



Published in final edited form as:

Am J Psychiatry. 2022 March ; 179(3): 226–241. doi:10.1176/appi.ajp.2021.21030299.

Genetics and brain transcriptomics of completed suicide.

Giovanna Punzi, M.D., Ph.D.^{1,*}, Gianluca Ursini, M.D., Ph.D.^{1,2}, Qiang Chen, Ph.D.¹, Eugenia Radulescu, M.D., Ph.D.¹, Ran Tao, Ph.D.¹, Louise A. Huuki, P.S.M.¹, Pasquale Di Carlo, M.D., Ph.D.¹, Leonardo Collado Torres, Ph.D.¹, Joo Heon Shin, Ph.D.¹, Roberto Catanesi, M.D.³, Andrew E. Jaffe, Ph.D.^{1,4}, Thomas M. Hyde, M.D., Ph.D.^{1,2,5}, Joel E. Kleinman, M.D., Ph.D.^{1,2}, Trudy F. C. Mackay, Ph.D.⁶, Daniel R. Weinberger, M.D.^{1,2,5,7,*}

¹Lieber Institute for Brain Development, Johns Hopkins University Medical Campus, Baltimore, Maryland, USA

²Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

³Section of Forensic Psychiatry and Criminology, Institute of Legal Medicine, D.I.M., University of Bari 'Aldo Moro', Bari, Italy

⁴Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

⁵Departments of Neurology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

⁶Department of Genetics and Biochemistry and Center for Human Genetics, Clemson University, Greenwood, South Carolina, USA

⁷Departments of Neuroscience, and Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Abstract

Objective: The authors sought to study the transcriptomic and genomic features of completed suicide by parsing the method chosen, to capture molecular correlates of the distinctive frame of mind of individuals who die by suicide while reducing heterogeneity.

Method: The authors analyzed gene expression (RNA sequencing) from postmortem dorsolateral prefrontal cortex of patients who died by suicide with violent *versus* non-violent means and other

*corresponding authors: Daniel R. Weinberger, Lieber Institute for Brain Development, Johns Hopkins University School of Medicine, 855 North Wolfe Street, Baltimore, Maryland, 21205 Tel. 410-955-1000; drweinberger@libd.org; Giovanna Punzi, Lieber Institute for Brain Development, Johns Hopkins University School of Medicine 855 North Wolfe Street, Baltimore, Maryland 21205 Tel. 410-955-1000; giovanna.punzi@libd.org.

Author contributions: GP, GU, and DRW conceived the project, designed the study, analyzed the data and interpreted all the results. GP performed behavioral analysis based on the violent suicide construct, to which RC contributed. ER performed WGCNA. RT performed the qPCR experiment. LH performed deconvolution. TMH, and JEK curated the brain collection. JHS organized and performed RNA sequencing analysis. QC and AEJ organized and performed genotyping and imputation. QC computed GRS. GP and GU analyzed the data, and PDC, LCT and AEJ contributed statistics. TFCM performed the fly study. GP, GU, and DRW drafted the manuscript, and all authors contributed to the final version of the article.

Competing interests: The authors declare no competing interests.

Data and materials availability: In order to protect the privacy of the study participants, the genetic, gene expression combined with manner of death / suicide means data generated and analyzed during the current study are available from the corresponding authors on reasonable request, together with the codes used for the analyses.

non-suicide patients with the same psychiatric disorders, and also of neurotypicals (total n=329). They then examined genomic risk-scores (GRS) for each psychiatric disorder included, and GRS for cognition (IQ) and for suicide attempt, testing how they predict diagnosis or traits (total n=888).

Results: Patients who died by suicide by violent means (vS-pt) showed a transcriptomic pattern remarkably divergent from each of the other patient groups but less from neurotypicals; consistently, their genomic profile of risk was relatively low for their diagnosed illness as well as for suicide attempt and relatively high for IQ, i.e., more similar to neurotypicals than other patients. Differentially expressed genes (DEGs) associated with vS-pt pointed to purinergic signaling in microglia, showing similarities to GWAS of *Drosophila* aggression. WGCNA revealed that these DEGs were co-expressed in a context of mitochondrial metabolic activation unique to vS-pt.

Conclusions: These findings suggest that vS-pt are in part biologically separable from other patients with the same diagnoses, and their behavioral outcome may be less dependent on genetic risk for conventional psychiatric disorders while associated with an alteration of purinergic signaling and mitochondrial metabolism.

Keywords

Suicide death; suicide method; postmortem brain; RNA sequencing; genomic risk; purinergic signaling; mitochondria

Introduction

Suicide is the 10th ranking cause of death in the United States for all age groups combined, the 2nd for younger samples, a trend that has steadily increased in recent years and may be worsening due to the ongoing pandemic(1). Aside from collateral anti-suicide effects of lithium(2) and clozapine(3), both underused medications, pharmacologic treatments specific to suicide do not exist, and medications mostly rely on targeting psychiatric disorders that are risk factors. However, death by suicide in absence of identifiable psychiatric disorders is not rare(4, 5), and most patients with psychiatric conditions at high-risk do not actually die by suicide. Notably, suicidal behavior aggregates in families, independent of the transmission of those high-risk disorders(6). Further, suicidal behavior has been associated with biological features and risk factors not necessarily shared by psychiatric disorders associated with it(5, 7, 8). In this regard, severe anxiety/agitation and poor impulse control may be better predictors of suicide plans and attempts than the presence of a psychiatric diagnosis(9). Suicidal behavior has therefore been proposed as a diagnostic entity in the classification of mental disorders(5, 7), though this is a matter of ongoing debate. Contrasting the biology of suicide patients with that of non-suicidal patients with similar psychiatric diagnoses, and separately with neurotypical controls not affected by mental illness, may be a way forward to elucidate molecular mechanisms underlying the choice of suicidal behavior.

In addition to recent popular genetic approaches leveraging sample size (e.g., GWAS), attention to stratifying the clinical phenotype can increase the power to detect genomic

effects in psychiatric conditions(10). Suicide is not a unitary entity and involves complex biological and psychosocial determinants, in which a particular mental state motivates and accompanies an extreme and definitive behavior; the biological readout of the emotional intensity of this *final* disposition might be observable in brain postmortem. Indeed, it has been argued that risk factors for suicide include those for suicidal ideation and those for progression to the actual behavior(11). Suicidal ideation factors account more for the motivational phase (the development of the intention or desire to die), and the latter for the volitional phase (the enactment of that intention). As noted above, aggression is among the critical moderators of the shift from suicidal ideation to action(12); notably, violence against the self and others show a shared association(13).

In principle, the decision to kill oneself using a violent method is less likely to be reversible, implicating suicide methods(14) that employ relatively high levels of aggression as potential hallmarks of individuals who do this. The state of mind of those subjects, as they employ violent methods, is reasonably expected to differ from those who choose non-violent methods; this has been related to altered decision-making(15, 16). Thus, the investigation of how suicide was performed might translate into a less heterogeneous readout in the biology *fixed* in the brain at death. Further supporting the distinction of suicide by violent or by non-violent means, we reported in two independent studies(14, 17) association of expression of a single gene, *LINC01268*, specifically with suicide by violent means. A genotype associated with relatively increased expression of *LINC01268* in brain was also linked to emotional regulation and aggressive behaviors even in living individuals not engaged in suicidal behaviors or thoughts.

Here, we investigate gene expression in completed suicides with RNA sequencing in DLPFC, a brain area that has been associated with behavioral regulation in general(18), with aggression control in particular(19), and with suicide and aggressive behavior in our previous hypothesis-driven single-gene level study(14). Our findings suggest that completed suicides by violent means may be in part dissociable trans-diagnostically, with the differentially expressed genes (DEGs) implicating purinergic signaling, and showing similarities to a GWAS of a *Drosophila* aggression model(20). We measure genomic risk-scores (GRSs) for each of the psychiatric disorders included in the sample, as well as for cognition and for suicide attempt, and test how they predict diagnosis or traits in patients who died by suicide with violence *versus* other patients and controls. Finally, we used Weighted Gene Co-Expression Analysis (WGCNA) to test for gene co-expression modules and module interactions specifically associated with suicide by violent means. Our data suggest that the genomic profiles of risk of suicides by violent means are not consistent with their primary psychiatric diagnoses, and that their DEGs may operate in the context of a unique energetic metabolism configuration that sets them apart from the other individuals.

Methods

Postmortem Data – RNA sequencing.

Subjects.—Samples were obtained from the Lieber Institute postmortem RNA sequencing (RNAseq) dataset(21, 22). Of the brain regions available in our repository and linked to aggression, the DLPFC (dorsolateral prefrontal cortex, i.e. Brodmann Area (BA) 46/9)

afforded us the largest N for the analysis. As in our previous study, only samples with RIN (RNA integrity number) ≥ 6.9 were used. The samples include tissue from donors of European ancestries (affected with schizophrenia [SZ], major depression [MD] or bipolar [BP] disorder) ≥ 13 years of age, to minimize effects on gene expression based on associations with ancestry or early developmental stages(23). This cohort of brains has been described in details in our previous single-gene study(14), with the difference that two samples have been dropped upon further QC. Indeed, after the hg38 realignment (see below) of our RNA-seq data using SPEAQeasy(24), these two samples did not satisfy inclusion criteria based on identity matching between genotypes from DNA genotyping and genotypes inferred from RNA variant calling. These criteria are based on a set of common expressed variants that can be inferred by both DNA and RNA, from which a correlation matrix can be computed to identify sample swaps(24). The resulting samples of 226 include non-suicide and suicide patients. Additionally, the samples in the present study include a group of non-suicide controls not affected with mental disorders (neurotypicals) from the same repository with similar characteristics (BA 46/9, of European ancestries, with age ≥ 13 years; N=103), who mostly died by natural death. Detailed information on the samples are reported in table S1.

Postmortem brain tissue.—Methods of collection follow an established protocol that has been reported elsewhere(21, 22). Briefly, grey matter tissue from the crown of the middle frontal gyrus was obtained from the coronal slab corresponding to the middle one-third immediately anterior to the genu of the corpus callosum; subcortical white matter was carefully trimmed from the area immediately below the middle frontal gyrus(22). Total RNA was extracted from ~ 100 mg of homogenate DLPFC grey matter tissue, using the RNeasy kit (Qiagen) according to the manufacturer's protocol.

Manner of death and suicide.—All case narratives were reviewed to establish the degree of violence associated with this form of extreme acting-out; the decision does not obey preset categories, but rests on the evaluation of all circumstances. Briefly, a suicide by violent means is a suicide where self-harm with lethal intent is carried out by causing likely painful injuries, examples are: hanging, gunshot, blunt force, sharp force, jumping from heights, self-imposed motor vehicle accident, et cetera. A suicide by non-violent means consists of actions that are more 'physiological', like swallowing or breathing something that is not hurting at that moment (i.e. drug overdose, CO monoxide), hence does not require the subject to produce injuries on their body to die. However, we do not adhere to fixed labels (e.g. 'poisoning' = always non-violent, others = always violent), but we consider the circumstances of each suicide. Examples of suicide cases (asphyxia, drowning et cetera) that can fall in either category have been detailed in(14), together with the methods employed in our sample). All diagnoses and decisions about manner of suicide were determined blind to any of the molecular data.

Gene expression.—As in our previous study(14), we analyzed BrainSeq PhaseI poly(A)⁺ library data(21, 25). RNA extraction and sequencing have been previously described(21, 25); in the present work, data were revised by aligning reads to the human genome UCSC

hg38 build(26). We additionally performed qPCR to validate the DE of a selected set of DEGs.

