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Broadening our horizons: gene expression profiling to help better understand the neurobiology of suicide and depression

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Abstract

The complexity of the neurobiological alterations underlying major depression and suicide is significant. Gene expression studies using high-throughput genomic technologies have provided important insight into novel genes and pathways displaying alterations associated with major depression and suicide, thereby providing a global view of the pathological changes taking place, as well as indicating potential new targets for therapeutic interventions. This review discusses the methodologies which have been used to profile gene expression patterns in depression and suicide, as well as examines several of the metabolic pathways which have been frequently implicated by studies using this approach. Future directions to be taken towards increasing our understanding of the origin and downstream effects of altered gene expression are also discussed.

Keywords: suicide, major depression, gene expression, microarray

Introduction

Major depressive disorder is a common and debilitating illness which accounts for significant decrements in health and disability [Moussavi et al., 2007]. Suicidal behaviors represent the most devastating consequences of this disorder, and suicide accounts for one million deaths worldwide each year [Nock et al., 2008, Kim et al., 2003]. Numerous family, twin, and adoption studies have suggested that heritable genetic factors increase predisposition for major depression and independently contribute to suicide risk [Arsenault-Lapierre et al., 2004, Brent et al., 1996, McGirr et al., 2009, Statham et al., 1998]. Although a history of psychiatric disorders is found in the majority of suicide completers, it has become clear that this relationship represents the involvement of both shared and distinct neurobiological risk factors, and that suicidal behaviors are distinct psychiatric phenotypes [Arsenault-Lapierre et al., 2004, McGirr et al., 2007, McGirr and Turecki, 2008]. Based on prevailing neurochemical hypotheses of major depression and suicide and mechanisms of action of available therapeutic agents, initial molecular genetic studies investigating these conditions focused mainly on candidate genes related to monoaminergic neurotransmission [Brezo et al., 2008]. However, although efforts to identify and characterize the molecular substrates of suicide and depression have been ongoing for decades, we have yet to obtain a comprehensive understanding of the biological processes underlying mood psychopathology.

In order to expand its focus, research has moved towards high-throughput technologies as a means to identify novel neurobiological mechanisms involved in psychopathology. Gene expression microarray studies, which compare samples obtained from cases to those of controls, provide the ability to obtain a functional profile of alterations in gene expression that occur in

relation to specific pathological conditions. By examining the expression of a vast number of genes in parallel, high-throughput technologies thus allow valuable insight into the overall biological processes underlying disease, and as such, this technology has been used by many groups over the last decade to identify gene expression profiles characteristic of suicide or depression. These studies are described in Tables 1 and 2. In addition to those reviewed here, many additional studies have included subjects who died by suicide, but without examining gene expression alterations specific to suicide outcome (reviewed in [Sequeira and Turecki, 2006, Iwamoto and Kato, 2006, Altar et al., 2009]). This review will first discuss the methodologies which have been used to profile gene expression alterations in depression and suicide, then will examine several of the neurobiological pathways which have been consistently implicated in their pathology. Following this, future directions to be taken towards increasing our understanding of the origin and downstream effects of altered gene expression will be discussed.

Methodology

This section will discuss methodological issues regarding the various microarray technologies which have been used, and experimental methods which have been employed to validate these findings. Subsequently, issues related to sample characteristics and tissue sources will be discussed.

Technology:

The most commonly used platform in gene expression studies of depression and suicide has been the Affymetrix one-colour arrays. These arrays are generated by photolithographically printing hundreds of thousands of 25 base pair oligonucleotides (probes). Fragmented biotinylated cRNA, produced from mRNA extracted from each sample following conversion to cDNA, is hybridized to each chip and the intensity of fluorescence at each probe is measured following immunochemical treatment of the chip. The Illumina BeadChip array uses similar methods, with the main exception being the use of larger hybridization probes. Historically, probes have been designed to target the 3' end of mRNA molecules in order to reduce the impact of quality and processing biases. Several generations of 3' in vitro transcription (IVT) microarray chips have now been produced, with the most important differences being the number of targeted sequences that are included. Several post-processing steps are used to examine intensity data, including normalization of intensity values across chips, and identification of factors affecting the quality of the data [Miron and Nadon, 2006]. The debate regarding the most appropriate algorithm is still ongoing [Steinhoff and Vingron, 2006], and a discussion of the strengths and weaknesses of these algorithms is beyond the scope of this review. In general, the most commonly used analysis methods have been Affymetrix's Microarray Analysis Suite (MAS), and Robust Multiarray Average (RMA) [Irizarry et al., 2003]. The two-colour cDNA microarrays produced by Agilent have also been used by several groups. This technology involves labelling cDNA with one of two fluorescent dyes, then hybridizing two differently-labelled samples to each array. As with one-colour arrays, factors relating to the selection of reference samples and analytical methods to examine the data are much debated [Steinhoff and Vingron, 2006]. Similar to the Affymetrix and Illumina arrays, targeted sequences are generally located at the 3' end of the mRNA molecule. Newer arrays have been designed to target larger

proportions of the transcriptome as well as allow more specific aspects of gene expression, such as differential splicing of exons, to be examined.

Statistics:

The capacity of microarrays to profile the expression of thousands of genes has allowed research to move away from candidate gene analyses towards less hypothesis-drive approaches. Yet at the same time, the vast quantity of data generated by these technologies has required the development of statistical methods to deal with the issues arising from multiple testing. While it is generally accepted that the historical methods to correct for multiple testing, such as the Bonferroni correction, are excessively stringent, failing to sufficiently correct will inevitably produce a large number of false positive results. Given the importance of this issue in microarray analysis, it is not surprising that this continues to be a hotly-debated topic, and statistical methods vary considerably between studies. In order to minimize both false positive and false negative findings, researchers have attempted to use algorithms or selection criteria which yield results lying somewhere in the middle of these two extremes [Gadbury et al., 2009]. The most typical methods involve pre-specified fold change (FC) and P-value cutoffs, or false discovery rate (FDR) corrections. While microarray studies have generally been designed to allow for simple group-wise comparisons, newer statistical analyses are being developed and employed in order to identify and control for confounding factors and other relevant clinical variables.

Technical Validation:

Following statistical analysis of microarray data to identify genes which appear to be differentially expressed in the disease group, technical validation of significant findings is generally required. However, the choice of which genes to validate, and the method by which to do this vary from study to study and may be based upon the statistical significance of the findings, the potential relevance of particular genes to psychiatric conditions, or their involvement in pathways of specific interest to the researcher. Quantitative real time polymerase chain reaction (RT-PCR) is considered to be the "gold-standard" method of validation for gene expression studies, although less precise methods such as semi-quantitative RT-PCR and immunohistochemistry have also been employed. While it was initially considered essential that alterations in mRNA expression be validated at the protein level, it has become increasingly recognized that a lack of correlation between these two does not necessarily signify a failure to validate, as mRNA and protein levels represent distinct aspects of cellular and intracellular functioning. Indeed, this is not unexpected given our expanding understanding of the extent to which genes are subjected to processes such as alternate splicing, nonsense mediated mRNA-decay, and microRNA-induced silencing.

