neurons. This opposite direction of effect on microglia and synapses is consistent with a growing literature suggesting that activated microglia have more frequent and prolonged contacts with, and may have increased phagocytosis of, dendritic spines (58). Since our work here is performed in the human postmortem brain, it is unclear whether the synaptic changes observed in MDD (decreased in men, increased in women) are driven by microglia changes, or vice versa. Additionally, it is unclear whether the opposite molecular signatures of MDD in men and women might drive sex differences in MDD symptomatology. To glean more definitive links, follow-up studies in rodent models would perturb immune function in both directions (in both sexes) and assess MDD-associated behavioral domains (e.g., anxiety-, anhedonia-, despair-related behavior).

Limitations of these results are inherent to studies involving human postmortem brains and the heterogeneity of psychiatric cohorts. Although many covariates could affect gene expression independently of psychiatric diagnosis, our statistical method only included the top two relevant covariates for each gene. This increased our statistical power, but might have ignored additional relevant covariates. We were not sufficiently powered for some cofactors, including recurrent/single episode MDD and comorbid drug abuse. Our meta-analysis/regression approach gave us statistical power to identify consistent molecular changes across brain regions in MDD. However, we note that this method might miss brain region-specific changes important for disease progression. Future studies will use large cohorts of MDD subjects and matched controls, with sufficient statistical power to detect potential sex-specific MDD changes. Although men and women with MDD tend to have

differential responses to antidepressants, the medications taken by our subjects were largely similar between men and women, and antidepressant usage was used as a potential cofactor; thus, our results were not driven by different medications between the sexes.

To conclude, our study reveals divergent corticolimbic molecular changes in men and women with MDD. Thus, it follows that potential novel treatments should target sex-specific pathology. For instance, our results suggest that treatments to suppress immune function might be more appropriate for men with MDD, while treatments which boost immune function might be more appropriate for women with MDD. Alternatively, future treatments might aim to target the limited shared pathology present in both men and women with MDD. The implications of MDD cell-specific changes between men and women remain to be further investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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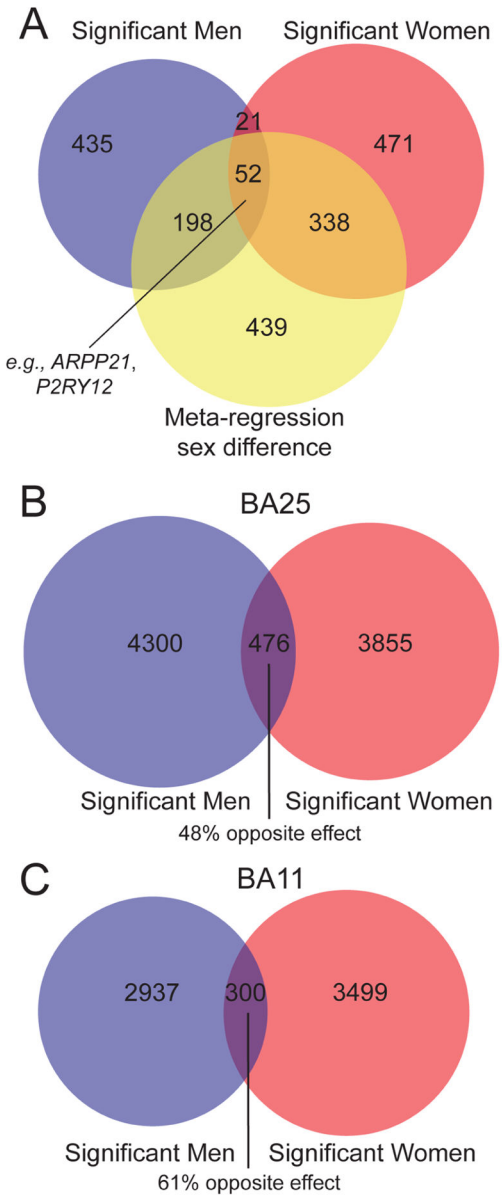

Figure 1. Distinct transcriptional changes in men and women with MDD

(A) Venn diagram displaying overlap in differentially expressed genes in men with MDD, in women with MDD, and in genes identified via meta-regression for sex ($q < 0.05$). We confirmed these results using an independent replication dataset ($p < 0.05$); there was very little overlap in DE genes identified in men and women with MDD in BA25 (**B**) and BA11 (**C**).

