

ORIGINAL ARTICLE

Gene expression associated with suicide attempts in US veterans

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According to a recent report from the Office of Suicide Prevention in the US Department of Veterans Affairs, veterans represent 8.5% of the US population, but account for 18% of all deaths from suicide. The aim of this study of psychiatric patients ($n = 39$; 87% male) was to compare blood gene expression data from veterans with a history of one or more suicide attempts to veterans who had never attempted suicide. The attempter and non-attempter groups were matched for age and race/ethnicity, and both groups included veterans with a diverse psychiatric history that included posttraumatic stress disorder (PTSD) and substance-use disorders. Veterans were interviewed for lifetime psychiatric history, including a detailed assessment of prior suicide attempts and provided a blood sample. Results of Ingenuity Pathway Analysis (IPA) identified several pathways associated with suicide attempts, including the mammalian target of rapamycin (mTOR) and WNT signaling pathways. These pathways are of particular interest, given their role in explaining pharmacological treatments for suicidal behavior, including the use of ketamine and lithium. These results suggest that findings observed in civilians are also relevant for veterans and provide a context for interpreting results observed in post-mortem samples. In conclusion, an emerging body of work that shows consistency in findings across blood and brain samples suggests that it might be possible to identify molecular predictors of suicide attempts.

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INTRODUCTION

Substantial progress has been made in identifying stable traits associated with suicidal behavior and concomitant biological markers, but there are no replicated clinical or biological predictors that distinguish acute or imminent risk from stable-risk traits.¹ Much of the work that has been conducted to identify potential suicide biomarkers has been conducted by examining post-mortem brain tissue.² Molecular markers or processes identified in post-mortem tissue may reflect long-standing risk and/or a more proximal precursor of the death from suicide. An unanswered question is whether the results obtained in post-mortem tissue generalize to blood samples obtained from people who attempted, but did not complete suicide. If the same molecules or processes are observed in blood from people who did not die from suicide, this suggests an association with a more stable trait of risk. In accord with this view, recent research on suicide risk has increasingly focused on identifying molecular processes and networks in blood.^{3–6}

Nearly one out of five people in the United States who die from suicide have served in the military,⁷ which indicates that this is a large proportion of the US population that requires monitoring and intervention for suicidal risk. Recent research on active duty service personnel and veterans has generally highlighted the importance of a diverse range of psychiatric disorders as contributing factors for attempts and suicide completion, including panic disorder and posttraumatic stress disorder (PTSD)⁸ as well as psychiatric problems that may have developed during

military service such as depression, bipolar disorder, alcohol use disorder⁹ and intermittent explosive disorder.⁸ However, because suicide attempts occur in the context of many different psychiatric diagnoses and are independent of any single diagnosis,¹⁰ it is important to identify factors associated with suicide versus underlying psychiatric symptoms. That is, many studies of suicide that examine molecular markers and processes are conducted comparing people in relatively discrete diagnostic groups (for example, people with mood disorder or psychosis) to psychiatrically healthy controls (although see Niculescu *et al.*⁵ who studied a sample of men with a range of mood and psychotic disorders). In the case of post-mortem tissue, a typical comparison group consists of people who died from accidents without underlying psychiatric symptomatology. Such comparisons make it difficult to determine whether the observed biological signal is specific to suicide *per se*, or whether it reflects the complex psychiatric background, both of which are present in suicide attempts and deaths.

The aim of the current study was to identify molecular pathways and networks associated with suicide attempts using genome-wide gene expression data obtained from peripheral blood samples of veterans. To minimize sources of heterogeneity that would make it difficult to detect molecular pathways in a relatively small sample, the study was conducted in veterans who had attempted suicide compared with veterans who were comparable by gender, race, ethnicity, age and psychiatric history but who had never made a suicide attempt. A psychiatric comparison group was chosen to maximize the clinical utility of the study as clinical providers are often faced with the dilemma of distinguishing

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between patients who will self-harm and patients who will not. In addition, the psychiatric comparison group allows for clearer interpretation as any observed biological differences would reflect suicide history rather than the confounding influence of psychopathology and associated features (for example, medication use).

MATERIALS AND METHODS

Participants

One hundred and thirty-one veterans (61 who had made one or more suicide attempts and 70 who had never made a suicide attempt) provided blood samples and completed a clinical interview. Patients were referred to the study by mental health providers at the James J Peters VAMC or were self-referred. All study procedures, including recruitment from the inpatient psychiatry unit, were approved by the Institutional Review Boards at the James J Peters VAMC, the Icahn School of Medicine at Mount Sinai and the US Army Medical Research and Materiel Command Human Research Protection Office; all study procedures, including screening and evaluation, were conducted after the veterans provided written, informed consent.

