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Changed Cortical Risk Gene Expression in Major Depression and Shared Changes in Cortical Gene Expression between Major Depression and Bipolar Disorders.

Running Title: Cortical gene expression in mood disorders

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ABSTRACT

Background: Mood disorders likely occur in someone with a genetic predisposition who encounter a deleterious environmental factor leading to dysregulated physiological processes due to genetic mutations and epigenetic mechanisms altering gene expression. To gain data to support this hypothesis we measured levels of gene expression in three cortical regions known to be affected by the pathophysiologies of major depression (MD) and bipolar disorders (BD).

Methods: Levels of RNA were measured using the Affymetrix™ Human Exon 1.0 ST Array in Brodmann's areas (BA) 9, 10 and 33 (left hemisphere) from individuals with MD, BD and age and sex matched controls with changed expression taken as a fold change in RNA ≥ 1.2 at $p < 0.01$. Data was analysed using JMP® genomics 6.0 and the probable biological consequences of changes in gene expression determined using Core and Pathway Designer Analyses in Ingenuity Pathway Analysis.

Results: There were altered levels of RNA in BA 9 (MD = 424; BD = 331), BA 10 (MD = 52; BD 24) and BA 33 (MD = 59 genes; BD = 38 genes) in mood disorders. No gene was differentially expressed in all three regions in either disorder. There was a high correlation between fold changes in levels of RNA from 112 genes in BA 9 from MD and BD ($r^2 = 0.91$, $p < 0.001$). Levels of RNA for 4 risk genes for MD were lower in BA 9 in that disorder.

Conclusion: Our data argues there are complex regional-specific changes in cortical gene expression in MD and BD that includes the expression of some risk genes for MD in those with the disorder. It could be hypothesised that the common changes in gene expression in MD and BD could be involved in the genesis of symptoms common to both disorders.

Keywords: major depression, bipolar disorders, cortex, postmortem CNS, gene expression

INTRODUCTION

Concordance rates between monozygotic twins for major depression (MD) (~ 40%) and bipolar disorders (BD) (~ 50%) argue that the causes of these disorders are not wholly genetic. To accommodate these observations, it is currently hypothesised that MD and BD occur in a genetically predisposed individual and are triggered by exposure to a deleterious environmental factor (Craddock, 2006). It is now known environmental factors can change the activity of biochemical pathways through the modulation of epigenetic mechanisms which act to change gene expression and therefore it is likely that the aetiologies of MD and BD involve changes in gene expression brought about by a combination of the actions of inherited risk genes and changes in epigenetic mechanisms occurring in response to environmental factors. This notion has led to studies to identify changes in gene expression in the CNS from individuals with MD and BD, particularly in the cortex as cortical dysfunction is present in both disorders (Drevets et al., 2008).

Following the posit that changes in cortical gene expression contribute to the aetiologies of mood disorders a number of studies used gene expression arrays to compared levels of gene expression in the cortex of individuals with MD and BD to that in individuals with no history of psychiatric disorders (Controls). These studies report changes in gene expression in the cortex from individuals with MD would impact on glutamatergic signalling, GABAergic signalling (Aston et al., 2004; Sequeira et al., 2009), synaptic function, oligodendrocyte function (Choudary et al., 2005), cell proliferation (Tochigi et al., 2008; Klempan et al., 2009), RNA processing (Sequeira et al., 2007), signal transduction, metabolism (Kang et al., 2007), gene transcription and myelination (Klempan et al., 2009). In BD changes in gene expression would impact on the transforming growth factor β1, caspase 8 (Bezchlibnyk et al., 2001), oligodendrocytes, myelination (Tkachev et al., 2003), mitochondrial function (Sun et al., 2006) and nervous system development (Nakatani et al., 2006). Additionally, some studies have argued there are commonalities in changes in

cortical gene expression between MD and BD (Tkachev et al., 2003; Iwamoto et al., 2004) which may reflect some shared aetiologies (Cuellar et al., 2005). Finally, studies have not find omnibus changes in gene expression across multiple cortical regions in MD (Evans et al., 2004; Choudary et al., 2005; Sequeira et al., 2007; Klempan et al., 2009; Sequeira et al., 2009) or BD (Evans et al., 2004). Hence, taken together, microarray-based expression studies suggest there are cortical-region specific changes in gene expression in MD and BD and that there are differences and commonalities in the underlying biochemical pathways driving those aetiologies of the disorders.

Recently, results from the merging of expression array data from 9 independent studies for re-analyses have been reported (Gandal et al., 2018a). The headline outcome from this re-analysis of data was that the is a shared molecular neuropathology across major psychiatric disorders. For example, it was suggested there were changes in gene expression in the cortex from subjects with autism spectrum disorder, schizophrenia and BD involved in glial cell differentiation and fatty acid metabolism. Notably, the re-analysis did suggest that in MD they were changes in gene expression enriched for genes involved with G-protein-coupled receptors, cytokine-cytokine interactions and hormone activity. The conclusion drawn from these finding was that the pathophysiology of MD, as has been suggested previously (Dean, 2011), involves inflammatory pathways and the hypothalamic-pituitary axis.

