Brain Gene Expression Pattern of Subjects with Completed Suicide and Comorbid Substance Use Disorder

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Keywords

Postmortem · Dorsolateral prefrontal cortex · Expression study · Microarray · Transcriptomics · Substance use disorder

Abstract

Background/Aim: Although individuals with substance use disorder (SUD) are at high risk of committing suicide, most studies of postmortem gene expression exclude subjects with SUD due to the potential confounding effect of drugs in the transcriptome. Thus, little is known about the gene expression profile in suicides with SUD. The identification of altered biological processes in suicides with SUD is crucial in the comprehension of the interaction between both pathologies. **Methods:** We evaluated the gene expression profile in

the dorsolateral prefrontal area of suicides and nonsuicides with and without SUD by microarrays. **Results:** We identified 222 differentially expressed genes, predominately enriched in cell proliferation in the comparison between suicides with and without SUD. When comparing the transcriptome of suicides with SUD to nonsuicides with SUD, we identified 550 differentially expressed genes, mainly enriched in oxidative phosphorylation. Differentially expressed genes (1,417) between suicides and nonsuicides without SUD were detected. Most of them were related to mitochondrial function. **Conclusion:** Interaction between suicide and SUD seems to influence the expression of genes involved in glial proliferation and glutamatergic neurotransmission. These results highlight, for the first time, that suicides with SUD have a gene expression profile distinct from that of subjects with only one of these disorders.

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Introduction

According to the World Health Organization's latest report, more than 800,000 people committed suicide in 2015, making it the 17th most frequent cause of death worldwide [1]. Suicide rates have been increasing in many countries, including Mexico, where they increased from 3.5 to 5.2 suicides per 100,000 inhabitants between 2000 and 2014 [2]. A first step in decreasing the suicide rate is to identify the factors associated with this behavior. There has been an association between suicidal behavior and certain sociocultural, situational, and individual factors, such as access to health services, interpersonal conflicts, divorce, unemployment, isolation, lack of social support, previous suicide attempt, and presence of mental disorders [1, 3].

Several postmortem studies using a psychological autopsy have confirmed the association between suicide and the presence of mental disorders. The well-known review of Fleischmann et al. [4] reported that 88.6% of the suicide victims suffered from a mental disorder, where mood disorders were the most frequent diagnosis (42.1%), followed very closely by substance use disorder (SUD) [4]. Similar rates of mental illnesses among individuals who successfully committed suicide have been recently reported [5, 6].

It is estimated that, compared to the general population, subjects with alcohol abuse are almost ten times more likely to commit suicide, whereas people with abuse of other substances are approximately fourteen times more likely to commit this act [7]. Dependence on several substances such as alcohol, inhalants, heroin [8], and tobacco [9] have also been associated with suicidal behavior. Although subjects with SUD are at an increased risk for suicidal behavior, most postmortem suicide gene expression studies have excluded this group of patients [10]. Therefore, little is known about the molecular basis of the association between SUD and suicidal behavior. A recent study by Gandal et al. [11] (2018) evaluated the transcriptome of five mental disorders; autism, schizophrenia, bipolar disorder, major depression, and alcohol use disorder (AUD), where they observed a lack of overlap between the AUD and the other mental disorders. This result suggests that a different gene expression profile exists in suicides with SUD compared to subjects with other mental pathologies.

The prefrontal cortex is the brain region responsible for decision making, inhibition and short-term memory, functions known to be altered in subjects with suicidal behavior [12, 13]. Structural and functional changes in

the prefrontal cortex, specifically the dorsolateral prefrontal cortex, as well as alterations in cognitive abilities associated with the prefrontal region, have been identified in subjects with suicidal behavior and in subjects with SUD [14]. Therefore, it is of particular interest to evaluate gene expression in this brain region in suicidal subjects with and without SUD, in order to have a better understanding of the interaction between suicide and SUD. The identified genes could be used as potential biomarkers of suicidal behavior in subjects with SUD and ultimately for the implementation of novel treatments for this population. In this preliminary study, we compared the gene expression profile in the dorsolateral prefrontal area of subjects who committed suicide with and without SUD to the transcriptome of subjects with and without SUD who died due to a cause other than suicide.