Scientific Validation:

While technical validation of microarray results is an important step in high-throughput studies, confirming that the technology has properly measured mRNA levels does not exclude the possibility that the differences in expression represent phenomena that are not associated to the phenotype being investigated. However, greater confidence in the scientific relevance of the findings can be obtained by examining through more detailed studies of the implicated genes or

pathways and by obtaining evidence of their external validity, for instance by replication in independent samples. While collaborations between groups have allowed the differential expression of particular genes to be replicated in independent samples, this is not always feasible. Publicly available resources, such as the Stanley Neuropathology Consortium Integrative Database (SNCID), which holds a large body of postmortem brain gene expression data and other information obtained from a psychiatric sample and population controls, can allow researchers to readily determine how well their results can be extended to other populations [Kim and Webster, 2010b]. Moreover, the extensive information available for these subjects has allowed researchers to combine expression data with other neurobiological measures [Kim and Webster, 2010b, Kim and Webster, 2010a], thus allowing even greater knowledge to be obtained. Several studies have also included animal models to further assess the role of genes identified in human studies [Kang et al., 2007, Sibille et al., 2009]. Hypothesis-driven pre-selection of genes or probesets prior to statistical analysis, based upon knowledge regarding a particular biological pathway [Lalovic et al., 2009, Morita et al., 2005] or chromosomal region [Fiori et al., 2009], has also been used as a means to both limit the penalties for multiple testing, as well as increase the likelihood that positive results represent biologically relevant findings. Finally, to extend findings beyond the level of mRNA expression, the involvement of implicated genes have also been examined through genetic association studies in larger samples [Yanagi et al., 2005, Sequeira et al., 2006].

Samples:

The ability to detect significantly differentially expressed genes, as well as the capacity to meaningfully interpret these results in reference to clinical phenotypes such as suicide and depression, is highly dependent upon both the number of samples within each group, and the methods by which diagnostic groups are defined.

Given the high cost of microarray studies and issues inherent in recruiting sufficient numbers of suitable samples, group sizes in these studies have generally been small, ranging from six [Yanagi et al., 2005] to ninety [Kim et al., 2007] subjects overall, with generally between ten and fifteen subjects within each experimental group. Moreover, differentiating the effects of suicide from those associated with psychiatric disorders has posed a significant problem, as a significant number of subjects recruited to studies examining gene expression patterns associated with specific Axis I disorders died by suicide, and conversely, the majority of suicide completers are diagnosed with an Axis I disorder.

Studies assessing gene expression profiles of suicide completers have included subjects with three major underlying Axis I diagnoses: major depression, bipolar disorder, and schizophrenia. A variety of methods have been used to attempt to identify suicide-specific gene expression changes, including 1) grouping together all suicides and comparing to a healthy control group [Sibille et al., 2004, Yanagi et al., 2005, Gwadry et al., 2005, Thalmeier et al., 2008, Ernst et al., 2008], 2) grouping suicide completers with each Axis I disorder separately then comparing each group to the healthy control group [Choudary et al., 2005, Sequeira et al., 2006, Sequeira et al., 2007, Tochigi et al., 2008, Klempan et al., 2009c], and 3) comparing individuals who died by suicide with those who died of other causes within groups of subjects with specific Axis I disorders [Kim et al., 2007]. Equally, studies designed to characterize gene expression profiles specific to depression face similar issues in that results may be confounded

by suicide. While some studies have attempted to assess or control for the effects of suicide within their depressed population [Aston et al., 2005, Kim and Webster, 2010b], the majority of studies have ignored the issue altogether. Furthermore, depression itself is a highly heterogeneous illness, comprised of individuals who differ greatly in terms of clinical presentation and treatment-responsiveness, as well as numerous other epidemiological, psychosocial, genetic, and neurobiological factors. The same is true of suicide completers who, in addition to differences in Axis I diagnoses, can differ considerably with respect to other sociodemographic and neurochemical variables, as well as the actual presentation of suicidal behaviors. Accordingly, variations in the distribution of different suicide or depressive subphenotypes between populations can impact microarray findings, and may partially account for difficulties in replicating results between studies. Given the limited availability of suitable samples, researchers must balance between excluding subjects to obtain a more homogenous population, and having sample sizes sufficiently large to have the necessary power to detect small magnitude effects.

Problems associated with other confounding factors such as alcohol, medication, and other drugs have been less well addressed. A few studies [Sequeira et al., 2007, Klempan et al., 2009c, Sequeira et al., 2009b, Kang et al., 2007, Kim et al., 2007] included drug or alcohol use as covariate, either in their initial models or as a secondary analysis exploring significantly differentially expressed probesets. One study examined the potential effects of antidepressants by performing a secondary analysis to examine how antidepressant use was related to their differentially expressed genes in medicated depressed individuals as well as controls and depressed subjects not taking antidepressants at the time of death [Evans et al., 2004]. Several studies assessed if the differentially expressed genes found in the entire sample were still

significant when subjects were grouped according to drug or alcohol use [Aston et al., 2005, Iwamoto et al., 2004], while one group also performed a gene expression study in a separate sample of alcohol abusers [Sequeira et al., 2009b]. Similarly, the effects of pharmacological treatments in animals have been assessed to determine the influence of drugs on human gene expression [Kang et al., 2007, Sequeira et al., 2009a, Ernst et al., 2009a]. Finally, one study combined information obtained from depressed humans with that from a microarray study performed in an antidepressant-treated mouse model of depression in order to identify genes displaying similar alterations in expression between the two datasets [Sibille et al., 2009].

Although gender is an important factor to consider in gene expression studies, and particularly in studies of psychiatric disorders, given the important gender effects on prevalence and manifestation of psychiatric disorders, including suicidal behavior and major depression [Hawton, 2000, Seedat et al., 2009, Ernst et al., 2009b, McGirr et al., 2006], very few studies have looked at gender differences in brain gene expression associated with mood psychopathology. In part, the lack of such studies results from logistical difficulties in recruiting female brain samples, as a consequence of the gender differences in suicide rates. As a result, researchers have been forced to use either exclusively male samples to avoid gender-specific effects, or to use mixed samples in which the numbers of female subjects are generally insufficient to fully examine the influence of gender. Although studies examining depression without considering suicide have been able to include more equivalent numbers of males and females, the effects of gender on gene expression have typically been ignored.

Tissue:

To date, all microarray studies examining suicide, and the majority of studies investigating depression, have used postmortem brain tissues. Due to their known involvement in other psychiatric conditions, studies have largely focused on tissues obtained from the prefrontal cortex (Brodmann areas (BA) 8, 9, 10, 11, 44, 45, 46, and 47) or the limbic area (amygdala, hippocampus, BA 24, and BA 29), although additional regions, including the temporal cortex [Aston et al., 2005], the motor cortex [Sequeira et al., 2006], the locus coeruleus [Bernard et al., 2010], and the thalamus [Chu et al., 2009], have also been examined. More recently, some studies have expanded their focus even further to obtain a more global view of altered gene expression across the brain [Ernst et al., 2009a, Sequeira et al., 2009b].

Two recent microarray studies were performed in peripheral blood samples to examine gene expression signatures associated with depression [Spijker et al., 2010, Segman et al., 2010]. There is growing support for the use of blood samples to identify peripheral biomarkers for gene expression in the brain [Glatt et al., 2005], and these samples provide an excellent resource to examine changes in gene expression over time, and allows a greater capacity to properly control for confounding variables by allowing the pre-selection of groups to address specific research questions.