More recently, RNA sequencing (RNAseq) has been used to identify changes in gene expression in the CNS from subjects with mood disorders. An early study examined gene expression in the dorsolateral prefrontal cortex from subjects with BD and particularly focussed on the lower level of expression of two genes, prominin 1 (PROM1) and ATP binding cassette subfamily G Member 2 (*ABCG2*), because these cells are strongly expressed in neural stem cells and other highly plastic tissues (Akula et al., 2014). These

data lead the authors to the posit that the pathophysiology of BD included reduced neuroplasticity. The finding of lower levels of mRNA encoding the gene Fli-1 proto-oncogene, ETS transcription factor (*FL1*), which is involved in neuronal crest development suggest BD may have a neurodevelopment component. One study found changes in levels of gene expression that would impact on the activity of lysosomes, Fc gamma receptor-mediated phagocytosis and regulation of the actin cytoskeleton pathways in the anterior cingulate cortex (BA 24) from subjects with BD (Zhao et al., 2015). The authors stressed the importance of changes in lysosomal function as these could impact on endocytosis, exocytosis, neuronal maturation and migration, neurite outgrowth, phagocytosis, synaptic density and plasticity as well as vesicle trafficking. Another study found that changes in gene expression in the anterior cingulate from subjects with BD would have a much more focussed impact resulting involving changes in expression of G protein-coupled receptors and their regulatory pathways (Cruceanu et al., 2015). In a study using tissue from subjects with MD, BD and Sz it was suggested that there was evidence for lower expression of neuron-specific genes and higher expression of astrocytic genes in the anterior cingulate cortex from subjects with BD and schizophrenia (Ramaker et al., 2017). This study was somewhat unusual as it found no significant changes in gene expression in the dorsolateral prefrontal cortex from subjects with BD and schizophrenia and found no significant changes in gene expression in any CNS region from subjects with MD. By contrast, another study using RNAseq reported large numbers of changes in gene expression in 6 CNS regions from subjects with MD (Labonte et al., 2017). Significantly, this study found that overall, changes in gene expression in MD would impact on ERK signalling but the study also found that many changes in gene expression were gender specific, with more changes in gene expression occurring in females compared to males with only relative-small (45-163 genes) gender independent changes in gene expression in the CNS regions studied. It was argued that the gender-specific nature of gene expression in MD could be due to differential changes in dual specificity phosphatase 6 (*DUSP6*) in the ventromedial cortex (BA 25) from female subjects with MD as that gene acts as a key hub gene affecting what the authors identify as a female

specific gene expression module. By contrast, the authors emphasise the potential important of a higher level of expression of empty spiracles homeobox 1 (*EMX1*) in males with MD as this is a gene of importance in the development of the male cortex.

A recent transcriptome-wide isoform level study has reported RNAseq data for two cortical regions in subjects with BP, schizophrenia and autism spectrum disorder (Gandal et al., 2018b). This study has generated a rich dataset with the authors suggest has a scope and complexity that means the data does not lend itself to "simple mechanistic reduction". However, their early analyses of the data confirmed previous findings suggesting changes in cortical gene expression should down-regulate neuronal function and synaptic signalling as well as up-regulating neuroinflammatory pathways. Changes in levels of expression of genes involved in microglial function were also reported as enriched in BD as well as schizophrenia. Notably, the authors concluded that whilst they study was a unique-large scale study, other studies profiling gene expression in additional cases across a number of CNS regions would still be informative on the pathophysiology of psychiatric disorders.

To add data to the number of CNS studied in mood disorders we decided to measure levels of cortical gene expression in tissue from subjects with MD and BD. We have recently reported extensive changes in gene expression across 3 cortical regions (the rostral prefrontal cortex (Brodmann's area (BA) 10), dorsolateral prefrontal cortex (BA 9) and the anterior cingulate (BA 33) from individuals with schizophrenia (Scarr et al., 2018a). Given it has been suggested there are similarities in changes in gene expression in the cortex of subjects with schizophrenia compared to those with mood disorders we decided to complete our studies on mood disorders in the same three cortical regions. We hypothesised such a study would have added value in that all of these three cortical regions have been shown to be dysfunctional in subjects with MD and BD (Drevets et al., 2008).

METHODS

Human Tissue Collection and Processing

Human CNS tissue was collected postmortem at the Victorian Institute of Forensic Medicine after gaining approval from the Ethics Committee at that Institute. For each case, written approval to collect tissue was obtained from the next-of-kin. Case history reviews where completed using the Diagnostic Instrument for Brain Studies (DIBS) leading to a diagnosis, made by consensus between two clinicians, according to DSM IV criteria (Scarr et al., 2018b). Using data from the DIBS, postmortem interval (PMI) was calculated as the time from death to autopsy. Where death was not witnessed, tissue was only collected when the donor had been seen alive 5 hours prior to being found dead and PMI was taken as the time from being found to autopsy plus half the time between the donor been last seen and found dead.

On removal, CNS tissue was rapidly processed using a standardised procedure (Scarr et al., 2018b), tissue pH was measured as describe previously as this is the best indicator of good tissue preservation.

Following our study design, tissue from BA 9, 10 and 33 was collected using the parameters described in our study in schizophrenia (Scarr et al., 2018a) but from CNS from individuals with MD, BD and age and sex matched controls.

Cortical Gene Expression

Total RNA was prepared from ~100 mg of frozen grey matter from each of the 3 cortical regions to be studied and RNA prepared as describe previously (Scarr et al., 2018a) (Supplementary Methods 1).

After another on-site check of RNA quality, signal intensity at each probe was measured for all cases in each cortical region using the Affymetrix™ Human Exon 1.0 ST Arrays (Affymetrix, Santa Clara, CA, USA) at the Ramaciotti Centre for Genomics using the manufacturers recommended procedures; the resulting data being processed as described previously (Scarr et al., 2018a) (Supplementary Methods).

RESULTS

Demographics and sample collection data

For this study, tissue was collected from 3 cortical regions from subjects with MD (n = 15), BD (n = 15) and controls (n = 15). There were no significant differences in age, gender ratio, PMI or brain weight between the diagnostic groups (Table 1). There was a significant variation of CNS pH with diagnoses due to higher CNS pH (+ 4%) in MD and lower CNS pH in BD (-0.6%) compared to controls. The ratio of suicide completion to other forms of death was higher in MD and BD compared to controls. In addition, the ratio of suicide completion to other forms of death were higher in MD compared to BD. RINs did not vary with diagnoses in any of the three CNS regions studied.