Materials and Methods

Subjects and Brain Samples

Postmortem brain samples were obtained from the Institute of Forensic Sciences (INCIFO) in Mexico City, Mexico, from subjects who committed suicide and subjects with sudden death in 2016 in Mexico City. Suicide completers are defined as those individuals whose causes of death were self-inflicted injuries, which correspond to X60–X84 codes of the International Classification of Diseases, 10th Revision (ICD-10). The nonsuicidal subjects presented sudden, not self-inflicted death (for example, a car accident) without a period of prolonged agonal state or prolonged illness. The Ethics Committee for Human Research at the National Institute of Genomic Medicine approved this research.

Fresh brain tissue samples from the dorsolateral prefrontal cortex (Brodmann area 9) were dissected using the middle frontal gyrus and the precentral sulcus anatomical references and stored in RNAlater, an RNA stabilization reagent (Qiagen, Singapore) at -80°C until its use. The postmortem interval (PMI) represents the time interval between the estimated time of death and the sample collection, which ranged from 06:50 to 29:30 h. A toxicology test for detecting substance consumption at the time of death was performed in both peripheral blood and brain tissue.

Subjects were psychiatrically characterized by a diagnostic estimation based on data obtained through from the legal medical records, which contain (i) declarations from the police, relatives, and witnesses, (ii) demographic information, (iii) acute and chronic stressful life situations, (iv) autopsy and toxicological reports, and (v) clinical records in case the individual received medical attention. A consensus diagnosis, based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria, was achieved between a pathologist, a psychologist, and a psychiatrist, and then reviewed and confirmed by an independent experienced psychiatrist. The complete methods for diagnostic estimation are described elsewhere [15].

Sixty-six samples were collected, considering the following experimental groups: 23 suicide completers with comorbid SUD (Suicidal SUD+ group), 20 suicide completers without SUD (Sui-

Table 1. Demographic, clinical, and suicide subjects' characteristics

SUD	Suicides		Nonsuicides		<i>p</i> value
	+	–	+	–	
Subjects	23	20	9	14	
Age, years	31.95±17.43	32.8±15.19	30.88±7.04	31.78±19.51	0.645
Gender (M:F)	21:2	12:8	8:1	8:6	0.028
PMI, h	14.91±3.91	15.03±4.81	17.76±7.07	16.84±4.44	0.128
AUD	17	0	8	0	
Positive result in toxicology test	16	3	4	0	
Cause of death ^a	19/0/2/1/1/0/0	16/1/1/1/1/0/0	1/2/0/3/2/1/0	4/4/0/2/0/1/4	

Values indicate number of subjects unless otherwise indicated. Continuous data is presented as mean ± standard deviation. +, positive; –, negative; SUD, substance use disorders; M, male; F, female; PMI, postmortem interval; AUD, alcohol use disorder. ^a Asphyxia/gunshot/intoxication/trauma/puncture wound/traffic accident/shock. Two-tailed *p* value test is described in Methods.

cidal SUD– group), 9 subjects with SUD whose cause of death was not suicide (Nonsuicidal SUD+ group), and 14 nonsuicide subjects without SUD (Nonsuicidal SUD– group); the latter two groups were named as nonsuicides for the purposes of this study. Demographic, clinical, and suicide subjects' characteristics are described in Table 1. Categorical variables between experimental groups, such as gender, were assessed by Fisher's exact test, and continuous variables, for instance age and PMI, using analysis of the variance (ANOVA) tests. The interactive comparison between the experimental groups is illustrated in Figure 1.

RNA Isolation

RNA isolation was performed using the RNeasy kit from Qia-gen™ following the manufacturer's instructions. The purity of the extracted RNA was evaluated on the NanoDrop1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and its integrity by electrophoresis with 1% agarose gel. Subsequently, the RNA integrity number of the samples was evaluated using the Agilent Bioanalyzer. Only samples with an RNA integrity number >7 were further processed.

