Archival Report

Biological Psychiatry: Celebrating 50 Years

Association of a Noncoding RNA Postmortem With Suicide by Violent Means and In Vivo With Aggressive Phenotypes

Giovanna Punzi, Gianluca Ursini, Giovanna Viscanti, Eugenia Radulescu, Joo Heon Shin, Tiziana Quarto, Roberto Catanesi, Giuseppe Blasi, Andrew E. Jaffe, Amy Deep-Soboslay, Thomas M. Hyde, Joel E. Kleinman, Alessandro Bertolino, and Daniel R. Weinberger

ABSTRACT

BACKGROUND: Previous findings suggest that differences in brain expression of a human-specific long intergenic noncoding RNA (*LINC01268*; GRCh37/hg19: *LOC285758*) may be linked to suicide by violent methods. We sought to replicate and extend these findings in a new sample and translate the results to the behavioral level in living healthy subjects.

METHODS: We examined RNA sequencing data in human brains to confirm the prior postmortem association of the long intergenic noncoding RNA specifically with suicide by violent means. In addition, we used a genetic variant associated with *LINC01268* expression to detect association in healthy subjects with trait aggression and with in vivo prefrontal physiology related to behavioral control. Finally, we performed weighted gene coexpression network analysis and gene ontology analysis to identify biological processes associated with a *LINC01268* coexpression network.

RESULTS: In the replication sample, prefrontal expression of *LINC01268* was again higher in suicides by violent means ($n = 65$) than in both nonsuicides ($n = 78$; $p = 1.29 \times 10^{-6}$) and suicides by nonviolent means ($n = 46$; $p = 1.4 \times 10^{-6}$). In the living cohort, carriers of the minor allele of a single nucleotide polymorphism associated with increased *LINC01268* expression in brain scored higher on a lifetime aggression questionnaire and show diminished engagement of prefrontal cortex (Brodmann area 10) when viewing angry faces during functional magnetic resonance imaging. Weighted gene coexpression network analysis highlighted the immune response.

CONCLUSIONS: These results suggest that *LINC01268* influences emotional regulation, aggressive behavior, and suicide by violent means; the underlying biological dynamics may include modulation of genes potentially engaged in the immune response.

Keywords: Aggression, Brain, Postmortem, RNA-seq, Suicide, Suicide methods

https://doi.org/10.1016/j.biopsych.2018.11.002

Suicide is the 10th leading cause of death in the United States for all age groups combined (1), a trend that has been steadily increasing in the last 15 years against the backdrop of generally declining mortality (2). Psychiatric disorders are strongly predictive of suicidal ideation but are less suitable in predicting which patients will actually act on such thoughts; in contrast, severe anxiety/agitation and poor impulse control are better indicators of higher risk for suicide plans and attempts (3). The choice of violence as a means of suicide has been considered a correlate of the cumulative amount of lifetime impulsive-aggressive behavior (4), and, consistently, an association between violent offending behavior and suicide has been suggested as well (5). The two phenomena can ultimately coexist in a single episode of acting-out (homicide-suicide or extended suicide) (6). Recent findings (7) also show that the choice of a violent method for a self-harm episode translates into a remarkably higher risk for suicide in the short term; further research suggests an increase in such self-inflicted injuries particularly among youths (8).

As suicidal ideation is a limited predictor of outcome, the detection of genetic markers of risk is a valuable insight for prevention (9), with impulsive aggression as a strong candidate endophenotype (10,11). One approach in this line of research has been to investigate genetic signaling involved in the action of drugs credited as effective against suicide, such as lithium (12). Intriguingly, levels of lithium in drinking water have been associated with lower suicidality (13,14) and with lesser rates of homicides (15). In a prior study of a small cohort of patients with schizophrenia (16), we found that the expression of *MARCKS*, a gene repeatedly implicated in suicidal behavior (9,17,18) and downregulated by lithium (19), is significantly increased in the dorsolateral prefrontal cortex (DLPFC) of

ISSN: 0006-3223

suicides, specifically by violent means. The association of *MARCKS* with suicide, however, was statistically conditioned on a long intergenic noncoding RNA (lincRNA) that maps adjacent to it—*LINC01268*; GRCh37/hg19: *LOC285758* (16)—suggesting that this lincRNA is responsible for the *MARCKS* association. Intriguingly, the non–protein-coding portion of the human genome has been increasingly recognized as a source of evolutionary organismal complexity (20). Whereas molecular mechanisms lie in the chain of causation from genome to behavior, aggressive and suicidal acts ultimately involve complex, human-specific behavior and emotional control, and indeed long-term risk for suicide at the level of higher-order brain function has been related to control dysfunction of PFC (21).

