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A Targeted Sequencing Study of Glutamatergic Candidate Genes in Suicide Attempters with Bipolar Disorder

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Abstract

Suicidal behavior has been shown to have a heritable component that is partly driven by psychiatric disorders [Brent and Mann, 2005]. However, there is also an independent factor contributing to the heritability of suicidal behavior. We previously conducted a whole exome sequencing study of bipolar suicide attempters and bipolar non-attempters to assess this independent factor [Monson et al., 2016]. This whole exome study implicated glutamatergic neurotransmission in attempted suicide, as did our genome-wide association study (GWAS) of the attempted suicide phenotype [Willour et al., 2012]. In the current study, we have conducted a targeted next-generation sequencing study of the glutamatergic N-methyl-D-aspartate (NMDA) receptor, neurexin, and neuroligin gene families in 476 bipolar suicide attempters and 473 bipolar non-attempters. The goal of this study was to gather sequence information from coding and regulatory regions of these glutamatergic genes to identify variants associated with attempted suicide. We identified 186 coding variants and 4,298 regulatory variants predicted to be functional in these genes. No individual variants were overrepresented in cases or controls to a degree that was statistically significant after correction for multiple testing. Additionally, none of the gene-level results were statistically significant following correction. While this study provides no direct support for a role of the examined glutamatergic candidate genes, further sequencing in expanded gene sets and datasets will be required to ultimately determine whether genetic variation in glutamatergic signaling influences suicidal behavior.

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Keywords

Bipolar disorder; suicidal behavior; candidate gene sequencing; *NLGN1*; *GRIN3A*

Introduction

Completed suicide is the tenth leading cause of death among all age groups in the United States and the second leading cause of death in 15-24 year olds [2015; Sullivan et al., 2015]. There are an additional 25 suicide attempts for each completed suicide, making suicidal behavior a common and devastating phenotype [2015].

Epidemiological studies have consistently shown that the risk for suicidal behavior is heritable, with the current heritability estimates ranging from 30% to 50% [Mann et al., 2009]. This heritability is comprised of two main factors. The first comes from a liability to psychiatric disorders [Brent and Mann, 2005]. The second genetic factor is independent and may span multiple psychiatric disorders. This independent factor is currently hypothesized to be impulsive aggression, suggesting that the highest risk individuals have both this trait and a psychiatric disorder [Brent and Mann, 2005; Mann et al., 2009].

The earliest studies searching for genetic susceptibility loci in suicidal behavior used candidate gene approaches, many of which focused on the serotonergic system [Brezo et al., 2008]. However, when these studies failed to produce consistent results, multiple large-scale studies, including linkage analyses and genome-wide association studies (GWAS), were done to identify genetic contributors to suicidal behavior [Hesselbrock et al., 2004; Zubenko et al., 2004; Willour et al., 2007; Sequeira et al., 2009; Butler et al., 2010; Willour et al., 2012; Nagy et al., 2014]. These studies have provided evidence for a role of glutamatergic signaling in the risk for such behavior [Sequeira et al., 2009; Willour et al., 2012; Nagy et al., 2014]. Additionally, lithium and ketamine, two drugs effective in treating suicidal behavior, are hypothesized to affect glutamatergic signaling. Both long-term lithium treatment and acute ketamine treatment result in the attenuation of glutamatergic signaling (Figure 1) [Dixon and Hokin, 1998; Li et al., 2010]. The involvement of these medications in treating suicidal behavior further supports the hypothesis that glutamatergic signaling has a role in this phenotype.

Mammals contain three neurexin (NRXN) genes, five neuroligin (NLGN) genes, and eight (NMDA) receptor genes (see materials and methods for complete gene list) [Lewis et al., 1996; Sudhof, 2008; Traynelis et al., 2010]. All three gene families are located at the synapse where they play a role in glutamatergic signaling. Presynaptic NRXN proteins and postsynaptic NLGN proteins form a cell adhesion complex involved in synapse stabilization and specification [Sudhof, 2008]. In addition, NLGN proteins interact with NMDA receptors, a type of ionotropic glutamate receptor, through scaffolding protein complexes (Figure 1) [Meldrum, 2000]. In this study, we present a targeted sequencing assessment of these three glutamatergic gene families in 476 bipolar suicide attempters and 473 bipolar non-attempters. This study provides a novel and in-depth examination of sequence variation in a set of candidate genes with the goal of identifying additional genetic susceptibility loci for suicidal behavior.