Microarray Detection

The gene expression of the 66 samples was analyzed using the Illumina HumanHT-12 v4 BeadChip microarray, which contains 47,231 probes. RNA samples were labeled into biotinylated cRNAs with the TargetAmp™-Nano Labeling Kit (Epicentre), followed by hybridization into the BeadChips according to the manufacturer's protocol. The BeadChips were scanned on an iScan Microarray Scanner (Illumina) immediately after the protocol.

Microarray Analysis

For data pre-processing the 47,231 probes contained in the microarray were annotated so that each one was assigned their respective EntrezID and gene symbol with the Bioconductor "IlluminaHumanv4.db" package [16]. Then, raw probe intensities were background corrected with the "normexp" method with "mle" maximum likelihood approximation in the "limma" package [17–19]. Subsequently, control, ERCC, and unannotated probes (without a valid EntrezID number) were filtered out. Low detection probes, namely those with a detection *p* value >0.05 and those not

Fig. 1. Venn diagrams for the interactive comparison between experimental groups. In this study, the gene expression profile of Brodmann area 9 of suicides with substance use disorder (Suicidal SUD+) and nonsuicides without SUD (Nonsuicidal SUD) were compared to suicides without SUD (Suicidal SUD–) and nonsuicides with SUD (Nonsuicidal SUD+). The complete lists of differentially expressed genes in these comparisons are shown in the online supplementary material.

present in at least 50% of arrays in each experimental group, were removed.

Gene expression values were normalized to compensate for systematic technical differences between the arrays by the quantile method and log₂-transformed [20]. Replicate probes within the arrays were summarized and replaced with their average value. To visualize the data and evaluate possible batch effects, we performed a principal component analysis where we could detect a batch effect associated with an unknown source of variation. A multivariate analysis with the ARSYN package was executed to remove such an effect [21].

The resulting data comprises a 2 × 2 factorial experimental design. The first factor is whether the individual committed suicide

Fig. 2. Principal component analysis (PCA) scatter plot of the studied subjects. **a** Using quantile-normalized gene expression levels. **b** After multi-dimensional normalization using ARSyN. It is worth noting that in **a** the experimental groups are confounded due to an excess of uncontrolled variability (batch effect). This effect was corrected in **b**, where the first two principal components (PC1 and PC2) demonstrated a clear separation of these groups. Experimental groups: suicide (Suicidal), nonsuicide (Nonsuicidal), with or without substance use disorder (SUD+/SUD-).

or not. The second factor is the absence or presence of SUD. The data was analyzed using a gene by gene independent linear model using the Bioconductor “limma” package [17, 18] according to equation (1):

$$y_{ijk} = \mu + \alpha I_{\text{Suicide}_i} + \beta \gamma I_{\text{Substance}_j} + \gamma I_{\text{Suicide}_i} \times I_{\text{Substance}_j} + \epsilon_{ijk} \quad (1)$$

where, y_{ijk} is the $\log_2$ gene expression level for the i -th suicide level, under the j -th SUD level, in the k -th biological replicate; μ is the global average gene expression; α , β , and γ are the suicide and SUD coefficients, respectively; I_{Suicide_i} and $I_{\text{Substance}_j}$ are the indicator dummy functions (0 or 1), for i -th suicide and j -th SUD levels, respectively; $\epsilon_{ijk} \sim N(0, \sigma^2)$ is the random error which is normally distributed with zero expected value and variance equal to σ^2 for the i -th suicide, j -th substance, and k -th biological replicate respective levels.

Principal effect and corresponding double interaction assessments were carried out using Type-III sum of squares. Biological marginal hypothesis tests between specific experimental groups were performed using a two-tailed F test using the complete estimated linear model parameters. p values associated with the differentially expressed genes were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate method [22]. For the purposes of this study, the significant level was set to 0.001. Visualization of the results from each comparison was car-

ried out by means of heatmaps using “gplot” [23]. The data preprocessing and processing were executed in R [24].