The main hypothesis underlying the present report is that the choice of a violent method of suicide represents a more precise feature to specifically target to detect genetic signatures for suicide in general. The aims of this study were 1) to replicate our earlier finding of a link between expression of *LINC01268* in PFC and suicide specifically by violent means (16) by analyzing a new sample of human brains, including patients with schizophrenia, depression, and bipolar disorder; 2) to translate this finding into a behavioral context via a single nucleotide polymorphism (SNP) associated with *LINC01268* expression, a measure of lifelong aggressive behavior, and a functional magnetic resonance imaging (fMRI) paradigm targeting prefrontal function related to negative emotion processing; and 3) to further explore the biological meaning of the transcript through analysis of the network of genes coexpressed with it in brain using weighted gene coexpression network analysis (WGCNA).

METHODS AND MATERIALS

Postmortem Data

Subjects. A large cohort of brains with an RNA integrity number ≥6.9 was obtained from the Lieber Institute for Brain Development brain repository, focusing on the available RNA sequencing (RNA-seq) dataset from DLPFC (Brodmann areas [BA] 9 and 46) (22). The relationship between suicide and the lincRNA was first explored in a diagnostically mixed sample of 189 adult Caucasian patients who met DSM-IV criteria for a lifetime diagnosis of schizophrenia, bipolar disorder, or major depressive disorder, individuals not included in our earlier study. The comparison between suicides and nonsuicides is best evaluated in patients only, to control for associations with a diagnostic state. A single race (Caucasian) was chosen to avoid confounding effects on gene expression based on racial genomic variation; only donors ≥13 years of age were selected, as gene expression displays strong nonlinear changes during developmental stages (23). One brain donor was discarded as an extreme outlier for *LINC01268* expression (see Statistical Analysis). Within this replication sample, 111 individuals completed suicide, and 78 were nonsuicidal. Within the suicide cohort, 46 were suicides by nonviolent means, and 65 were suicides by violent means. In addition to testing for association between suicide by violent means and our lincRNA in this new sample, we also analyzed the results after adding patients from the previous study. Following the described criteria for inclusion, the total brain sample comprised 228 subjects. Table S1 in Supplement 1 provides a summary of the combined patient sample. The demographics portion of Table S1 in Supplement 1 shows that suicide subjects were younger than nonsuicide subjects ($p = .002$), especially those who committed suicide by violent means ($p < .002$), and that among suicide subjects, more male subjects chose violent means than female subjects ($p < .006$). These data support the involvement of both age-related and sex-related factors in impulsive/aggressive behaviors.

Postmortem Brain Tissue. Postmortem brains from the Lieber Institute for Brain Development were collected at autopsy from several medical examiners; some of these brain tissues were transferred under a Material Transfer Agreement from the National Institute of Mental Health. A similar uniform procedure was employed in the acquisition, processing, and dissection of all samples regardless of source and affiliation; detailed methods relating to the collection have been reported elsewhere (22,24).

Manner of Death and Suicide. Cause and manner of death and contributory causes or medical conditions related to death were obtained from medical examiner documents (22). Nonsuicidal deaths included natural deaths, accidents, and homicides. In regard to the suicide group, attribution of suicide method (i.e., violent vs. nonviolent) was determined blind to the postmortem RNA-seq data. For such classification, we did not simply rely on the medical examiner labeling and fixed classes as in previous literature [e.g., poisoning or asphyxia = nonviolent, others = violent (25)], but performed a case-by-case in-depth evaluation of all available information, including interviews with next of kin. Supplement 1 contains a detailed discussion of violent and nonviolent methods. Table 1 provides details about the suicides in the whole sample of 228 subjects (see also Subjects above).

Gene Expression. RNA extraction, sequencing, and data processing were performed as previously described (26); details are provided in Supplement 1.

Table 1. Details of Suicides in the Whole Sample (*N* = 228 Subjects)

Types of Suicide	No. of Cases
Suicides by Violent Means	
Hanging	38
Gunshot	15
Blunt force	13
Sharp force	5
Asphyxia (violent method)	4
Motor vehicle accident	1
Low-voltage electrocution	1
Suicides by Nonviolent Means	
Drug poisoning	31
Overdose	13
Carbon dioxide poisoning	4
Asphyxia (suicide bag)	1
Asphyxia (positional)	1

Genotyping. Genomic DNA was extracted from cerebellum of the same samples using standard procedures with FlexiGene DNA kits (QIAGEN GmbH, Hilden, Germany). Genotyping was performed as previously described (22,27,28), and an independent set of SNPs obtained by linkage disequilibrium (LD) pruning (29) was used to perform genome-wide clustering to obtain multidimensional scaling components for quantitative measures of ancestry. For our expression quantitative trait loci (eQTL) analysis, we removed SNPs showing genotype missing rate >5%, deviation from Hardy-Weinberg equilibrium ($p < .0001$), or minor allele frequency <15% (to optimize sample size in genotypic groups). Additional quality control 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.

eQTL Identification. We searched for SNPs associated with *LINC01268* expression in a sample of healthy subjects ($N = 105$) from the Lieber Institute for Brain Development brain repository to avoid confounding effects of diagnosis and treatment. Similar to the patients sample, these subjects were Caucasian and ≥13 years of age for design homogeneity. The age criterion was further supported by the evidence that the brain expression of the lincRNA did show strong nonlinear changes before the age of 13 years in the healthy subject cohort (Figure S1 in Supplement 1).