Differential expression across manners of death.—We fitted a main model to test association of suicide with gene expression, adjusting for quality surrogate variables (qSVs) (27) accounting for RNA quality based on an experimental paradigm (described in the next sub-section), sex and age, as follows:

$$Expression \sim 4Groups + 9PCs(qSVs) + Age + Sex$$

The groups in the model were classified as: (i) non-suicide patients with psychiatric disorders (“non-suicide patients”, N=99); (ii) patients with psychiatric disorders who died by suicide by non-violent means (“non-violent suicide patients”, N=50); (iii) patients with psychiatric disorders who died by suicide by violent means (“violent suicide patients”, N=77); each compared with the baseline condition of (iv) non-suicide individuals not affected by psychiatric disorders (“neurotypicals”, N=103), and between each other. A sensitivity analysis fitting a model that excluded neurotypicals, using non-suicide patients as baseline condition, was done in order to also adjust for diagnosis as a potential confounder (SM), as follows:

$$Expression \sim 3Groups + 6PCs(qSVs) + Age + Sex + Diagnosis$$

In the DE analyses, after removing low-expressed genes (mean RPKM <0.17) using the “expression_cutoff” function in *segmented*, we performed voom-normalization in the *limma* package(28), and we then used the “lmTest” and “ebayes” functions in the package to fit the statistical models to estimate log2 fold-changes, moderated *t*-statistics and corresponding *P*-values. We extracted statistics of the different coefficients of interest, and we employed the function “makeContrasts” for further comparisons.

Multiple-testing correction via the false discovery rate (FDR) was applied using the set of expressed genes, i.e. 23,346 genes. All DEG results shown in this report are FDR <0.05.

RNA quality correction.—In addition to selecting samples only with RIN ≥6.9, we estimated and removed from the DE analysis potential further biases of RNA quality by using qSVA, i.e. quality surrogate variable analysis. This method(27), developed using data from RNA degradation experiments, has been shown to improve rates of replication in DEG analyses across postmortem human brain studies. The qSVA model approach and its implementation have been described in detail elsewhere(25, 27). PCA (principal component analysis) was performed on the log2-transformed degradation matrix (with an offset of 1) and the top 9 PCs were selected using the BE algorithm, and extracted. The set of these PCs (i.e. quality surrogate variables, qSVs) was included as adjustment variables in DE analyses. 6 PCs were selected and included in DE analysis that fit the sensitivity analysis model, based on BE algorithm output on this subset. We note that such qSVs are also highly correlated with pH in our sample: this further excludes the possibility that DE results may be confounded by different agonal state, of which pH may be considered a proxy, as a marker

of terminal hypoxia. Additionally, qSVs are also highly correlated with RIN and postmortem interval (pmi), as shown in figure S1 and table S2).

Gene Ontology.—The list of DEGs generated in each contrast, and the genes in each WGCNA module, were analyzed through the GO Consortium toolkit(29) against a custom background of all genes expressed in the DE model. We chose a p-value calculation based on the FDR method of accounting for multiple testing, as provided by the Consortium. We complemented this analysis with an upstream regulator analysis in IPA (Ingenuity Systems; Qiagen China Co., Ltd.) against a background of genes expressed in the CNS provided by the tool. The GO Consortium toolkit(29) was also employed for enrichment on the list of genes linked to *Drosophila* aggression, against the related background.

Cellular proportion deconvolution.—Deconvolution was performed with the ReferenceBasedDecomposition function from the R package *BisqueRNA* version 1.0.4(30), using the use.overlap = FALSE option. The single-cell reference dataset used was single nucleus RNA-seq from the 10X Genomics protocol, which includes tissue from 8 donors and 5 brain regions(31). The 9 cell-types considered in the deconvolution of the tissue were Astrocytes (Astro), Endothelial cells (Endo), Microglia (Micro), Mural cells, Oligodendrocytes (Oligo), Oligodendrocyte Progenitor Cells (OPC), T cells, Excitatory Neurons (Excit), and Inhibitory Neurons (Inhib). Marker genes were selected by first filtering for genes common between the bulk data and the reference data, then calculating the ratio of the mean expression of each gene in the target cell-type over the highest mean expression of that gene in a non-target cell-type. The 25 genes with the highest ratios for each cell-type were selected as markers, from which estimates of cellular proportion were derived as described in(32).

Postmortem Data – Genomic Risk Scores (GRSs).

Subjects.—Samples were obtained from the Lieber postmortem repository for the calculation of polygene or genomic risk scores (GRSs)(21). This cohort, an extension of the expression dataset with less than 1/3 subjects overlapping, includes 895 samples (888 samples after removing extreme outliers for the studied GRSs) from: donors who were controls of European ancestries (i.e., neurotypicals: non-suicide, non-affected with mental disorders, N=247) or patients (non-suicide or suicide, affected with SZ, N=147; MD N=310; or BP, N=184) ≥ 13 years of age, similarly to the RNA-seq cohort. The samples were classified for suicide and suicide methods similarly to the DE dataset (table S1). Of note, while the DE analysis does not warrant dividing patients within diagnostic groups for power issues, we did each GRS analysis in the appropriate diagnostic group.

Genotyping.—Genomic DNA was extracted from cerebellum of the same samples using standard procedures with Flexi Gene DNA kits (Qiagen, Germany). Genotyping was performed as previously described(21, 33, 34), and an independent set of SNPs obtained by LD-pruning(35) was used to perform genome-wide clustering to obtain multidimensional scaling (MDS) components for quantitative measures of ancestry. We removed SNPs showing genotype missing rate $>10\%$, deviation from Hardy-Weinberg equilibrium ($p < 1e-06$), or minor allele frequency $<0.05\%$. Additional QC was performed

on individual genotyping results. Individuals were removed if their overall genotyping rate was below 97%. The data were checked for sample duplications and cryptic relatedness.

Derivation of GRSs.—Genomic risk scores (i.e. GRSs, also known as Polygenic Risk Scores or PRSs)(36) were calculated for each individual, using summary statistics of GWAS for each diagnosis or trait under consideration as described elsewhere(37). In brief, GRSs are a measure of cumulative genomic risk(36) calculated as the sum of alleles weighted by the corresponding effects for the diagnosis or trait identified by the respective GWAS study (i.e. SZ(38), SZ resilience(39), MD(40), BP(41), IQ(42), and suicide attempt(43) GWAS). Consistent with the standard procedure for GRS calculation(37, 38), only autosomal SNPs were included in the analysis, to prevent bias related to sex. We performed a linkage disequilibrium (LD) clumping of the SNPs by removing variants that have $r^2 \geq 0.1$ with the index SNP within 500 kb, as reported elsewhere(37, 38). We multiplied beta or the natural log of the OR of each index SNP, obtained from the respective GWAS, by the imputation probability for the effective allele of each index SNP, and summed the products over all index SNPs. For each diagnosis or trait, ten GRSs (GRS1–10) were calculated using subsets of SNPs selected according to the GWAS *P*-value thresholds of association with each disorder: 5e–08 (GRS1), 1e–06 (GRS2), 1e–04 (GRS3), 0.001 (GRS4), 0.01 (GRS5), 0.05 (GRS6), 0.1 (GRS7), 0.2 (GRS8), 0.5 (GRS9), and 1 (GRS10). SNPs in sets with lower *P* values are also in sets with higher *P* values (for example, SNPs in GRS1 are included in GRS2, SNPs in GRS2 are included in GRS3, and so on). GRS6 was employed in the analyses in this report, following previous evidence(38) that it has the highest accuracy in predicting the respective disease or trait.

Postmortem Data – Weighted Gene Co-Expression Analysis (WGCNA).

WGCNA was performed on the residuals from the linear model used in the DE analysis that accounted for death modality and removed the unwanted variance associated with age, sex and qSVs as previously described^{67,68}. For this purpose, we fit a model through the *cleaningY* function (<https://github.com/LieberInstitute/jaffelab>) by which the variable *4Groups* was *protected* (estimated, but not marginalized), whereas variance explained by the other variables (age, sex, qSVs) was removed. A co-expression network from the expression residuals calculated as explained above was constructed for all 329 samples by using a standard WGCNA pipeline as previously described(44, 45). Briefly, we computed gene pairwise correlations (method “*bi-weight*”), and adjacency matrices (parameters: β power = 4 estimated with the *sft* function, network type “*signed hybrid*”), and detected modules of co-expressed genes with hierarchical clustering (*cutreeDynamic* function, parameters: minimum module size = 20, merge cut height = 0.15, pam stage = TRUE). We calculated module e igengenes (MEs) for the detected modules with a WGCNA routine (<https://cran.r-project.org/web/packages/WGCNA/WGCNA.pdf>) that implements a “*singular value decomposition*” (svd) algorithm. We used MEs in comparisons between the main groups, and in pairwise correlations (i.e., “module eigengene network analysis”(46)) moderated by the death modality (*4Groups*).

See SM for details on additional statistical analyses.

Results

A brain transcriptional landscape related to suicide method.

The comparison of non-suicide patients with neurotypicals yielded 80 DEGs (34 down and 46 up in non-suicide patients, figure 1a); there was no significant GO term enrichment for these genes (table S3a). The comparison of non-violent suicide patients with neurotypicals yielded 362 DEGs (149 down and 213 up) in non-violent suicide patients, figure 1b), with the genes upregulated being 6.74-fold enriched for the Huntington disease pathway (i.e. P00029, FDR=0.05, table S3b). The comparison of non-violent suicide patients with non-suicide patients yielded 44 DEGs (21 down and 23 up in non-violent suicide patients, figure 1c), there was no significant GO enrichment for these genes (table S3c).

In contrast, the comparison of violent suicide patients with neurotypicals identified only 8 DEGs (3 down and 5 up in violent suicide patients, figure 1d), and there was no significant GO enrichment for these genes (table S3d). The comparison of violent suicide patients with non-suicide patients yielded 189 DEGs (67 down and 122 up in violent suicide patients, figure 1e); there was no significant enrichment for the down-regulated DEGs, while the analysis on the up-regulated DEGs returned several terms of enrichment (table S3e). Specifically, analysis on these DEGs showed a robust enrichment for biological and molecular processes involving the G protein-coupled purinergic nucleotide receptor signaling pathway (GO:0035589, 76.5-fold enrichment at FDR=0.01) and activity (GO:0045028, 76.5-fold enrichment at FDR=0.003). DEGs overlapping these terms include: *P2RY12*, *P2RY13*, *GPR34* (a paralog of *P2RY14*), and *PTAFR*; notably, *P2RY13* and *P2RY12* are in the top 10 DEGs in this contrast. *P2RY13* and *P2RY12* have been previously shown as the most highly correlated genes with *LINC10268*, the gene highlighted in our previous studies(14, 17). In addition, the top DEG in this contrast was *LINC00996* (FDR=3.80E-06), a long non-coding RNA, which is transcribed from a locus on chr7 with GWAS-significant association with brain oscillatory activity(47). In this locus, *LINC00996* flanks several GTPase, IMAP family genes (*GIMAP*), some of which have been previously linked to completed suicide cases within high-risk families, regardless of co-occurring psychopathology(48). *GIMAP6* and *GIMAP8* were also DEGs in this contrast.

The most dramatic differences in DEG analyses involved the comparison of violent suicide patients against non-violent suicide patients, yielding 843 DEGs, (417 down and 426 up in violent suicide patients, figure 1f). The DEGs with greater expression in non-violent suicide patients showed enrichment for terms related to GTP binding (including binding of purine nucleoside and nucleotide) and, again, Huntington disease (5.49-fold enriched at FDR<0.0007) as well as cytoskeletal proteins related to Huntington disease, i.e. tubulin (PC00228, 22.05-fold enriched at FDR<0.00003, table S3f). The analysis for DEGs with greater expression in violent suicide patients again showed robust enrichment for terms regarding the G protein-coupled purinergic nucleotide receptor activity (GO:0045028, 26.08-fold enrichment at FDR=0.03), produced by the same top DEGs emerged in the previous contrast (*P2RY12*, *P2RY13*, *GPR34*, *PTAFR*) with the addition of *P2RY14*. *LINC00996* was again among the top DEGs (FDR=0.0003), and several *GIMAP* isoforms in its locus were DE as well: *GIMAP6*, *GIMAP7*, *GIMAP2*, *GIMAP1*, *GIMAP4* (all

of them previously linked to completed suicide cases regardless of psychopathology(48)) plus *GIMAP8*, though at FDR=0.08. The upstream regulator analysis revealed a consistent inhibition of EIF4E, a translation regulator, in the violent suicide condition.

Overall, these results suggest that at least at the transcriptional level in DLPFC, patients who die by suicide by violent means are clearly separable from patients with analogous psychiatric conditions who do not die by suicide as well as from patients who die by suicide by non-violent means. Additionally, non-violent suicide patients are minimally different in these analyses from non-suicide patients; this and the other results are strengthened in sensitivity analyses performed in a patient-only design, adjusting for diagnosis (SM and figures S2a–b). In contrast, these data show that patients who die by suicide by violent means are relatively less differentiated from neurotypicals, as very few DEGs emerge in the comparison between these two groups. Figures 2a–e graphically display these data in regard to the expression of the purinergic genes and of *LINC00996*; table S4a–f contains full results of DE for each contrast. Finally, we validated these principal results with qPCR (SM, figure S3).