Functional Annotation Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID), version 6.8, was used for over-representation analysis [25, 26]. The annotation categories were restricted to use: Kyoto Encyclopedia of Gene and Genomes (KEGG), Gene Ontology (GO), and Reactome. The up- and downregulated genes were analyzed separately in DAVID, since this approach is more powerful for finding significant functional pathways documented in the databases [27]. Only the resulting 6,862 transcripts were used as background reference. Biological pathways with a modified Fisher exact p value < 0.05 (EASE score) were considered enriched [28]. In addition, Ingenuity® Pathway Analysis software (IPA®, QIAGEN Redwood City) was used to complement the analysis [29].

Results

Subjects and Brain Samples

According to the demographic, clinical, and suicide subjects’ characteristics presented in Table 1, no signifi-

Fig. 3. Substance use comparison heatmap for suicide subjects. The 222 differentially expressed genes (in columns) between suicide subjects with substance use disorder (Suicidal SUD+) and suicide subjects without SUD (Suicidal SUD-), with red and blue color, respectively (in rows). Gene expression levels are depicted in red-green spectrum for down-/upregulated genes.

cant differences were observed between the experimental groups in terms of age (p value = 0.645) and PMI (p value = 0.128). However, there was an association between gender and experimental group (p value = 0.028). In this context, more men than women successfully committed suicide across all the groups. This fact is consistent with previous reports in the Mexican population, where it is known that the majority of those who commit suicide or suffer from SUD belong to the male gender [2].

In the suicide with SUD group, 73.91% (20/23) of the subjects had AUD, whereas in the nonsuicidal group with SUD, 88.88% (8/9) of the subjects had AUD. From the subjects who obtained a positive result in the toxicology test from the suicide with SUD group, 87.5% were positive for alcohol only and 12.5% were positive for alcohol and cocaine. Subjects from the other groups were only positive for alcohol.

The quantile-normalized gene expression levels were inspected for batch effects using principal component analysis (PCA) over the subjects (Fig. 2a). Together, the first two components (PC1 and PC2) represented 50.98% of the total variability. Unfortunately, the experimental groups were mixed together whereas one would have ex-

pected that they clustered. However, after applying AR-SyN (Fig. 2b), a noteworthy separation along the PC1 axis formed three clusters: (i) Suicidal SUD- subjects, (ii) Suicidal SUD+ subjects, and (iii) nonsuicides (Nonsuicidal SUD+ and Nonsuicidal SUD-), using only 13.44% of the total gene expression variance.

Suicidal SUD+ versus Suicidal SUD-

In the comparison between subjects who committed suicide and had SUD ($n = 23$) and subjects who committed suicide without SUD ($n = 20$), 222 differentially expressed genes were detected, yielding 112/110 genes up-/downregulated, respectively. Visual inspection over the corresponding heatmap showed that the two experimental groups clustered together (Fig. 3). The complete list of differentially expressed genes in this comparison is shown in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000493940).

The functional analysis results of the downregulated genes showed that GO terms were significantly enriched in cation transport, neuron part, signaling, synapse, immune response receptors, behavior, learning or memory, and cellular respiration. The upregulated genes showed

Fig. 4. Top 5 enriched canonical pathways from IPA. Canonical pathways related to upregulated genes (red bars) and downregulated genes (green bars) detected when comparing suicides with substance use disorder (Suicidal SUD+) to suicides without SUD (Suicidal SUD–).

Fig. 5. Suicide comparison heatmap for subjects with substance use disorder (SUD). The 550 differentially expressed genes (in columns) between suicides with SUD (Suicidal SUD+) and nonsuicidal subjects with SUD (Nonsuicidal SUD+), with red and blue color, respectively (in rows). Gene expression levels are depicted in red-green spectrum for down-/upregulated genes.

Fig. 6. Top 5 enriched canonical pathways from IPA. Canonical pathways related to upregulated genes (red bars) and downregulated genes (green bars) detected when comparing suicides with substance use disorder (Suicidal SUD+) to nonsuicides with SUD (Nonsuicidal SUD+).

enrichment for GO terms related to epithelium development, cell adhesion, regulation of nervous system development, regulation of gene expression, and phosphatidylinositol-mediated signaling. The complete list of related GO terms is available in online supplementary Table 1.