Clinical assessment

Interviews included the Mini international Neuropsychiatric Interview for the assessment of DSM-IV Axis I psychiatric diagnoses¹¹ and the Scale of Suicidal Ideation¹² for the assessment of suicidal ideation in the past 7 days, administered by a masters or doctoral level psychologist. For veterans with a history of a suicide attempt, the Columbia Suicide History Form¹³ and the Suicide Intent Scale¹⁴ were administered to characterize intention to die during the most recent attempt and lethality of all attempts. All suicide history forms were discussed in consensus, including ratings of lethality. Clinicians also collected basic demographic information, including use of current medications, and reviewed the electronic medical chart for confirmation of medication use and diagnosis. Trauma history was obtained using the Childhood Trauma Questionnaire.¹⁵

The process used to create comparable groups of suicide attempters and non-attempters consisted of first selecting individuals who had made the most recent and lethal attempts and then identifying a comparable control based on race/ethnicity, gender and age (within 5 years). An additional consideration included psychiatric diagnosis and medication status, resulting in 20 pairs. Data from one individual were excluded following gene expression assay because the sample showed signs of poor quality and DNA contamination in the reverse transcriptase polymerase chain reaction (RT-PCR) validation, resulting in a final sample size of 39.

Blood drawing and processing

Forty milliliters of blood were drawn via venipuncture between 0900 and 1000 hours, and collected into PAXgene RNA tubes, centrifuged immediately and stored for further extraction of RNA at the Integrative Systems Biology Lab at the US Army Center for Environmental Health Research. Total RNA was isolated using PAXgene Blood miRNA kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). RNA quality and quantity were determined using NanoDrop 2000 spectrometer (Thermo Fisher, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

mRNA array hybridization and image processing

Whole-Human Genome Microarray 60 mer Oligo slides printed with Agilent SurePrint Technology were obtained from Agilent Technologies (Agilent Technologies). The slides contained ~41 000 genes and transcripts. Agilent Low Input Quick Amp Labeling Kit, along with Agilent Two-Color Spike-Mix (Agilent Technologies) was used to label experimental (Cy5-labeled) and reference samples—Stratagene Human universal reference RNA (Cy3-labeled, Agilent Technologies). All labeled sample reactions were then subjected to fragmentation followed by 17 h hybridization using the Agilent Gene Expression Hybridization Kit.

mRNA data acquisition and analysis

Following scanning and extraction, individual mRNA microarrays were LOESS-normalized¹⁶ and the bottom 30% of the distribution of probes were filtered out¹⁷ as were those that did not have a corresponding RefSeq

gene annotation. Quality-control examination of arrays, normalization and primary identification of differentially regulated molecules were performed using custom R scripts (R Development Core Team) with Bioconductor library packages.¹⁸ No outlier samples or technical batch effects were identified.

Microarray validation

Twenty-nine mRNA probes with the lowest *P*-values were selected for further validation using RT-PCR. In addition to the sample that was removed because of low signal and DNA contamination, seven samples showed issues with RT-PCR internal controls and were removed for validation purposes. In re-analyzing mRNA microarray values on the remaining 32 individuals, 27 of the 29 probes retained a *P*-value below 0.05. Four probes showed attempter versus control differences in the RT-PCR data with a *P*-value below 0.05, all in directional agreement with the microarray results. Overall, 18 of the 29 probes moved in the same direction between microarray and RT-PCR.

Statistical and pathway analyses

t-tests and χ^2 -analyses were used to evaluate group differences between attempters and controls on demographic and clinical variables. Principal component (PC) analyses were conducted on gene expression data to identify outlier samples and any clinical or sample-processing factors (that is, confounds) that were associated with global gene expression patterns. Moderated *t*-test with a Benjamini and Hochberg false discovery rate correction was used to quantify individual gene differences between attempters and controls. IPA (QIAGEN Redwood City, www.qiagen.com/ingenuity), which uses open source databases and proprietary hand curation to annotate gene functions, was used for primary pathway analysis for differentially regulated mRNA between attempters and non-attempters and to identify activated or suppressed upstream regulators. Cytoscape,¹⁹ with networkanalyst²⁰ visualization, incorporating data from Kyoto Encyclopedia of Genes and Genomes (KEGG),²¹ wikipathways²² and reactome,²³ was used to find alternative connections and annotation enrichment between differentially expressed genes.

RESULTS

Table 1 summarizes demographic and clinical characteristics of veterans who had a history of suicide attempt(s) compared to psychiatric controls to further demonstrate the comparability of the samples. As expected, the groups did not differ by age, gender, race or ethnicity. The two groups reported comparable levels of childhood trauma exposure and had similar rates of mood disorder, PTSD and alcohol-use disorder. Veterans who had attempted suicide were also more likely to have a substance use disorder, reflected by a higher rate of narcotic or marijuana dependence. The groups were similar with respect to use of psychotropic medications or use of any type of medication.

PC analyses of mRNA probes revealed a strong separation of attempters from non-attempters in the first two PC. These two components accounted for 34.8 and 16.8% of the variance in the gene expression data, respectively, and the top five PCs accounted for 68% of total variance. To investigate the influence of possible confounding clinical variables on mRNA expression levels, clinical variables and demographic variables with at least a 10% frequency were correlated with loadings for the first two PC dimensions. Suicide attempter status was most strongly correlated with the first two components, whereas gender was correlated with the second component. Other potential confounds including age, use of medication for Type II diabetes, PTSD, bipolar disorder and major depressive disorder were not strongly associated with the major PCs of the mRNA expression data.