Weighted Gene Coexpression Network Analysis. WGCNA (30) was performed on RNA-seq data from DLPFC of subjects completing suicide by violent means, with gene ontology analysis on each module identified. Validation of the modules was further achieved with module preservation. Details of this method are provided in Supplement 1.

Behavioral Data

Subjects. The Group of Psychiatric Neuroscience at the University of Bari, Italy, recruited 72 Italian healthy, unrelated Caucasian adults (all >18 years of age), nonoffenders, for this study. Participants provided written informed consent; protocols and procedures were approved by the local institutional review board. All subjects underwent the Structured Clinical Interview for DSM-IV to exclude Axis I psychiatric disorders. None of the subjects had a history of significant drug or alcohol abuse (no active drug use in the 6 months before the study), head trauma with loss of consciousness, or significant medical illness or psychiatric disorders in first-degree relatives. One outlier (see Statistical Analysis) for adulthood aggressiveness (based on the Brown-Goodwin [BG] questionnaire score related to adulthood [Adult BG]; see below) was removed, and 2 more subjects were excluded because the aggressive behaviors uncovered by the interview did not fully qualify them as nonoffenders. The final sample consisted of 69 subjects (32 male; mean ± SD age = 26.12 ± 4.84), of whom 51 were TT, 13 were CT, and 5 were CC for the genotype of interest (rs7747961, the eQTL identified in our analysis; see Results). From this cohort, 58 individuals underwent 3T fMRI. They were matched for age, sex, handedness (Edinburgh Inventory), socioeconomic status (Hollingshead Scale), and IQ (Wechsler Adult Intelligence Scale–Revised).

DNA Extraction and Genotyping. DNA was extracted from whole blood using standard procedures, with QIAamp DNA Blood Midi Kit (QIAGEN GmbH); genotyping and quality control were performed as described for the postmortem sample.

Life History of Aggression. All subjects were evaluated by a psychiatrist using the revised version of the BG questionnaire consisting of 11 items (31), which measures actual behavioral manifestations rather than temperament. The interview provides distinct scores for each phase of life (≤12 years, 12–18 years, and ≥18 years, i.e., adulthood) and a total score. For the purposes of this study, only the BG score related to adulthood (Adult BG; mean ± SD value = 15.38 ± 3.16) was analyzed as an index of current aggressiveness.

fMRI Paradigm. The event-related fMRI paradigm (32) consisted of presentation of faces with angry, fearful, happy, and neutral emotional expressions from a validated set of facial pictures (NimStim Face Stimulus Set; https://www.macbrain.org/resources.htm) (33). Subjects were asked whether they would approach or avoid the faces presented during the task [explicit emotional processing (32)]. In the present study, we focused on brain responses during processing of angry faces (angry stimuli vs. crosshairs), as they are considered signals of threat and tend to evoke hostile feelings (34).

fMRI Data Acquisition. Details are reported in Supplement 1.

Statistical Analysis

All data processing was performed using R statistical language (35). Grubbs' tests and 3× interquartile range method (36) were used to identify outliers. Normality test (Shapiro-Wilk) showed that *LINC01268* expression did not exhibit a normal distribution; therefore, logarithmic values (log2) were calculated and employed in each analysis, with an offset of 1 to avoid issues with 0s. Two-sample *t* test and χ^2 test for differences were used to compare demographic data across manners of death in the postmortem cohort and across genotypes in the Italian sample. χ^2 test was also used to test the effect of comorbidity on suicide by violent means versus nonsuicide. Linear models (i.e., linear regressions) were used for comparisons of RNA expression between groups, with manner of death as an independent variable (or comorbidity in the sensitivity analyses) and with age, sex, and RNA integrity number as covariates (age and RNA integrity number in the analysis by sex). Linear models were used for the eQTL analysis, with 10 gene expression heterogeneity principal components and 3 genetic principal components as covariates (28). Linear models were used to investigate the relationship between Adult BG and genotype, covarying for age and sex. In all analyses, age was coded in years as a continuous variable.

fMRI Data Analysis and WGCNA. Details are reported in Supplement 1.

