Correspondence

Suicide Is a Confounder in Postmortem Studies on Depression

To the Editor:

Over the past decade, pioneering studies have reported changes in various genes and proteins in postmortem brain samples of major depressive disorder (MDD) patients [e.g., (1–14); see also (15–17)] (Table 1). Even though clear inferences were made based on these results regarding underlying disease mechanisms, the results differ clearly between different studies, often even in opposite directions, and even if the patient groups had been carefully matched for factors like age, sex, and postmortem delay. As a result, it is difficult to distill a uniform picture from these unique studies, let alone obtain a better insight in the neurobiological changes underlying MDD or suicide.

Based on a careful review of the literature (see Table 1) and on our own studies (18–20), we propose that part of this confusion results from the fact that suicide has always been considered a symptom, and thus an integral part of MDD (21), whereas in these studies, the group of depressed patients often contained both MDD patients who died by suicide (MDD-S) and MDD patients who died from other causes and were not suicidal (MDD-NS). As a result, postmortem studies that claimed to have determined molecular alterations in relation to MDD had selected a sample of depressed patients that consisted almost entirely of those who had died from suicide. Notably, these patients were commonly compared with control subjects who had no psychiatric disorder, thereby not controlling for the fact that suicide per se may have influenced gene expression in the postmortem brain as well, and may thus have confounded findings that were interpreted as being specific for depression alone. Studies that claimed to have shown changes in relation to suicide had generally compared cases who had committed suicide with matched controls without any psychiatric disorder, thereby disregarding the fact that suicide occurs in many psychiatric disorders and conditions, including mood and anxiety disorders, but also in schizophrenia, personality disorder, or substance abuse disorder (21,22).

To date, very few articles studied (molecular) differences between suicide and depression. Turecki's group (23) attempted to disentangle alterations due to either depression or suicide by comparing three groups: 1) patients who committed suicide during an episode of MDD, 2) suicide victims without a lifetime history of MDD, and 3) age-matched control subjects with no history of suicidal behavior and without a major psychiatric diagnosis. Consistent with a contribution of this confounder, they indeed found several differentially expressed genes in the hippocampus when comparing the two groups of suicide completers (23). Follow-up studies showed that all differentially expressed gamma-aminobutyric acid (GABA)–related genes were clearly upregulated in suicide completers with MDD but were downregulated in suicide completers without MDD, suggesting a depression-specific effect on GABAergic genes (11). Furthermore, Pandey's group (24) tried to divide the suicide group into a subgroup with depression and one with other psychiatric disorders, thereby separating effects of depression on suicide-related changes from contributions by other psychiatric conditions. Scifo *et al.* (25) further used statistics to assess a role for suicide in MDD. We applaud and highlight these approaches. Ideally, as a next step, future studies could assess which molecular changes relate to suicide per se, i.e., are independent of MDD. This would involve including a fourth comparison group of patients that had experienced MDD but did not die from suicide.

Also, other studies on MDD show clear differences when suicide is being taken in account; Gray *et al.* (26) analyzed MDD-S and MDD-NS patients and found increased dorsolateral prefrontal cortex expression of several glutamate receptor–related genes in MDD-S subjects. Furthermore, the use of other approaches illustrates the importance of distinguishing suicide from MDD per se; e.g., Miller *et al.* (27) used positron emission tomography imaging to study live subjects, comparing 1) control subjects without a brain disorder, 2) MDD patients without a history of suicide attempts, and 3) MDD patients with a history of suicide attempts. They found that only the suicide attempters had lower serotonin transporter levels. A group of patients (depressed or otherwise) that had actually died by suicide was lacking for obvious reasons. Also, in our own studies, we found differences in GABA/glutamate-related genes between MDD-S and MDD-NS patients in the prefrontal cortex (20). Furthermore, a recent meta-analysis showed that it is possible to distinguish suicidal from non-suicidal patients based on cytokine levels (28).