Given the limited sample size and low power to detect differences, no individual gene expression probes emerged with a false discovery rate-corrected *P*-value below 0.05. For the purposes of conducting further downstream pathway enrichment and network analysis, 853 probes that had absolute fold change greater than 0.5 and an uncorrected *P*-value below 0.01 were considered to represent differentially expressed probes. This

Table 1. Demographic and clinical characteristics in veterans without (–) or with (+) a history of suicide attempts

	Suicide history– (n=21)	Suicide history+ (n=18)	Test statistic
Age	45.19 (2.94)	45.33 (3.17)	F(1,37) = 0.001, P = ns
Men # (%)	18 (85.7)	16 (88.9)	$\chi^2(1) = 0.087$, P = ns
Black # (%) ^a	7 (33.3)	9 (50.0)	$\chi^2(2) = 3.24$, P = ns
Hispanic # (%)	9 (42.9)	6 (33.3)	$\chi^2(1) = 0.37$, P = ns
Childhood Trauma Questionnaire—mean (s.d.) ^b	9.05 (0.68)	11.59 (1.12)	U = 114, (n ₁ = 19n ₂ = 18), P = ns
Mood disorder # (%)	16 (76.2)	16 (88.9)	$\chi^2(1) = 1.06$, P = ns
Posttraumatic stress disorder # (%)	7 (33.3)	7 (38.9)	$\chi^2(1) = 0.13$, P = ns
Current alcohol use disorder # (%)	2 (9.5)	3 (16.7)	$\chi^2(1) = 0.44$, P = ns
Lifetime alcohol use disorder # (%)	13 (61.9)	15 (83.3)	$\chi^2(1) = 2.20$, P = ns
Current substance-use disorder # (%)	3 (14.3)	10 (55.6)	$\chi^2(1) = 7.43$, P = 0.006
Lifetime substance-use disorder # (%)	7 (33.3)	15 (83.3)	$\chi^2(1) = 9.85$, P = 0.002
Current medication use # (%)	18 (85.7)	14 (77.8)	$\chi^2(1) = 0.42$, P = ns
Current psychotropic use # (%)	13 (61.9)	14 (77.8)	$\chi^2(1) = 0.28$, P = ns

Abbreviations: CTQ, Childhood Trauma Questionnaire; ns, not significant. p = ns, no significant difference at $P \leq 0.05$. ^aTwo people self-identified as bi-racial. ^bTwo people did not complete the CTQ. A non-parametric test was used because of a skewed distribution.

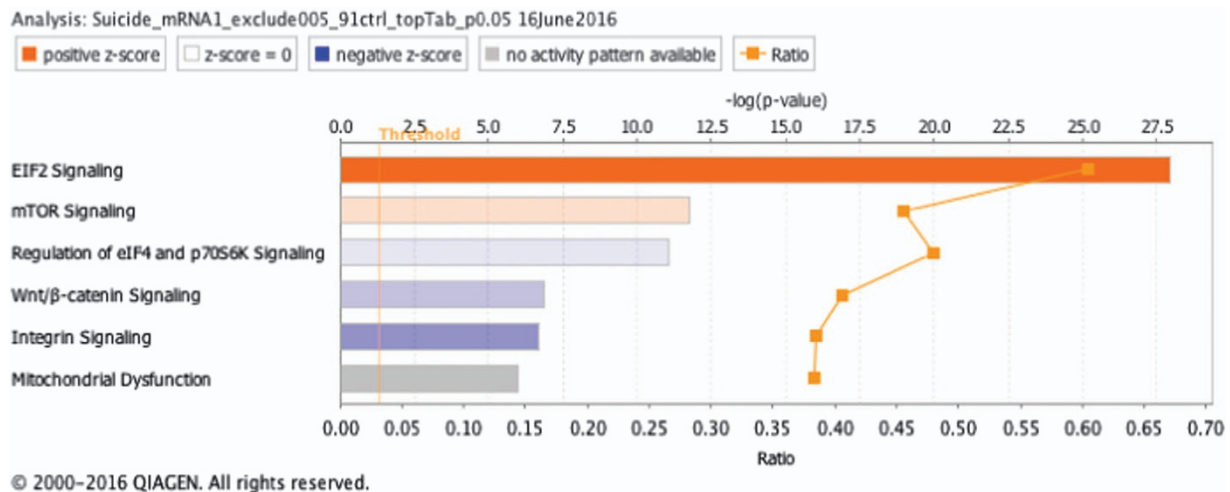


Figure 1. Top 5 enriched IPA canonical pathways ($P < 0.01$) using differentially expressed genes ($P < 0.05$, absolute $\log_2fc > 0.5$). The copyright refers to the software program. The figure has not been published previously. IPA, Ingenuity Pathway Analysis.

cutoff P -value corresponded to a false discovery rate of 11.9%. Lower gene expression was more common in veterans who had attempted suicide relative to those who had not (that is, 607 of the 853 probes). Higher gene expression in attempters was observed in 246 probes.

Several highly enriched canonical pathways were identified with IPA including eukaryotic initiation factor (eIF) 2 signaling, mammalian target of rapamycin (mTOR) signaling and regulation of eIF4 and p70S6K signaling (Figure 1). Three additional pathways, including Wnt/ β -catenin signaling, Integrin signaling and mitochondrial dysfunction, were less highly enriched, but still had P -values $< 10^{-5}$. A network analysis of genes associated with the three most highly enriched pathways noted above showed that several genes associated with the mTOR pathway were highly connected downregulated genes, including *AKT1*, *AKT1S1*, *TSC2* and *eIF4G1*. In contrast, upregulated genes included a large number of ribosomal proteins and eIF family members (see Figure 2). Additional analyses in Cytoscape using reactome, KEGG and wikipathway annotations also identified the WNT signaling pathway as well as Focal Adhesion, Oxidative Phosphorylation, mRNA processing and Ribosomal Proteins.