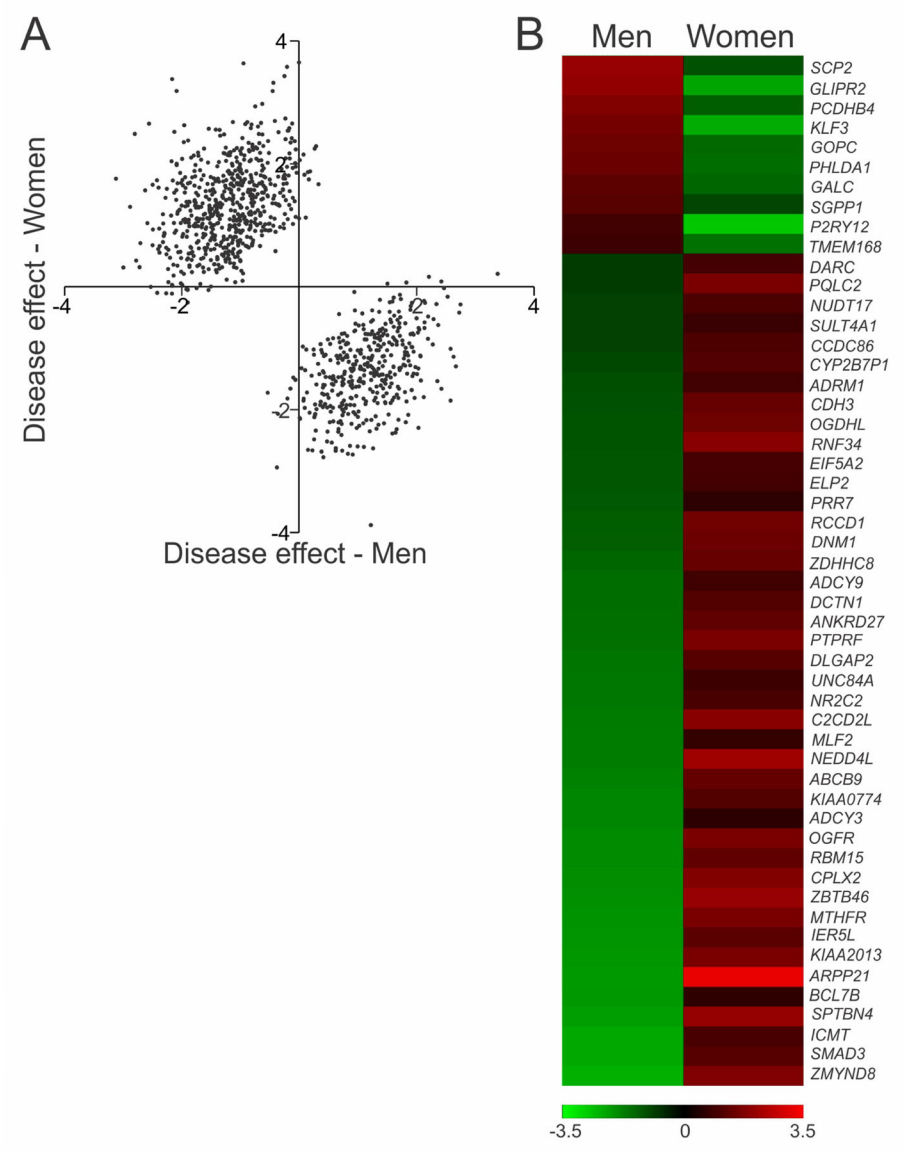

Figure 2. Genes affected in opposite directions in men and women with MDD

(A) Scatterplot indicating the overall pattern of opposite effect size directions for the full meta-regression by sex gene list (1027 genes). **(B)** Heatmap indicating opposite effect sizes of the 52 genes significantly ($q < 0.05$) changed in opposite directions in men and women with MDD. These genes were identified in both the meta-regression dataset as well as in the sex-specific meta-analysis datasets.