Gene expression in Major Depression

Compared to controls, the level of RNA for 424 genes (Figure 1A, Supplementary Table 1) differed significantly (43 higher; Figure 1B) in BA 9 from individuals with MD compared to changed levels RNA for 52 genes in BA 10 (41 higher; Figure 1C) and 59 genes in BA 33 (7 higher; Figure 1D). Levels of RNA for 3 genes where different between MD and controls in BA 9 and 10 from individuals with MD (Higher: ankyrin repeat domain 18D, pseudogene (*ANKRD18DP*), syndecan 4 (*SDC4*); Lower: purinergic receptor P2Y12 (*P2RY12*)). Levels of RNA for 5 genes differed in BA 9 and 33 (Lower: family with sequence similarity 182 member A (*FAM182A*), galactose-3-O-sulfotransferase 4 (*GAL3ST4*), olfactory receptor family 1 subfamily F member 1 (*OR1F1*), prostate and testis expressed 2 (*PATE2*), solute carrier family 5 member 10 (*SLC5A10*)) and levels of RNA for 1 gene was different in BA 10 and 33 (Higher: haemoglobin

subunit beta (*HBB*)) between MD and controls. Levels of no RNA differed significantly in all the cortical regions studied.

IPA core analysis showed an over representation of genes with altered levels of expression in BA 9 from individuals with MD in canonical pathways involved in estrogen biosynthesis, ceramide biosynthesis, antioxidant actions of vitamin C and bupropion degradation, glutamate receptor signalling in BA 10 and glutathione redox reactions and communications between immune cells in BA 33 (Supplementary Table 2). Notably, in all three cortical regions from individuals with MD changes in levels of RNA would be expected to be associated with organismal injury and abnormalities. Only in BA 9 were changes in levels of RNA in MD associated with inflammatory disease whereas in BA 10 they are associated with neurological and psychological disorders (Supplementary Table 2).

Whilst there were no changes in the expression of the same gene across three cortical regions from individuals with MD, the differential changes in levels of RNA in each cortical region would impact on cell morphology (Supplementary Table 2). In addition, changes in gene expression would impact on molecular transport (BA 9 and BA 10) and cell signalling (BA 9 and BA 33) in two cortical regions. However, a number of the changes in gene expression would have within cortical-region effect on cellular development and cellular growth and proliferation in BA 9, cellular function and maintenance, cellular compromise and nucleic acid metabolism in BA 10 and carbohydrate metabolism, cell death and survival and cell-to-cell signaling and interaction in BA 33.

Notably, IPA Pathway Designer analysis showed no substantial interactions between genes with altered levels expression within any of the three cortical regions in MD.

There were lower levels of RNA from 4 MD risk genes (cleavage polyadenylation factor subunit CLP1 (*CLP1*), MHC class I polypeptide-related sequence B (*MICB*), psoriasis susceptibility 1 candidate 2 (*PSORS1C2*) and transmembrane protein 42 (*TMEM42*)) identified in a large gene wide association study (GWAS) in MD (Wray et al., 2018) in BA 9, but not 10 or 33. Further analyses of gene interactions in BA 9 using IPA Pathway Designer showed that, allowing one connecting gene (epidermal growth factor receptor (*EGFR*)), allowed *MICB* to be incorporated into an interactome of 54 genes with altered levels of expression in BA 9 from individuals with MD (Figure 2; Supplementary Table 3). Creating an *EGFR* containing interactome could be justified as it clearly past the fold criteria for significance (Ratio = 1.24) but fell marginally short of the probability criteria ($p = 0.03$).

The 54 genes present in the interactome of genes containing the risk gene *MICB* were subjected to a core analysis which showed the genes in the interactome would have a marked impact on inflammatory related pathways and that cytokines interleukin 4, interleukin 6 and colony stimulating factor 2 were key drivers in the pathway (Supplementary Table 4). At the molecular and cellular levels these genes would strongly impact on cell death and survival, cellular development, cellular growth and proliferation, cell morphology and cellular movement.

Gene expression in Bipolar Disorder

In BD, levels of RNA for 331 genes (Figure 1E, Supplementary Table 5: 35 higher; Figure 1F) differed significantly in BA 9 compared to 24 (16 higher; Figure 1G) genes in BA 10 and 38 (12 higher; Figure 1H) genes in BA 33; levels of RNA for no gene differed in more than one cortical region. By contrast to MD, there we no genes with changed levels of expression in the cortex of subjects with BD listed as being associated with an altered risk for the disorder in a large GWAS study (Stahl et al., 2019).

Notably, many of the canonical pathways with an over-representation of genes with a changed level of expression in BA 9 from individuals with BD were related to inflammation and immunity (Supplementary Table 6). In BA 10, neuroinflammation and circadian rhythm were canonical pathways that could be affected by gene changes in BD. In addition, changes in gene expression in BA 33 would affect ceramide biosynthesis and the effects of estrogen. In BD, changes in levels of RNA in BA 9 and BA 10 would be expected where there was organismal injury. In addition, changes in gene expression in BA 9 from individuals with BD would be expected in inflammatory disease whereas in BA 10 they would be expected in those with neurological or psychological disorders (Supplementary Table 6).

Despite the absence of changes in expression of any gene across the three cortical regions studied, changes in gene expression in BD would be expected to affect cell death and survival (BA 9 and 10) and cell morphology (BA 10 and 33) in two cortical regions (Supplementary Table 6). Other outcomes from changes in gene expression seem restricted to a single cortical region and would include cell signalling, molecular transport, vitamin and mineral metabolism as well as nucleic acid metabolism in BA 9, cellular function and maintenance, carbohydrate metabolism and cell cycle in BA 10 and protein synthesis, cell-to-cell signalling and interaction, cellular movement and cellular assembly and organisation in BA 33.

