Prefrontal gene expression changes in mood disorders and suicide

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Publication date
2018
Document Version
Other version
License
Other

CHAPTER 2

TOWARDS AN IDENTIFICATION OF THE MOLECULAR DIFFERENCES BETWEEN DEPRESSION AND SUICIDE

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Submitted

Over the past decade, there has been a rise in studies reporting on various molecular changes in postmortem brain tissue of psychiatric patients who had suffered from mood disorders and/or suicide. However, when compared to each other, many of these studies often presented clear discrepancies, even though matching for factors like age and postmortem delay was often carefully performed. As a result, it is still hard to distill a uniform picture from them, let alone obtain a better insight in the neurobiological mechanisms underlying mood disorders. We here propose that part of this confusion results from the fact that suicide has always been considered a feature and symptom, and thus an integral part of mood disorders, whereas it may represent a different entity from a behavioural and also molecular point of view. Actively engaging in the act of ending one's own life may represent a whole different "state of mind" with terms of inhibitory behaviour or impulse control, then experiencing prolonged mood problems, or feelings of guilt, worthlessness and sadness per se. Yet, in many previous studies, major depressive disorder (MDD) patients who committed suicide have generally been included in the same group as MDD patients who died of other causes, but did not idealize or commit suicide. Same for the bipolar disorder (BD) patients.

To start addressing this possible difference, we have focused in our own molecular studies in the prefrontal cortex (PFC). In elderly mood disorder patients who did not commit suicide, we found significant changes in two (i.e. GABRB2 and PSD-95) out of thirty-two gamma-aminobutyric acid (GABA) and glutamate related transcripts, in the anterior cingulate cortex (ACC) (Zhao et al., 2012). For the exactly same target gene set in our recent study, we found an enhanced expression and for the most genes in the ACC in the depressed suicide MDD patient group, while gene expression levels in the non-suicide MDD patient group were decreased (Chapter 4). Previously, we report that the expression levels of corticotropin-releasing hormone (CRH) and neuronal nitric oxide synthase (NOS)-interacting DHHC domain-containing protein with dendritic mRNA (NIDD) were increased in the ACC of the suicide MDD patient group (Zhao et al., 2015). Also, there was a significant increase in neuronal located components (EAAT3, EAAT4, ASCT1, SNAT1, SNAT2) of the glutamate-glutamine cycle in the ACC, whereas the astroglia located components (EAAT1, EAAT2, GLUL) were decreased in the dorsolateral PFC (DLPFC) of suicide MDD patients (Zhao et al., 2016).

We next set out to investigate whether the earlier reported alterations in literature were also related to either mood disorder per se, or to a mood disorder

that ended with suicide. We found that most previously published postmortem studies did not distinguish mood disorder per se from mood disorder with suicide alterations and may thus have presented mixed results. We list several recent papers to further illustrate this topic in Table 1.

Firstly, postmortem studies that claimed to have determined molecular alterations in relation to *mood disorders* had in fact very often selected mood disorder patients that had died all, or for a major part, from *suicide*. They were compared to control subjects without any psychiatric disorder, and thus disregarded that suicide may have confounded the findings that were now proposed to be due to mood disorder per se.

Secondly, the studies that claimed to have shown changes in relation to *suicide* had generally compared cases who committed suicide to matched controls without any psychiatric disorder, thereby disregarding the fact that suicide is not MDD or BD-specific as many suicide cases have suffered from various psychiatric disorders, including anxiety, but also often from substance abuse, schizophrenia and personality disorders (Hawton and van Heeringen, 2009). Martins-de-Souza et al. in Table 1 has touched upon this problem. They stated in their paper that suicide might be a confounder, but that they could not explore this any further due to the small group size (Martins-de-Souza et al., 2012).

It is further important to note that, in turn, for studies into suicide per se, mood disorder represents a confounder. This is particularly relevant for biological analyses of suicide behaviour, where distinct, disease-related contributions to suicide risk are generally not well defined (Lutz et al., 2017) and e.g. no suicide-specific behavioural changes, possible biomarkers or other indicators for impending suicide are known, although some developments in this direction utilizing polyphenic risk scores and questionnaires, are underway (Le-Niculescu et al., 2013; Levey et al., 2016; Niculescu and Le-Niculescu, 2017).