In addition to those discussed above, other important variables that act as potential confounders include postmortem interval (PMI), pH, and RNA quality. These factors should be considered during the selection of subjects and tissue samples, as well as during subsequent statistical analyses. While important, PMI is imprecise and varies according to the unique environmental conditions in which the body of the brain donor was following death. In fact, PMI provides primarily an indirect measure of RNA quality and pH, which are both important variables, differentially affecting the expression of various gene transcripts. Tissue pH can be

influenced by a number of factors including antemortem agonal state, cause of death, antemortem medical interventions and metabolic and respiratory functions [Hynd et al., 2003]. pH affects not only RNA quality, but can have specific effects on the expression of many genes, including those implicated in psychiatric disorders [Vawter et al., 2006]. Finally, RNA quality likely represents the most important confounding variable as it can be influenced by postmortem and laboratory-specific factors, such as sample storage and RNA extraction methods, and most importantly, the RNA degradation state can directly impact the results. RNA quality is typically assessed by two related measures: the RNA Integrity Number (RIN) [Auer et al., 2003], and the 5'/3' ratios of specific housekeeping genes. RIN measurements are obtained prior to microarray analysis and indicate overall levels of RNA degradation, while 5'/3' ratios are obtained from gene expression data and indicate the relative levels of the 5' and 3' ends of specific gene transcripts. The impact of low 5'/3' ratios on gene expression data can vary, depending on the microarray technology being used (3' targeted probesets vs exon arrays) as well as the method used for cDNA synthesis (oligo(dT) priming vs random primers).

Dysregulated Genes and Pathways

The comparison of significant findings between studies is hampered by differences in the reporting of data and the tendency to focus upon genes within known and literature-relevant pathways. Moreover, owing to the difficulties in obtaining sufficient numbers of the elusive group of non-suicide psychiatric controls, it has been difficult to extricate the gene expression changes associated with suicidal behaviors from those pertaining to underlying psychiatric disorders, including depression. Nonetheless, several pathways have been consistently implicated

in these studies, including the glutamatergic and γ-amino-butyric acid (GABA)-ergic neurotransmitter systems, growth factors, the polyamine system, neuronal vesicle-mediated release, and glial cell functioning. It is noteworthy that studies have failed to indicate evidence of differential expression of genes from serotonergic and noradrenergic pathways, which are thought to be etiologically relevant to suicide and major depression.

Glutamate and GABA:

Evidence of altered expression of genes related to GABA and glutamate signalling are among the strongest findings arising from microarray studies both examining suicide and depression, strongly emphasizing the importance of these two pathways in psychiatric neurobiology. These two systems are intricately tied, as glutamate is a precursor for GABA synthesis by glutamic acid decarboxylase (GAD), and following its release into synapses, GABA is transported into astrocytes and converted to glutamine, which is converted into glutamate by glutaminase (GLS) [Daikhin and Yudkoff, 2000, Bak et al., 2006]. A summary of GABA and glutamate-related genes found to be significantly differentially expressed in suicide or depression is shown in Table 3.

GABA is the primary inhibitory neurotransmitter in the CNS, and acts upon two classes of receptors: ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors. Microarray studies have consistently found alterations in the expression of GABA receptor subunits in both prefrontal and limbic areas [Choudary et al., 2005, Sequeira et al., 2007, Kim et al., 2007, Klempan et al., 2009c, Sequeira et al., 2009b]. Alterations in the expression of a GABA transporter (SLC6A1) [Sequeira et al., 2009b] and an enzyme involved in GABA metabolism

(ALDH9A1) [Kang et al., 2007] have also been observed in suicide, and depression, respectively. Additionally, one study from our group found that 16 and 36% of the probesets annotated as involved in GABAergic signalling were significantly differentially expressed in suicide completers in BA 44 and 46, respectively [Klempan et al., 2009c]. Altered expression of GABA-related genes was also found in a candidate gene studies which identified significant decreases in GABRA1, $GABA_A$, α3 (GABRA3), GABRA4, and GABRD subunits in the frontopolar region of suicide victims [Merali et al., 2004]. Interestingly, a recent study also found differences in the interrelations between different $GABA_A$ subunits across the brain of depressed suicide completers relative to controls [Poulter et al., 2010].

Glutamate is the primary excitatory neurotransmitter in the brain, and acts at four classes of receptors: the ionotropic α-amino-3-hydroxy-5-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptors, as well as the metabotropic glutamate receptors. As with GABA, alterations in the expression of several glutamate receptor subunits have been observed in both suicide completers and depressed subjects [Thalmeier et al., 2008, Klempan et al., 2009c, Sequeira et al., 2009b, Bernard et al., 2010]. Additionally, several studies have observed downregulated expression of glutamate-ammonia ligase (glutamine synthetase) (GLUL), the enzyme responsible for removing glutamate from synapses, GLS, and glial high-affinity glutamate transporters SLC1A2 and SLC1A3 [Kim et al., 2007, Klempan et al., 2009c, Sequeira et al., 2009b, Choudary et al., 2005, Kang et al., 2007, Bernard et al., 2010]. Findings of alterations in the expression of glutamatergic genes, including the vesicular glutamate transporter 1 (VGLUT1) and NMDA receptor subunit 1 (GRIN1), have also been observed in candidate gene studies of depressive disorders [Uezato et al., 2009, Law and Deakin, 2001].

Growth factors:

Growth factors are cell signalling molecules with essential roles in cell proliferation, differentiation, and survival, which are dependent upon their binding to specific cell surface receptors [Pawson, 1994]. Altered expression of genes involved in the signalling pathways for two classes of growth factors, the neurotrophins and fibroblast growth factor (FGF), have consistently been observed in gene expression studies of suicide and depression.

The neurotrophin brain-derived neurotrophic factor (BDNF) plays an important role in neuronal growth and survival through its binding to the receptor neurotrophic tyrosine kinase, type 2 (NTRK2). Previous evidence has implicated this pathway in depression, as antidepressant treatment increases both the peripheral and brain expression of BDNF [Sen et al., 2008, Chen et al., 2001], which is associated with improvement of depressive symptoms [Brunoni et al., 2008]. It is thus of interest that several microarray studies have found altered expression of both BDNF and NTRK2 in the brains of depressed subjects [Evans et al., 2004, Aston et al., 2005, Kim and Webster, 2010b, Bernard et al., 2010]. Although BDNF expression has not been found to be altered in microarray expression studies of suicide completers, decreased expression of NTRK2 has been observed in several brain regions [Sequeira et al., 2007, Kim et al., 2007, Ernst et al., 2009a]. This decrease appears to be due to the downregulation of one specific NTRK2 isoform, TrkB.T1, which is expressed exclusively in astrocytes [Ernst et al., 2009a].

Alterations in the expression of components of FGF signalling pathways have also been consistently observed in suicide and depression. This growth factor is involved in cell proliferation and differentiation during development, and has essential roles in adult neuronal

signal transduction [Ornitz and Itoh, 2001]. There are over twenty FGF ligands in humans, which act upon four different tyrosine kinase receptors (FGFR1-4) [Ornitz and Itoh, 2001]. Dysregulated expression of genes involved in FGF signalling have been observed in a number of studies examining suicide completers [Kim et al., 2007, Ernst et al., 2008]. The same is true in studies of depression, where significant differences in the expression of several FGF ligands and receptors, particularly FGFR2 and FGFR3, has been observed [Evans et al., 2004, Aston et al., 2005, Kang et al., 2007, Tochigi et al., 2008, Bernard et al., 2010]. Similar to BDNF, FGF has also been shown to be upregulated following antidepressant treatment [Bachis et al., 2008].