Gene expression in enriched pathways. Gene expression magnitude and significance for the top 50 probes representing

individual genes within the eIF2 signaling pathway that distinguished attempters from non-attempters showed that many were shared between the eIF4 and eIF2 pathways (see Table 2). While the majority of top probes were downregulated (33 and 37 out of 50, respectively, for eIF2 and eIF4), most eIF family genes were upregulated. Downregulated probes in these pathways represent bone morphogenetic proteins and several growth factors, including heparin binding growth factor, connective tissue growth factor and several vascular endothelial growth factor and platelet derived growth factor family members.

Gene expression magnitude and significance for the top 50 probes representing individual genes within the mTOR pathway showed that 12 of the top 50 probes were more highly expressed in attempters. The *DVL1* gene was represented by both significantly up- and downregulated probes. The mTOR KEGG pathway showed a pattern of downregulation for genes in and around mTOR complex 1 and, including mTOR itself. RICTOR in the mTOR complex 2 and several downstream eIF genes were upregulated. Closer examination of the genes that were downstream of mTOR showed that most were moving in the direction predicted by mTOR suppression (see Figure 3).

With respect to the WNT signaling pathway, six separate members of the proteasome subunit gene family were

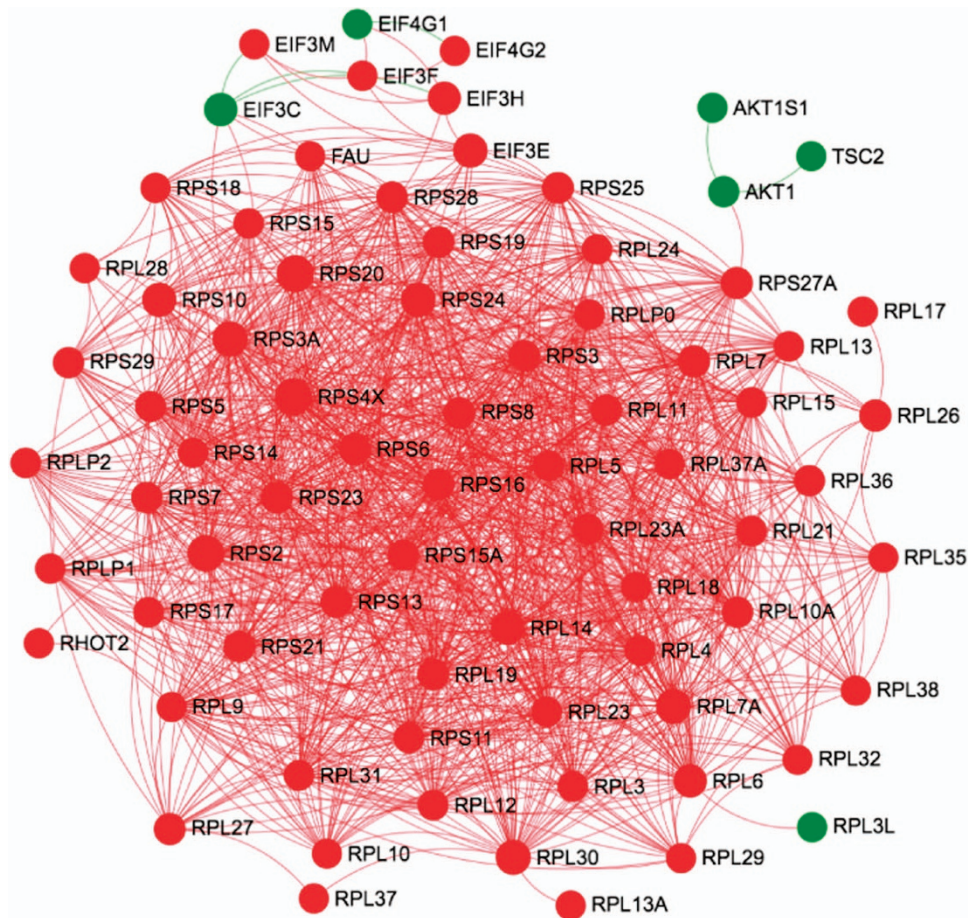


Figure 2. The corresponding networks for the top three canonical pathways. Pathway generated in Cytoscape using Fisher exact test at FDR (q) < 0.05 and visualized using NetworkAnalyst. Genes upregulated in veterans with a history of suicide attempt relative to veterans without a history of attempt are shown in red and downregulated genes are shown in green. FDR, false discovery rate.

upregulated (at $P < 0.012$). Four cadherin (CHD), four g-protein (GN) and two LRP gene family members were downregulated in this pathway. Of the 280 probes representing genes identified with IPA in the eIF2 pathway, 31 (11%) had a P -value less than 0.01. Of these 31 probes, only seven showed higher gene expression in attempters, and five of these genes represent eIF3 and eIF4 family members. Downregulated probes in this pathway represent bone morphogenetic protein and several growth factors including VEGFB, heparin binding growth factor, connective tissue growth factor and platelet derived growth factor family members.