Table 1 Findings of postmortem studies in 'Depression' and 'Suicide' populations

Journal (Year)	First author	Title	Main methods	Brain area	Subjects	Conclusion by authors
Altherations in 'Depression'						
J Psychiatry Neurosci. (2016)	Clark	Reduced kynurenine pathway metabolism and cytokine expression in the prefrontal cortex of depressed individuals	Q-PCR; Histology; immunohisto-chemistry	VLPFC	45 MDD (25 S), 36 C	Depression, in the absence of medical illness or an overt inflammatory process, is associated with compromised, rather than increased kynurenine pathway metabolism in the VLPFC.
Am J Psychiatry (2015)	Kunii	CHRNA7 and CHRFAM7A mRNAs: Co-Localized and Their Expression Levels Altered in the Postmortem Dorsolateral Prefrontal Cortex in Major Psychiatric Disorders	Q-PCR; in-situ	DLPFC	176 SZ, 61 BD, 138 MDD, 326 C	These data show preferential fetal CHRFAM7A expression in the human PFC and suggest abnormalities in the CHRFAM7A/ CHRNA7 ratios in SZ and BD, due mainly to overexpression of CHRFAM7A.
Neurobiol Dis (2015)	Reinhart	Evaluation of TrkB and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder	Q-PCR	DLPFC	15 SZ (7 S), 15 BD (8 S), 15 MDD (7 S), 15 C	No difference on mRNAs encoding TrkB, total BDNF, and the four most abundant BDNF transcripts (I, IIc, IV, and VI)
Plos one (2014)	Smalheiser	Expression of microRNAs and Other Small RNAs in Prefrontal Cortex in Schizophrenia, Bipolar Disorder and Depressed Subjects	Q-PCR	PFC	15 SZ (7 S), 15 BD (8 S), 15 MDD (7 S), 15 C	Discrete miRNA alterations were observed in all disorders, as well as in suicide subjects (pooled across diagnostic categories) compared to all non-suicide subjects
Psychiatry Res (2014)	Hamazaki	Fatty acid composition of the postmortem prefrontal cortex of patients with schizophrenia, bipolar disorder, and major depressive disorder	lipid acid compar-ison	BA8	15 SZ (3 S), 15 BD (4 S), 15 MDD (13 S), 15 C	No significant differences in the levels of PUFAs or other fatty acids in the PFC (BA8) between patients and controls. No differences in any individual fatty acids between suicide and non-suicide cases.

Table 1 Continued, Findings of postmortem studies in 'Depression' and 'Suicide' populations

Journal (Year)	First author	Title	Main methods	Brain area	Subjects	Conclusion by authors
Nat Med (2014)	Ota	REDD1 is essential for stress-induced synaptic loss and depressive behavior.	Q-PCR	PFC	1st cohort: 36 MDD (no suicide information), 36 C 2nd cohort: 37 MDD (no suicide information), 35 C	REDD1 levels are increased in the postmortem PFC of human subjects with MDD relative to matched controls.
Transl Psychiatry (2012)	Martins-de-Souza	Identification of proteomic signatures associated with depression and psychotic depression in post-mortem brains from major depression patients	Proteomics	BA9	24 MDD (17 S), 12 C	Protein changes in energy metabolism, synaptic function, histidine triad nucleotide binding protein 1 in depression.
Prog Neuropsychopharmacol Biol Psychiatry (2011)	Jernigan	The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder.	WB	PFC	12 MDD (10 S), 12 C	Deficits in mTOR-dependent translation initiation in MDD particularly via the p70S6K/eIF4B pathway.
J Affect Disord (2011)	Gittins	A morphometric study of glia and neurons in the anterior cingulate cortex in mood disorder.	immunoautoradiography	ACC	5 MDD + 2 BD (4 S), 9 C	Reduced mTOR, p70S6K, eIF4B and p-eIF4B protein expression in MDD subjects relative to controls. No group differences were observed in eIF4E, p-eIF4E or actin levels.
Am J Psychiatry (2011)	Deschwanden	Reduced metabotropic glutamate receptor 5 density in major depression determined by [(11)C]ABP688 PET and postmortem study	WB	BA10	15 MDD (11 S), 15 C	Reduced mGluR5 protein expression in depression.
Mol Psychiatry (2011)	Shelton	Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression	Microarray	BA10	14 MDD (7 S), 14 C	Evidence of inflammatory, apoptotic, and oxidative stress in depression.
Prog Neuropsychopharmacol Biol Psychiatry (2009)	Feyissa	Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression.	WB	PFC	14 MDD (10 S), 10 C	Reduced expression of NR2A and NR2B, and PSD-95 protein level, with no change in the NR1 subunit.