Finally, it is of interest that some of the genes with changed levels of expression in BA 9 and BA 10 from individuals with BD are involved in cholinergic activity as well as dopaminergic activity in BA 9 (Supplementary Table 6).

Gene expression in Major Depression and Bipolar Disorder

Levels of RNA for 112 genes (9 higher) differed in BA 9 from both MD and BD (Supplementary Table 7) and there was a high level of correlation between fold change in levels of expression of these genes between diagnoses ($r^2 = 0.91$, $p < 0.001$: Figure 3). By contrast, the level RNA for only 1 gene, BICD cargo adaptor 1 (*BICD1*), was lower in BA 10 and another gene ITPRIP like 1 (*ITPRIPL1*) was lower in BA 33 from individuals with MD and BD. IPA Core Analyses suggested the genes with altered levels of expression in BA 9 from individuals with MD and BD had an over-representation in canonical pathways involved in bupropion degradation and estrogen biosynthesis. Finally, at the level of diseases and disorders it was notable that the changes in gene expression in BA 9 that were common to MD and BD would be expected in an inflammatory response and neurological disease (Supplementary Table 8).

At the molecular level, the genes with changed level of expression in BD and MD would affect cell-to-cell signalling, lipid metabolism, small molecule biochemistry, cell morphology and cellular assembly and organisation (Supplementary Table 8).

DISCUSSION

This study has resulted in three major findings. First, changes in cortical gene expression in mood disorders differ between cortical regions, with by far the largest number of changes being in BA 9. This differs from our finding in the same three cortical regions from individuals with schizophrenia where by far and away the greatest number of changes in gene expression were in BA 10 (Scarr et al., 2018a). Second, the levels of expression of 4 risk genes (*CLP1*, *MICB*, *PSORS1C2* and *TMEM42*) for MD (Wray et al., 2018) are lower in BA 9, but not 10 or 33, from individuals with that disorder. Third, there were highly correlated changes in levels of expression of 112 genes in BA 9, but not BA 10 or 33, from individuals with MD and BD.

Showing regionally specific changes in cortical gene expression agrees with previous studies (Evans and Heather, 2016; Choudary et al., 2005; Sequeira et al., 2007; Klempan et al., 2009; Sequeira et al., 2009; Labonte et al., 2017; Ramaker et al., 2017) and BD (Evans et al., 2004; Ramaker et al., 2017). Given these data, it can be concluded that unique changes in gene expression within many cortical regions are probably contributing to the region-specific changes in cortical dysfunction known to occur in MD and BD (Drevets et al., 2008). Of interest in our study were differences in the prevalence in gene expression between BA 9 and 10, adjacent cortical regions, from subjects with MD and BD with many more changes in gene expression identified in BA 9. These data differ markedly from our data in schizophrenia where we showed by far and away most prevalent changes in gene expression were in BA 10. Such regional differences are important because BA 10 is now recognised to control diverse functions that include risk and decision making, odor evaluation, reward and conflict, pain, and working memory (Fuster, 2015). Our data now suggests such functions could be more affected by changes in gene expression in schizophrenia compared

to individuals with MD or BD. By contrast, BA 9 is important in memory, evaluating recency, overriding automatic responses, verbal fluency, error detection, auditory verbal attention, inferring the intention of others, inferring deduction from spatial imagery, inductive reasoning, attributing intention and sustained attention (Fuster, 2015) and therefore abnormalities in these cortical functions may be more affected by changes in gene expression in MD and BD. In addition, the high prevalence of regionally-specific changes in gene expression in different psychiatric disorders means the outcomes from pooling gene expression data from multiple cortical regions for re-analysis (Gandal et al., 2018a) should be treated with some caution.

In our study, at the level of canonical pathways, changes in gene expression are predicted to affect the biosynthesis of estrogen and ceramide biosynthesis, the antioxidant actions of vitamin C and the degradation of bupropion. These findings suggest that gene expression changes in BA 9 may be important in linking the impact of oestrogen on cognitive and emotional disfunction in MD (Albert and Newhouse, 2019). Ceremide is an important component of sphingolipids and therefore gene expression changes affecting ceremide biosynthesis could be contributing to the changes in lipid biosynthesis in MD (Walther et al., 2018). In addition, changes in gene expression affecting the actions of vitamin C and the degradation of bupropion could be important in regulating how individuals with MD respond to treatment with those two compounds. By contrast, in BA 10 changes in gene expression would be expected to affect glutamate receptor signalling which is thought to be important in the aetiology of the disorder (Niciu et al., 2014). In BA 33 the impact of gene expression on glutathione redox reactions suggests oxidative stress could be a component of the aetiology of MD and why treatment with antioxidants can lesson symptom severity (Berk et al., 2014). Given existing data implicating inflammatory pathways in MD (Dean, 2011) it was surprising that inflammation-related pathways were not suggested to be affected by changes in gene expression in any of the three cortical regions studied. In a Core Analyses, there needs to be a change in the level of expression of more genes in a pathway than expected by chance. Thus, in our overall data from BA 9 from

subjects with MD there were changes in expression of inflammatory-related genes such as interleukins 4, 16 and 17F and TNRSF 4, 13C and 14 (Supplementary 1) must have not been numerous enough to clear such a hurdle. However, as can been seen below, more focussed analyses of changes in gene expression in BA 9 from subjects with MD suggests changes in inflammatory-related pathways cannot be excluded from contributing to the pathophysiology of the disorder.