RESULTS

Expression of *LINC01268* and Suicide

To evaluate the relationship between DLPFC expression of the lincRNA and suicidal behavior in general (i.e., violent and nonviolent methods together) in a new sample of subjects (N = 189), the two main groups of suicidal and nonsuicidal brain donors were first compared. The analysis (Figure S2A in Supplement 1) confirmed the association of *LINC01268* RNA expression levels (reads per kilobase per million) with suicide, in that people who completed suicide had significantly higher levels of *LINC01268* in their DLPFC than nonsuicidal donors (t = 2.750, p = .006). Subsequently, the subgroup of suicides by violent means was compared with nonsuicides and suicides by nonviolent means. This analysis (Figure S2B in Supplement 1) confirmed that the association between *LINC01268* and suicide was driven exclusively by the suicides by violent means: the violent suicide group had higher *LINC01268* expression in DLPFC than both nonsuicide and suicide by nonviolent means groups (t = 5.064, $p = 1.29 \times 10^{-6}$; t = 5.116, $p = 1.4 \times 10^{-6}$, respectively). Nonsuicides and suicides by nonviolent means did not differ in *LINC01268* expression values (t = −0.505, p = .614). The analysis yields similar results by adding diagnosis to the model as a covariate. Figure 1A and B displays our cumulative findings by including the subjects that we have previously studied (16): the combined sample comprises brains from 228 individuals, thus increasing statistical power.

We further tested this larger sample in a number of sensitivity analyses. Additional boxplots show the effect of suicide by violent means versus nonsuicide on DLPFC expression of *LINC01268*, divided by sex and diagnosis (Figures S3 and S4 in Supplement 1). The expression of the lincRNA is significantly higher in the suicide by violent means group than in the nonsuicide group in each sex (Figure S3 in Supplement 1) and in each diagnosis (Figure S4 in Supplement 1), although in the bipolar disorder group there is only a statistical trend, likely owing to the relatively smaller size of this subcohort (Table S1 in Supplement 1). Moreover, a codiagnosis of alcohol and/or substance use is not associated with suicides by violent means ($\chi^2_1 = 1.0789$, p = .299) and is not associated with brain expression of *LINC01268* ($t_{4,223} = -0.917$, p = .3604). Furthermore, to minimize artifacts such as occult RNA quality differences that may affect the results and partially explain the differences between the three groups, we repeated the analysis with the addition of adjusting covariates from a principal components analysis, as reported in Additional Sensitivity Analyses in Supplement 1. These analyses, confirming all the results, excluded also the potential effect on gene expression of the injuries resulting from the violent action, i.e., it is suicide per se that relates to RNA-seq differences rather than the physical damage.

rs7747961 as a Candidate eQTL for *LINC01268*

We searched for *cis*-acting eQTL possibly associated with expression of the *LINC01268* transcript by evaluating SNPs within 100,000 bp upstream and downstream of the gene coordinates. Of the SNPs tested in a dataset from brains of healthy subjects, none survived correction for multiple comparisons across the genome (false discovery rate); however, SNPs with nominally significant p values (< .05) were further explored. An additive model uncovered five SNPs that fit this criterion: three of them are independent in our sample ($r^2 < .1$, rs503593, rs9481396, and rs7747961); two others were in strong LD with rs9481396 (rs73547709 [R^2 = .964, D′ = 1.000] and rs59034807 [R^2 = .892, D′ = 0.962]). Whereas rs9481396 showed overdominance, thus not allowing us to identify the allele clearly associated with increased expression, the associations of rs503593 and rs7747961 with gene expression had more linear distributions ($p < .05$). In further analysis employing a dominant model, only rs7747961 showed nominally significant association with *LINC01268* levels (p = .041): carriers of the minor C allele (CT and CC genotypes) showed significantly higher values of *LINC01268* transcript than homozygotes for the major allele (Figure 2A). rs7747961 lies 2205 bases upstream of the *MARCKS* transcription start site and is linked with regulatory element markers at the SNP site in chromatin immunoprecipitation experiments from publicly available datasets from brain (e.g., https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php). As this SNP did not reach multiple testing–corrected levels of significance, we performed additional sensitivity analyses. We selected the 23 LD-independent SNPs within the 200-kb window of our original SNP expression analyses using pruning of the genotype data (LD $R^2 < .1$) (29). rs7747961 had stronger eQTL statistics than