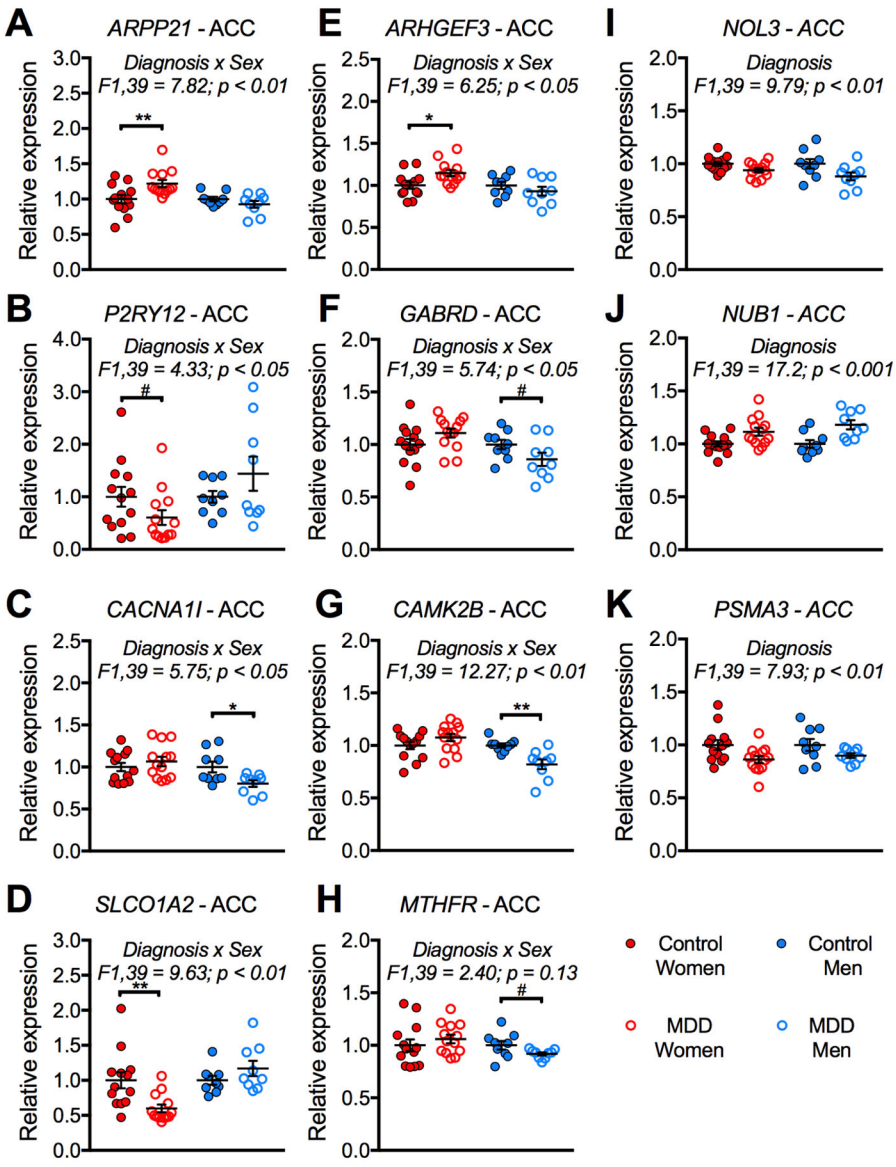

Figure 3. Verification of meta-regression results using arrays in the ACC

The MD2 ACC microarray experiments were performed at the same time in men and women with MDD, allowing us to directly compare expression changes from the microarray studies. There were significant sex x diagnosis interactions for *ARPP21* (**A**), *P2RY12* (**B**), *CACNA1I* (**C**), *SLCO1A2* (**D**), *ARHGEF3* (**E**), *GABRD* (**F**), and *CAMK2B* (**G**). There was a significant main effect of diagnosis on expression of *NOL3* (**I**), *NUB1* (**J**), and *PSMA3* (**K**). (**A**) There was a significant increase in *ARPP21* expression in only women with MDD. (**B**) There was a trend for a decrease in *P2RY12* expression in only women with MDD. (**C**) There was a significant decrease in *CACNA1I* expression in only men with MDD. (**D**) There was a decrease in *SLC01A2* expression in only women with MDD. (**E**) There was an increase in *ARHGEF3* expression in only women with MDD. (**F**) There was a trend for a decrease in *GABRD* expression in only men with MDD. (**G**) There was a decrease in *CAMK2B* expression in only men with MDD. (**H**) There was a trend for a decrease in

MTHFR expression in only men with MDD. (**I**) There was a significant decrease in *NOL3* expression in both men and women with MDD. (**J**) There was a significant increase in *NUB1* expression in both men and women with MDD. (**K**) There was a significant decrease in *PSMA3* expression in men and women with MDD. *, $p < 0.05$; **, $p < 0.01$; #, $p < 0.1$.