Table 1 Continued, Findings of postmortem studies in 'Depression' and 'Suicide' populations

Journal (Year)	First author	Title	Main methods	Brain area	Subjects	Conclusion by authors
Int J Geriatr Psychiatry (2009)	Van Otterloo	Reductions in neuronal density in elderly depressed are region specific.	Cell counting	BA9	10 MDD (> 60 yrs, 5 S), 10 C	Neither the overall nor laminar density of pyramidal or non-pyramidal neurons was significantly different between groups. The cortical and laminar widths were also not affected.
Br J Psychiatry (2009)	Khundakar	Morphometric analysis of neuronal and glial cell pathology in the dorsolateral prefrontal cortex in late-life depression.	Nissl staining	DLPFC	17 MDD (1 S), 10 C	Reduced volume of pyramidal neurones in the whole cortex, which was also present in layer 3 and more markedly in layer 5. There were no comparable changes in non-pyramidal neurones and no glial differences.
Neuropsychopharmacology (2007)	Rajkowska	GABAergic neurons immunoreactive for calcium binding proteins are reduced in the prefrontal cortex in major depression.	Cell counting	DLPFC	14 MDD (9 S), 11 C	A significant reduction in the density and size of GABAergic interneurons immunoreactive for calcium binding proteins in the PFC in MDD.
J Neurosci (2007)	Kang	Gene expression profiling in postmortem prefrontal cortex of major depressive disorder.	Microarray	PFC	15 MDD (no suicide information), 15 C	Stresscopin and FoxD3 are increased in neurons of DLPFC gray matter of MDD subjects.
Altherations in 'Suicide'						
Transl Psychiatry (2016)	Schiavone	The NADPH oxidase NOX2 as a novel biomarker for suicidality: evidence from human *post mortem* brain samples	Immunostaining	Cortex	26 AS, 6 NSA, 10 C	Increased NOX2, interleukin-6 and 8-hydroxy-2'-deoxyguanosine expression in the cortex of AS subjects compared with control and NSA subjects.
Psychoneuroendocrinology (2014)	Pandya	Glucocorticoid regulates TrkB protein levels via c-Cbl dependent ubiquitination: A decrease in c-Cbl mRNA in the prefrontal cortex of suicide subjects	Q-PCR	BA10	15 S (no psychiatric information), 13C	A significant decrease in c-Cbl mRNA levels in the PFC of suicide subjects.

Table 1 Continued, Findings of postmortem studies in 'Depression' and 'Suicide' populations

Journal (Year)	First author	Title	Main methods	Brain area	Subjects	Conclusion by authors
Mol Neurobiol (2014)	Monsalve	Abnormal Expression Pattern of *Notch* Receptors, Ligands, and Downstream Effectors in the Dorsolateral Prefrontal Cortex and Amygdala of Suicidal Victims	Q-PCR	DLPFC	13 S (no psychiatric information), 13C	DLL1 levels were increased, whereas DLL4, JAGGED1, and JAGGED2 were significantly decreased in the DLPFC. HES1 was significantly reduced.
Synapse (2013)	Bach	Elevated serotonin and 5-HIAA in the brainstem and lower serotonin turnover in the prefrontal cortex of suicides	HPLC	PFC	6 S (mixed psychiatric subjects), 8 C	No significant differences in 5-HT or 5-HIAA in suicides, the mean 5-HIAA/5-HT ratio was much lower in suicides
Psychoneuroendocrinology (2013)	Pandey	Region-specific alterations in glucocorticoid receptor expression in the postmortem brain of teenage suicide victims	Proteomics	PFC	24 S (mixed psychiatric subjects), 24 C	Decreased GRa and GR inducible genes in the PFC in the suicide victims.
Plos One (2012)	Kekesi	Altered functional protein networks in the PFC and amygdala of victims of suicide	Proteomics	PFC	6 S (no psychiatric diagnosis information), 6 C	Altered functional protein network in the PFC of suicide victims.
Biol Psychiatry (2008)	Poulter	GABA-A receptor promoter hypermethylation in suicide brain: implications for the involvement of epigenetic processes	DNA methylation mapping	FPC	10 S (11 MDD, male), 13 C (male), 10 S (MDD, female), 10 C (female)	Increased DNA methyltransferase transcript in suicide, and the change in the FPC was related to the increased methylation of a gene whose mRNA expression has previously been shown to be reduced.