DISCUSSION

Results of Ingenuity Pathway Analyses identified several pathways of interest that may be associated with suicide attempts, adding to a growing body of research that examines molecular markers of suicidal behavior in blood. Some of these pathways, or specific genes in the pathways, have been linked to suicide in prior studies of both blood and brain tissue. A review of the differentially expressed gene lists from three recently published studies that examined gene expression in blood³⁻⁵ shows overlap with some of the differentially expressed genes (DEGs) observed in the current study. In addition, examination of the common DEGs shows that the direction of gene expression (for example, upregulated versus downregulated in association with suicide) was nearly always the same. For example, the TNFSF1 (Tumor

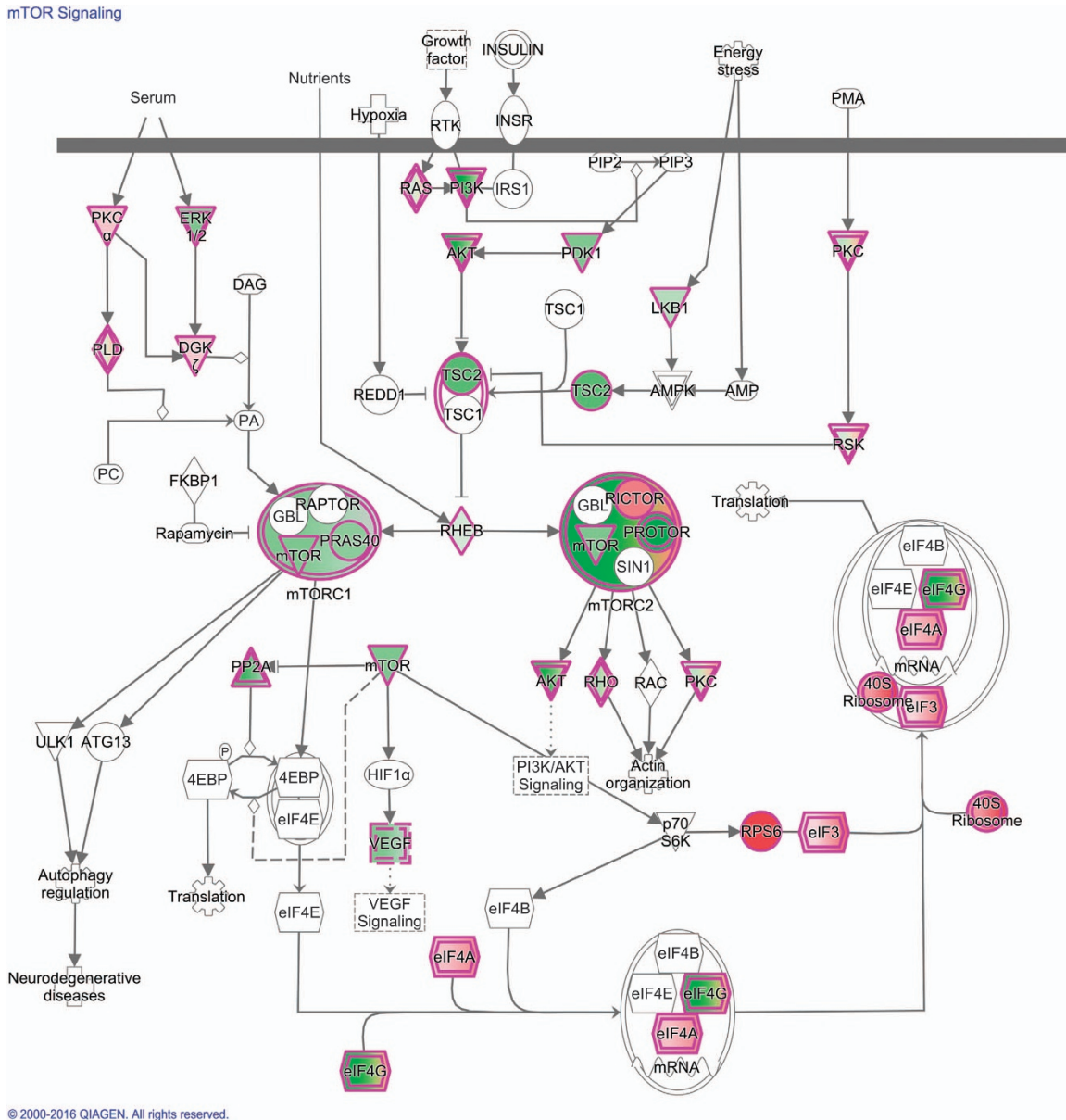
Necrosis Factor (Ligand) Superfamily, Member 10) gene was upregulated in attempters versus non-attempters, an association that was also observed by Le-Niculescu *et al.*³ in men reporting a high level of suicidal ideation. Kim *et al.*²⁴ also reported that this gene was overexpressed in prefrontal cortex post-mortem tissue in people with schizophrenia who had died from suicide. This gene codes for a cytokine that is part of the tumor necrosis factor family. In addition, the PIK3C3 (Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3) was upregulated in suicide attempters, consistent with results reported by Levey *et al.*⁴ in a sample of women with high suicidal ideation. A single-nucleotide polymorphism in this gene was also associated with antidepressant treatment-emergent suicidal ideation in a genome wide association study of depressed inpatients.²⁵ These results provide partial replication of gene expression associations with suicidal behavior, extending the research in civilian samples to US veterans.