In BD changes in gene expression in BA 9 and BA 10 would affect inflammation-related pathways that have been strongly implicated in the aetiology of that disorder (Stertz et al., 2013). Interestingly, changes in gene expression in BA 10 are predicted to affect circadian rhythm which is known to be affected in individuals with BD (Allison G. Harvey 2008). Finally, changes in gene expression in BA 33 in BD, rather than BA 9 as in MD, would affect ceramide biosynthesis and the effects of estrogen with both these being implicated in the aetiology of BD (Schwarz et al., 2008; Meinhard et al., 2014). Thus, at least at the level of canonical pathways our data is giving a new insight into the changes in gene expression that could impact on the genesis of specific symptoms and drug responsiveness in individuals with mood disorders.

It is particularly significant that our data shows lower levels of expression for 4 risk genes for MD in BA 9 from individuals with the disorder. One of the risk genes with lower level of expression in MD is *CLP1* which encodes a multifunctional kinase which is a component of the tRNA splicing endonuclease complex and a component of the pre-mRNA cleavage complex II and is therefore implicated in tRNA, mRNA and siRNA maturation (Hanada et al., 2013). *MICB* activates cells which express the NKG2D type II receptor which we have now shown can be incorporated into an interactome of genes with altered levels of expression in BA 9 from individuals with MD which will impact on cortical inflammatory pathways which have been suggested to be important in the aetiology of MD (Dean, 2011). Although little seems to be known about the function of *PSORS1C2* its was reported as increased in mice after Rb family inactivation

which forces neurons to undergo S-phase leading to cell death (Oshikawa et al., 2013) and hence *PSORA1C2* could be important in the development of the human CNS. Finally, *TMEM42* is a transmembrane protein of no known function that is ubiquitously expressed but is more highly expressed in the cortex during foetal development (Guedj et al., 2015). Thus, our data showing decreased expression of 4 risk gene for MD in the BA 9 from individuals with the disorder is of interest as all of these genes appear to have potential to affect the development of the cortex. Our cohort size was too small to determine if the decrease in expression of the 4 risk genes for MD were associated with the risk variant of those genes but given the low frequency of the risk variants our data could argue that there is changed cortical expression of risk genes for MD and that these changes could be further modify by the inheritance of a risk variant.

We also show a strong correlation between levels of expression of genes between individuals with MD and BD. This raises the possibility that the same genes are contributing to a cortical pathology or some intrinsic functional connectivity patterns that have been reported in both disorders. At the level of canonical pathways, the genes with correlated levels of expression across mood disorders would have effects on immunity and inflammation which have been associated with the aetiologies of such disorders (Dean, 2011). Whatever the outcome, our data is the first to suggest that a significant number of the same genes could be contributing to the aetiologies of both MD and BD but this commonality of gene action may be limited to specific cortical regions such as BA 9.

Focussing on changes in gene expression in the cortex of individuals with MD at the level of molecular and cellular functions, our data shows some agreement with other studies predicting changes in cell signalling (Sequeira et al., 2009; Kang et al., 2007), molecular transport (Sequeira et al., 2009; Klempan et al., 2009; Zhurov et al., 2012), cell-to-cell signalling (Sequeira et al., 2009; Aston et al., 2005; Kang et al., 2007;

Zhurov et al., 2012), cellular development (Klempan et al., 2009; Aston et al., 2005; Kang et al., 2007), carbohydrate metabolism (Klempan et al., 2009). In BD, our data agrees with other studies that suggests pathways involved in cell death (Shao and Vawter, 2008), cellular growth and proliferation (Shao and Vawter, 2008) would be affected by changed levels of gene expression.

There are some limitations to this study. As with any study using tissue from individuals with MD and BD who have been treated, there is a possibility the changes in gene expression reported are associated with drug treatment before death. However, studies examining gene CNS expression changes in human cells and rodents after treatment with antidepressant drugs (Lin and Tsai, 2016) or mood stabilisers (Squassina et al., 2010) have not shown that such drug treatments alter levels of expression of the genes we have shown to be differentially expressed in BA 9 from MD or BD suggesting our data is not simply due to drug affects. Cohort sizes in this study are equivalent to others examining cortical gene expression in mood disorders but are relatively small. There was a 3.35% study wide difference in CNS pH with diagnoses which is unlikely to be a significant confound. Suicide rates varied across diagnostic cohorts which is a confound that could not be resolved because of our cohort sizes. Hence, our findings should be considered as preliminary but suggesting that understanding the impact of changes in cortical gene expression in mood disorders will advance understanding of their aetiologies.

Despite the limitations of our study, like others, we show differential changes in gene expression are present in different cortical regions from individuals with MD (Evans et al., 2004; Choudary et al., 2005; Sequeira et al., 2007; Klempan et al., 2009; Sequeira et al., 2009) and BD (Evans et al., 2004; Choudary et al., 2005). We also report that the highest number of genes with altered levels of expression in both MD and BD is in BA 9 and that there are highly correlated changes in the expression of 112 genes in BA 9 from individuals with both disorders. In addition, for the first time, we have linked a risk gene for MD to an interactome of

genes with altered levels of expression in BA 9 from individuals with MD. This interactome suggests this gene, *MICB*, can have profound effects on inflammation-related pathways in the dorsolateral prefrontal cortex. This finding is also significant because a large scale transcriptomic study using postmortem CNS from subjects with psychiatric disorders argued the data from that study did not lend itself to mechanistic reduction (Gandal et al., 2018b). By contrast, our study has provided a pathway of immediate interested that can be the starting point for establishing some of the biological underpinnings of MD, confirming the proposition ongoing studies of gene expression in the CNS from subjects with psychiatric disorders are of value (Gandal et al., 2018b). In this pathway in particular, what remains to be established is whether the impact of inheritance of a risk genotype of *MICB* acts to accentuate the impact of an already changed level of gene expression.

References

Akula N, Barb J, Jiang X, et al. (2014) RNA-sequencing of the brain transcriptome implicates dysregulation of neuroplasticity, circadian rhythms and GTPase binding in bipolar disorder. *Mol Psychiatry* 19: 1179-1185.