Polyamines:

The polyamines are ubiquitous aliphatic molecules comprising agmatine, putrescine, spermidine, and spermine. The polyamine system has been identified in all organisms, and plays an important role in numerous essential cellular functions, including growth, division, and signalling cascades, as well as stress responses at both the cellular and behavioral levels [Minguet et al., 2008, Tabor and Tabor, 1984, Gilad and Gilad, 2003, Seiler and Raul, 2005, Rhee et al., 2007]. Although early research regarding the polyamine system in psychiatric disorders focused on its involvement in schizophrenia and stress responses [Fiori and Turecki, 2008], its involvement in suicide and depression was not suspected until microarray studies identified spermidine/spermine N1-acetyltransferase (SAT1) as one of the genes displaying the strongest and most consistently altered expression in suicide completers [Sequeira et al., 2006]. Evidence for its downregulation in suicide completers has now been extended to additional brain regions and populations [Sequeira et al., 2007, Klempan et al., 2009b, Klempan et al., 2009c,

Guipponi et al., 2009], and results have now been extended to animal models of depression [Karssen et al., 2007]. Dysregulated expression of other polyamine-related genes, including spermine synthase (SMS), spermine oxidase (SMOX), and ornithine aminotransferase-like 1, has also been identified [Sequeira et al., 2007, Klempan et al., 2009c]. We recently performed a global brain analysis of polyamine-related probesets in suicide completers, and identified fourteen genes, including SAT1, SMS, and SMOX, displaying significantly altered expression [Fiori et al., In Preparation].

Dysregulated expression of this system has also been implicated in depression. In particular, altered SAT1 expression was more pronounced in brains of depressed suicide completers compared to non-depressed suicide completers [Sequeira et al., 2006]. Moreover, we recently identified a promoter polymorphism in SAT1 which was significantly associated with suicide, but only in individuals diagnosed with depressive disorders [Fiori and Turecki, 2009], strongly suggesting that altered polyamine metabolism is a hallmark of both depression and suicide. Additionally, SAT1 was recently shown to display altered synchrony of expression between the amygdala and cingulate in depressed individuals [Gaiteri et al., 2010]. Finally, decreased expression of S-adenosylmethionine decarboxylase (AMD1) has been identified in the temporal cortex of depressed subjects [Aston et al., 2005].

Synaptic Vesicles:

In addition to the well-studied factors affecting neurotransmitter metabolism, synaptic transmission is highly regulated by presynaptic factors controlling the vesicle-mediated release of neurotransmitters. Considerable evidence is now emerging implicating altered function of this

system in depression and suicide. Altered expression of numerous synapse-related genes have been identified in suicide completers, including vesicle-associated membrane protein 3 (VAMP3), synaptotagmin I (SYT1), synaptotagmin IV (SYT4), synaptotagmin V (SYT5), synaptotagmin XIII (SYT13), synaptophilin (SNPH), synaptophysin-like protein (SYPL), synapsin II (SYN2), synaptosomal-associated protein (23 kDa) (SNAP23), synaptosomal-associated protein (25 kDa) (SNAP25), synaptosomal-associated protein (29 kDa) (SNAP29), synaptic vesicle glycoprotein 2B (SV2B), synaptopodin 2 (SYNPO2) [Sequeira et al., 2006, Sequeira et al., 2007, Klempan et al., 2009c, Sequeira et al., 2009b]. Microarray studies in depression have identified altered expression of several of these genes, including VAMP3 and SNA25 [Aston et al., 2005, Kim and Webster, 2010a], as well as synaptogyrin 2 (SYNGR2), synaptojanin 2 (SYNJ2), N-ethylmaleimide-sensitive factor attachment protein, alpha (NAPA), syntaxin 1A (STX1A), synaptotagmin I (SYT1), and syntaxin binding protein 1 (STXBP1) [Aston et al., 2005, Tochigi et al., 2008, Kim and Webster, 2010a].

Glial cells:

Glial cells play many distinct roles in the CNS, including the development and maintenance of the nervous system, processing of synaptic transmission, regulation of cerebral blood flow, and immune responses in the brain [Araque, 2008, Pfrieger, 2009, Koehler et al., 2009, Gehrmann et al., 1995]. In part due to the long-held belief that glial cells did little more than support neurons, traditional research in psychiatric disorders largely ignored the importance of these cells until histopathological studies discovered altered numbers and densities of glia in mood disorders [Hercher et al., 2009]. As shown in Table 4, alterations in the expression of glial-

specific genes have now been observed in numerous gene expression studies in both depression and suicide.

Astrocytes form the largest group of cells in the CNS [O'Kusky and Colonnier, 1982], and have multiple functions, including roles in the synthesis, release and uptake of neurotransmitters, development of synapses, and formation of the blood brain barrier [Fiacco et al., 2009, Montana et al., 2006, Stevens, 2008]. In addition, as mentioned above, astrocytes play essential roles in glutamate neurotransmission [Daikhin and Yudkoff, 2000, Bak et al., 2006]. Astrocytic functioning appears to be affected in both suicide and depression, which both display differential expression of astrocyte-specific genes in microarray studies [Ernst et al., 2009a, Kim et al., 2007, Kang et al., 2007, Choudary et al., 2005, Sequeira et al., 2009b, Bernard et al., 2010], as well as candidate gene studies [Barley et al., 2009].

While the best-known function of oligodendrocytes is their role in axon myelination, they also particulate in neurotransmission, synaptic function, neuronal development and neuronal survival [Deng and Poretz, 2003]. Downregulated expression of oligodendrocyte-specific genes has been observed in both suicide and depression, and include genes involved in myelin synthesis and formation as well as transcription factors related to oligodendrocyte-specific genes [Aston et al., 2005, Sibille et al., 2009, Bernard et al., 2010, Klempan et al., 2009c, Sequeira et al., 2006, Klempan et al., 2009a].

Future Steps

While it is clear that microarray studies have shed considerable light on the nature of gene expression changes that occur in depression and suicide, we are still far from understanding

either the origin of these alterations, nor the nature of their short- and long-term effects on brain function. However, continued advances in the fields of both molecular biology and high-throughput technologies will provide the means to answer these questions. By combining gene expression data with information obtained through other measures, such as clinical, epigenetic, genomic, proteomic, or metabolic studies, a more comprehensive view of these processes will be possible. In addition, continued sample collection and collaborations between research groups will allow for a greater capacity to investigate the effects of variables such as medication, gender, and the environment, as well as to properly differentiate the effects of suicide from those of depression and other psychiatric disorders.