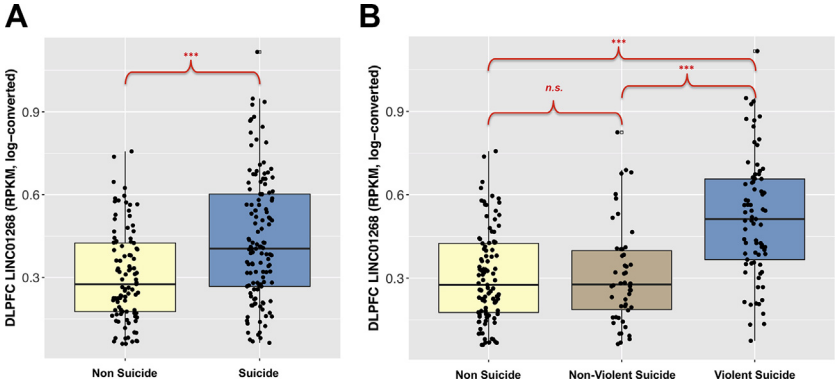

Figure 1. **(A, B)** Boxplots of the effect of manner of death on the dorsolateral prefrontal cortex (DLPFC) expression of *LINC01268* in the total sample (N = 228, all patients, with age, sex, and RNA integrity number as covariates). Manner of death is significantly associated with *LINC01268* expression, which is greater in suicidal samples compared with nonsuicidal samples **(A)** and specifically in suicides by violent means (n = 77) compared with nonsuicides (n = 101) and suicides by nonviolent means (n = 50) **(B)**. Nonsuicides and suicides by nonviolent means do not differ. Statistics: **(A)** Nonsuicide vs. suicide: $t_{1,223} = 3.979$, $p = 9.37 \times 10^{-5}$; **(B)** nonsuicide vs. violent suicide: $t_{1,173} = 6.527$, $p = 7.16 \times 10^{-10}$; nonsuicide vs. nonviolent suicide: $t_{1,146} = 0.056$, $p = .955322$; nonviolent suicide vs. violent suicide: $t_{1,122} = 5.450$, $p = 2.66 \times 10^{-7}$. RPKM, reads per kilobase per million. ***$p < .001$. n.s., not significant.

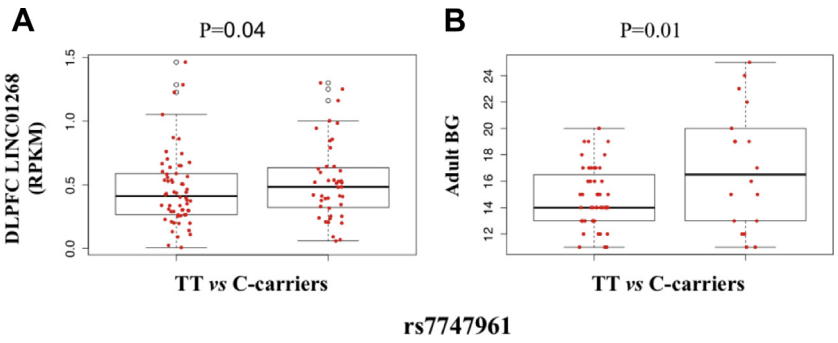

Figure 2. **(A)** Boxplots of the association of rs7747961 genotype with dorsolateral prefrontal cortex (DLPFC) expression of *LINC01268*. Statistics for expression quantitative trait loci linear model (10 expression heterogeneity principal components and 3 genetic principal components) are reported at the top of the graphic. In both additive (not shown) and dominant models, rs7747961 genotype is nominally significantly associated with *LINC01268* expression, which is greater in C carriers (right) compared with homozygotes for the major allele T (left). **(B)** Boxplots of the effect of rs7747961 genotype on levels of adult ratings of aggressiveness (based on Brown-Goodwin [BG] questionnaire score related to adulthood [Adult BG]). Statistics for linear model (with age and sex as covariates) are reported at the top of the graphic. rs7747961 genotype is significantly associated with aggressiveness in adulthood, which is greater in C carriers (right) compared with homozygotes for the major allele T (left). RPKM, reads per kilobase per million.

all 23 independent SNPs (*p* value range: .1–.9) in the discovery dataset. Lastly, we tested rs7747961 as a potential eQTL for *LINC01268* in DLPFC in the independent CommonMind Consortium (37) control sample dataset (N = 216) and confirmed this association, which was also directionally concordant (in both additive and dominant models, $p < .05$). Hence, rs7747961 was considered suitable as an eQTL for interrogation of a potential proxy of *LINC01268* expression in in vivo clinical genetic studies.

Association of rs7747961 With Behavioral Measures of Aggressiveness

To translate *LINC01268* transcription to the behavioral level and expand our inquiry to living subjects, an independent sample of living healthy subjects was assessed for a trait measure of aggressiveness and genotyped for rs7747961. The relationship between Adult BG and genotype was tested using the same dominant model used with the postmortem expression results. Remarkably, the same genotypes (the carriers of the C allele) associated with increased expression of *LINC01268* in postmortem brains were also associated with more aggressiveness in healthy subjects (p = .01) (Figure 2B), with the same allelic directionality. We further considered the 23 LD SNPs described above and found that rs7747961 was more strongly associated with Adult BG than any of them (*p* value range: .1–.9), suggesting that this SNP or less frequent variants in LD with it may play a role in aggressive behavior. Finally, in the largest sample exploring aggressive behavior in children to date (N = 18,988) and the first genome-wide association study on the subject, the minor allele for rs7747961 was consistently associated with higher aggression in children, although with a p value below the threshold for genome-wide association significance (false discovery rate p = .091) (38).