Albert KM and Newhouse PA. (2019) Estrogen, Stress, and Depression: Cognitive and Biological Interactions. *Annual Review of Clinical Psychology* 15: null.

Allison G. Harvey PD. (2008) Sleep and Circadian Rhythms in Bipolar Disorder: Seeking Synchrony, Harmony, and Regulation. *American Journal of Psychiatry* 165: 820-829.

Aston C, Jiang L and Sokolov BP. (2004) Microarray analysis of postmortem temporal cortex from patients with schizophrenia. *Journal of Neuroscience Research* 77: 858-866.

Aston C, Jiang L and Sokolov BP. (2005) Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol Psychiatry* 10: 309-322.

Berk M, Dean OM, Cotton SM, et al. (2014) The efficacy of adjunctive N-acetylcysteine in major depressive disorder: a double-blind, randomized, placebo-controlled trial. *Journal of Clinical Psychiatry* 75: 628-636.

Bezchlibnyk YB, Wang JF, McQueen GM, et al. (2001) Gene expression differences in bipolar disorder revealed by cDNA array analysis of post-mortem frontal cortex. *Journal of Neurochemistry* 79: 826-834.

Choudary PV, Molnar M, Evans SJ, et al. (2005) Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proceedings of the National Academy of Sciences of the United States of America* 102: 15653-15658.

Craddock N. (2006) Genetics of mood disorders. *Psychiatry* 5: 170-174.

Cruceanu C, Tan PP, Rogic S, et al. (2015) Transcriptome sequencing of the anterior cingulate in bipolar disorder: dysregulation of G protein-coupled receptors. *Am J Psychiatry* 172: 1131-1140.

Cuellar AK, Johnson SL and Winters R. (2005) Distinctions between bipolar and unipolar depression. *Clinical Psychology Review* 25: 307-339.

Dean B. (2011) Understanding the role of inflammatory-related pathways in the pathophysiology and treatment of psychiatric disorders: evidence from human peripheral studies and CNS studies. *International Journal of Neuropsychopharmacology* 14: 997-1012.

Drevets WC, Price JL and Furey ML. (2008) Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Structure & Function* 213: 93-118.

Evans RD and Heather LC. (2016) Metabolic pathways and abnormalities. *Surgery (Oxford)*.

Evans SJ, Choudary PV, Neal CR, et al. (2004) Dysregulation of the fibroblast growth factor system in major depression. *Proceedings of the National Academy of Sciences of the United States of America* 101: 15506-15511.

Fuster JM. (2015) *The Prefrontal Cortex*, New York: Academic Press.

Gandal MJ, Haney JR, Parikshak NN, et al. (2018a) Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. *Science* 359: 693-697.

Gandal MJ, Zhang P, Hadjimichael E, et al. (2018b) Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science* 362.

Guedj F, Pennings JLA, Ferres MA, et al. (2015) The fetal brain transcriptome and neonatal behavioral phenotype in the Ts1Cje mouse model of Down syndrome. *American Journal of Medical Genetics. Part A* 167A: 1993-2008.

Hanada T, Weitzer S, Mair B, et al. (2013) CLP1 links tRNA metabolism to progressive motor-neuron loss. *Nature* 495: 474.

Iwamoto K, Kakiuchi C, Bundo M, et al. (2004) Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Molecular Psychiatry* 9: 406-416.

Kang HJ, Adams DH, Simen A, et al. (2007) Gene expression profiling in postmortem prefrontal cortex of major depressive disorder. *Journal of Neuroscience* 27: 13329-13340.

Klempan TA, Sequeira A, Canetti L, et al. (2009) Altered expression of genes involved in ATP biosynthesis and GABAergic neurotransmission in the ventral prefrontal cortex of suicides with and without major depression. *Mol Psychiatry* 14: 175-189.

Labonte B, Engmann O, Purushothaman I, et al. (2017) Sex-specific transcriptional signatures in human depression. *Nature Medicine* 23: 1102-1111.

Lin E and Tsai S-J. (2016) Genome-wide microarray analysis of gene expression profiling in major depression and antidepressant therapy. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 64: 334-340.

Meinhard N, Kessing LV and Vinberg M. (2014) The role of estrogen in bipolar disorder, a review. *Nordic Journal of Psychiatry* 68: 81-87.

Nakatani N, Hattori E, Ohnishi T, et al. (2006) Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: relevance to neuronal network perturbation. *Hum.Mol.Genet.* 15: 1949-1962.

Niciu MJ, Ionescu DF, Richards EM, et al. (2014) Glutamate and its receptors in the pathophysiology and treatment of major depressive disorder. *Journal of neural transmission (Vienna, Austria : 1996)* 121: 907-924.

Oshikawa M, Okada K, Nakajima K, et al. (2013) Cortical excitatory neurons become protected from cell division during neurogenesis in an Rb family-dependent manner. *Development* 140: 2310-2320.

Ramaker RC, Bowling KM, Lasseigne BN, et al. (2017) Post-mortem molecular profiling of three psychiatric disorders. *Genome Medicine* 9: 72.

Scarr E, Udawela M and Dean B. (2018a) Changed frontal pole gene expression suggest altered interplay between neurotransmitter, developmental, and inflammatory pathways in schizophrenia. *NPJ Schizophrenia* 4: 4.

Scarr E, Udawela M, Thomas EA, et al. (2018b) Changed gene expression in subjects with schizophrenia and low cortical muscarinic M1 receptors predicts disrupted upstream pathways interacting with that receptor. *Molecular Psychiatry* 23: 295-303.

Schwarz E, Prabakaran S, Whitfield P, et al. (2008) High Throughput Lipidomic Profiling of Schizophrenia and Bipolar Disorder Brain Tissue Reveals Alterations of Free Fatty Acids, Phosphatidylcholines, and Ceramides. *Journal of Proteome Research* 7: 4266-4277.