That gene expression is altered at specific genes and gene pathways in suicide and depression is abundantly clear from both the microarray-based studies described in this review as well as numerous candidate gene expression studies. However, identifying the molecular mechanisms responsible for differences in gene expression represents an equally essential step for understanding the etiology of depression and suicidal behaviors, as well as for providing targets by which to treat these conditions. Genetic factors, particularly those within gene regulatory regions, are important determinants of gene expression, and genetic variants, such as single nucleotide polymorphisms (SNPs), can have a large impact on expression. While many functional SNPs have been identified and putatively associated with psychiatric conditions, these studies have been mainly confined to candidate genes. By integrating information obtained from high-throughput genotyping platforms with gene expression data, we will be able to identify the functional variants underlying gene expression differences. In addition, these studies will allow the identification of copy number variations (CNV), large segments of chromosomal deletions

and amplifications, which have been primarily investigated in other psychiatric conditions, most notably bipolar disorder, schizophrenia, and autism [Cook and Scherer, 2008].

The potential role of epigenetics in psychiatry is also becoming increasingly clear, and represents an important bridge between the environment and heritable genetic factors [McGowan et al., 2008, McGowan et al., 2009, Zhang and Meaney, 2010]. The field of epigenetics involves factors which influence the expression of genes without altering the DNA sequence itself, and comprise DNA methylation, histone modifications, and microRNAs. To date, published epigenetic studies in depression and suicide have focused only on genes of interest. However, as with gene expression, high-throughput methods targeting epigenetic factors have now become available, thus opening the door to the systematic investigation of epigenetic effects that may be involved in conferring risk to suicide or depression. Analysis of gene expression patterns can be used to initiate hypothesis-driven studies to investigate the involvement of specific epigenetic modifications in altered gene expression, and furthermore, integration of gene expression and epigenetic data can provide insight into the molecular mechanisms mediating dysregulated gene expression.

As mentioned above, the majority of microarray studies in suicide and depression date have used 3’ expression arrays, which typically probe only the 3’ end of mRNAs. Newer arrays have now been developed which can specifically examine the expression of each exon within a gene. These will allow the identification of genes which show specific splicing differences, which may improve our understanding of how the function of these genes may be altered in suicide and depression. Although high-throughput methods to examine the proteome have not advanced as quickly as other fields, the technology continues to be improved. In the future, combining

mRNA expression with protein expression data will allow for a better understanding of how alterations in gene expression can confer particular psychiatric phenotypes.

Finally, the importance of better examining the relationship between clinical variables and gene expression cannot be over-stressed, as this represents the critical point at which this research can be translated into medicine. Gender is an essential consideration in studies of depression and suicide, and sample recruitment practices and statistical analyses must be adjusted in order to examine gender-specific factors. Also, increasing sample sizes and the use of well-characterized and appropriately-selected control groups are important for enabling the identification of phenotype-specific gene expression changes, a particularly important challenge in neurobiological studies of suicide. Finally, developing a more comprehensive view of the means by which psychopharmacological agents affect gene expression is an important step in understanding the mechanisms by which they exert their therapeutic effects, which will greatly assist in the development of better treatments.

Conclusions

The complexity of the neurobiological alterations associated with psychiatric conditions such as major depression and suicide is evident, and the necessity of widening our focus beyond the pathways classically investigated is clear. Gene expression profiling of suicide and depression has provided a much-needed tool for identifying new targets, and has allowed us to obtain a more global view of the pathological mechanisms taking place in the brain. Perplexingly, relatively few studies have found alterations in the expression of genes related to the pathways classically associated with depression and suicide, such as the monoaminergic

systems. While this could be seen with some concern given the large body of evidence supporting their involvement in these conditions, instead, it is enticing to speculate that gene expression studies have provided us with information on pathways and systems that, when combined with information from other neurobiological and psychological studies, may yield a better understanding of the underlying neurobiology and eventually help develop better treatments.

References

Altar CA, Jurata LW, Charles V, Lemire A, Liu P, Bukhman Y et al. Deficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. Biol Psychiatry 2005;58(2):85-96.

Altar CA, Vawter MP, Ginsberg SD. Target identification for CNS diseases by transcriptional profiling. Neuropsychopharmacology 2009;34(1):18-54.

Araque A. Astrocytes process synaptic information. Neuron Glia Biol 2008;4(1):3-10.

Arsenault-Lapierre G, Kim C, Turecki G. Psychiatric diagnoses in 3275 suicides: a meta-analysis. BMC Psychiatry 2004;4:37.

Aston C, Jiang L, Sokolov BP. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. Mol Psychiatry 2005;10(3):309-22.

Auer H, Lyianarachchi S, Newsom D, Klisovic MI, Marcucci U, Kornacker K. Chipping away at the chip bias: RNA degradation in microarray analysis. Nat Genet 2003;35(4):292-3.

Bachis A, Mallei A, Cruz MI, Wellstein A, Mocchetti I. Chronic antidepressant treatments increase basic fibroblast growth factor and fibroblast growth factor-binding protein in neurons. Neuropharmacology 2008;55(7):1114-20.

Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J Neurochem 2006;98(3):641-53.

Barley K, Dracheva S, Byne W. Subcortical oligodendrocyte- and astrocyte-associated gene expression in subjects with schizophrenia, major depression and bipolar disorder. Schizophr Res 2009;112(1-3):54-64.

Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD et al. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. Mol Psychiatry 2010.

Brent DA, Bridge J, Johnson BA, Connolly J. Suicidal behavior runs in families. A controlled family study of adolescent suicide victims. Arch Gen Psychiatry 1996;53(12):1145-52.

Brezo J, Klempan T, Turecki G. The genetics of suicide: a critical review of molecular studies. Psychiatr Clin North Am 2008;31(2):179-203.

Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. Int J Neuropsychopharmacol 2008;11(8):1169-80.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001;50(4):260-5.

Choudary PV, Molnar M, Evans SJ, Tomita H, Li JZ, Vawter MP et al. Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. Proc Natl Acad Sci USA 2005;102(43):15653-8.

Chu TT, Liu Y, Kemether E. Thalamic transcriptome screening in three psychiatric states. J Hum Genet 2009;54(11):665-75.

Cook EH, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. Nature 2008;455(7215):919-23.

Daikhin Y, Yudkoff M. Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr 2000;130(4S Suppl):1026S-31S.

Deng W, Poretz RD. Oligodendroglia in developmental neurotoxicity. Neurotoxicology 2003;24(2):161-78.

Ernst C, Bureau A, Turecki G. Application of microarray outlier detection methodology to psychiatric research. BMC Psychiatry 2008;8:29.

Ernst C, Deleva V, Deng X, Sequeira A, Pomarenski A, Klempan T et al. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. Arch Gen Psychiatry 2009a;66(1):22-32.

Ernst C, Mechawar N, Turecki G. Suicide neurobiology. Prog Neurobiol 2009b;89(4):315-33.

Evans SJ, Choudary PV, Neal CR, Li JZ, Vawter MP, Tomita H et al. Dysregulation of the fibroblast growth factor system in major depression. Proc Natl Acad Sci USA 2004;101(43):15506-11.

Fiacco TA, Agulhon C, McCarthy KD. Sorting out astrocyte physiology from pharmacology. Annu Rev Pharmacol Toxicol 2009;49:151-74.

Fiori LM, Turecki G. Implication of the polyamine system in mental disorders. J Psychiatry Neurosci 2008;33(2):102-10.

Fiori LM, Turecki G. Association of the SAT1 In/del Polymorphism with Suicide Completion. Am J Med Genet B Neuropsychiatr Genet 2009;153B(3):825-9.