Materials and Methods

Sample Collection

Subject information was collected as part of the National Institute of Mental Health (NIMH) Genetics Initiative, <https://www.nimhgenetics.org/> [1997]. For this study, we used subjects from our attempted suicide GWAS, which included individuals of European-American descent (Table 1) [Willour et al., 2012]. All subjects were unrelated and with a diagnosis of bipolar I (BPI) or schizoaffective disorder, bipolar type (SABP). Attempters were defined by their positive answer to the question in the Diagnostic Interview for Genetic Studies (DIGS): “Have you ever tried to kill yourself?” [Nurnberger et al., 1994] We selected only attempters that indicated they had definite or serious intent to die as determined by the DIGS questionnaire. A total of 476 suicide attempters (224 males and 252 females) and 476 non-attempters (224 males and 252 females) were included for sequencing. Institutional Review Board-approved written informed consent was provided by all subjects prior to enrollment.

Target Determination

Our target regions were derived from the following genes: *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*, *GRIN3A*, *GRIN3B*, *GRINA*, *NRXN1*, *NRXN2*, *NRXN3*, *NLGN1*, *NLGN2*, *NLGN3*, *NLGN4X*, and *NLGN4Y*. For each of these genes, both coding and non-coding (regulatory) regions were included in the sequencing target. The coding regions consisted of all exons of all alternative transcripts defined by Ensembl, GENCODE, RefSeq, or the “UCSC Genes” track of the UCSC Genome Browser [Kent et al., 2002; Harrow et al., 2012; Pruitt et al., 2014; Cunningham et al., 2015]. The regulatory genomic regions were comprised of all alternative promoter regions (defined as 2kb upstream of the transcription start site), intron-exon boundaries (± 50 bp), and any putative ENCODE regulatory elements [2012]. These regulatory elements included any genomic regions, either intronic or within a 10kb flanking sequence of each gene, that were defined by ENCODE as both a transcription factor binding site and a DNase hypersensitivity site (UCSC Genome Browser tables wgEncodeRegDnaseClusteredV2 and wgEncodeRegTfbsClusteredV2) [Quinlan, 2014]. Sequencing probes for these regions were designed using the SureDesign Custom Design Tool software from Agilent (Santa Clara, CA).

Next-Generation Sequencing

We used Agilent's SureSelect^{XT} Target Enrichment next-generation sequencing technology to obtain sequencing data for these 16 genes. To start, 3 μ g of high quality genomic DNA was sheared using the Covaris (Woburn, MA) E220 ultrasonicator. Library preparation (end repair, A-tail addition, and adapter ligation), hybridization, and target selection were carried out using standard protocols on the SciClone Next-Generation Sequencing (NGS) Robotics Workstation (PerkinElmer: Waltham, MA) at the Iowa Institute of Human Genetics Genomics Division. The final prepped samples were run on an Illumina (San Diego, CA) HiSeq 2000 in multiplexed pools of 16 samples per lane.

The 100bp paired-end reads were aligned to the hg19 reference human genome using the Burrows-Wheeler Aligner (BWA: v0.6.2) [Li and Durbin, 2009]. SAMtools (v0.1.18) [Li et al., 2009], BAMtools (v2.2.3) [Barnett et al., 2011], and Picard (v1.88; <http://>

picard.sourceforge.net) were used for indexing and duplicate removal. The Genome Analysis Tool Kit (GATK: v3.1.1) was used to determine quality scores, coverage depth, and genotypes [McKenna et al., 2010]. The GATK HaplotypeCaller was used to call indels and single nucleotide polymorphisms (SNPs) [McKenna et al., 2010]. All samples were called as a single group. Quality control tests checking for quality-to-depth ratio, mapping quality, strand bias, and haplotype score were run for each variant via GATK, and any variants that failed these quality control tests were removed. We also removed variants that were missing calls in > 10% of subjects or that failed Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$). Variants that had a depth of coverage < 10X (Table S1) or a genotyping quality score < 20 were set to missing, as were any heterozygous calls in the X chromosome of male subjects (excluding the pseudoautosomal region) or Y chromosome calls in female subjects. We only included variants with a minor allele frequency (MAF) ≥ 0.05 in our dataset, the European 1000 Genomes dataset [Abecasis et al., 2012], the Exome Aggregation Consortium dataset (Cambridge, MA: <http://exac.broadinstitute.org>), and the NHLBI GO Exome Sequencing Project dataset (Seattle, WA: <http://evs.gs.washington.edu/EVS/>). We also compared subject calls to our existing attempted suicide GWAS data and removed three non-attempter subjects with > 20% genotype mismatch, making our final number of subjects 476 bipolar suicide attempters and 473 bipolar non-attempters [Willour et al., 2012]. Overall, the variant calls in this study had a 99.75% concordance rate with our GWAS results. Finally, we examined the 1000 Genomes SNPs covered in our study [Auton et al., 2015]. We covered 96.1% (317 out of 330) of the 1000 Genomes SNPs with a MAF between 0.01 and 0.05. For SNPs with a MAF > 0.05, we covered 98.5% (870 out of 883) of the 1000 Genomes SNPs.