Sequeira A, Klempan T, Canetti L, et al. (2007) Patterns of gene expression in the limbic system of suicides with and without major depression. *Mol Psychiatry* 12: 640-655.

Sequeira A, Mamdani F, Ernst C, et al. (2009) Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. *PloS One* 4: e6585.

Shao L and Vawter MP. (2008) Shared gene expression alterations in schizophrenia and bipolar disorder. *Biological Psychiatry* 64: 89-97.

Squassina A, Manchia M and Del Zompo M. (2010) Pharmacogenomics of Mood Stabilizers in the Treatment of Bipolar Disorder. *Human Genomics and Proteomics : HGP* 2010: 159761.

Stahl EA, Breen G, Forstner AJ, et al. (2019) Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nature Genetics* 51: 793-803.

Stertz L, Magalhães PVS and Kapczinski F. (2013) Is bipolar disorder an inflammatory condition? The relevance of microglial activation. *Current opinion in psychiatry* 26: 19-26.

Sun X, Wang JF, Tseng M, et al. (2006) Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *Journal of Psychiatry and Neuroscience* 31: 189-196.

Tkachev D, Mimmack ML, Ryan MM, et al. (2003) Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 362: 798-805.

Tochigi M, Iwamoto K, Bundo M, et al. (2008) Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. *Neuroscience Research* 60: 184-191.

Walther A, Cannistraci CV, Simons K, et al. (2018) Lipidomics in Major Depressive Disorder. *Frontiers in Psychiatry* 9: 459-459.

Wray NR, Ripke S, Mattheisen M, et al. (2018) Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature Genetics* 50: 668-681.

Zhao Z, Xu J, Chen J, et al. (2015) Transcriptome sequencing and genome-wide association analyses reveal lysosomal function and actin cytoskeleton remodeling in schizophrenia and bipolar disorder. *Mol Psychiatry* 20: 563-572.

Zhurov V, Stead JD, Merali Z, et al. (2012) Molecular pathway reconstruction and analysis of disturbed gene expression in depressed individuals who died by suicide. *PloS One* 7: e47581.

FIGURE LEGENDS

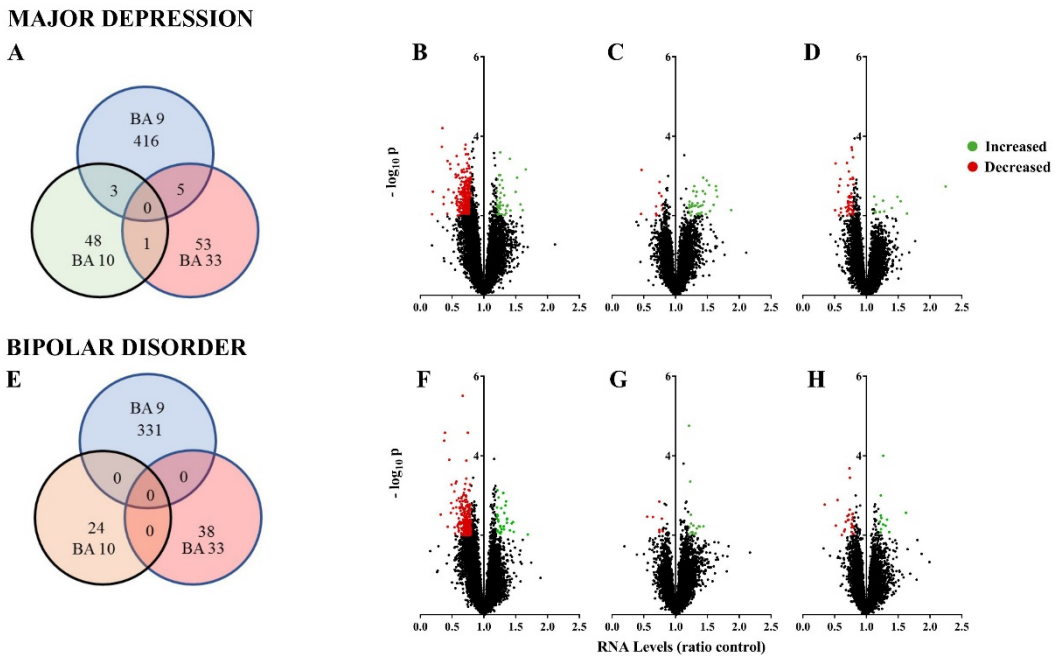


Figure 1: Cortical gene expression in mood disorders:

A: Venn diagram showing the number of genes differentially expressed in Brodmann's areas (BA) 9, 10 and 33 from individuals with major depressive (MD).

B-D: Volcano plots of changes in gene expression in BA 9 (B), BA 10 (C) and BA 3 (D) from individuals with MD.

E: Venn diagram showing the number of genes differentially expressed in BA 9, 10 and 33 from individuals with bipolar disorders (BD).

F-H: Volcano plots of changes in gene expression in BA 9 (F), BA 10 (G) and BA 3 (H) from individuals with BD.