Fiori LM, Zouk H, Himmelman C, Turecki G. X Chromosome and Suicide. Mol Psychiatry 2009. In Press.

Gadbury GL, Garrett KA, Allison DB. Challenges and approaches to statistical design and inference in high-dimensional investigations. Methods Mol Biol 2009;553:181-206.

Gaiteri C, Guilloux JP, Lewis DA, Sibille E. Altered gene synchrony suggests a combined hormone-mediated dysregulated state in major depression. PLoS ONE 2010;5(4):e9970.

Garbett K, Gal-Chis R, Gaszner G, Lewis DA, Mirnics K. Transcriptome alterations in the prefrontal cortex of subjects with schizophrenia who committed suicide. Neuropsychopharmacol Hung 2008;10(1):9-14.

Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 1995;20(3):269-87.

Gilad GM, Gilad VH. Overview of the brain polyamine-stress-response: regulation, development, and modulation by lithium and role in cell survival. Cell Mol Neurobiol 2003;23(4-5):637-49.

Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, Khanlou N et al. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc Natl Acad Sci USA 2005;102(43):15533-8.

Guipponi M, Deutsch S, Kohler K, Perroud N, Le Gal F, Vessaz M et al. Genetic and epigenetic analysis of SSAT gene dysregulation in suicidal behavior. Am J Med Genet B Neuropsychiatr Genet 2009;150B(6):799-807.

Gwadry FG, Sequeira A, Hoke G, Ffrench-Mullen JM, Turecki G. Molecular characterization of suicide by microarray analysis. Am J Med Genet C Semin Med Genet 2005;133C(1):48-56.

Hawton K. Sex and suicide. Gender differences in suicidal behaviour. Br J Psychiatry 2000;177:484-5.

Hercher C, Turecki G, Mechawar N. Through the looking glass: Examining neuroanatomical evidence for cellular alterations in major depression. J Psychiatr Res 2009;43(11):947-61.

Hynd MR, Lewohl JM, Scott HL, Dodd PR. Biochemical and molecular studies using human autopsy brain tissue. J Neurochem 2003;85(3):543-62.

Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4(2):249-64.

Iwamoto K, Kakiuchi C, Bundo M, Ikeda K, Kato T. Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. Mol Psychiatry 2004;9(4):406-16.

Iwamoto K, Kato T. Gene expression profiling in schizophrenia and related mental disorders. Neuroscientist 2006;12(4):349-61.

Kang HJ, Adams DH, Simen A, Simen BB, Rajkowska G, Stockmeier CA et al. Gene expression profiling in postmortem prefrontal cortex of major depressive disorder. J Neurosci 2007;27(48):13329-40.

Karssen AM, Her S, Li JZ, Patel PD, Meng F, Bunney WE et al. Stress-induced changes in primate prefrontal profiles of gene expression. Mol Psychiatry 2007;12(12):1089-102.

Kim C, Lesage A, Seguin M, Chawky N, Vanier C, Lipp O et al. Patterns of co-morbidity in male suicide completers. Psychol Med 2003;33(7):1299-309.

Kim S, Choi KH, Baykiz AF, Gershenfeld HK. Suicide candidate genes associated with bipolar disorder and schizophrenia: an exploratory gene expression profiling analysis of post-mortem prefrontal cortex. BMC Genomics 2007;8:413.

Kim S, Webster MJ. Correlation analysis between genome-wide expression profiles and cytoarchitectural abnormalities in the prefrontal cortex of psychiatric disorders. Mol Psychiatry 2010a;15(3):326-36.

Kim S, Webster MJ. The Stanley Neuropathology Consortium Integrative Database: a novel, web-based tool for exploring neuropathological markers in psychiatric disorders and the biological processes associated with abnormalities of those markers. Neuropsychopharmacology 2010b;35(2):473-82.

Klempan TA, Ernst C, Deleva V, Labonte B, Turecki G. Characterization of QKI Gene Expression, Genetics, and Epigenetics in Suicide Victims with Major Depressive Disorder. Biol Psychiatry 2009a;66(9):824-31.

Klempan TA, Rujescu D, Merette C, Himmelman C, Sequeira A, Canetti L et al. Profiling brain expression of the spermidine/spermine N(1)-acetyltransferase 1 (SAT1) gene in suicide. Am J Med Genet B Neuropsychiatr Genet 2009b;150B(7):934-43.

Klempan TA, Sequeira A, Canetti L, Lalovic A, Ernst C, Ffrench-Mullen J et al. 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 2009c;14(2):175-89.

Koehler RC, Roman RJ, Harder DR. Astrocytes and the regulation of cerebral blood flow. Trends Neurosci 2009;32(3):160-9.

Lalovic A, Klempan T, Sequeira A, Luheshi G, Turecki G. Altered expression of lipid metabolism and immune response genes in the frontal cortex of suicide completers. J Affect Disord 2009. In Press.

Law AJ, Deakin JFW. Asymmetrical reductions of hippocampal NMDAR1 glutamate receptor mRNA in the psychoses. Neuroreport 2001;12(13):2971-4.

McGirr A, Alda M, Seguin M, Cabot S, Lesage A, Turecki G. Familial Aggregation of Suicide Explained by Cluster B Traits: A Three-Group Family Study of Suicide Controlling for Major Depressive Disorder. Am J Psychiatry 2009;166(10):1124-34.

McGirr A, Paris J, Lesage A, Renaud J, Turecki G. Risk factors for suicide completion in borderline personality disorder: a case-control study of cluster B comorbidity and impulsive aggression. J Clin Psychiatry 2007;68(5):721-9.

McGirr A, Seguin M, Renaud J, Benkelfat C, Alda M, Turecki G. Gender and risk factors for suicide: evidence for heterogeneity in predisposing mechanisms in a psychological autopsy study. J Clin Psychiatry 2006;67(10):1612-7.

McGirr A, Turecki G. What is specific to suicide in schizophrenia disorder? Demographic, clinical and behavioural dimensions. Schizophr Res 2008;98(1-3):217-24.

McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 2009;12(3):342-8.

McGowan PO, Sasaki A, Huang TC, Unterberger A, Suderman M, Ernst C et al. Promoter-wide hypermethylation of the ribosomal RNA gene promoter in the suicide brain. PLoS ONE 2008;3(5):e2085.

Merali Z, Du L, Hrdina P, Palkovits M, Faludi G, Poulter MO et al. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. J Neurosci 2004;24(6):1478-85.

Minguet EG, Vera-Sirera F, Marina A, Carbonell J, Blazquez MA. Evolutionary diversification in polyamine biosynthesis. Mol Biol Evol 2008;25(10):2119-28.

Miron M, Nadon R. Inferential literacy for experimental high-throughput biology. Trends Genet 2006;22(2):84-9.

Montana V, Malarkey EB, Verderio C, Matteoli M, Parpura V. Vesicular transmitter release from astrocytes. Glia 2006;54(7):700-15.

Morita K, Saito T, Ohta M, Ohmori T, Kawai K, Teshima-kondo S et al. Expression analysis of psychological stress-associated genes in peripheral blood leukocytes. Neurosci Lett 2005;381(1-2):57-62.

Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet 2007;370(9590):851-8.