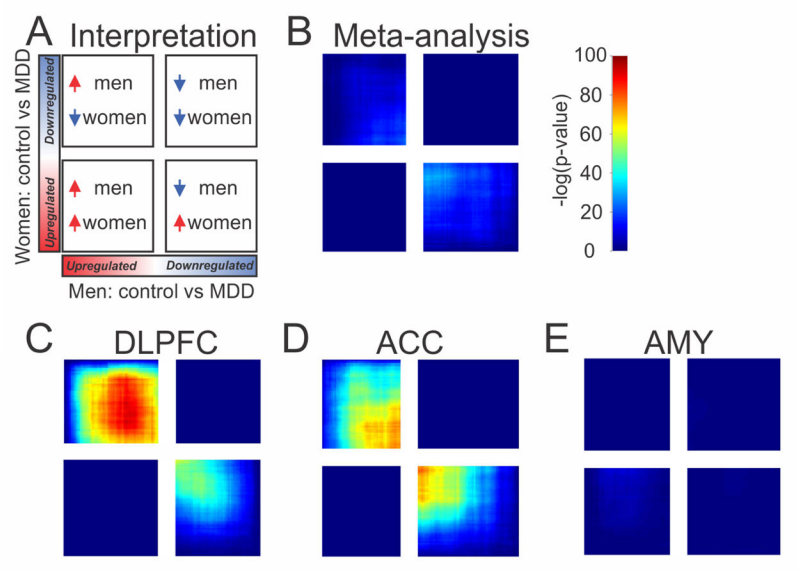

Figure 4. Threshold-free differential expression patterns reveal that men and women with MDD have opposite molecular signatures

(A) Schematic indicating interpretation of RRHO plots. A hot spot in the bottom left corner indicates overlap in genes up in both men and women with MDD. A hot spot in the top right corner indicates overlap in genes down in both men and women with MDD. A hot spot in the top left indicates overlap in genes up in men and down in women with MDD. A hot spot in the bottom right indicates overlap in genes down in men and up in women with MDD. Note that in the RRHO plots, the quadrants are not always of equal size; this is due to the fact that there is typically not an even split in the number of genes that are up and down regulated. **(B)** There was no significant overlap in genes that were up in both men and women with MDD or down in both men and women with MDD. However, there was a weak overlap in genes that were changed in opposite directions in men and women with MDD. There was a strong overlap in genes that were affected in opposite directions in the DLPFC (**C**) and ACC (**D**) of men and women with MDD. **(E)** There was no overlap in gene expression profiles in the AMY.