Additional sensitivity analyses (SM) excluded length of illness (as a proxy of illness severity), minor age, brain trauma (table S5, figure S4a–b), and exposure to various substances as per toxicological screening (figure S5a–b), and to potential therapeutics as potential confounders, or interacting factors, in these results. Table S3h summarizes the DE statistics (contrast of violent suicide patients against non-suicide patients, and against non-violent suicide patients) for the main genes of interest as well as the enrichment of the purinergic related terms, in each sensitivity analysis.

Finally, analysis performed separately by sex suggests that the DEGs may be driven by the male sample, although in the absence of a significant interaction with sex, an increased female sample would be necessary for a firm conclusion (SM, figure S6).

Evidence of a linear continuum of gene expression in the context of suicide.

Because the results indicate a higher number of DEGs when contrasting violent suicide patients with other patients, rather than with neurotypicals, we further explored the transcriptomic divergence between the four groups. Specifically, we analyzed how differences in genes expression between these four groups were related, also using a threshold-free algorithm for detecting and visualizing overlap trends between gene-expression profiles(49) (SM: Violent suicide patients are less differentiated from neurotypicals than from other patients in DEGs).

The analysis shows that the genes up-regulated in violent suicide patients compared with non-suicide patients tended also to be up-regulated in violent suicide patients compared with neurotypicals (figure S7a–b, g), and in neurotypicals compared with non-suicide patients (figure S7c–d, h); the same was true for the down regulated DEGs (figure S7a–d, g–h). These results support the intriguing possibility that, at a transcriptional level in DLPFC, the neurotypical condition lies between non-suicide patients and violent suicide patients. In addition, the genes up-regulated in violent suicide patients compared to non-violent suicide patients tended also to be up-regulated in non-suicide patients compared with non-violent

suicide patients and vice versa (figure S7e–f, i). In other words, these results suggest that the non-violent suicide patient and the violent suicide patient conditions represent the opposite tails of a potential continuum of gene expression in DLPFC.

To test this possibility more directly, we investigated whether gene expression changed linearly from non-violent suicide to non-suicide patients to neurotypicals to violent suicide patients. We modelled a linear relationship between gene expression and an ordinal variable, where the four conditions of non-violent patient, non-suicide patient, neurotypical, and violent suicide patient were coded respectively as “0”, “1”, “2” and “3”. Remarkably, we found that 936 genes were significantly ($FDR < 0.05$) associated with the ordinal scale: in particular, 493 genes showed a linear increase and 443 genes a linear decrease of gene expression from non-violent suicide to non-suicide patients to neurotypicals to violent suicide patients. The genes with a significant linear increase of expression included *LINC00996*, the top DEG lincRNA in violent suicide patients, and, notably, the genes driving the enrichment for purinergic signaling associated with suicide by violent means. Indeed, the genes with linearly decreasing expression were significantly enriched for the Huntington disease pathway and related terms, while the genes with linearly increasing expression were significantly enriched for the G protein-coupled purinergic nucleotide receptor signaling pathway and activity (i.e., top term of enrichment at 23.36-fold, table S7g).

Taken together, these findings further support the conclusion that violent and non-violent suicides are considerably different in DLPFC gene expression, defining the opposite tails of a transcriptional continuum in the samples we studied. However, we cannot exclude that the possible acute ingestion of large quantities of one or more toxicants by the non-violent suicide patients may have affected their brain levels of gene expression. Conversely, the violent suicide cases do not pose this risk, so the observation remains that those genes that are less expressed in non-suicide patients compared to neurotypicals are more expressed in violent suicide patients compared to non-suicide patients (figure S7c–d, h), and *marginally* more expressed (i.e., at a genome-wide marginal level of significance) in violent suicide patients compared to neurotypicals (figure S7a–b, g), and vice versa (figure S7a–d, g–h).

Cellular deconvolution.

We performed deconvolution to test for potential differences between groups in the estimated proportion of 9 cell types. The analysis shows a relative reduction in the estimated proportion of OPCs in violent compared with non-violent suicide (figure S8); however, the DE results were not affected by cellular composition (figure S9).

Drosophila aggressive behavior.—Because suicide by violent means has been associated with aggression(14, 17, 50, 51), we compared our data on suicide by violent means with two GWAS analyses of aggressive behavior in inbred and selected outbred strains of *Drosophila melanogaster*(20). A total of 473 *Drosophila* genes were associated with aggressive behavior in these analyses, of which 298 had human orthologs with DIOPT(52) score >3 . The genes implicated in the *Drosophila* GWAS analyses were enriched for many Gene Ontology terms related to the purinergic pathway (top terms listed by fold

enrichment in table S3i), remarkably consistent with our results in human brain. We also explored GWAS data of aggression in other animals, such as chickens(53) and dogs(53), but were unable to confirm purinergic-specific signaling in the gene lists based on those studies. However, it is of note that those studies employ animals that are outbred or only partially inbred, and involving diverse phenotypes and underpowered sample sizes.

Divergence in genomic risk between patients who died by suicide by violent means and patients with similar diagnoses.—The transcriptome analyses show that

completed suicide by violent means represents a potentially distinct clinical group, at least in terms of displaying a profile of gene expression that is different from non-suicide and non-violent suicide patients with similar diagnoses. We therefore investigated whether these expression differences were reflected at the genome level, by analyzing genomic risk-scores (GRSs) for each of the psychiatric disorders in the patient samples, and for other traits associated with suicide (see table S1 for sample details).

GRS in patients with schizophrenia: As expected, the schizophrenia GRS calculated based on the latest GWAS data (szGRS(38)) and a GWAS p-value threshold of 0.05, was significantly higher in all patients affected with schizophrenia (N=147) compared to neurotypicals (N=247), ($t=6.673$, $p=8.87e-11$), and the variance of case-control status explained by szGRS at this p-value threshold was 11.6% (figure 3a). When dividing patients with schizophrenia into the three subsets of patients related to suicide (non-suicide, non-violent suicide, and violent suicide), non-suicide patients and non-violent suicide patients had higher szGRS compared with neurotypicals (respectively $t=6.847$, $p=3.07e-11$ and $t=2.820$, $p=0.005$). However, the scores of violent suicide patients were not significantly different from those of neurotypicals ($t=1.429$, $p=0.154$), and were significantly lower than those of non-suicide patients with the same diagnosis ($t=-2.311$, $p=0.021$) (figure 3b). Indeed, the szGRS was significantly associated with case-control status when considering only non-suicide cases, and explained 12.8% variance of case-control status. The variance of case-control status was down to 3% when considering non-violent suicide cases. When considering only violent suicide cases, szGRS was not associated with case-control status, as szGRS explained only 0.7% of the variance. In other words, the liability of schizophrenia explained by szGRS was more than 18 times higher in non-suicide patients compared to violent suicide patients, and more than 4 times higher in non-violent suicide patients compared to violent suicide patients. These results were not affected by the inclusion or the exclusion of patients with schizoaffective disorder (see SM). These data echo the transcriptomic analysis in suggesting that patients with schizophrenia who died by suicide by violent means are different in terms of genomic risk for their own given diagnosis compared with other patients with schizophrenia.

In light of these results, we considered that an additional insight to risk for a disease is how some people avoid illness despite other risk factors. Since variants associated with schizophrenia resilience are not significantly associated with risk(39), we tested how suicide patients with schizophrenia behave in terms of schizophrenia resilience GRS (resGRS). Consistent with what we obtained with the szGRS, we found that resGRS was significantly higher in violent-suicide patients compared with neurotypicals ($t=2.034$, $p=0.043$), with a

similar trend in violent suicide patients compared with non-violent suicide patients ($t=1.704$, $p=0.089$), while there were no other significant comparisons across the other various groups (figure S10).

Because cognitive impairment is a relevant feature of schizophrenia risk and illness(54), and cognition may be relevant to suicide, we investigated differences in GRS for Intelligence Quotient (iqGRS(42)) between groups. We found that, while iqGRS was significantly lower in non-suicide schizophrenia patients compared with neurotypicals ($t=-2.469$, $p=0.0140$), there were no significant differences in iqGRS between neurotypicals and violent suicide schizophrenia patients ($t=1.042$, $p=0.2982$), who indeed had higher iqGRS compared with non-suicide patients ($t=2.282$, $p=0.0230$) (figure 3c). There was no significant difference in iqGRS between non-violent suicide patients and the other groups, though this may be a power issue. Although iqGRS was inversely correlated with szGRS ($r=-0.1708447$, $p=0.0007$), we found consistent results (figure S11) in additional analyses on iqGRS adjusting for szGRS.

GRS in patients with major depression: Similarly to szGRS, GRS for major depression (mdGRS(40)) was significantly, although modestly, higher in all patients affected with major depression ($N=310$) compared to neurotypicals ($N=247$). The variance of case-control status explained by mdGRS was 1.6% when considering the whole group of patients with this diagnosis ($t=2.839$, $p=0.0047$ figure 3d). However, when dividing them into the three subsets (non-suicide, non-violent suicide, and violent suicide), mdGRS was significantly associated with case-control status only when considering non-suicide cases ($t=2.803$, $p=0.005$) and non-violent suicide cases ($t=2.485$, $p=0.013$), but not when considering violent suicide patients, whose mdGRSs were not significantly different from those of neurotypicals ($t=0.867$, $p=0.38646$) (figure 3e). Indeed, the variance of case-control status explained by mdGRS was up to 2.3% when considering only non-suicide patients; 1.8% when considering non-violent suicide patients; down to 0.2% when considering patients with depression who died by violent suicide. In other words, the liability of major depression explained by genomic risk is more than 10 times higher in non-suicide than in violent suicide cases.

Similar to the analysis on the sample with schizophrenia, we investigated differences in iqGRS(42) between the patient groups. Again, we found that, while iqGRS was significantly lower in non-suicide patients compared with neurotypicals ($t=-2.830$, $p=0.004$), there was no significant difference in iqGRS between neurotypicals and violent suicide patients ($t=-0.328$, $p=0.743$), who indeed trended to have higher iqGRS compared with non-suicide patients ($t=1.901$, $p=0.057$) (figure 3f). In these data, non-violent suicide patients were not significantly different from the other groups, which may be an issue of sample size. Additional analysis on the relationship of case-control status with iqGRS, adjusting for mdGRS, provided the same results (figure S12).

GRS in patients with bipolar disorder: As expected, GRS for bipolar disorder (bpGRS(41)) was significantly higher in all patients affected with bipolar disorder ($N=184$) compared to controls ($N=247$), explaining 3.4% of the liability to illness ($t=3.644$, $p=0.0003$) (figure 3g). In contrast to the results based on diagnoses of schizophrenia and

depression, GRS shows less clear dispersion based on suicide or method in the context of a diagnosis of bipolar disorder. Bipolar patients who died by suicide using violent methods ($t=2.544$, $p=0.0113$) and non-suicide patients ($t=3.217$, $p=0.0014$) had higher bpGRS compared with neurotypical controls. bpGRS explained 3.2% and 2% of the variance of case-control status, respectively. There was a similar trend for non-violent suicide patients ($t=1.512$, $p=0.1313$). Indeed, bpGRS were not significantly different between non-suicide patients and non-violent and violent suicide patients with bipolar disorder (figure 3h). Additionally, violent suicide patients and non-violent suicide patients had similar iqGRS, which was in fact not significantly different across all four groups, neurotypicals included (figure S13). While bpGRS and iqGRS were not correlated in the whole sample ($r=-0.0060$, $p=0.90$), they showed a negative correlation selectively in the subsample of violent suicide patients ($r=-0.47$, $p=0.002$, figure S14), suggesting an interaction between the two scores. Therefore, we divided the sample into high- and low-iqGRS (defined as above or below the median iqGRS), and detected an interaction between the two scores on violent suicide case-control status ($t=3.016$, $p=0.003$). In the presence of low iqGRS, bpGRS was able to predict 11.7% of the variance of violent suicide case-control status ($t=4.004$, $p=0.0001$ figure 3i); while in the presence of high iqGRS, the variance of violent case-control status explained by bpGRS was dramatically reduced to 0.17% ($t=-0.464$, $p=0.644$). Thus, within the bipolar diagnosis group, reduced genomic risk for the disorder was found in the violent suicide group having relatively higher GRS for intellectual capacity, analogous in part to findings in schizophrenia and depression.