It is also of great functional value to identify pathways or consider how gene families are regulated together in association with suicide. IPA identified the eIF2 pathway as a highly enriched canonical pathway associated with suicide attempter status. Of note, over 10% of the probes representing the genes of this pathway were differentially expressed in attempters versus non-attempters. Moreover, several members of the eIF3 and eIF 4 gene families were significantly upregulated in suicide attempters (also represented in Table 2). Proteins coded by these genes form the mammalian ribosome initiation complex at the 5' end of an mRNA

Table 2. Gene expression significance (uncorrected *P*-value) and magnitude (fold change for suicide attempters versus controls) for the top 50 probes representing individual genes within the (a) EIF2, (b) mTOR, (c) EIF4 and (d) WNT signaling pathways.

a) EIF2			b) mTOR			c) EIF4			d) Wnt		
Gene	P-value	Fold Change	Gene	P-value	Fold Change	Gene	P-value	Fold Change	Gene	P-value	Fold Change
AGO2	0.00186	-0.55053	AGO2	0.00186	-0.55053	AKT2	0.011218	0.240797	AKT2	0.011218	0.240797
AKT2	0.011218	0.240797	AKT1	0.0063	-0.77535	AKT2	0.022638	-0.21768	ATF4	0.0005	0.43022
BMP2	0.015846	-0.15567	AKT1S1	0.00102	-0.41588	BMP2	0.015846	-0.15567	CCND1	0.00184	-0.51052
BMP6	0.00568	-0.26287	AKT2	0.011218	0.240797	BMP6	0.00568	-0.26287	CDH1	0.010447	-0.4222
BMP7	0.00977	-0.4345	BMP6	0.00568	-0.26287	BMP7	0.00977	-0.4345	CDH13	0.00314	-0.31498
BMP7	0.019082	-0.11312	BMP7	0.00977	-0.4345	BMP7	0.019082	-0.11312	CDH15	0.011275	-0.30127
CTGF	0.0068	-0.48802	BRAF	0.00156	-0.19061	BRAF	0.00156	-0.19061	CDH2	0.0052	-0.33736
EIF1AX	0.00687	0.34505	CCND1	0.00184	-0.51052	CCL20	0.025718	-0.22866	CSNK1G2	0.00756	-0.66428
EIF2B1	0.00858	0.17333	CTGF	0.0068	-0.48802	CCL22	0.010406	-0.09535	CTNNB1	0.00058	-0.36115
EIF3E	0.001	0.53851	DVL1	0.00251	-0.34642	CXCL8	0.018666	-0.22451	CXCC4	0.00673	-0.11367
EIF3F	0.00589	0.21209	DVL1	0.008	0.18387	EIF1AX	0.00687	0.34505	DVL1	0.00251	-0.34642
EIF3H	0.00008	0.5597	EIF2B1	0.00858	0.17333	EIF3D	0.039902	0.135943	DVL1	0.008	0.18387
EIF4A2	0.014015	0.423604	FZD10	0.01003	-0.36231	EIF3E	0.001	0.53851	FZD10	0.01003	-0.36231
EIF4G2	0.00811	0.37953	FZD5	0.00821	-0.27683	EIF3F	0.00589	0.21209	FZD5	0.00821	-0.27683
EIF5A	0.017084	-0.19797	FZD9	0.00867	-0.33053	EIF3H	0.00008	0.5597	FZD9	0.00867	-0.33053
FGF20	0.017406	0.095063	GAS1	0.00198	-0.23391	EIF4A1	0.028557	0.206369	GNA12	0.00965	-0.34595
GAS1	0.00198	-0.23391	GAS6	0.0008	-0.35416	EIF4A2	0.014015	0.423604	GNAZ	0.00457	-0.61638
GAS6	0.0008	-0.35416	GDF5	0.012558	-0.8038	EIF4G2	0.00811	0.37953	GNG8	0.00629	-0.48214
GDF10	0.016568	0.133903	HDGF	0.00375	-0.4699	FGF20	0.017406	0.095063	GPC4	0.010363	-1.04602
GDF5	0.012558	-0.8038	HDGF	0.00664	-1.10536	HRAS	0.015756	-0.15722	LEFTY2	0.00476	-0.18169
GMFG	0.018321	0.295479	IGF2	0.00501	-0.54315	IL13	0.00877	-0.09513	LRP5	0.00903	-0.27136
GPC6	0.018914	-0.36262	LEFTY2	0.00476	-0.18169	IL23A	0.00047	0.40808	LRP6	0.00565	-0.70367
HDGF	0.00375	-0.4699	MAP2K2	0.00187	-0.30382	ITGA2B	0.039129	-0.21977	MAP2K2	0.00187	-0.30382