Abbreviations

ACC, anterior cingulate cortex; BA, Brodmann area; AS, asphyctic suicide; BD, bipolar disorder C, non-psychiatric control subjects; DLPFC, dosolateral prefrontal cortex; FPC, frontopolar cortex; GABA, gamma-Aminobutyric acid; MDD, major depressive disorder; NSA: Non-suicidal asphyxia OFC, orbital frontal cortex; PFC, prefrontal cortex; S, suicide victims, SZ, schizophrenia or schizoaffective disorder; VLPFC, ventrolateral prefrontal cortex; WB, western blot.

During the past few years, more and more scientists are realizing this issue, as summarized in several recent papers and reviews (Aleman et al., 2014). A Canadian research group was one of the first that tried to disentangle alterations due to either depression or suicide (Klempan et al., 2009; Sequeira et al., 2007; Sequeira et al., 2009). In their studies, postmortem tissues from three patients groups were compared: 1) patients who committed suicide during an episode of major depression, 2) suicide victims with no lifetime history of major depression and 3) age-matched controls with no history of suicidal behaviour, and without a major psychiatric diagnosis. They found a large number of differentially expressed genes in the hippocampus mainly when comparing the two groups of suicide completers that seemed to be associated with major depression (Sequeira et al., 2007). Further studies indeed showed that all differentially expressed GABAergic genes were clearly up-regulated in suicide completers who suffered from major depression, and down-regulated in suicide completers without a history of depressive disorders, suggesting a depression-specific effect (Sequeira et al., 2009). However, to identify molecular processes related to suicide per se, and thus independent of major depression, was problematic, since a comparison group that had suffered from major depression, but did not die by suicide, was lacking (Sequeira et al., 2007).

In contrast, in more imaging studies, designed to distinguish between depressed patients with and without suicide attempters, clear differences were found in relation to suicide (Miller et al., 2013; van Heeringen et al., 2011). A good example illustrating the importance of distinguishing suicide from depression per se, is the PET study by Miller et al, who studied three groups of patients: 1) controls without a brain disorder, 2) major depressed patients who did not have a history of suicide attempts, and 3) major depressed patients who did have a history of suicide attempts (Miller et al., 2013) and found that only the suicide attempters had lower serotonin transporter levels. In this respect, it would have been very informative if a group of patients (depressed or otherwise) that had actually died of suicide had been included as well.

Thus, in order to improve our understanding of the underlying neurobiological mechanisms of psychiatric disorders in general, and to identify possibly different molecular signatures between mood disorder and mood disorder with suicide in particular, it is important we realize that the results of previous studies have likely been obscured by the analysis of mixed groups which may have prevented a correct interpretation of the true changes at hand. We therefore propose that

in future studies, at least four groups are ideally distinguished: 1) age-matched controls without a brain disorder, 2) mood disorder patients who did not commit suicide, nor had suicide ideations, nor attempted suicide, but died from other causes instead, 3) mood disorder patients for whom suicide attempts or ideations were present, but who died from other causes, and 4) mood disorder patients who did commit suicide.

As the above-mentioned papers and the overview of earlier work on this subject (Table 1) indicate, studies with such a 4-groups design still have to be performed and tissues from such distinct groups may not be easy to collect. Yet, it will be a first very important step towards a better understanding of the molecular changes, and from there, towards better risk assessment, patient stratification and future treatment and prevention strategies of the 2 severe, life threatening, and likely inherently different, psychiatric disorders and suicide.

ACKNOWLEDGEMENTS

We wish to thank Mrs. W. Verweij for correcting the English.

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