Suicidal intent: GWASs on human aggression of adequately powered sample size are to date lacking(55). However, since suicide attempt is considered a risk factor for suicide completion(56), we used summary statistics from a recent GWAS on attempted suicide(43) to calculate GRS for suicide attempt (saGRS) and we tested, in our total sample, the association with suicide completion. Compared with neurotypicals, non-violent suicide patients had higher saGRS ($t=2.503$, $p=0.01251$), as did non-suicide patients ($t=3.072$, $p=0.00219$), while violent suicide patients were, once again, not significantly different from neurotypicals ($t=0.900$, $p=0.36844$). In other words, saGRS was higher in all patients, except violent suicide patients.

Finally, we excluded the possibility that the differences in gene expression between groups are driven by the GRSs, since in our sample there are no genes with expression significantly associated with any of the tested GRSs ($FDR > 0.05$). Indeed, when adding to the set of predictors each of the 3 main diagnosis GRS and iqGRS, the results are not affected, as shown in figure S15–16. We can therefore conclude that genomic scores do not drive the transcriptomic results.

Weighted Gene Co-Expression Analysis (WGCNA).—To gain further insight into the biological processes potentially involved in the brain correlates of violent suicide while reducing the dimensionality of the dataset, we used Weighted Gene Co-Expression Analysis (WGCNA), an approach accounting for the coordinated expression among genes. A co-expression network based on all 329 brain samples produced 44 modules, and we used their module eigengenes (ME) in downstream analysis. We first searched for

modules associated with suicide by violent means in comparison to the other groups. The only module significantly associated with violent suicide patients, compared to all others (Bonferroni p-value = 0.0484), was *sienna3*, a module enriched for synaptic transmission and GABA synthesis (table S6a, figure S16). We next searched for modules enriched for violent suicide DEGs; as expected, the most enriched module was *green* (purinergic signaling, including *P2RY12*, *P2RY13*, *GPR34*, *PTAFR*, table S6b), other modules were *salmon* (defense response, immunity), *orange* (negative regulation of MAP kinase activity), *white* (angiogenesis) and again *sienna3* (*geneset* tests in figure S17, table S6). Within the *green* module, the 4 purinergic receptors of interest show features of hub genes, i.e., higher intra-module connectivity (table S6h–i); moreover, 33 of the hubs in this module are also DEGs in violent suicide (table S6h–i), further supporting an alteration of the purinergic signaling.

Finally, we investigated relationships between modules that appear specific for violent suicide, by prioritizing MEs pairwise correlations that were significantly affected by the 4 groups condition, that is, MEs that interacted with death modality in predicting expression of other MEs. In this way, we identified two MEs interactions that were nominally significant in all groups compared with violent suicide: *yellowgreen* (not enriched for any term) with *salmon* (figure S18), and *green* (purinergic) with *lightcyan1* (figure 4). Notably, *lightcyan1* was highly enriched for terms related to cellular respiration in mitochondria (i.e. TCA cycle or Krebs cycle, electric transport et cetera), and includes one gene (*MT-ND6*) whose peripheral expression has been previously associated with suicide(57). This result suggests that in violent suicide, as gene connectivity in the *green* module increases, so does connectivity in the *lightcyan1*, while the opposite is true in all other individuals. Finally, we further verified that the main modules eigengenes of interest (i.e., green, lightcyan1, salmon, sienna3 and white) emerging in this analysis are not significantly associated with the covariates age, sex, diagnosis (p-value > 0.05).

Discussion

By leveraging RNAseq and genomic data from a large sample of DLPFC tissue from completed suicide cases, non-suicide patients and healthy controls, and a focus on the suicidal method, we identified signatures associated with suicide death and aspects of its phenomenology. We show that those who die by suicide by violent method may be a separate clinical condition from other suicide cases and from non-suicide psychiatric patients with similar diagnoses. G protein-coupled purinergic signaling may play a role in this distinction and in related aggressive phenotypes, in a specific mitochondrial background of altered energy metabolism.

A previous attempt at genome-wide expression profiling in suicide via next generation sequencing(58), not taking into account means of suicide, failed to observe FDR-significant effects specific to suicide and not to major depression, likely due to limited sample size (i.e., total n=59, of which 21 were cases of suicide). This study, however, suggested potential deficits in microglia and ATPase activity in suicide. Additionally, an earlier microarray investigation(59), comparing suicides with non-suicides with the same diagnosis (schizophrenia), found up-regulation of purinergic signaling (including *P2RY13*) in suicide,

as did a recent study(60) looking at a selected pool of glia-related genes (including *P2RY12*) using real time qPCR. By studying a larger sample with RNA sequencing, we have been able to parse out the effect of suicide on the brain transcriptome in the context of a psychiatric diagnosis and highlight the signaling involved, while providing novel insight into this extreme behavior.

Suicide by violent means may be a distinct condition.

The possibility that suicide by violent methods is a biologically meaningful designation, that is, relatively orthogonal to conventional psychiatric disorders, is first suggested by our analyses of gene expression. We showed that in the patients who died by suicide by violent means, the expression patterns are different not only from other patients diagnosed with the same psychiatric disorders, but also from patients who died by suicide by non-violent means.

We also investigated genomic risk, which is less likely confounded by the experience of the psychiatric condition, or by postmortem tissue artifacts. The GRS analyses largely mirror the transcriptomic findings that cases who died by suicide by violent means show only marginal if any differences from neurotypicals in genomic risk, as well as divergence from non-suicide patients and non-violent suicide patients in genomic risk for their clinical diagnoses. Patients affected with schizophrenia or depression who died by suicide by violent means are less “genetically susceptible” to their diagnosed disorder, and more “genetically inclined” to higher IQ than other patients. Additionally, patients affected with schizophrenia who died by suicide by violent means are also more “genetically resilient” to their diagnosed disorder. These results militate towards the conclusion that individual with the diagnosis of schizophrenia or depression who select a violent means of suicide are distinguishable biologically from similarly diagnosed patients who do not make this extreme choice.

In contrast to the findings in cases diagnosed with either schizophrenia or depression, violent suicide patients with the diagnosis of bipolar disorder do not differ in diagnosis-GRS compared with other patients with the same diagnosis. That those patients evince genetic risk more in line with others similarly diagnosed with bipolar disorder may be explained by evidence that BP as a diagnosis is highly associated with impulsive behavior and with suicide, with suicide rates in some studies up to 20–30-fold greater than in general population(61). However, the possibility that, in general, violent suicide is associated with higher iqGRS and low diagnosis-GRS is supported also by the analysis in bipolar patients where, in presence of high iqGRS, violent suicide patients have lower bpGRS compared with other patients.

Perhaps surprisingly, the genomic profile of the cases who died by violent suicide is not associated with suicide attempt GRS, which complies with the notion that genetic studies on suicide attempt may not catch the genetic architecture of the actual completed behavior, especially by violent method. This is likely because they include by design(43) people who survived a suicide attempt, thus excluding the more than 50% of suicidal patients who die in their first attempt(56) and who may better represent those at highest genetic risk for suicide completion(62).

The potential role of purinergic signaling and mitochondrial function.

Our molecular data further suggest that suicide by violent means, while less biased by genetic risk for conventional psychiatric disorders, may share characteristics with entities such as aggression, as supported by the aggressive phenotype in *Drosophila* and by our previous findings in humans(14). Indeed, the DE analysis implicates purinergic signaling, and genes linked to aggressive behavior in two GWAS analyses in *D. melanogaster*(20) also point to purinergic-related terms as the most enriched processes. The purinergic DEGs belong to the co-expression network studied in our previous publication, with *P2RY13* being the top gene most strongly related to *LINC01268* (see(14)). *P2RY13* codes for a trans-membrane receptor, enriched in microglia(63) and involved in microglia-mediated hippocampal neurogenesis(64). Included in the same network is also *P2RY12*, a purinergic DEG and microglia-enriched gene as well(63), whose knockout produces behavioral alterations in mice brain(65). P2Y12 receptors are involved in lithium pharmacodynamics(66), an interesting notion in light of lithium anti-suicide properties. P2Y12 receptor signaling is also required for physiological microglia–neuron communication at somatic junctions(67, 68); notably, other human microglia signatures appear at the top of violent suicide DEGs, including *CX3CR1*, *TMEM119*, and *SELPLG*.

Intriguingly, the potential involvement of purine signaling in suicide by violent method and in aggressive behaviors resonates with aspects of the syndrome caused by mutations in the gene *HPRT1*, i.e. Lesch-Nyhan Disease (LND)(69). The most defining behavioral manifestation of the disease is the irresistible impulse to self-injury, including lips and fingers biting, eye poking and head banging⁴⁷. The etiopathogenesis of LND is poorly understood; however, as a result of *HPRT1* deficiency, purine recycling to their nucleosides is not functional, leading to enhanced de novo purine synthesis(70). Of note, a recent study has shown that in mice brain and plasma, prophylaxis with ketamine following stress is associated with long-term alterations of purine and pyrimidine metabolism(71); ketamine is also known to transiently reduce suicidal ideation with a single dose(72).

We searched for upstream regulators of the DEGs in violent suicide patients against non-suicide patients, and against non-violent suicide patients, and the analysis pointed to *EIF4E*, a critical regulator of translation. Intriguingly, a recent study has found that cell-specific translation via eIF4E is central to the antidepressant activity of ketamine(73). According to these data, ketamine would exert its therapeutic action by activating eIF4E; consistently, the directionality of our DEGs would suggest an upstream inhibition of this translational regulator.

Among violent suicide DEGs, the most significant is the functionally unknown *LINC00996*, which flanks several GTPases, IMAP family genes (GIMAP), some of which are also violent suicide DEGs. GIMAP genes in this region have been previously linked to completed suicide cases within high-risk families, regardless of co-occurring psychopathology(48). *LINC00996*'s expression is also restricted to microglia(74); importantly, an independent bioinformatic investigation(75) showed the G-protein coupled purinergic nucleotide receptor signaling as one of the top pathways enriched for the genes related to *LINC00996*.

The DE results posed the challenging question about how the relatively fewer biological differences between violent suicide patients and neurotypicals identified here translate into their markedly different behavior. We cannot rule out that more substantial differences will emerge in transcriptional analyses of other brain regions, or in single cellular phenotypes, which are the subject of future investigation, or at epigenetic and proteomic levels, consistent with the enrichment of upstream signal to DEGs in violent suicide for translation regulators such as *EIF4E*. Meanwhile, the DE based on this contrast highlights only 8 DEGs, the top of which is *ELFN2*, a novel adhesion molecule that selective binds metabotropic glutamate receptors (mGluRs). *Elfn2* knockout mice show susceptibility to seizure, anxiety/compulsivity, and hyperactivity(76). However, this gene is not DE in violent suicide patients compared with non-suicide patients, which suggests that the result may be driven by case-control status.

According to the linear model results, neurotypicals are in the middle of a continuum of gene expression, featuring the purinergic DEGs, with the two extremes being violent suicide and non-suicide / non-violent suicide patients. One possible explanation of this evidence is that purinergic signaling functions optimally within a narrow window of expression, where lower expression is associated to disease status, and higher to health - but only within a certain threshold, as has been reported for other genes linked to neurotypism(77).

Interestingly, the WGCNA results suggest that, in the context of suicide by violent means, the purinergic DEGs may function in an opposite state of energy metabolism to that observed in all other individuals. Indeed, purinergic DEGs tend to be more co-expressed in the context of higher energy metabolism in the violent suicide cases, in contrast to lower energy metabolism co-expression in neurotypicals and the other groups. As mentioned, recruitment of microglial processes to somatic junctions is linked to mitochondria neuronal activity in neuron-microglia communication(67, 68). Recent evidence(78, 79) has further outlined a key role for microglia in neuronal inhibition, by converting ATP to ADP which stimulates P2Y12 microglia receptors. In this light, another finding of the WGCNA data is the association with violent suicide of a module enriched for GABA synthesis. Thus, our data point to violent suicide, in contrast to the other three conditions, as involving a state of excessive neuronal-microglia communication that may be detrimental to brain function.