The joint functional analysis, including both up- and downregulated genes, indicated that the genes identified as differentially expressed genes between suicidal with SUD (Suicidal SUD+) and suicidal without SUD (Suicidal SUD-) groups were associated with cell differentiation, cell death, cell adhesion, and neurogenesis. Since cell differentiation was the GO term associated with the highest percentage of genes, we further investigated terms related to this GO category in this comparison and found “neuron and glial cell differentiation” with a significant p value. The complete list of related GO terms resulting from this joint analysis is available in online supplementary Table 2.

Moreover, the top five canonical pathways identified by IPA ranked by their p value related to the up- and downregulated genes are illustrated in Figure 4. The top ten canonical pathways derived from the analysis of all

differentially expressed genes is available in online supplementary Figure S1.

Suicidal SUD+ versus Nonsuicidal SUD+

The gene expression profile for suicides with SUD ($n = 23$) with respect to suicides without SUD ($n = 9$) showed 550 differentially expressed genes, of which 438 were upregulated and 112 downregulated. Visual inspection over the corresponding heatmap showed that the two experimental groups clustered together (Fig. 5). The complete list of differentially expressed genes in this comparison is in the online supplementary material.

In this comparison, the downregulated genes showed enriched GO terms related to signal peptides, receptor, zinc finger proteins, and metabolic processes. The upregulated genes were associated with the mitochondrial membrane, antigen processing and presentation, and cytoskeleton. The complete list of GO terms is available in online supplementary Table 3.

When analyzing the complete list of differentially expressed genes between suicidal individuals with SUD and nonsuicidal individuals with SUD, we found that they were associated with immune response, oxidative phosphoryla-

Fig. 7. Suicide comparison heatmap for subjects without substance use disorder (SUD). The 1,417 differentially expressed genes (in columns) between suicides without SUD (Suicidal SUD-) and nonsuicidal subjects without SUD (Nonsuicidal SUD-), with red and blue color, respectively (in rows). Gene expression levels are depicted in red-green spectrum for down-/upregulated genes.

tion, Parkinson's disease, and mitotic cell cycle phase transition. The complete list of GO terms obtained from this joint analysis is available in online supplementary Table 4.

The top five canonical pathways identified by IPA ranked by *p* value related to differentially expressed genes are illustrated in Figure 6. The top ten canonical pathways resulting from the joint analysis of differentially expressed genes identified in this comparison are available in online supplementary Figure S2.

Suicidal SUD- versus Nonsuicidal SUD-

In the comparison between suicides without SUD ($n = 20$) and nonsuicides without SUD ($n = 14$), 1,417 differentially expressed genes were detected, yielding 923/494 genes up-/downregulated, respectively. The corresponding heatmap showed that the two experimental groups clustered together (Fig. 7). The complete list of differentially expressed genes identified in this comparison is available in the online supplementary material.

In this comparison, the downregulated genes exhibited enriched GO terms associated with cell communication, regulation of gene expression, neurogenesis, and programmed cell death. Regarding the last term, it is

noteworthy that it includes genes of both positive and negative regulation of this process. The upregulated genes were related to mitochondria, programmed cell death, ion transport, and neuron projection. The complete list of GO terms from this analysis is available in online supplementary Table 5.

In the joint functional analysis, with both up- and downregulated genes, we observed that most of the genes identified as differentially expressed genes between suicidal without SUD and nonsuicidal without SUD groups were enriched in GO terms related to mitochondrion, regulation of cell communication, programmed cell death, and neuron differentiation (online suppl. Table 6).

The top five canonical pathways identified by IPA ranked by *p* value related to differentially expressed genes are illustrated in Figure 8. The top ten canonical pathways resulting from the analysis of all differentially expressed genes identified in this comparison is available in online supplementary Figure S3.