Nock MK, Borges G, Bromet EJ, Cha CB, Kessler RC, Lee S. Suicide and suicidal behavior. Epidemiol Rev 2008;30:133-54.

O'Kusky J, Colonnier M. A laminar analysis of the number of neurons, glia, and synapses in the adult cortex (area 17) of adult macaque monkeys. J Comp Neurol 1982;210(3):278-90.

Ornitz DM, Itoh N. Fibroblast growth factors. Genome Biol 2001;2(3):REVIEWS3005.

Pawson T. Tyrosine kinase signalling pathways. Princess Takamatsu Symp 1994;24:303-22.

Pfrieger FW. Roles of glial cells in synapse development. Cell Mol Life Sci 2009;66(13):2037-47.

Poulter MO, Du L, Zhurov V, Palkovits M, Faludi G, Merali Z et al. Altered Organization of GABA(A) Receptor mRNA Expression in the Depressed Suicide Brain. Front Mol Neurosci 2010;3:3.

Rhee HJ, Kim EJ, Lee JK. Physiological polyamines: simple primordial stress molecules. J Cell Mol Med 2007;11(4):685-703.

Seedat S, Scott KM, Angermeyer MC, Berglund P, Bromet EJ, Brugha TS et al. Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Arch Gen Psychiatry 2009;66(7):785-95.

Segman RH, Goltser-Dubner T, Weiner I, Canetti L, Galili-Weisstub E, Milwidsky A et al. Blood mononuclear cell gene expression signature of postpartum depression. Mol Psychiatry 2010;15(1):93-100, 2.

Seiler N, Raul F. Polyamines and apoptosis. J Cell Mol Med 2005;9(3):623-42.

Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry 2008;64(6):527-32.

Sequeira A, Mamdani F, Ernst C, Vawter MP, Bunney WE, Jr., Klempan T et al. Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. PLoS ONE 2009a.

Sequeira A, Gwadry FG, Ffrench-Mullen JM, Canetti L, Gingras Y, Casero RA et al. Implication of SSAT by gene expression and genetic variation in suicide and major depression. Arch Gen Psychiatry 2006;63(1):35-48.

Sequeira A, Klempan T, Canetti L, Ffrench-Mullen J, Benkelfat C, Rouleau GA et al. Patterns of gene expression in the limbic system of suicides with and without major depression. Mol Psychiatry 2007;12(7):640-55.

Sequeira A, Mamdani F, Ernst C, Vawter MP, Bunney WE, Klempan T et al. Global Brain Gene Expression Analysis Links Glutamatergic and GABAergic Alterations to Suicide and Major Depression. PLoS ONE 2009b;4(8):e6585.

Sequeira A, Turecki G. Genome wide gene expression studies in mood disorders. OMICS 2006;10(4):444-54.

Shelton RC, Claiborne J, Sidoryk-Wegrzynowicz M, Reddy R, Aschner M, Lewis DA et al. Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. Mol Psychiatry 2010. In Press.

Sibille E, Arango V, Galfalvy HC, Pavlidis P, Erraji-Benchekroun L, Ellis SP et al. Gene expression profiling of depression and suicide in human prefrontal cortex. Neuropsychopharmacology 2004;29(2):351-61.

Sibille E, Wang Y, Joeyen-Waldorf J, Gaiteri C, Surget A, Oh S et al. A molecular signature of depression in the amygdala. Am J Psychiatry 2009;166(9):1011-24.

Spijker S, Van Zanten JS, De Jong S, Penninx BWJH, Van Dyck R, Zitman FG et al. Stimulated Gene Expression Profiles as a Blood Marker of Major Depressive Disorder. Biol Psychiatry 2010. In Press.

Statham DJ, Heath AC, Madden PA, Bucholz KK, Bierut L, Dinwiddie SH et al. Suicidal behaviour: an epidemiological and genetic study. Psychol Med 1998;28(4):839-55.

Steinhoff C, Vingron M. Normalization and quantification of differential expression in gene expression microarrays. Brief Bioinform 2006;7(2):166-77.

Stevens B. Neuron-astrocyte signaling in the development and plasticity of neural circuits. Neurosignals 2008;16(4):278-88.

Tabor CW, Tabor H. Polyamines. Annu Rev Biochem 1984;53:749-90.

Thalmeier A, Dickmann M, Giegling I, Schneider B, Hartmann M, Maurer K et al. Gene expression profiling of post-mortem orbitofrontal cortex in violent suicide victims. Int J Neuropsychopharmacol 2008;11(2):217-28.

Tochigi M, Iwamoto K, Bundo M, Sasaki T, Kato N, Kato T. Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. Neurosci Res 2008;60(2):184-91.

Uezato A, Meador-Woodruff JH, McCullumsmith RE. Vesicular glutamate transporter mRNA expression in the medial temporal lobe in major depressive disorder, bipolar disorder, and schizophrenia. Bipolar Disord 2009;11(7):711-25.

Vawter MP, Tomita H, Meng F, Bolstad B, Li J, Evans S et al. Mitochondrial-related gene expression changes are sensitive to agonal-pH state: implications for brain disorders. Mol Psychiatry 2006;11(7):663-79.

Yanagi M, Shirakawa O, Kitamura N, Okamura K, Sakurai K, Nishiguchi N et al. Association of 14-3-3 epsilon gene haplotype with completed suicide in Japanese. J Hum Genet 2005;50(4):210-6.

Zhang TY, Meaney MJ. Epigenetics and the Environmental Regulation of the Genome and Its Function. Annu Rev Psychol 2010;61:439-66.

Table 1: Microarray studies examining gene expression in suicide completers.

Study	Institution	Platform	Tissue (s)	Groups	Gender (s)
[Sibille et al., 2004]	New York State Psychiatric Institute, USA	HG-U133A	BA 9, 47	MD-S, C	M/F
[Yanagi et al., 2005]	Kobe University, Japan	HG-U95A	Amygdala	S, C	M
[Gwadry et al., 2005]	QSBB, Canada	HG-U133A/B	BA 11	MD-S, C	M
[Choudary et al., 2005]	UC Davis/ UC Irvine/ University of Michigan, USA	HG-U95Av2	BA 9/46, 24	MD-S, BD-S, C	M/F
[Sequeira et al., 2006]	QSBB, Canada	HG-U133A/B	BA 4, 8/9, 11	MD-S, S, C	M
[Sequeira et al., 2007]	QSBB, Canada	HG-U133A/B	Amygdala, hippocampus, BA 24, BA 29	MD-S, S, C	M
[Kim et al., 2007]	SNCID, USA	HG-U133A	BA 46/10	BD-S, BS-NS, SCZ-S, SCZ-NS	M/F
[Garbett et al., 2008]	CNMD, USA	HG-U133A/B	BA 46	SCZ-S, SCZ-NS, C	M/F
[Thalmeier et al., 2008]	Johann Wolfgang Goethe University, Germany.	HumanRef-8 BeadChip	BA 11	S, C	M/F
[Tochigi et al., 2008]	SNCID, USA	HG-U95Av2	BA 10	MD-S, SCZ-S, BD-S, C	M/F
[Ernst et al., 2008]	QSBB, Canada	HG-U133A/B	BA 8/9, 11	MD-S, C	M
[Klempan et al., 2009b]	QSBB, Canada	HG-U133A/B	BA 44, 45, 46, 47	MD-S, S, C	M
[Ernst et al., 2009a]	QSBB, Canada	HG-U133 plus 2	BA 4, 6, 8/9, 10, 11, 45, 46, 47, cerebellum	S, C	M
[Sequeira et al., 2009b]	QSBB, Canada	HG-U133A/B	BA 4, 6, 8/9, 10, 11, 20, 21, 38, 24, 29, 44, 45, 46, 47, amygdala, hippocampus, nucleus accumbens	MD-S, S, C	M
[Kim and Webster, 2010b]	SNCID, USA	HG-U133A, HG-U95Av2, HG-	Frontal cortex, hippocampus, cingulate cortex, temporal cortex,	BD, MD, SZ, C	M/F