rs7747961, Aggressiveness, and Brain Activity During Explicit Emotion Processing

Given the association between *LINC01268* RNA brain levels and rs7747961 and between the latter and aggressiveness, data from an fMRI study of response to emotionally charged stimuli (i.e., angry faces) in the healthy subject sample were analyzed. The factorial regression analysis indicated no main effect of rs7747961 or Adult BG on the imaging data but uncovered an interaction between rs7747961 and Adult BG in right BA 10 (Montreal Neurological Institute coordinates: x = 36, y = 50, z = 28; Z = 3.53; familywise error–corrected p = .05) (Figure 3A). In particular, post hoc t tests revealed that a negative correlation between right BA 10 activity during explicit processing of angry faces and Adult BG scores was present in C carriers, whereas a positive correlation was present in TT carriers (all familywise error–corrected $p < .05$) (Figure 3B). This interaction explains the lack of a major genotype effect.

Gene Coexpression Network Analysis

Lastly, to gain potential insight into the biological processes related to *LINC01268*, we explored coexpression gene networks in postmortem DLPFC that contained *LINC01268*

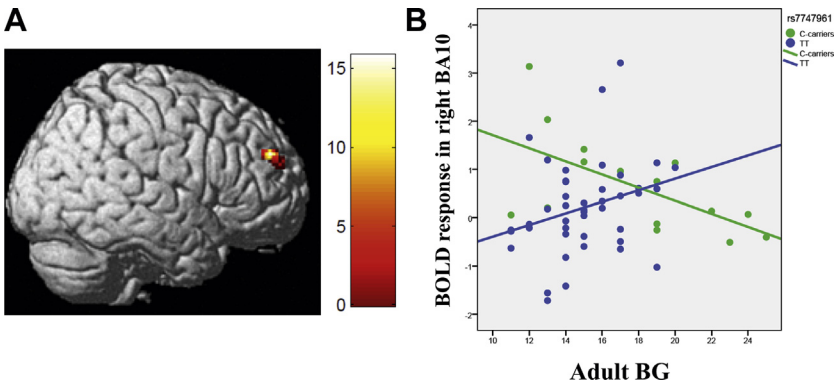

Figure 3. Functional magnetic resonance imaging study. **(A)** Rendered image showing the interaction between adulthood aggressiveness (based on Brown-Goodwin [BG] questionnaire score related to adulthood [Adult BG]) and rs7747961 in right Brodmann area (BA) 10. **(B)** Scatterplot showing the significantly different relationship in C carriers and TT carriers between Adult BG and blood oxygen level–dependent (BOLD) response in right BA 10 during explicit processing of angry faces. See text for statistics.

using the WGCNA algorithm (30). WGCNA was performed on the RNA-seq dataset from the sample of suicide by violent means and identified 15 modules of coexpressed genes. These modules (randomly color-coded for discussion purposes) varied in size from 41 to 2130 genes. Using our conservative approach to RNA quality correction, 13,341 expressed genes were not allocated to any module (gray genes). Our module of primary interest (i.e., the module including *LINC01268*) was labeled as magenta and contained 224 genes. Figure 4 shows the genes within the magenta module for which the weights of interconnectivity (edges) were greater than 0.1, an applied threshold for visualization purposes (figure created with VisANT; http://visant.bu.edu/). Within the magenta module, *LINC01268* had the strongest connection with *P2RY13*, a purinergic receptor present both in the peripheral immune system and in brain (39), whereas the most connected gene in the whole module (also known as the hub) was *LAPTM5*, a gene mainly expressed in hematopoietic cells and localized to the lysosome and shown to modulate inflammatory response by macrophages (40). The magenta module, containing *LINC01268*, was significantly enriched ($p < .01$, $q < .05$ after Benjamini-Hochberg correction) for 480 gene ontology biological processes gene sets related to immunological functions, such as positive regulation of immune response (Table S2 in Supplement 2). Module preservation analysis used to support network validity showed that the magenta module was strongly preserved in nonsuicide and in suicide by nonviolent means expression datasets ($Z_{summary} > 40$ and median rank ≈ 1) (Figure S5 in Supplement 1), indicating that *LINC01268* belongs to a network associated with immunity, not limited to suicide by violent means. However, we cannot exclude potential differences between groups in gene connectivity strengths (e.g., differences between groups concerning intramodular, extramodular, or total connectivity for *LINC01268*), which may be investigated in the future with more detailed analyses (41).

DISCUSSION

We report a molecular association in human brain with suicide by violent means that is both strong and replicable, identifying the first noncoding RNA consistently associated with suicidal behavior/aggression. Data from a new sample confirm the presence of a significant difference in DLPFC expression levels of *LINC01268* between suicide and nonsuicide deceased subjects and specifically increased *LINC01268* expression when violent means of suicide are chosen. Moreover, an SNP associated with its expression, rs7747961, is associated with personal ratings of aggressiveness in a cohort of living healthy subjects; the same SNP interacts with a prefrontal physiological assay of processing emotionally charged and potentially aggressive stimuli on fMRI. Finally, *LINC01268* is significantly coexpressed in brain with genes related to the immune response, at least in the periphery.