Nonsuicidal SUD+ versus Nonsuicidal SUD-

When comparing nonsuicides with SUD to nonsuicides without SUD, we identified only one gene to be

Fig. 8. Top 5 enriched canonical pathways from IPA. Canonical pathways related to upregulated genes (red bars) and downregulated genes (green bars) detected when comparing suicides with substance use disorder (Suicidal SUD-) to nonsuicides with SUD (Nonsuicidal SUD-).

downregulated, *LOC285074*, which encodes for the anaphase promoting complex subunit 1 pseudogene.

Interaction between Suicide and SUD

The interaction term (suicide by SUD) exhibited influence in the expression of 55 genes; 26 of these genes were downregulated and 29 were upregulated. The functional enriched GO terms related to these genes are shown in Table 2. Results of IPA for such genes are available in on-line supplementary Figure S4.

Discussion

To the best of our knowledge, this is the first transcriptome-level study comparing gene expression profiles in the dorsolateral prefrontal cortex of suicides with SUD and suicides without this disorder, as SUD has been considered in most studies as a confounder and hence excluded from the analysis. Two studies identified multiple probesets whose expression was influenced by alcohol or cocaine use in the ventral prefrontal cortex [30], limbic system, and posterior cingulate gyrus [31] in suicides

with major depression of French-Canadian origin. One of these studies [32] included drug or alcohol use in their statistical models, without finding an association between substance abuse and the expression of differentially expressed genes in Brodmann area 46. The other examined the effects of alcohol on genes identified as differentially expressed in a larger French-Canadian sample and in a murine model of acute and chronic alcohol use, discarding the fact that the pattern of gene expression observed in the study was due to the use of substances [33]. Most of the studies have either excluded subjects based on toxicology reports, which only provide information about the use of certain drugs at the moment of death, or ignored the issue altogether [10].

Suicidal SUD+ versus Suicidal SUD-

When comparing suicides with SUD to suicides without SUD, an evident overexpression of genes participating in pathways involved in the regulation of cellular proliferation and migration, such as Rho family GTPases, glioma invasiveness signaling, and SP1 signaling, known for its determinant role in cell survival and proliferation, was identified [34, 35]. Parkinson's signaling is one of the

Table 2. GO functional terms associated with the genes participating in the interaction between SUD and suicide

GO ID	GO name	<i>n</i> (%)	EASE*	Upregulated genes	Downregulated genes
GO:0016740	Transferase activity	14 (25.92)	0.037	<i>CDK19, PACSIN3, PTAR1, RASSF2, YES1, PTPN11</i>	<i>HSP90AA1, MAP2K4, POLR3A, SMS, UHMK1, ISCU, KLHL28, GTF2H3</i>
GO:0016301	Kinase activity	9 (16.66)	0.007	<i>CDK19, HSP90AA1, PACSIN3, RASSF2, GTF2H3, YES1, UHMK1, PTPN11</i>	<i>MAP2K4</i>
GO:0010001	Glial cell differentiation	5 (9.25)	0.005	<i>SOX10, OLIG1, NDRG1, CNP, PTPN11</i>	
GO:0006950	Response to stress	18 (33.33)	0.027	<i>CDK19, HMGB1, HSP90AA1, SNX6, AP2S1, GTF2H3, COPS8, CNOT7, PTPN11, LSM14A, PCBP4, RASSF2, NDRG1, YES1,</i>	<i>UCHL1, MAP2K4, POLR3A, AACCS</i>
GO:0002682	Regulation of immune system process	9 (16.66)	0.016	<i>LSM14A, HMGB1, HSP90AA1, SNX6, AP2S1, RASSF2, YES1, PTPN11</i>	<i>MAP2K4</i>

GO, Gene Ontology; SUD, substance use disorder. * Modified Fisher exact *p* value.

enriched pathways for the downregulated genes in this comparison, which include the pro-apoptotic genes *CYCC*, *UCHL1*, and *SNCA* [36]. Of note, the abnormal expression of genes involved in cell proliferation and its regulation, such as *MAPK*, suggests that suicides with SUD may have an increased cell proliferation, which could lead to structural abnormalities in the prefrontal cortex of suicides with SUD and lead to an impaired function of this brain area.