Table 1

List of top 5 gene ontology pathways identified in men with MDD.[a]

Pathway	Men		Women	
	p-value	AUROC	p-value	AUROC
Regulation of synapse structure or activity[b]	$< 10^{-9}$	0.373 ↓	< 0.01	0.559 ↑
Regulation of synaptic plasticity[b]	$< 10^{-6}$	0.378 ↓	< 0.15	0.544 ↑
Positive regulation of synapse assembly[b]	$< 10^{-5}$	0.312 ↓	< 0.01	0.618 ↑
Inner mitochondrial membrane protein complex	$< 10^{-5}$	0.375 ↓	$< 10^{-6}$	0.347 ↓
G-protein coupled amine receptor activity	$< 10^{-5}$	0.169 ↓	< 0.15	0.622 ↑

Abbreviation: AUC, area under the curve.

[a] AUC < 0.5 indicates a pathway is enriched in genes that were downregulated in MDD in that sex. AUC > 0.5 indicates a pathway is enriched in genes that were upregulated in MDD in that sex.

[b] The synapse-related pathways have highly overlapping gene lists (see Figure S7). Bold indicates pathways affected in opposite directions in men and women with MDD.

Table 2

List of top 5 gene ontology pathways identified in women with MDD.[a]

Pathway	Women		Men	
	p-value	AUROC	p-value	AUROC
Antigen processing & presentation[b]	$< 10^{-10}$	0.353 ↓	NS	0.522
Antigen processing & presentation of exogenous peptide antigen[b]	$< 10^{-10}$	0.343 ↓	NS	0.515
Antigen processing & presentation of exogenous antigen[b]	$< 10^{-9}$	0.346 ↓	NS	0.511
Antigen processing & presentation of peptide antigen[b]	$< 10^{-9}$	0.354 ↓	NS	0.516
Mitochondrial translational termination	$< 10^{-9}7$	0.337 ↓	<0.05	0.428 ↓

Abbreviation: AUC, area under the curve.

[a]AUC < 0.5 indicates a pathway is enriched in genes that were downregulated in MDD in that sex. AUC > 0.5 indicates a pathway is enriched in genes that were upregulated in MDD in that sex.

[b]The antigen-related pathways have highly overlapping gene lists (see Figure S8).

Table 3

List of top 5 gene ontology pathways identified by metaR dataset.

Pathway	p-value	Men Effect size	Women Effect size
Regulation of synapse structure or activity[a]	$< 10^{-8}$	− 0.50	0.20
Antigen processing & presentation[b]	$< 10^{-7}$	− 0.003	− 0.50
MHC protein complex[b]	$< 10^{-7}$	0.50	− 1.20
Regulation of synapse organization[a]	$< 10^{-7}$	− 0.46	0.51
Antigen processing & presentation of exogenous peptide antigen[b]	$< 10^{-7}$	− 0.04	− 0.56

[a]The synapse-related pathways have highly overlapping gene lists.

[b]The antigen-related pathways pathways have highly overlapping gene lists (see Figure S9).

Table 4

Sex-specific associations of transcriptomic cell-type enriched gene sets.[a]

Cell type	Men		Women	
	q-value	AUC	q-value	AUC
Oligodendrocytes	< 0.005	0.763 ↑	< 0.1	0.319 ↓
Astrocytes	< 0.005	0.734 ↑	NS	0.434
Microglia	< 0.05	0.710 ↑	$< 10^{-4}$	0.134 ↓
Neurons	< 0.05	0.330 ↓	NS	0.525
Oligodendrocyte precursor cells	< 0.12	0.672 ↑	< 0.19	0.682 ↑
Endothelial cells	NS	0.559	NS	0.542

Abbreviation: AUC, area under the curve.

[a]AUC > 0.5 indicates a cell type is enriched in genes that were downregulated in MDD in that sex. AUC < 0.5 indicates a cell type is enriched in genes that were upregulated in MDD in that sex. Bold indicates cell-types affected in opposite directions in men and women with MDD.