HDGF	0.00664	-1.10536	MAPK3	0.00254	-0.4603	ITGA4	0.025251	0.456798	MAP3K7	0.0034	0.29253
HRAS	0.015756	-0.15722	MTOR	0.011145	-0.45814	ITGA8	0.00262	-0.11278	MMP7	0.00444	-0.12257
IFNW1	0.020607	0.074076	MYC	0.00204	0.29655	ITGA9	0.010823	-0.34228	MTOR	0.011145	-0.45814
IGF2	0.00501	-0.54315	NRG4	0.013033	-0.23796	ITGB3	0.00283	-0.88142	MYC	0.00204	0.29655
LEFTY2	0.00476	-0.18169	PDGFA	0.00136	-0.36254	ITGB4	0.032953	-0.3586	PIK3C3	0.00118	0.19415
MAP2K2	0.00187	-0.30382	PDGFC	0.012787	-0.22032	ITGB5	0.0008	-0.56547	PIK3CD	0.00217	-0.87041
MAPK3	0.00254	-0.4603	PDGFRA	0.012414	-0.31814	ITGB7	0.021558	0.221828	PORCN	0.00625	-0.75798
NRG4	0.013033	-0.23796	PDGF	0.010431	-0.38815	ITGB8	0.032573	-0.19144	PRKAR2B	0.00037	-0.76029
PDGFA	0.00136	-0.36254	PIK3C3	0.00118	0.19415	MAP2K2	0.00187	-0.30382	PRKCH	0.00333	0.39429
PDGFC	0.012787	-0.22032	PIK3CD	0.00217	-0.87041	MAPK11	0.035801	-0.29299	PSMA1	0.00877	0.26481
PDGFRA	0.012414	-0.31814	POLR3GL	0.00537	0.24082	MAPK3	0.00254	-0.4603	PSMA2	0.00504	0.3657
PGF	0.010431	-0.38815	PRKCB	0.00891	0.35047	MAPK4	0.032047	-0.18705	PSMA6	0.011933	0.486707
PIK3C3	0.00118	0.19415	PRKCH	0.00333	0.39429	MTOR	0.011145	-0.45814	PSMB1	0.00058	0.49947
PIK3CD	0.00217	-0.87041	PTN	0.01134	-0.23216	PABPC1	0.023295	0.379411	PSMB1	0.00274	0.29203
PPP1R11	0.01379	-0.44635	RAC3	0.00539	-0.49664	PIK3C3	0.00118	0.19415	PSMB4	0.00385	0.386
PPP1R13B	0.01404	-0.62991	RPS6	0.00064	0.69643	PIK3CD	0.00217	-0.87041	PSMD7	0.00071	0.34143
PPP1R13B	0.02252	-0.156	RPS6KA2	0.011295	-0.11759	PPP2R1A	0.026053	-0.4662	RAC3	0.00539	-0.49664
PPP1R3C	0.01584	-0.41523	RRAGA	0.00513	0.18879	PPP2R3A	0.023997	-0.22469	SERPINF1	0.00893	-0.43807
PTN	0.01134	-0.23216	TIMP4	0.00741	-0.17434	PPP2R4	0.018889	-0.23986	SKP1	0.010321	0.38239
SEMA4B	0.016797	-0.40522	TSC2	0.00245	-0.57672	PPP2R5B	0.00367	-0.65055	SRC	0.00707	-0.26791
SHC3	0.0086	-0.18462	VEGFA	0.0009	-0.3118	RPS6	0.00064	0.69643	TBL1X	0.00274	-0.31402
TGFB2	0.01793	-0.0772	VEGFA	0.00569	-0.38333	RRAS2	0.039096	0.290135	UBC	0.00796	0.45331
TIMP4	0.00741	-0.17434	VEGFB	0.010388	-0.46313	SHC2	0.027165	-0.22177	VEGFB	0.010388	-0.46313
UTS2	0.014793	-0.52121	WNT1	0.00645	0.22703	SHC3	0.0086	-0.18462	WIF1	0.00523	-0.10827
UTS2	0.016805	-0.38736	WNT11	0.010541	-0.1559	TNFSF10	0.03364	0.329012	WNT1	0.00645	0.22703
VEGFB	0.010388	-0.46313	WNT3	0.00395	-0.30926	VEGFB	0.010388	-0.46313	WNT11	0.010541	-0.1559
VEGFC	0.020082	-0.34295	YWHAQ	0.00309	0.31245	VEGFC	0.020082	-0.34295	WNT3	0.00395	-0.30926

Genes are listed alphabetically to emphasize shared family memberships. Positive fold changes are shaded red and negative fold changes are shown in green with larger magnitudes brighter.



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Figure 3. mTOR KEGG pathway colored by log fold change values for genes expressed at absolute $\log_2\text{fc} > 0.5$ and $P < 0.05$ (red upregulated and green downregulated). The copyright refers to the software program. The figure has not been published previously. KEGG, Kyoto Encyclopedia of Genes and Genomes; mTOR, mammalian target of rapamycin.