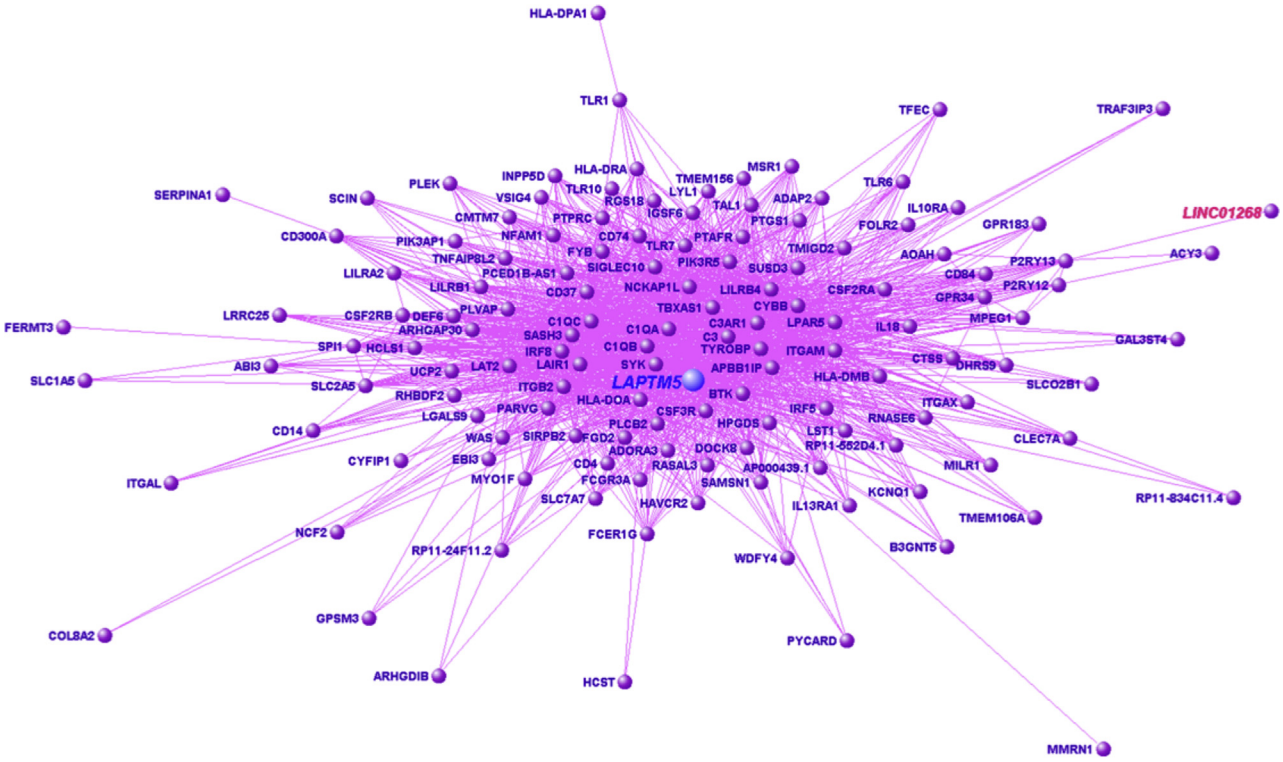

Figure 4. Weighted gene coexpression network analysis findings. Magenta module subnetwork containing *LINC01268* (note: owing to graphical constraints, only genes with strength of connectivity >0.1 are shown, to avoid cluttering). The hub (i.e., most connected) gene in the module, *LAPTM5*, is labeled in blue, and the gene of interest, *LINC01268*, is labeled in red. Within this group of top most connected genes, *LINC01268* has the strongest connectivity only with *P2RY13*. (Figure created with VisANT.)

In contrast to current clinical studies, our findings are from completed suicide cases. Most suicide research looks at survivors, which means studying subjects who did not actually carry out the very behavior being studied (suicide), i.e., who attempted—or thought about—various degrees of self-harm, in a range of time that may precede the study from weeks to few years. Yet, in an actual suicide, death is the result of willful actions, where the subject hurts himself or herself physically and effectively, in the context of a specific mental state. In our opinion, that state of mind, i.e., how that person was feeling at that very moment, may relate to a more salient biology that we can then observe in the brain. We expect that such biology would better stand out from the background when more violent and lethal methods were employed. Indeed, by adopting such precise classification, we were able to strongly replicate the association of *LINC01268* with suicide by violent means.