Finally, our data may have potential clinical implications: addressing suicide by violent means as a separate condition may prove decisive to inform prevention. Violent suicide DEGs in brain specimens, if mimicked by peripheral biomarkers, may potentially differentiate a suicide candidate from a patient who is not shifting from considering death to actually pursuing it, offering novel and more precise targets for therapy in short-term risk for suicide. In conclusion, our results converge in suggesting that violent suicide patients may form a group whose biology in brain and whose genetic profile of risk is less related to that of other similarly diagnosed psychiatric patients in the context of an alteration of the purinergic signaling and mitochondrial metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

We are grateful for the contributions of the Office of the Chief Medical Examiner of the State of Maryland, Office of the Chief Medical Examiner of Kalamazoo County Michigan, Office of the Chief Medical Examiner University of North Dakota School of Medicine, Gift of Life of Michigan, and Office of the Chief Medical Examiner of Santa Clara County California in assisting the Lieber Institute for Brain Development in the acquisition and curation of brain tissue donations for this study. We are deeply grateful to the brave and generous families that consented for the brain donation of their deceased next of kin. We thank Richard Straub, Ph.D., and Shizhong Han, Ph.D., for insightful discussions.

Funding:

We thank the Lieber and Maltz families for their visionary support that funded the acquisition of brain tissue and the analytic work of this project.

References

1. Reger MA, Stanley IH, Joiner TE. Suicide Mortality and Coronavirus Disease 2019-A Perfect Storm? JAMA psychiatry 2020.
2. Post RM. The New News about Lithium: An Underutilized Treatment in the United States. Neuropsychopharmacology 2018;43:1174–1179. [PubMed: 28976944]
3. Meltzer HY, Alphas L, Green AI, Altamura AC, Anand R, Bertoldi A, Bourgeois M, Chouinard G, Islam MZ, Kane J, Krishnan R, Lindenmayer JP, Potkin S, International Suicide Prevention Trial Study G. Clozapine treatment for suicidality in schizophrenia: International Suicide Prevention Trial (InterSePT). Archives of general psychiatry 2003;60:82–91. [PubMed: 12511175]
4. Phillips MR, Yang G, Zhang Y, Wang L, Ji H, Zhou M. Risk factors for suicide in China: a national case-control psychological autopsy study. Lancet 2002;360:1728–1736. [PubMed: 12480425]
5. Oquendo MA, Baca-Garcia E. Suicidal behavior disorder as a diagnostic entity in the DSM-5 classification system: advantages outweigh limitations. World Psychiatry 2014;13:128–130. [PubMed: 24890057]
6. Qin P, Agerbo E, Mortensen PB. Suicide risk in relation to family history of completed suicide and psychiatric disorders: a nested case-control study based on longitudinal registers. Lancet 2002;360:1126–1130. [PubMed: 12387960]
7. Oquendo MA, Baca-Garcia E, Mann JJ, Giner J. Issues for DSM-V: suicidal behavior as a separate diagnosis on a separate axis. The American journal of psychiatry 2008;165:1383–1384. [PubMed: 18981069]
8. Hill NTM, Robinson J, Pirkis J, Andriessen K, Krysinaka K, Payne A, Boland A, Clarke A, Milner A, Witt K, Krohn S, Lampit A. Association of suicidal behavior with exposure to suicide and suicide attempt: A systematic review and multilevel meta-analysis. PLoS Med 2020;17:e1003074. [PubMed: 32231381]
9. Nock MK, Hwang I, Sampson N, Kessler RC, Angermeyer M, Beautrais A, Borges G, Bromet E, Bruffaerts R, de Girolamo G, de Graaf R, Florescu S, Gureje O, Haro JM, Hu C, Huang Y, Karam EG, Kawakami N, Kovess V, Levinson D, Posada-Villa J, Sagar R, Tomov T, Viana MC, Williams DR. Cross-national analysis of the associations among mental disorders and suicidal behavior: findings from the WHO World Mental Health Surveys. PLoS Med 2009;6:e1000123. [PubMed: 19668361]
10. Geschwind DH, Flint J. Genetics and genomics of psychiatric disease. Science 2015;349:1489–1494. [PubMed: 26404826]
11. Klonsky ED, Saffer BY, Bryan CJ. Ideation-to-action theories of suicide: a conceptual and empirical update. Curr Opin Psychol 2018;22:38–43. [PubMed: 30122276]
12. Turecki G. Dissecting the suicide phenotype: the role of impulsive-aggressive behaviours. J Psychiatry Neurosci 2005;30:398–408. [PubMed: 16327873]
13. Mok PL, Pedersen CB, Springate D, Astrup A, Kapur N, Antonsen S, Mors O, Webb RT. Parental Psychiatric Disease and Risks of Attempted Suicide and Violent Criminal Offending in Offspring: A Population-Based Cohort Study. JAMA psychiatry 2016;73:1015–1022. [PubMed: 27580483]

14. Punzi G, Ursini G, Viscanti G, Radulescu E, Shin JH, Quarto T, Catanesi R, Blasi G, Jaffe AE, Deep-Soboslay A, Hyde TM, Kleinman JE, Bertolino A, Weinberger DR. Association of a Noncoding RNA Postmortem With Suicide by Violent Means and In Vivo With Aggressive Phenotypes. *Biological psychiatry* 2019;85:417–424. [PubMed: 30600091]
15. Jollant F, Bellivier F, Leboyer M, Astruc B, Torres S, Verdier R, Castelnau D, Malafosse A, Courtet P. Impaired decision making in suicide attempters. *The American journal of psychiatry* 2005;162:304–310. [PubMed: 15677595]
16. Perrain R, Dardennes R, Jollant F. Risky decision-making in suicide attempters, and the choice of a violent suicidal means: an updated meta-analysis. *Journal of affective disorders* 2021;280:241–249. [PubMed: 33220560]
17. Punzi G, Ursini G, Shin JH, Kleinman JE, Hyde TM, Weinberger DR. Increased expression of MARCKS in post-mortem brain of violent suicide completers is related to transcription of a long, noncoding, antisense RNA. *Molecular psychiatry* 2014;19:1057–1059. [PubMed: 24821221]
18. Roberts AC, Robbins TW, Weiskrantz L: *The prefrontal cortex : executive and cognitive functions* Oxford, Oxford University Press; 1998.
19. Achterberg M, van Duijvenvoorde ACK, van IMH, Bakermans-Kranenburg MJ, Crone EA. Longitudinal changes in DLPFC activation during childhood are related to decreased aggression following social rejection. *Proc Natl Acad Sci U S A* 2020;117:8602–8610. [PubMed: 32234781]
20. Shorter J, Couch C, Huang W, Carbone MA, Peiffer J, Anholt RR, Mackay TF. Genetic architecture of natural variation in *Drosophila melanogaster* aggressive behavior. *Proc Natl Acad Sci U S A* 2015;112:E3555–3563. [PubMed: 26100892]
21. BrainSeq AHBGCeadlo, BrainSeq AHBGC. BrainSeq: Neurogenomics to Drive Novel Target Discovery for Neuropsychiatric Disorders. *Neuron* 2015;88:1078–1083. [PubMed: 26687217]
22. Lipska BK, Deep-Soboslay A, Weickert CS, Hyde TM, Martin CE, Herman MM, Kleinman JE. Critical factors in gene expression in postmortem human brain: Focus on studies in schizophrenia. *Biological psychiatry* 2006;60:650–658. [PubMed: 16997002]
23. Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT, Colantuoni EA, Elkahoul AG, Herman MM, Weinberger DR, Kleinman JE. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. *Nature* 2011;478:519–523. [PubMed: 22031444]
24. Eagles NJ, Burke EE, Leonard J, Barry BK, Stolz JM, Huuki L, Phan BN, Serrato VL, Gutierrez-Millan E, Aguilar-Ordonez I, Jaffe AE, Collado-Torres L. SPEAQeasy: a scalable pipeline for expression analysis and quantification for R/bioconductor-powered RNA-seq analyses. *BMC Bioinformatics* 2021;22:224. [PubMed: 33932985]
25. Jaffe AE, Straub RE, Shin JH, Tao R, Gao Y, Collado-Torres L, Kam-Thong T, Xi HS, Quan J, Chen Q, Colantuoni C, Ulrich WS, Maher BJ, Deep-Soboslay A, BrainSeq C, Cross AJ, Brandon NJ, Leek JT, Hyde TM, Kleinman JE, Weinberger DR. Developmental and genetic regulation of the human cortex transcriptome illuminate schizophrenia pathogenesis. *Nature neuroscience* 2018;21:1117–1125. [PubMed: 30050107]
26. Collado-Torres L, Burke EE, Peterson A, Shin J, Straub RE, Rajpurohit A, Semick SA, Ulrich WS, BrainSeq C, Price AJ, Valencia C, Tao R, Deep-Soboslay A, Hyde TM, Kleinman JE, Weinberger DR, Jaffe AE. Regional Heterogeneity in Gene Expression, Regulation, and Coherence in the Frontal Cortex and Hippocampus across Development and Schizophrenia. *Neuron* 2019;103:203–216 e208. [PubMed: 31174959]
27. Jaffe AE, Tao R, Norris AL, Kealhofer M, Nellore A, Shin JH, Kim D, Jia Y, Hyde TM, Kleinman JE, Straub RE, Leek JT, Weinberger DR. qSVA framework for RNA quality correction in differential expression analysis. *Proc Natl Acad Sci U S A* 2017;114:7130–7135. [PubMed: 28634288]
28. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004;3:Article3.
29. Gene Ontology C. Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015;43:D1049–1056. [PubMed: 25428369]
30. Jew B, Alvarez M, Rahmani E, Miao Z, Ko A, Garske KM, Sul JH, Pietilainen KH, Pajukanta P, Halperin E. Accurate estimation of cell composition in bulk expression through robust integration of single-cell information. *Nat Commun* 2020;11:1971. [PubMed: 32332754]