	U133 plus 2	striatum, thalamus, occipital cortex, entorhinal cortex, amygdala, cerebellum, parietal cortex, other

BA – Brodmann area; BD – bipolar disorder; C – healthy control; CNMD- University of Pittsburgh's Center for the Neuroscience of Mental Disorders Brain Bank; MD - major depressive disorder; QSBB - Quebec Suicide Brain Bank; S – suicide completer; SCZ – schizophrenia; SNCID - Stanley Neuropathology Consortium Integrative Database

Table 2: Microarray studies examining gene expression patterns in depression.

Study	Institution	Platform	Tissue (s)	Groups	Gender (s)
[Evans et al., 2004]	UC Davis/ UC Irvine/ University of Michigan, USA	HG-U133A	BA 24, 46	BD, MD, C	M/F
[Iwamoto et al., 2004]	SNCID, USA	HG-U95A	BA 10	BD, MD, SZ, C	M/F
[Choudary et al., 2005]	UC Davis/ UC Irvine/ University of Michigan, USA	HG-U95Av2	BA 9/46, 24	MD-S, BD-S, C	M/F
[Altar et al., 2005]	SNCID, USA	Agilent Human 1 cDNA	Hippocampus	BD, MD, SZ, C	M/F
[Aston et al., 2005]	SNCID, USA	HG-U95A	BA 21	MD, C	M/F
[Kang et al., 2007]	Cuyahoga County Coroner's Office, USA	Agilent Human 1A Oligo chip	BA 9	MD, C	M/F
[Tochigi et al., 2008]	SNCID, USA	HG-U95Av2	BA 10	MD-S, SCZ-S, BD-S, C	M/F
[Chu et al., 2009]	SNCID, USA	HG-U133 plus 2	Dorsomedial thalamus (neurons)	BD, MD, SZ, C	M/F
[Sibille et al., 2009]	Allegheny County Medical Examiner's Office, USA	HG-U133 plus 2	Amygdala, BA 24	MD, C	M
[Kim and Webster, 2010a]	SNCID, USA	HG-U133A, HG-U95Av2	BA 9, BA 46	BD, MD, SZ, C	M/F
[Kim and Webster, 2010b]	SNCID, USA	HG-U133A, HG-U95Av2, HG-U133 plus 2	Frontal cortex, hippocampus, cingulate cortex, temporal cortex, striatum, thalamus, occipital cortex, entorhinal cortex, amygdala, cerebellum, parietal cortex, other	BD, MD, SZ, C	M/F
[Segman et al., 2010]	Hadassah Mt. Scopus Hospital, Israel	GeneChip Human Exon 1.0 ST	peripheral blood mononuclear cells	Postpartum depression	F
[Gaiteri et al., 2010]	Allegheny County	HG-U133 plus 2	Amygdala, anterior cingulate cortex	MD, C	M

	Medical Examiner's Office, USA				
[Bernard et al., 2010]	UC Davis/ UC Irvine/ University of Michigan, USA	HG-U133 plus 2	Locus coeruleus	BD, MD, C	M/F
[Spijker et al., 2010]	Vrije Universiteit Amsterdam, Leiden University, Netherlands	Agilent 44 K Whole Human Genome array	Whole blood	MD, C	M/F
[Shelton et al., 2010]	University of Pittsburg / Vanderbilt University, USA	GeneChip Human Exon 1.0 ST	BA 10	MD, C	M/F

BA – Brodmann area; BD – bipolar disorder; C – healthy control; MD - major depressive disorder; S – suicide completer; SCZ – schizophrenia; SNCID - Stanley Neuropathology Consortium Integrative Database; UC – University of California

Table 3: Differentially expressed genes in suicide and depression associated with GABA and glutamate neurotransmission.

System	Phenotype	Genes	Study
Glutamate	depression	GLUL	[Kang et al., 2007]
	depression	GRIA1, GRIA3, GRIK1, GRIK5, GRM3, SLC1A2, SLC1A3, GLUL	[Choudary et al., 2005]
	depression	GRIA1, GRIK1, GRM1, GRM5, VGLUT2, SLC1A2, SLC1A3, GLUL	[Bernard et al., 2010]
	suicide	GLUL, SLC1A3	[Kim et al., 2007]
	suicide	GRIK1	[Thalmeier et al., 2008]
	suicide	GRIN2A, GRINL1A, GRIA3, GRM3	[Klempan et al., 2009b]
	suicide	GRIA1, GRIA2, GRIA3, GRIA4, GRIK1, GRINA, GRIN2A, GRINL1A, GRM3, SLC1A2, SLC1A3, GLS, GLUL, GLUD1	[Sequeira et al., 2009b]
GABA	depression	GABRB2	[Aston et al., 2005]
	depression	GABARB3, GABRD, GABARG2	[Choudary et al., 2005]
	depression	ALDH9A1, GABBR1	[Kang et al., 2007]
	suicide	GABRA1, GABRB3	[Choudary et al., 2005]
	suicide	GABARAPL1, GABRA4, GABRA5, GABRB1, GABRB3, GABRR1, GABRD, GABRG1, GABRG2, SLC6A1	[Sequeira et al., 2009b]

Table 4: Differentially expressed genes in suicide and depression associated with glial cells.

Glial cell	Phenotype	Genes	Study
Oligodendrocyte	depression	CNP, MAG, MAL, MOG, MOBP, PMP22, PLLP, PLP1, ASPA, UGT8, ENPP2, EDG2, TF, KLK6, SOX10, OLIG2, ERBB3	[Aston et al., 2005]
	depression	MBP, MOBP, PLLP	[Sibille et al., 2009]
	depression	S100B	[Bernard et al., 2010]
	suicide	OLIG1	[Sequeira et al., 2006]
	suicide	GPM6B, S100B, QKI	[Klempan et al., 2009b]
	suicide	CNP, MBP, MOG, PMP22, S100B	[Sequeira et al., 2009b]
Astrocyte	depression	FGFR3	[Evans et al., 2004]
	depression	GLUL, SLC1A2, SLC1A3	[Choudary et al., 2005]
	depression	FGFR3, GLUL	[Kang et al., 2007]
	depression	AQP4	[Tochigi et al., 2008]
	depression	AQP4, FGFR3, GFAP, GJA1, GJB6, GLUL, SLC1A2, SLC1A3	[Bernard et al., 2010]
	suicide	FGFR3, GJA1, GLUL, SLC1A3	[Kim et al., 2007]
	suicide	TrkB.T1	[Ernst et al., 2009a]
	suicide	GLUL, SLC1A2, SLC1A3	[Sequeira et al., 2009b]