This probable increased cell proliferation, if corroborated by other studies, could lead to a higher cell density which would be different from the decreased neuronal and glial density in the prefrontal cortex reported for subjects with AUD in suicides [37–39]. Lower neuronal density has been reported in Brodmann area 9 [40]; however, the glial density has only been evaluated in the white matter of this brain area, where abnormal density of microglia was found in subjects who committed suicide [41].

Among the genes identified as differentially expressed between suicides with SUD and suicides without SUD are *SLC1A2*, *SLC1A3*, and *GLUD1*, which encode for proteins involved in glutamate metabolism. The first two genes are transporters of this neurotransmitter; *GLUD 1*, glutamate dehydrogenase, catalyzes the oxidative deamination of glutamate. The alteration of glutamatergic neurotransmission in subjects with suicidal behavior is well documented; however, an interesting finding from our

study is the overexpression of these genes in suicide subjects with SUD, contrary to previous postmortem studies of suicide with depression [42] and bipolar disorder [43] in the same brain area. This overexpression could be due to the chronic exposure to ethanol in most of the suicide subjects with SUD in our sample, as alterations in glutamatergic neurotransmission have been reported in subjects with alcohol dependence [44] and in animal models of alcoholism [45]. Our finding suggests that suicide with SUD present alterations in glutamatergic neurotransmission, and these abnormalities are different from those found in suicide without this comorbidity.

Another interesting finding is the overexpression of genes that encode for connexins 30 and 43 in suicide with SUD. Reduced expression of these genes has been reported in suicide cases [33] in addition to a reduced expression of its protein in subjects with alcohol dependence [46]. The finding that the expression of genes involved in glutamatergic neurotransmission and genes for connexins 30 and 43 are in the opposite direction to that previously reported in suicide with other psychiatric pathologies indicates that the presence of SUD leads to unique changes in gene expression in suicide subjects. If replicated in other populations, and corroborated by functional analysis, these findings could have important therapeutic implications, leading to treatment strategies focused on specific alterations in patients with SUD who could be at risk of committing suicide.

Suicidal SUD+ versus Nonsuicidal SUD+

An overexpression of genes involved in oxidative phosphorylation and mitochondrial function was detected in suicides with SUD compared to nonsuicide subjects with SUD, which may indicate an increased energy demand with a potential impact on synaptic and neuronal function. Since mitochondrially formed oxidants activate several signaling pathways related to apoptosis [47], this GO term was associated with the differentially expressed genes identified in this comparison, as expected.

In addition, mitochondrial function is crucial in neural migration, an especially relevant process, given the nature of the tissue studied [48–50]. Other canonical pathways associated with the differentially expressed genes from this comparison are the clathrin-mediated endocytosis [51] and chondroitin sulfate synthesis [52], which are also implicated in neural migration. This suggests a dysregulation in this function in suicides with SUD, perhaps as part of a synaptic refinement process [53].

Suicidal SUD– versus Nonsuicidal SUD–

As in the previous comparison, we detected an overexpression of genes involved in mitochondrial function. This observation is interesting, since both comparisons are between suicidal and nonsuicidal subjects in the presence of SUD and in the absence of this disorder, respectively. This suggests that the increased expression of mitochondrial genes could be relevant in the pathophysiology of suicide independently of the comorbidity with SUD.

Pantazatos et al. [54] reported an increased expression of genes involved in “DNA-dependent ATPase activity” in suicides compared to nonsuicides ($p < 0.1$ corrected). Therefore, we anticipate that future studies focusing on the mitochondrial function of subjects with suicidal behavior may help to clarify the role of this process in suicide.

Nonsuicidal SUD+ versus Nonsuicidal SUD–

When we compared the gene expression profile of nonsuicides with SUD to nonsuicides without SUD we detected the downregulation of *LOC285074*, which encodes for a pseudogene. Our criteria for considering a transcript as differentially expressed between groups was probably especially strict in order to detect more differences in this comparison. In the future, with the knowledge generated from other studies, we may be able to confirm if this pseudogene is relevant in the SUD pathology in nonsuicidal subjects.