31. Tran MN, Maynard KR, Spangler A, Collado-Torres L, Sadashivaiah V, Tippani M, Barry BK, Hancock DB, Hicks SC, Kleinman JE, Hyde TM, Martinowich K, Jaffe AE. Single-nucleus transcriptome analysis reveals cell type-specific molecular signatures across reward circuitry in the human brain. *bioRxiv* 2020:2020.2010.2007.329839.
32. Hoffman GE, Ma Y, Montgomery KS, Bendl J, Jaiswal MK, Kozlenkov A, Peters MA, Dracheva S, Fullard JF, Chess A, Devlin B, Sieberts SK, Roussos P. Sex Differences in the Human Brain Transcriptome of Cases With Schizophrenia. *Biological psychiatry* 2021.
33. Jaffe AE, Gao Y, Deep-Soboslay A, Tao R, Hyde TM, Weinberger DR, Kleinman JE. Mapping DNA methylation across development, genotype and schizophrenia in the human frontal cortex. *Nature neuroscience* 2016;19:40–47. [PubMed: 26619358]
34. Li M, Jaffe AE, Straub RE, Tao R, Shin JH, Wang Y, Chen Q, Li C, Jia Y, Ohi K, Maher BJ, Brandon NJ, Cross A, Chenoweth JG, Hoepfner DJ, Wei H, Hyde TM, McKay R, Kleinman JE, Weinberger DR. A human-specific AS3MT isoform and BORCS7 are molecular risk factors in the 10q24.32 schizophrenia-associated locus. *Nat Med* 2016;22:649–656. [PubMed: 27158905]
35. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575. [PubMed: 17701901]
36. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Res* 2007;17:1520–1528. [PubMed: 17785532]
37. International Schizophrenia C, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, Sklar P. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 2009;460:748–752. [PubMed: 19571811]
38. Schizophrenia Working Group of the Psychiatric Genomics C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511:421–427. [PubMed: 25056061]
39. Hess JL, Tylee DS, Mattheisen M, Schizophrenia Working Group of the Psychiatric Genomics C, Lundbeck Foundation Initiative for Integrative Psychiatric R, Borglum AD, Als TD, Grove J, Werge T, Mortensen PB, Mors O, Nordentoft M, Hougaard DM, Byberg-Grauholm J, Baekvad-Hansen M, Greenwood TA, Tsuang MT, Curtis D, Steinberg S, Sigurdsson E, Stefansson H, Stefansson K, Edenberg HJ, Holmans P, Faraone SV, Glatt SJ. A polygenic resilience score moderates the genetic risk for schizophrenia. *Molecular psychiatry* 2019.
40. Howard DM, Adams MJ, Clarke TK, Hafferty JD, Gibson J, Shirihi M, Coleman JRI, Hagenaars SP, Ward J, Wigmore EM, Alloza C, Shen X, Barbu MC, Xu EY, Whalley HC, Marioni RE, Porteous DJ, Davies G, Deary IJ, Hemani G, Berger K, Teismann H, Rawal R, Arolt V, Baune BT, Dannlowski U, Domschke K, Tian C, Hinds DA, and Me Research T, Major Depressive Disorder Working Group of the Psychiatric Genomics C, Trzaskowski M, Byrne EM, Ripke S, Smith DJ, Sullivan PF, Wray NR, Breen G, Lewis CM, McIntosh AM. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nature neuroscience* 2019;22:343–352. [PubMed: 30718901]
41. Stahl EA, Breen G, Forstner AJ, McQuillin A, Ripke S, Trubetskoy V, Mattheisen M, Wang Y, Coleman JRI, Gaspar HA, de Leeuw CA, Steinberg S, Pavlidis JMW, Trzaskowski M, Byrne EM, Pers TH, Holmans PA, Richards AL, Abbott L, Agerbo E, Akil H, Albani D, Alliey-Rodriguez N, Als TD, Anjorin A, Antilla V, Awasthi S, Badner JA, Baekvad-Hansen M, Barchas JD, Bass N, Bauer M, Belliveau R, Bergen SE, Pedersen CB, Boen E, Boks MP, Boocock J, Budde M, Bunney W, Burmeister M, Bybjerg-Grauholm J, Byerley W, Casas M, Cerrato F, Cervantes P, Chambert K, Charney AW, Chen D, Churchhouse C, Clarke TK, Coryell W, Craig DW, Cruceanu C, Curtis D, Czerski PM, Dale AM, de Jong S, Degenhardt F, Del-Favero J, DePaulo JR, Djurovic S, Dobbyn AL, Dumont A, Elvsashagen T, Escott-Price V, Fan CC, Fischer SB, Flickinger M, Foroud TM, Forty L, Frank J, Fraser C, Freimer NB, Frisen L, Gade K, Gage D, Garnham J, Giambartolomei C, Pedersen MG, Goldstein J, Gordon SD, Gordon-Smith K, Green EK, Green MJ, Greenwood TA, Grove J, Guan W, Guzman-Parra J, Hamshire ML, Hautzinger M, Heilbronner U, Herms S, Hipolito M, Hoffmann P, Holland D, Huckins L, Jamain S, Johnson JS, Jureus A, Kandaswamy R, Karlsson R, Kennedy JL, Kittel-Schneider S, Knowles JA, Kogevinas M, Koller AC, Kupka R, Lavebratt C, Lawrence J, Lawson WB, Leber M, Lee PH, Levy SE, Li JZ, Liu C, Lucae S, Maaser A, MacIntyre DJ, Mahon PB, Maier W, Martinsson L, McCarroll S, McGuffin P, McInnis MG, McKay JD, Medeiros H, Medland SE, Meng F, Milani L, Montgomery GW, Morris DW, Muhleisen TW, Mullins N, Nguyen H, Nievergelt CM, Adolfsson AN, Nwulia EA, O'Donovan C,

Loohuis LMO, Ori APS, Oruc L, Osby U, Perlis RH, Perry A, Pfennig A, Potash JB, Purcell SM, Regeer EJ, Reif A, Reinbold CS, Rice JP, Rivas F, Rivera M, Roussos P, Ruderfer DM, Ryu E, Sanchez-Mora C, Schatzberg AF, Scheftner WA, Schork NJ, Shannon Weickert C, Shehktman T, Shilling PD, Sigurdsson E, Slaney C, Smeland OB, Sobell JL, Soholm Hansen C, Spijker AT, St Clair D, Steffens M, Strauss JS, Streit F, Strohmaier J, Szelinger S, Thompson RC, Thorgeirsson TE, Treutlein J, Vedder H, Wang W, Watson SJ, Weickert TW, Witt SH, Xi S, Xu W, Young AH, Zandi P, Zhang P, Zollner S, e QC, Consortium B, Adolfsson R, Agartz I, Alda M, Backlund L, Baune BT, Bellivier F, Berrettini WH, Biernacka JM, Blackwood DHR, Boehnke M, Borglum AD, Corvin A, Craddock N, Daly MJ, Dannlowski U, Esko T, Etain B, Frye M, Fullerton JM, Gershon ES, Gill M, Goes F, Grigoriu-Serbanescu M, Hauser J, Hougaard DM, Hultman CM, Jones I, Jones LA, Kahn RS, Kirov G, Landen M, Leboyer M, Lewis CM, Li QS, Lissowska J, Martin NG, Mayoral F, McElroy SL, McIntosh AM, McMahon FJ, Melle I, Metspalu A, Mitchell PB, Morken G, Mors O, Mortensen PB, Muller-Myhsok B, Myers RM, Neale BM, Nimgaonkar V, Nordentoft M, Nothen MM, O'Donovan MC, Oedegaard KJ, Owen MJ, Paciga SA, Pato C, Pato MT, Posthuma D, Ramos-Quiroga JA, Ribases M, Rietschel M, Rouleau GA, Schalling M, Schofield PR, Schulze TG, Serretti A, Smoller JW, Stefansson H, Stefansson K, Stordal E, Sullivan PF, Turecki G, Vaaler AE, Vieta E, Vincent JB, Werge T, Nummerger JI, Wray NR, Di Florio A, Edenberg HJ, Cichon S, Ophoff RA, Scott LJ, Andreassen OA, Kelsoe J, Sklar P, Bipolar Disorder Working Group of the Psychiatric Genomics C. Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nat Genet* 2019;51:793–803. [PubMed: 31043756]

42. Sniekers S, Stringer S, Watanabe K, Jansen PR, Coleman JRI, Krapohl E, Taskesen E, Hammerschlag AR, Okbay A, Zabaneh D, Amin N, Breen G, Cesarini D, Chabris CF, Iacono WG, Ikram MA, Johannesson M, Koellinger P, Lee JJ, Magnusson PKE, McGue M, Miller MB, Ollier WER, Payton A, Pendleton N, Plomin R, Rietveld CA, Tiemeier H, van Duijn CM, Posthuma D. Genome-wide association meta-analysis of 78,308 individuals identifies new loci and genes influencing human intelligence. *Nat Genet* 2017;49:1107–1112. [PubMed: 28530673]
43. Mullins N, Bigdeli TB, Borglum AD, Coleman JRI, Demontis D, Mehta D, Power RA, Ripke S, Stahl EA, Starnawska A, Anjorin A, M.R.C.Psych, Corvin A, Sanders AR, Forstner AJ, Reif A, Koller AC, Swiatkowska B, Baune BT, Muller-Myhsok B, Penninx B, Pato C, Zai C, Rujescu D, Hougaard DM, Quesed D, Levinson DF, Binder EB, Byrne EM, Agerbo E, Dr.Med.Sc, Streit F, Mayoral F, Bellivier F, Degenhardt F, Breen G, Morken G, Turecki G, Rouleau GA, Grabe HJ, Volzke H, Jones I, Giegling I, Agartz I, Melle I, Lawrence J, M.R.C.Psych, Walters JTR, Strohmaier J, Shi J, Hauser J, Biernacka JM, Vincent JB, Kelsoe J, Strauss JS, Lissowska J, Pimm J, M.R.C.Psych, Smoller JW, Guzman-Parra J, Berger K, Scott LJ, Jones LA, Azevedo MH, Trzaskowski M, Kogevinas M, Rietschel M, Boks M, Ising M, Grigoriu-Serbanescu M, Hamshere ML, Leboyer M, Frye M, Nothen MM, Alda M, Preisig M, Nordentoft M, Boehnke M, O'Donovan MC, Owen MJ, Pato MT, Renteria ME, Budde M, Dipl P, Weissman MM, Wray NR, Bass N, M.R.C.Psych, Craddock N, Smeland OB, Andreassen OA, Mors O, Gejman PV, Sklar P, McGrath P, Hoffmann P, McGuffin P, Lee PH, Mortensen PB, Kahn RS, Ophoff RA, Adolfsson R, Van der Auwera S, Djurovic S, Kloiber S, Heilmann-Heimbach S, Jamain S, Hamilton SP, McElroy SL, Lucae S, Cichon S, Schulze TG, Hansen T, Werge T, Air TM, Nimgaonkar V, Appadurai V, Cahn W, Milanesechi Y, Major Depressive Disorder Working Group of the Psychiatric Genomics C, Bipolar Disorder Working Group of the Psychiatric Genomics C, Schizophrenia Working Group of the Psychiatric Genomics C, Fanous AH, Kendler KS, McQuillin A, Lewis CM. GWAS of Suicide Attempt in Psychiatric Disorders and Association With Major Depression Polygenic Risk Scores. *The American journal of psychiatry* 2019;176:651–660. [PubMed: 31164008]
44. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 2008;9:559. [PubMed: 19114008]
45. Radulescu E, Jaffe AE, Straub RE, Chen Q, Shin JH, Hyde TM, Kleinman JE, Weinberger DR. Identification and prioritization of gene sets associated with schizophrenia risk by co-expression network analysis in human brain. *Molecular psychiatry* 2020;25:791–804. [PubMed: 30478419]
46. Langfelder P, Horvath S. Eigengene networks for studying the relationships between co-expression modules. *BMC Syst Biol* 2007;1:54. [PubMed: 18031580]

47. Smit DJA, Wright MJ, Meyers JL, Martin NG, Ho YYW, Malone SM, Zhang J, Burwell SJ, Chorlian DB, de Geus EJC, Denys D, Hansell NK, Hottenga JJ, McGue M, van Beijsterveldt CEM, Jahanshad N, Thompson PM, Whelan CD, Medland SE, Porjesz B, Lacono WG, Boomsma DI. Genome-wide association analysis links multiple psychiatric liability genes to oscillatory brain activity. *Hum Brain Mapp* 2018;39:4183–4195. [PubMed: 29947131]
48. Coon H, Darlington TM, DiBlasi E, Callor WB, Ferris E, Fraser A, Yu Z, William N, Das SC, Crowell SE, Chen D, Anderson JS, Klein M, Jerominski L, Cannon D, Shabalin A, Docherty A, Williams M, Smith KR, Keeshin B, Bakian AV, Christensen E, Li QS, Camp NJ, Gray D. Genome-wide significant regions in 43 Utah high-risk families implicate multiple genes involved in risk for completed suicide. *Molecular psychiatry* 2018.
49. Cahill KM, Huo Z, Tseng GC, Logan RW, Seney ML. Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. *Sci Rep* 2018;8:9588. [PubMed: 29942049]
50. Dumais A, Lesage AD, Lalovic A, Seguin M, Tousignant M, Chawky N, Turecki G. Is violent method of suicide a behavioral marker of lifetime aggression? *Am J Psychiatry* 2005;162:1375–1378. [PubMed: 15994723]
51. Ludwig B, Dwivedi Y. The concept of violent suicide, its underlying trait and neurobiology: A critical perspective. *Eur Neuropsychopharmacol* 2018;28:243–251. [PubMed: 29254658]
52. Hu Y, Flockhart I, Vinayagam A, Bergwitz C, Berger B, Perrimon N, Mohr SE. An integrative approach to ortholog prediction for disease-focused and other functional studies. *BMC Bioinformatics* 2011;12:357. [PubMed: 21880147]
53. Zapata I, Serpell JA, Alvarez CE. Genetic mapping of canine fear and aggression. *BMC genomics* 2016;17:572. [PubMed: 27503363]
54. Schaefer J, Giangrande E, Weinberger DR, Dickinson D. The global cognitive impairment in schizophrenia: consistent over decades and around the world. *Schizophrenia research* 2013;150:42–50. [PubMed: 23911259]
55. Odintsova VV, Roetman PJ, Ip HF, Pool R, Van der Laan CM, Tona KD, Vermeiren R, Boomsma DI. Genomics of human aggression: current state of genome-wide studies and an automated systematic review tool. *Psychiatr Genet* 2019;29:170–190. [PubMed: 31464998]
56. Bostwick JM, Pabbati C, Geske JR, McKean AJ. Suicide Attempt as a Risk Factor for Completed Suicide: Even More Lethal Than We Knew. *Am J Psychiatry* 2016;173:1094–1100. [PubMed: 27523496]
57. Le-Niculescu H, Levey DF, Ayalew M, Palmer L, Gavrion LM, Jain N, Winiger E, Bhosrekar S, Shankar G, Radel M, Bellanger E, Duckworth H, Olesek K, Vergo J, Schweitzer R, Yard M, Ballew A, Shekhar A, Sandusky GE, Schork NJ, Kurian SM, Salomon DR, Niculescu AB 3rd. Discovery and validation of blood biomarkers for suicidality. *Molecular psychiatry* 2013;18:1249–1264. [PubMed: 23958961]
58. Pantazatos SP, Huang YY, Rosoklija GB, Dwork AJ, Arango V, Mann JJ. Whole-transcriptome brain expression and exon-usage profiling in major depression and suicide: evidence for altered glial, endothelial and ATPase activity. *Molecular psychiatry* 2017;22:760–773. [PubMed: 27528462]
59. Kim S, Choi KH, Baykiz AF, Gershenfeld HK. Suicide candidate genes associated with bipolar disorder and schizophrenia: an exploratory gene expression profiling analysis of postmortem prefrontal cortex. *BMC genomics* 2007;8:413. [PubMed: 17997842]
60. Zhang L, Verwer RWH, Lucassen PJ, Huitinga I, Swaab DF. Prefrontal cortex alterations in glia gene expression in schizophrenia with and without suicide. *Journal of psychiatric research* 2020;121:31–38. [PubMed: 31739114]
61. Plans L, Barrot C, Nieto E, Rios J, Schulze TG, Papiol S, Mitjans M, Vieta E, Benabarre A. Association between completed suicide and bipolar disorder: A systematic review of the literature. *Journal of affective disorders* 2019;242:111–122. [PubMed: 30173059]
62. Lopes FL, McMahon FJ. The Promise and Limits of Suicide Genetics. *Am J Psychiatry* 2019;176:600–602. [PubMed: 31366231]
63. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O’Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA,