Thus, to improve our understanding of the underlying neurobiological mechanisms and molecular "signatures" of depression, it is important to realize that earlier postmortem studies may have analyzed mixed groups, in which patients with and without suicide attempts were combined. This may have overlooked brain changes that are specific for suicide per se and thereby obscured a correct interpretation of the changes specific for depression alone.

Therefore, we propose that in future postmortem studies on mood disorders, ideally, at least four groups be distinguished: 1) age-matched control subjects without a brain disorder; 2) depressed patients who did not commit suicide and did not have suicidal ideations or attempts, but died from other causes; 3) depressed patients with suicide attempts or ideations, but who died from other causes; and 4) depressed patients with accomplished suicide. While brain material for such studies may not be easy to collect, it will form a very important next step toward a better understanding of the true molecular changes at play in MDD per se and, from there, may enable a better risk assessment, patient stratification, and future treatment strategies for the two severe, life-threatening, and likely inherently different, psychiatric disorders: MDD with suicide and MDD without suicide.

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Table 1. An Overview of Recent Postmortem Studies on Major Depressed and Suicide Populations

Citation	Main Methods	Brain Area	Subjects	Conclusion by Authors
Alterations in "Depression"				
Zhou *et al.* (29)	ncRNA expression analysis, WGCNA	ACC	26 Dep-S, 24 C	lncRNAs are differentially expressed in MDD patients who died by suicide and may represent regulators of important molecular functions and biological processes
Cobb *et al.* (1)	Immunostaining	Hipp	17 MDD (10 S), 17 C	Decreased density of GFAP-immunoreactive astrocytes in left hippocampus in MDD
Gajewski *et al.* (30)	WB	PFC, Hipp	14 MDD nonmedicated (no suicide info), 13 MDD medicated, 11 C	Downregulation of ΔFosB and other FosB isoforms in the hippocampus, but not the PFC, in the brains of both depressed and addicted individuals
Clark *et al.* (2)	RT-PCR, histology, immunohistochemistry	VLPFC	45 MDD (25 S), 36 C	Depression, in the absence of medical illness or an overt inflammatory process, is associated with compromised kynurenine pathway metabolism in the VLPFC
Kunii *et al.* (4)	RT-PCR, in situ	DLPFC	176 SZ, 61 BD, 138 MDD, 326 C	Abnormalities in the *CHRFAM7A*/*CHRNA7* ratios in SZ and BD, mainly owing to overexpression of *CHRFAM7A*
Reinhart *et al.* (5)	RT-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)
Rivero *et al.* (31)	Radio binding assay	BA9	26 MDD (24 S), 26 C	Upregulation of brain α_2- and β_1-adrenoceptors in depression
Smalheiser *et al.* (32)	RT-PCR	PFC	15 SZ (7 S), 15 BD (8 S), 15 MDD (7 S), 15 C	Altered discrete microRNA in all disorders, as well as in suicide subjects (pooled across diagnostic categories) compared with all nonsuicide subjects
Hamazaki *et al.* (33)	Lipid acid comparison	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 nonsuicide cases.
Ota *et al.* (34)	RT-PCR	PFC	First cohort: 36 MDD (no suicide info), 36 C; second cohort: 37 MDD (no suicide info), 35 C	Increased REDD1 levels in the PFC of MDD
Martins-de-Souza *et al.* (7)	Proteomics	BA9	24 MDD (17 S), 12 C	Altered protein level in energy metabolism, synaptic function, histidine triad nucleotide binding protein 1 in MDD
Jernigan *et al.* (35)	WB	PFC	12 MDD (10 S), 12 C	Deficits in p70S6K/eIF4B pathway in MDD
Gittins and Harrison (17)	Immunoautoradiography	ACC	5 MDD + 2 BD (4 S), 9 C	Reduced mTOR, p70S6K, eIF4B, and p-eIF4B protein expression in MDD subjects relative to control subjects. No group differences were observed in eIF4E, p-eIF4E, or actin levels.
Deschwanden *et al.* (36)	WB	BA10	15 MDD (11 S), 15 C	Reduced mGluR5 protein expression in depression
Shelton *et al.* (9)	Microarray	BA10	14 MDD (7 S), 14 C	Altered gene expression in inflammation and apoptosis in frontal cortex in MDD
Feyissa *et al.* (12)	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 in MDD
van Otterloo *et al.* (37)	Cell counting	BA9	10 MDD (>60 years of age, 5 S), 10 C	No difference on overall or laminar density of pyramidal or nonpyramidal neurons
Khundakar *et al.* (38)	Nissl staining	DLPFC	17 MDD (1 S), 10 C	Reduced volume of pyramidal neurons in the whole cortex (also in layer 3 and layer 5). No comparable changes in nonpyramidal neurons and no glial differences.
Rajkowska *et al.* (39)	Cell counting	DLPFC	14 MDD (9 S), 11 C	Reduced density and size of GABAergic interneurons immunoreactive for calcium binding proteins in the PFC in MDD
Kang *et al.* (40)	Microarray	PFC	15 MDD (no suicide info), 15 C	Increased stresscopin and FoxD3 in neurons of DLPFC of MDD