The significant increase of *LINC01268* DLPFC expression in suicide compared with nonsuicide potentially may offer a tool to differentiate suicidal candidates from patients who, even though affected by the same conditions, will not shift from contemplating death to actually pursuing it. In this regard, levels of *LINC01268*, and related genes, measured in peripheral blood—if linked with brain levels—may represent a further step for in vivo studies, leading to prevention and treatment strategies. Indeed, *MARCKS* levels in peripheral blood have been reported to show association with suicidal ideation (9). The presence of higher lincRNA expression specifically in suicides by violent means suggests an association of the noncoding RNA with aggressive behaviors, which our data from a questionnaire of aggressive behavior support. The association between an eQTL for the gene with a history of aggressive behaviors in healthy subjects substantiates further the connection and set the stage for our fMRI study.

In the fMRI protocol, the explicit emotional processing of facial expressions of anger was chosen as a proxy for an aggressive stimulus. Interestingly, C carriers, who are associated with increased *LINC01268* expression in brain and increased aggressiveness, show a negative correlation between DLPFC, specifically BA 10 activity, and aggressiveness, whereas the opposite is true for TT carriers. BA 10 has been implicated in complex cognitive operations (42) and found in both violent offending behavior (43) and suicide (44–47). A previous report (48), similarly testing anger as a stimulus, also found a negative relationship between aggressive behavior and response of the orbitofrontal cortex (including BA 10) in healthy control subjects, arguing that greater activity facilitates more controlled behaviors. In the present study, we found that this negative relationship between aggressiveness and anger-related BA 10 activity (i.e., lower activity with more aggressiveness) is found only in the context of C carrier rs7747961 genotypes, suggesting that BA 10 activity of individuals with major allele TT genotypes is indeed associated with relatively more controlled behavior.

Finally, the biological function of *LINC01268* in brain is largely unknown. To shed light on a potential role, we performed coexpression analysis in brain based on the assumption that genes coexpressed with *LINC01268* represent a molecular network that shares at least in part a common function. Weighted network analysis (WGCNA) revealed a *LINC01268*-related network containing genes involved in immunological responses, though such a conclusion is based largely on the function of the coexpressed genes in peripheral immune cells. Interestingly, the gene most highly correlated with *LINC01268* DLPFC expression is *P2RY13*, which is a recently identified and largely unknown purinergic receptor present both in the peripheral immune system and in brain (39).

In conclusion, our findings converge on implicating a role for *LINC01268* in emotional regulation, aggressive behaviors, and suicides by violent means. The biological mechanisms may involve the modulation of genes known to be engaged in immunity and drug response and may be relevant from a preventive, diagnostic, and therapeutic perspective.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the Lieber Institute for Brain Development and by the BrainSeq Consortium. This work was also supported by a grant by Regione Puglia, and by a "Capitale Umano ad Alta Qualificazione" grant by Fondazione con il Sud.

GP, GU, and DRW conceived the project, designed the study, and interpreted all the results. AD-S, TMH, and JEK curated the brain collection. JHS organized and performed RNA sequencing analysis. GU and AEJ organized and performed genotyping and imputation. GP and GU carried out differential expression and expression quantitative trait loci analyses, and AEJ contributed statistics. ER performed weighted gene coexpression network analysis. GP and GV together with TQ, GB, and AB organized and carried out recruitment of living subjects. GP together with GV acquired and analyzed behavioral data under the supervision of RC. GV, TQ, GB, and AB acquired functional magnetic resonance imaging data, which GV and TQ analyzed. GP, GU, and DRW drafted the manuscript, and all authors contributed to the final version of the article.

We thank Qiang Chen, Ph.D., and Rahul Bharadwaj, Ph.D., for insightful discussions; the Lieber and Maltz families; and all the brave and generous families that consented for the brain donation of their deceased next of kin.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Lieber Institute for Brain Development (GP, GU, ER, JHS, AEJ, AD-S, TMH, JEK, DRW), Johns Hopkins University Medical Campus; Departments of Psychiatry and Behavioral Sciences (GU, DRW), Neurology (TMH, DRW), and Neuroscience (DRW) and McKusick Nathans Institute of Genetic Medicine (DRW), Johns Hopkins School of Medicine; Department of Mental Health (AEJ), Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; and Section of Forensic Psychiatry and Criminology (GP, RC), Institute of Legal Medicine, Interdisciplinary Department of Medicine, and Group of Psychiatric Neuroscience (GP, GU, GV, TQ, GB, AB), Department of Basic Medical Science, Neuroscience and Sense Organs, Aldo Moro University, Bari, Italy.

Address correspondence to Daniel R. Weinberger, M.D., Lieber Institute for Brain Development, Johns Hopkins Medicine, 855 North Wolfe Street, Baltimore, MD 21205; E-mail: drweinberger@libd.org.

Received Aug 22, 2018; revised Oct 29, 2018; accepted Nov 6, 2018.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2018.11.002.

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