- Wu JQ. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2014;34:11929–11947. [PubMed: 25186741]
64. Stefani J, Tschesnokowa O, Parrilla M, Robaye B, Boeynaems JM, Acker-Palmer A, Zimmermann H, Gampe K. Disruption of the Microglial ADP Receptor P2Y₁₃ Enhances Adult Hippocampal Neurogenesis. *Frontiers in cellular neuroscience* 2018;12:134. [PubMed: 29867367]
 65. Zheng F, Zhou Q, Cao Y, Shi H, Wu H, Zhang B, Huang F, Wu X. P2Y₁₂ deficiency in mouse impairs noradrenergic system in brain, and alters anxiety-like neurobehavior and memory. *Genes Brain Behav* 2019;18:e12458. [PubMed: 29341465]
 66. Zhang Y, Hansson KM, Liu T, Magnell K, Huang Y, Carlson NG, Kishore BK. Genetic deletion of ADP-activated P2Y₁₂ receptor ameliorates lithium-induced nephrogenic diabetes insipidus in mice. *Acta Physiol (Oxf)* 2018:e13191. [PubMed: 30257062]
 67. Cserep C, Posfai B, Lenart N, Fekete R, Laszlo ZI, Lele Z, Orsolits B, Molnar G, Heindl S, Schwarcz AD, Ujvari K, Kornyei Z, Toth K, Szabadits E, Sperlagh B, Baranyi M, Csiba L, Hortobagyi T, Magloczky Z, Martinecz B, Szabo G, Erdelyi F, Szipocs R, Tamkun MM, Gesierich B, Duering M, Katona I, Liesz A, Tamas G, Denes A. Microglia monitor and protect neuronal function through specialized somatic purinergic junctions. *Science* 2020;367:528–537. [PubMed: 31831638]
 68. Nimmerjahn A Monitoring neuronal health. *Science* 2020;367:510–511. [PubMed: 32001643]
 69. Lesch M, Nyhan WL. A Familial Disorder of Uric Acid Metabolism and Central Nervous System Function. *Am J Med* 1964;36:561–570. [PubMed: 14142409]
 70. Torres RJ, Prior C, Garcia MG, Puig JG. A review of the implication of hypoxanthine excess in the physiopathology of Lesch-Nyhan disease. *Nucleosides Nucleotides Nucleic Acids* 2016;35:507–516. [PubMed: 27906640]
 71. McGowan JC, Hill C, Mastrodonato A, LaGamma CT, Kitayev A, Brachman RA, Narain NR, Kiebish MA, Denny CA. Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress. *Neuropsychopharmacology* 2018;43:1813–1821. [PubMed: 29599484]
 72. Wilkinson ST, Ballard ED, Bloch MH, Mathew SJ, Murrough JW, Feder A, Sos P, Wang G, Zarate CA Jr., Sanacora G. The Effect of a Single Dose of Intravenous Ketamine on Suicidal Ideation: A Systematic Review and Individual Participant Data Meta-Analysis. *The American journal of psychiatry* 2018;175:150–158. [PubMed: 28969441]
 73. Aguilar-Valles A, De Gregorio D, Matta-Camacho E, Eslamizade MJ, Khlaifia A, Skaleka A, Lopez-Canul M, Torres-Berrio A, Bermudez S, Rurak GM, Simard S, Salmaso N, Gobbi G, Lacaille JC, Sonenberg N. Antidepressant actions of ketamine engage cell-specific translation via eIF4E. *Nature* 2021;590:315–319. [PubMed: 33328636]
 74. Gandal MJ, Zhang P, Hadjimichael E, Walker RL, Chen C, Liu S, Won H, van Bakel H, Varghese M, Wang Y, Shieh AW, Haney J, Parhami S, Belmont J, Kim M, Moran Losada P, Khan Z, Mleczko J, Xia Y, Dai R, Wang D, Yang YT, Xu M, Fish K, Hof PR, Warrell J, Fitzgerald D, White K, Jaffe AE, Psych EC, Peters MA, Gerstein M, Liu C, Iakoucheva LM, Pinto D, Geschwind DH. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science* 2018;362.
 75. Ge H, Yan Y, Wu D, Huang Y, Tian F. Potential role of LINC00996 in colorectal cancer: a study based on data mining and bioinformatics. *Onco Targets Ther* 2018;11:4845–4855. [PubMed: 30147336]
 76. Dunn HA, Zucca S, Dao M, Orlandi C, Martemyanov KA. ELFN2 is a postsynaptic cell adhesion molecule with essential roles in controlling group III mGluRs in the brain and neuropsychiatric behavior. *Molecular psychiatry* 2019;24:1902–1919. [PubMed: 31485013]
 77. Koyama R, Yamada MK, Fujisawa S, Katoh-Semba R, Matsuki N, Ikegaya Y. Brain-derived neurotrophic factor induces hyperexcitable reentrant circuits in the dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2004;24:7215–7224. [PubMed: 15317847]
 78. Badimon A, Strasburger HJ, Ayata P, Chen X, Nair A, Ikegami A, Hwang P, Chan AT, Graves SM, Uweru JO, Ledderose C, Kutlu MG, Wheeler MA, Kahan A, Ishikawa M, Wang YC, Loh YE, Jiang JX, Surmeier DJ, Robson SC, Junger WG, Sebra R, Calipari ES, Kenny PJ, Eyo

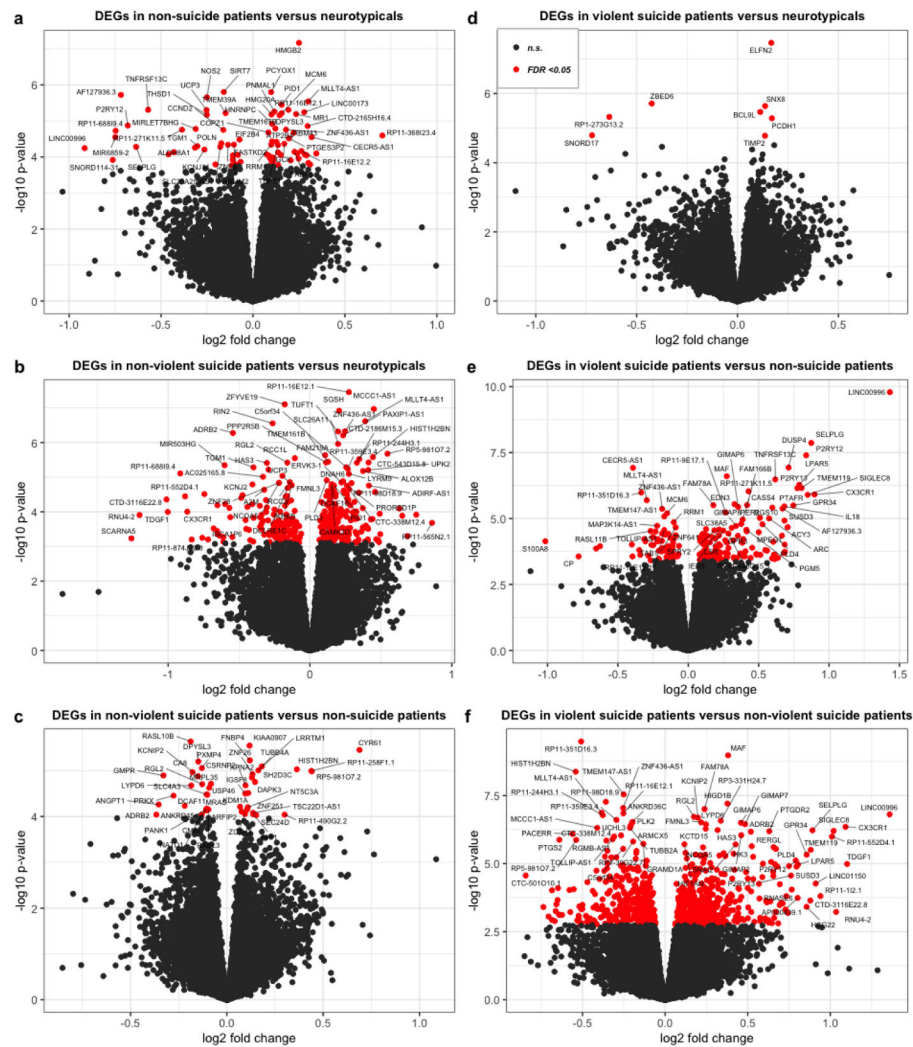
- UB, Colonna M, Quintana FJ, Wake H, Gradinaru V, Schaefer A. Negative feedback control of neuronal activity by microglia. *Nature* 2020;586:417–423. [PubMed: 32999463]
79. Merlini M, Rafalski VA, Ma K, Kim KY, Bushong EA, Rios Coronado PE, Yan Z, Mendiola AS, Sozmen EG, Ryu JK, Haberl MG, Madany M, Sampson DN, Petersen MA, Bardehle S, Tognatta R, Dean T, Jr., Acevedo RM, Cabrera B, Thomas R, Coughlin SR, Ellisman MH, Palop JJ, Akassoglou K. Microglial Gi-dependent dynamics regulate brain network hyperexcitability. *Nature neuroscience* 2021;24:19–23. [PubMed: 33318667]