Of note, results obtained from studies in neurodegenerative diseases suggest that pseudogenes and other non-

coding transcripts could participate in the regulation of gene expression through competition for miRNA-occupied sites [55].

Interaction between Suicide and SUD

In addition to finding an overexpression of genes involved in cell proliferation in the comparison between suicides with SUD and suicides without this comorbidity, genes involved in this process were detected among the probes influenced by the interaction between SUD and completed suicide, including *CNOT*, *ATP6VOC*, *MARCKSL1*, *NDRG1*, and *RAN*, suggesting that this process may be relevant in the interaction of both disorders. The overexpression of genes that participate in the differentiation of glial cells, such as *SOX10*, *OLIG1*, *NDRG1*, *CNP*, and *PTPN11*, may be potentially relevant in suicide with SUD. Suicide subjects with alcohol dependence have been shown to present higher glial density in the anterior cingulate cortex compared with suicide subjects without dependence [56].

Although we did not evaluate gene expression in this specific brain area, these results indicate that substances, such as alcohol, in interaction with completed suicide may influence glial density. A possible mechanism that could contribute to the increase in the expression of genes related to glial proliferation is the combination of immunomodulatory effects and factors related to stress, which would lead to higher glial density [56]. This also coincides with the GO categories of other genes that participate in the interaction of both disorders, such as the response to stress and the regulation of immune system processes.

It has been suggested that the interaction between depression and SUD could lead to glial dysfunction and a consequent alteration in glutamatergic neurotransmission [57, 58], so the pharmacological modulation of this circuit may play a key role in the treatment of SUD in comorbidity with other mental disorders. Ketamine, a glutamate-modulating agent, has been shown to reduce suicide in high-risk populations [58–60], so the inclusion of this medication in the treatment of subjects with SUD with suicidal tendencies is an option that would be worth exploring.

It should be noted that this is the first study to carry out a transcriptome analysis of suicide in a Latino population. Suicide is a phenomenon where different individual, sociodemographic, and situational factors interact, and these factors can vary according to place and ethnicity. The environmental factors to which subjects in the Mexican population are exposed may lead to epigenetic changes that are different from those of other popula-

tions, and this could lead to unique influences on gene expression.

The present study has some limitations, such as the relatively small sample size, especially in the nonsuicidal groups; it would be desirable to increase the sample number and include more subjects with abuse to substances other than alcohol, so we could explore the effect of these substances in interaction with suicide. Another limitation is the lack of analysis in other brain regions potentially relevant for suicide and SUD. Future studies should focus on epigenetic changes and/or genotype changes that might be underlying the transcriptome alterations observed in this study, as well as the inclusion of other potential confounding clinical characteristics not examined in this study (for instance, medications and co-occurring drug dependence not detected by our methods).

Conclusion

Our study suggests that suicide with SUD presents alterations in gene expression that are different from those of suicide without this comorbidity and nonsuicide subjects with SUD. Therefore, our results suggest that the interaction of suicide with SUD leads to a unique expression profile. Our results also suggest an alteration in the expression of genes implicated in glial differentiation and glutamatergic neurotransmission in suicides with SUD.

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Statement of Ethics

The Ethics Committee for Human Research at the National Institute of Genomic Medicine approved this research (CEI 2016/33).

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

B.C. participated in the conception of the work, sample processing, analysis and interpretation of the data. She also wrote the present paper; this work is part of her PhD program. N.M.-J. and G.R.F. participated in the design of the work, sample processing, acquisition of the data (microarrays), and results interpretation. In addition, they revised the present manuscript, providing essential ideas, and approved its final version. R.C.M.-M., F.G.-D., A.M.-L., and C.D.-O. participated in the sample acquisition and revised the present paper, providing relevant content. C.F. contributed to the data analysis, results interpretation, and writing of the present paper. C.W.-B., D.C.G., H.N., and P.O.-W. contributed to the conception and design of the work, and interpretation of the data. They participated actively in the writing of the present paper and provided key ideas for results interpretation. All the authors revised and approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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