(reviewed in Hinnebusch and Lorsch²⁶), which is regulated by mTOR, another pathway of interest identified in the current study. Although eIF2 and eIF4 pathways have not been linked to suicide in prior work, gene expression level of eIF2 (and mTOR and WNT) was downregulated in one published report examining post-mortem tissue in people who had a schizophrenia diagnosis relative to non-psychiatric controls.²⁷

The mTOR pathway also emerged as an important pathway in the present study, consistent with prior suicide research. Niculescu *et al.*⁵ reported that KEGG analyses identified the mTOR pathway as the top pathway associated with suicide in a sample of men with mood and psychotic disorders. As this pathway has been identified in analyses of both blood and brain tissue, this suggests that it may be important for prediction both suicide attempts and completion. The mTOR pathway has been linked to broad constructs important for psychiatric conditions including learning and memory and brain development.^{28,29} With respect to more specific diagnoses, Jernigan *et al.*³⁰ reported that protein

expression for mTOR and related genes in the pathway (p70S6K, eIF4B and p-eIF4B) were lower in post-mortem tissue of people with depression compared with psychiatric controls. The mTOR pathway has also been implicated in the action of ketamine, a fast-acting antidepressant that has been shown to reduce suicidal ideation in open label and clinical trials.³¹ In rats, ketamine administration is associated with an increase in synaptic signaling proteins in the mTOR pathway and altered behavior in putative depressogenic paradigms.³² Blockade of mTOR signaling with rapamycin completely blocked ketamine-induced synaptogenesis and alterations in behavior.³²

The WNT pathway was also identified as relevant for suicide in the current analyses. This pathway is also critical for neuronal development³³ and has been implicated in prior molecular studies of suicide. For example, Ren *et al.*³⁴ examined protein and gene expression of *GSK-3 β* and *β -catenin*, two genes in the WNT pathway and reported that protein and gene expression of both genes was lower in prefrontal cortex tissue of adolescents who

died from suicide, relative to control participants who died from other causes. Similarly, in another study of prefrontal cortex tissue, protein levels of β -catenin and pGSK3 β were lower in people who died from suicide, regardless of mood disorder diagnoses.³⁵ Willour *et al.*³⁶ also identified a region on Chromosome 2 (2p25) that was associated with suicide attempts in a sample of patients with bipolar disorder. The single-nucleotide polymorphisms in this region are in a linkage disequilibrium block that includes the *ACP1* (acid phosphatase 1) gene, which influences WNT signaling. Follow-up analyses in an independent sample (post-mortem tissue) showed that *ACP1* gene expression was higher in people with bipolar disorder who had completed suicide. Finally, WNT signaling is also regulated by lithium, which has long been used to prevent suicidal behavior in people with bipolar disorder and other psychiatric diagnoses.³⁷

Limitations of the results reported here must be acknowledged. First, the sample size was small and not sufficiently powered to draw conclusions for individual genes. Second, the DEG significance cutoffs were lowered for pathway enrichment analysis. This introduces the danger of adding noise rather than signal at the pathway level, but the top multiple comparison-corrected pathway-level *P*-values remained highly significant. Moreover, as noted above, the results reported here are in accord with previously published results in studies that incorporated blood and post-mortem samples. Third, rather than examining gene expression in the larger sample of veterans who were phenotyped, we opted to match groups based on race, age and psychiatric background. The increase in efficiency of the design with the ability to rule out potential confounding influences was thought to outweigh any loss of statistical power. The results of the PCA suggest that many of the other potential confounds (for example, medication use) were not strongly associated with the major PCs of the mRNA expression data. In particular, we note that linear regression analyses showed that when attempter status and current substance-use disorder were included in the same model, only the attempter status variable was significantly associated with PC1, $t=2.36$, $P=0.0238$. Current substance use $t=0.70$, $P=0.49$. Similar results were observed when attempter status and lifetime substance-use disorder were included in the same model for PC1, attempter status $t=2.47$, $P=0.018$; lifetime substance use $t=0.368$, $P=0.71$. The results of these analyses suggest that substance-use diagnoses do not account for the association between gene expression and suicide history. However, we acknowledge that the relatively low frequency of some of these clinical and demographic variables is associated with reduced power to observe a significant relationship. Fourth, it should be acknowledged that the group of patients who did not report a history of suicide attempts in the past may make an attempt in the future, which highlights the importance of conducting longitudinal research. Finally, with respect to methodology, blood samples were drawn into PAXgene tubes, which raised the question of whether white blood cell count could explain group differences in results. Future studies in well-defined subpopulations of blood cells should be conducted.

In summary, gene expression analyses conducted in a small study of veterans with diverse psychiatric diagnoses identified three pathways of interest that may be associated with suicide attempts. The mTOR and WNT signaling pathways have also been implicated in treatments for suicide, suggesting further research on these pathways may lead to newer or more improved means of treating suicidal ideation and behavior. Notably, the mTOR and WNT pathways are associated with suicide completion, and their prominence in our results using peripheral tissue suggests that the pathways may represent treatment targets and or trait markers of risk for suicide attempts and completion. The results reported here represent an extension of previous work in several important ways. First, to our knowledge, this is the first sample reported in the literature that included only US veterans. Second, the

psychiatric profile of the research participants was diverse, including people with PTSD and substance-use disorders. This enhances the clinical utility of the findings and enabled a comparison of suicide attempts rather than background psychiatric symptomatology. To conclude, the overall high level of pathway and gene-level agreement between this study and prior work demonstrate that the major molecular signatures of suicide attempts in veterans are shared with other populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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