Author Manuscript

Author Manuscript

Author Manuscript

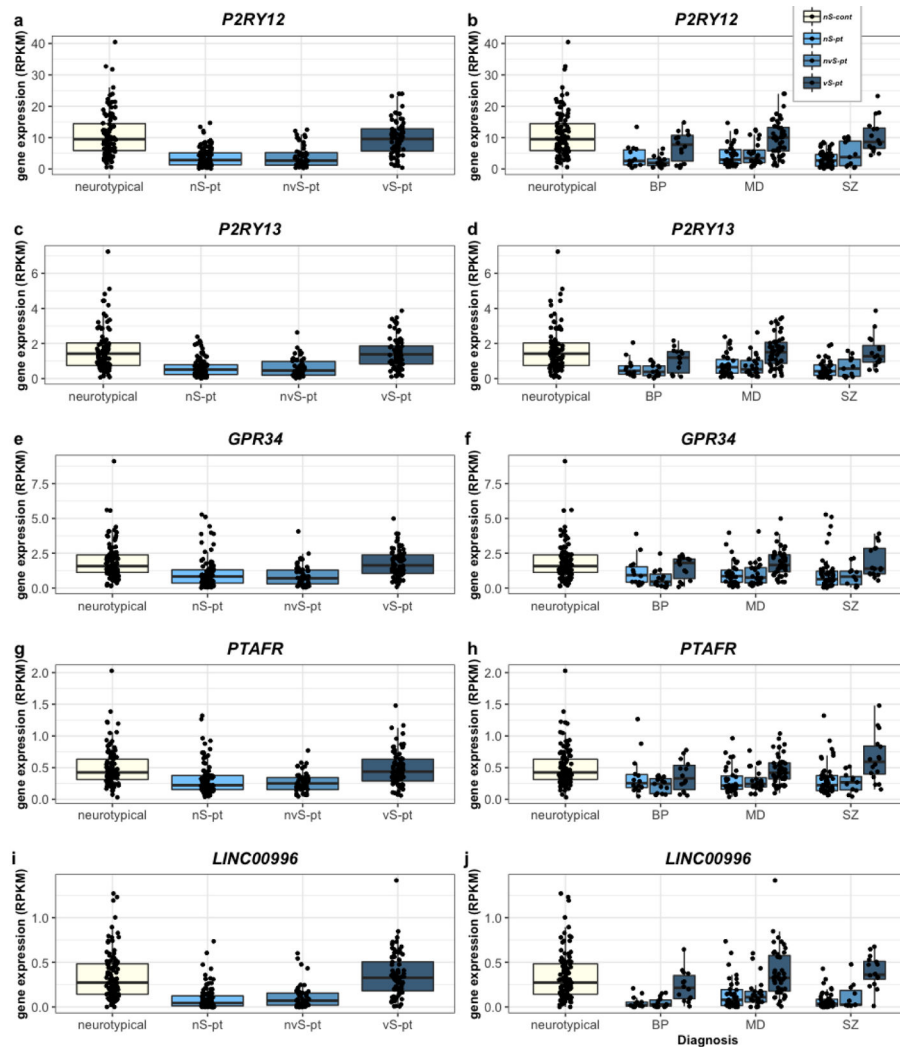
Author Manuscript



333x385mm (72 x 72 DPI)

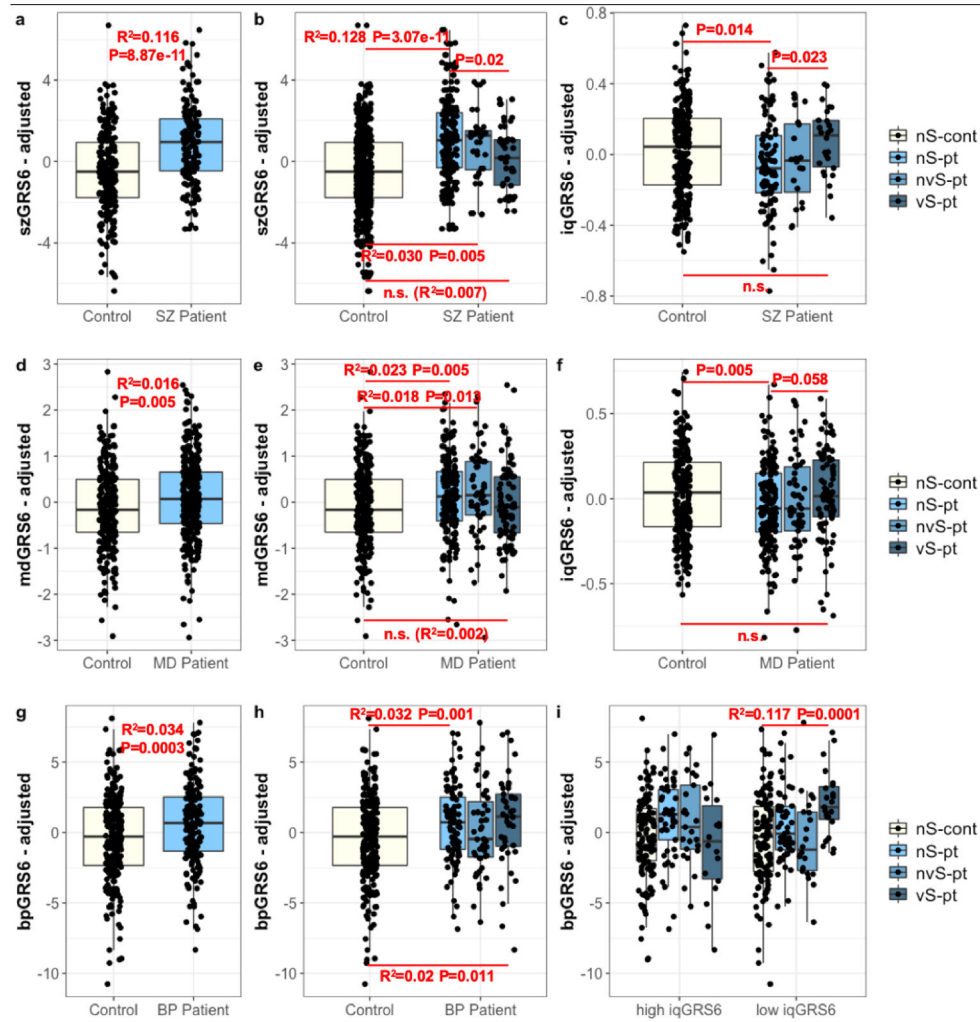
Fig. 1 |. Differential expression analysis.

Volcano plots of the differentially expressed genes (DEGs) in DLPFC (RNAseq) obtained comparing: **a.** non-suicide patients with neurotypicals **b.** non-violent suicide patients with neurotypicals; **c.** non-violent suicide patients with non-suicide patients; **d.** violent suicide patients with neurotypicals; **e.** violent suicide patients with non-suicide patients; **f.** violent suicide patients with non-violent suicide patients. DEGs at FDR<0.05 are shown in red, top DEGs are labeled with gene name. All the statistics were obtained from the DE analyses using a linear model adjusting for sex, age, and quality surrogate variables accounting for RNA quality.



333x385mm (72 x 72 DPI)

Fig. 2 |. Transcription differences between violent suicide patients and neurotypicals. Boxplots of expression of the purinergic genes (a-b. *P2RY12*; c-d. *P2RY13*; e-f. *GPR34*; g-h. *PTAFR*) and of i-j. *LINC00996*, in the combined patient sample (left) and by diagnosis (right). Neurotypical = non-suicide control; nS-pt = non-suicide patient; nvS-pt = non-violent suicide patient; vS-pt = violent suicide patient. BP = bipolar disorder; MD = major depression disorder; SZ = schizophrenia. The purinergic genes and *LINC00996* are consistently up-regulated in patients who died of suicide by violent means, and in neurotypicals, compared with other patients, in each diagnostic group.

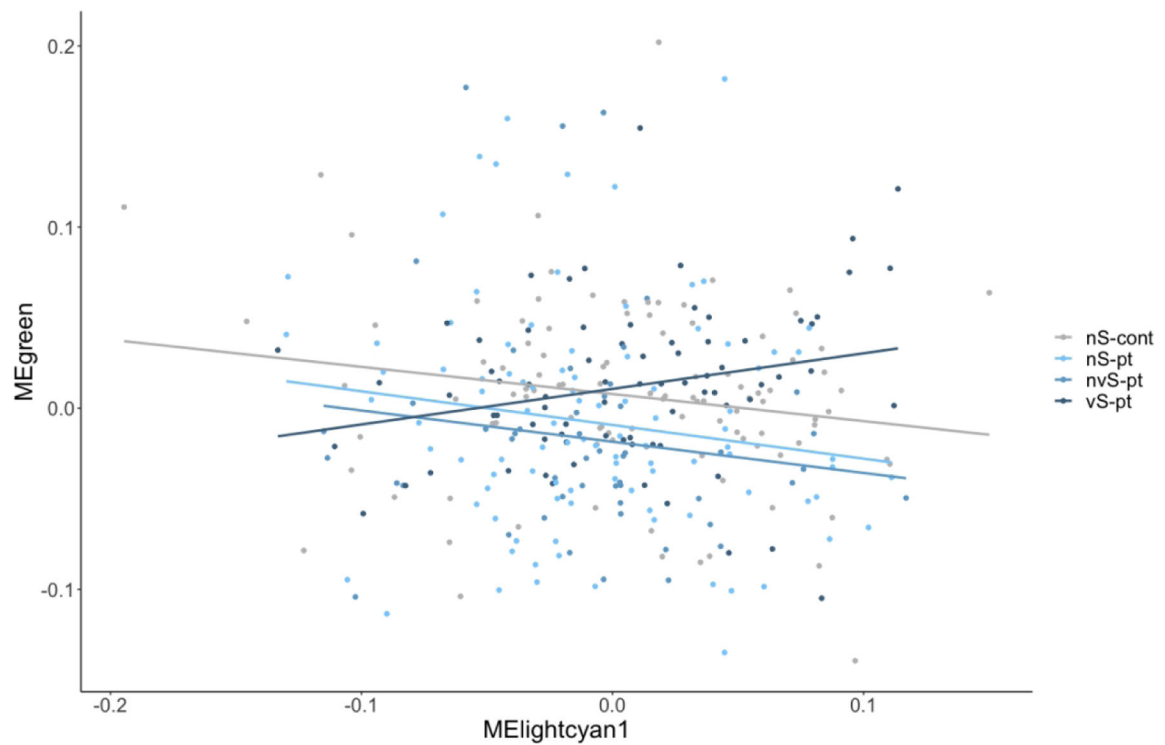


182x192mm (150 x 150 DPI)

Fig. 3 |. Analysis of genomic risk.

Boxplots of the relationship between diagnosis-GRS and IQ GRS with case-control status in each of the three diagnostic groups, that is: schizophrenia (**a-c**), major depression (**d-f**), and bipolar disorder (**g-i**). **a-c**, Subsample of neurotypicals and patients with schizophrenia (SZ): **a**. GRS6 for schizophrenia (szGRS6 is GRS at .05 P-value) and SZ case-control status; **b**. szGRS6 and SZ case-controls status, with SZ patients divided in the three manner of death categories: non-suicide patients (nS-pt), non-violent suicide patients (nvS-pt), violent suicide patients (vS-pt); **c**. GRS6 for IQ (iqGRS6) and SZ case-controls status, with SZ patients divided in the three categories. **d-f**, Subsample of neurotypicals and major depression (MD): **d**. GRS6 for major depression (mdGRS6) and MD case-control status; **e**. mdGRS6 and MD case-controls status, with MD patients divided in the three categories; **f**. iqGRS6 and MD case-controls status, with MD patients divided in the three categories. **g-i**, Subsample of neurotypicals and patients with bipolar disorder: **g**. GRS6 for bipolar disorder (bpGRS6) and

BP case-control status; **h.** bpGRS6 and BP case-controls status, with BP patients divided in the three categories; **i.** Interaction between bpGRS6 and iqGRS6 on BP case-controls status, with patients divided in the three categories. N.B. control = non-suicide controls (a.k.a. neurotypicals). See main text for comment and detailed statistics. All the statistics were generated using multiple logistic regression (a-b, d-e, g-i) and multiple regression (c-f), adjusting for population stratification (ten PCs), sex, and age.



444x298mm (72 x 72 DPI)

Fig. 4 |. WGCNA, interaction between *lightcyan1* ME and groups on *green* ME.

Boxplots of the interaction between *lightcyan1* (i.e. energy metabolism in mitochondria) module eigengene (ME) and the 4 main groups on *green* (G protein-coupled purinergic signaling) ME. Interaction statistics: neurotypicals (a.k.a. nS-cont)*violent suicide patients $t=-2.852$, $p=0.00462$; non-suicide patients*violent suicide patients $t=-2.482$, $p=0.01358$; non-violent suicide patients*violent suicide patients $t=-2.095$, $p=0.03693$. All the statistics were generated using multiple regression with the interaction term ME*group.