Table 1. Continued

Citation	Main Methods	Brain Area	Subjects	Conclusion by Authors
Alterations in "Suicide"				
Weissmann *et al.* (3)	Capillary electrophoresis single-stranded conformational polymorphism	BA9, BA24	8 S (8 MDD), 8 C	Region-specific changes in RNA editing of 5-HT_{2C} receptor mRNA in MDD or suicide subjects
Schiavone *et al.* (41)	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
Pandya *et al.* (42)	RT-PCR	BA10	15 S (no psychiatric info), 13 C	Decreased c-Cbl mRNA levels in the PFC of suicide subjects
Monsalve *et al.* (43)	RT-PCR	DLPFC	13 S (no psychiatric info), 13 C	Increased DLL1, decreased DLL4, JAGGED1, and JAGGED2 in the DLPFC
Bach *et al.* (14)	HPLC	PFC	6 S (mixed psychiatric subjects), 8 C	No significant differences in 5-HT or 5-HIAA, but lower mean 5-HIAA/5-HT ratio in suicides
Pandey *et al.* (44)	Proteomics	PFC	24 S (mixed psychiatric subjects), 24 C	Decreased GRa- and GR-inducible genes in the PFC of suicide subjects
Kekesi *et al.* (8)	Proteomics	PFC	6 S (no psychiatric diagnosis info), 6 C	Altered functional protein network in the PFC of suicide subjects
Poulter *et al.* (13)	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 subjects

A detailed comparison of the patient descriptions highlights that in many studies, the depression group contains both cases that did and did not die of suicide (see Subjects column) and the same applies for articles in which suicide per se was studied, in which depression is often a confounder in many cases.

5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; ACC, anterior cingulate cortex; AS, asphyctic suicide; BA, Brodmann area; BD, bipolar disorder; BDNF, brain-derived neurotrophic factor; C, nonpsychiatric control subjects; Dep, depression; DLPFC, dorsolateral prefrontal cortex; eIF, eukaryotic translation initiation factor; FPC, frontopolar cortex; GABA, gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; GR, glucocorticoid receptor; Hipp, hippocampus; info, information; HPLC, high-performance liquid chromatography; lncRNA, long noncoding RNA; MDD, major depressive disorder; mGluR5, metabotropic glutamate receptor 5; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; ncRNA, noncoding RNA; NOX2, nicotinamide adenine dinucleotide phosphate oxidase isoform 2; NSA, nonsuicidal asphyxia; p-eIF, phosphorylated eukaryotic translation initiation factor; PFC, prefrontal cortex; PSD, postsynaptic density protein; PUFA, polyunsaturated fatty acids; RT-PCR, reverse transcriptase polymerase chain reaction; S, suicide subjects; SZ, schizophrenia; TrkB, tropomyosin receptor kinase B; VLPFC, ventrolateral prefrontal cortex; WB, Western blot; WGCNA, weighted gene co-expression network analysis.

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Article Information

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