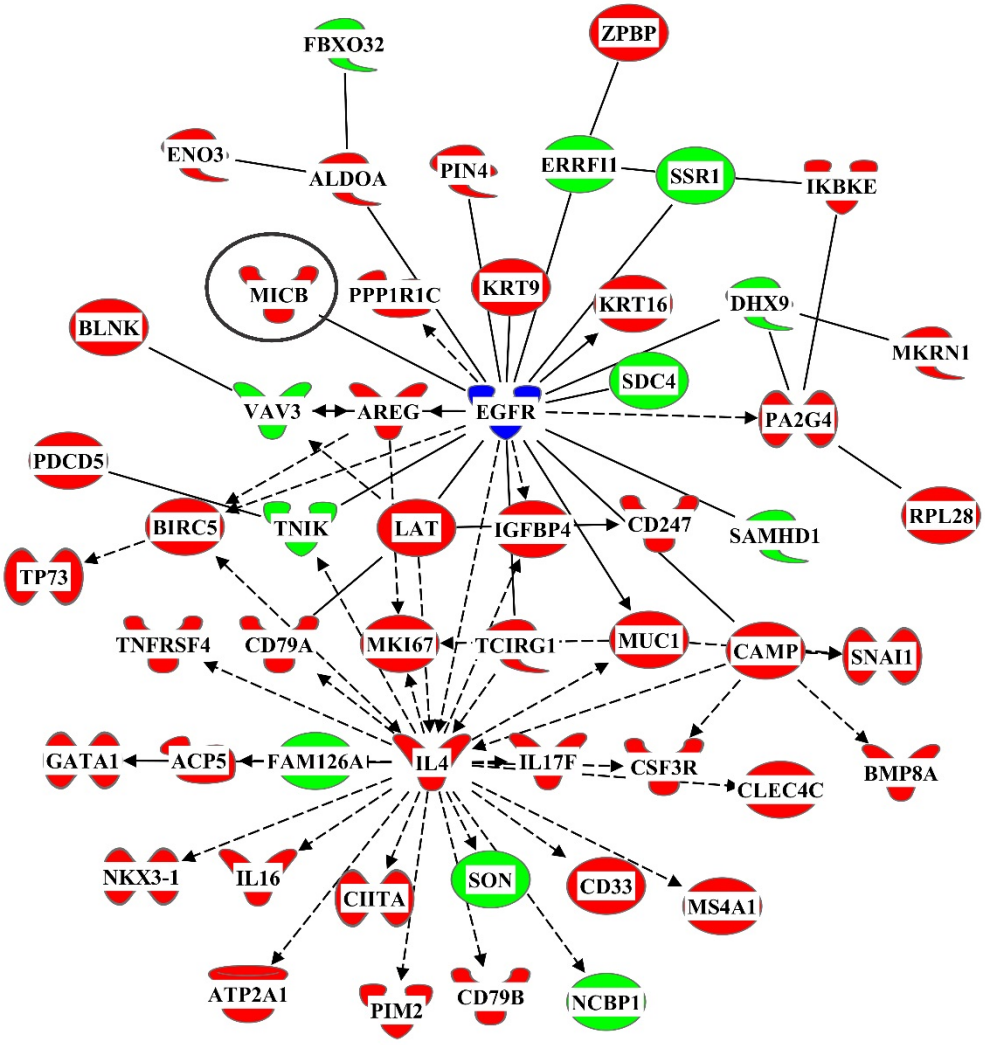


Figure 2: An interactome of genes that were differentially expressed in Brodmann's area 9 from individuals with major depression that includes the risk gene MHC class 1 polypeptide-related sequence B (*MICB*) and the connecting gene epidermal growth factor receptor (*EGFR*).

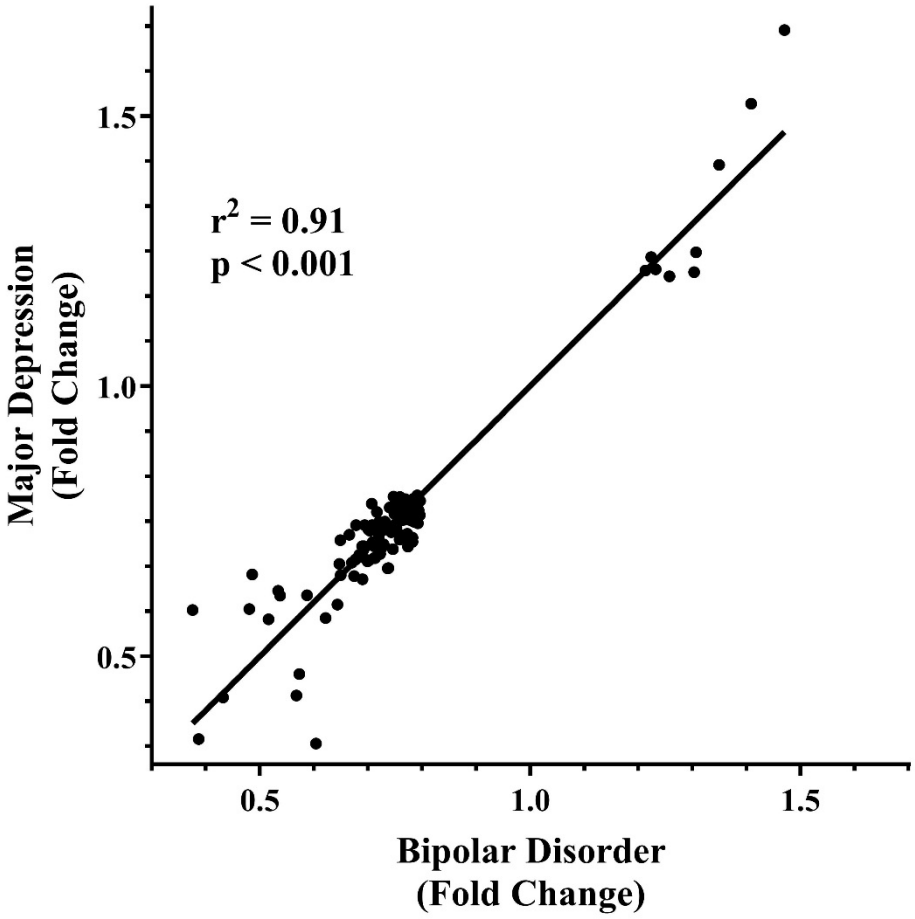


Figure 3: The correlation between changes in gene expression of 112 genes in Brodmann's area 9 from individuals with major depression and bipolar disorders.

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