Statistical Analyses

We performed both individual-variant and gene-level tests. The individual-variant tests were done using logistic regression with Firth's correction method [Firth, 1993] within the “logistf” R package (v1.21) [Heinze and Puh, 2010]. The individual-variant tests were corrected for sex and the first three principal components from our attempted suicide GWAS as covariates [Willour et al., 2012]. Because alcohol dependence and duration of illness differed significantly between our attempters and non-attempters (Table 1), we also ran secondary analyses in which alcohol dependence and duration of illness were included as covariates. However, these analyses did not significantly affect the top findings from our primary analysis (data not shown). Because we examined 5,209 variants with MAF ≥ 0.05 (Tables S2, S3), the threshold for study-wide significance following the conservative Bonferroni correction for multiple testing was 9.6×10^{-6} . For a less conservative multiple testing correction, we also used Haploview (v4.2) [Barrett et al., 2005] with the “confidence intervals” method [Gabriel et al., 2002] to determine the number of linkage disequilibrium (LD) blocks within our 16 genes. We identified a total of 118 LD blocks across our genes, making the threshold for study-wide significance (when corrected for the number of LD blocks rather than the number of variants) 4.2×10^{-4} .

We also performed gene-level assessments for each of the 16 genes examined. These gene-level assessments were run separately on coding regions and regulatory regions. We also looked at two different groups of variants classified as disruptive and broad [Purcell et al., 2014]. The disruptive variants for coding regions included any frameshift, stopgain/

nonsense, or essential splice site variants identified by ANNOVAR [Wang et al., 2010]. Coding variants that received a broad classification included all disruptive coding variants and any additional non-synonymous variants that were predicted to be damaging by at least one of six bioinformatics packages (SIFT [Ng and Henikoff, 2003], Polyphen2 HVAR [Adzhubei et al., 2010], Polyphen2 HDIV [Adzhubei et al., 2010], MutationTaster [Schwarz et al., 2010], LRT [Chun and Fay, 2009], VEST [Carter et al., 2013]).

For the regulatory regions, the disruptive classification included any variants that received a score of 1 or 2 from the Regulome database (v1.1), meaning that they are likely to interfere with the binding of regulatory proteins [Boyle et al., 2012]. The broad classification for regulatory regions included all regulatory disruptive variants plus any variants that received a score of 3-6 from the Regulome database [Boyle et al., 2012].

The gene-level assessment of these variant groups included two types of tests: a gene-burden test and a sequence kernel association test (SKAT: v1.0.9) [Wu et al., 2011]. The gene-burden test was done using a previously described collapsing method [Li and Leal, 2008] to collapse rare, functional variants by subject over each gene. Subjects that have one or more rare, functional variants within the assessed gene are given a burden value of “1” for that gene, while subjects with no rare, functional variants are given a burden value of “0” for that gene. The resulting burden values were assessed using the “logistf” R package (v1.21) [Heinze and Pühr, 2010] based on logistic regression with Firth's correction method [Firth, 1993] to determine whether the presence of variant burden across the gene was associated with attempters or non-attempters. SKAT was a complementary gene-level approach run specifically on autosomal chromosomes (i.e. all genes except *NLGN3*, *NLGN4X*, and *NLGN4Y*). SKAT uses a multivariate regression model that takes the directionality of variant effect into account. The gene-level tests were corrected for sex and the first three principal components from our attempted suicide GWAS as covariates [Willour et al., 2012]. We also ran secondary analyses in which alcohol dependence and duration of illness were included as covariates. However, these analyses did not significantly affect the top findings from our primary analysis (data not shown). Because we ran a total of eight tests per gene, the study-wide significance threshold for the gene-level tests was 3.9×10^{-4} .

Pathway analyses were also done on the set of 16 genes using PLINK/SEQ (v0.10) [Purcell et al., 2007] and SKAT. PLINK/SEQ was done using the “--burden” method with 10,000 permutations. SKAT analyses were done using the “davies” model for the regression framework of the autosomal genes within our gene set. A total of eight tests were run for the pathway analysis, so the threshold for study-wide significance was 6.3×10^{-3} . The PLINK/SEQ analysis was corrected for sex, and the SKAT analysis was corrected for sex and the first three principal components from our attempted suicide GWAS as covariates [Willour et al., 2012].

Results

We examined the NMDA receptor, NRXN, and NLGN gene families using both individual-variant and gene-level tests. The individual-variant tests look for specific variants that are

significantly more common in attempters or non-attempters, while the gene-level tests look for an enrichment of variants in a given gene in attempters or non-attempters.

We identified a total of 5,209 variants (Table S3), of which 46 reached nominal significance ($p < 0.05$) for association with suicide. Of these variants, 186 coding variants and 4,298 regulatory variants were predicted to be functional. While our Q-Q plot shows that common variants do follow the expected distribution (Figure S1), variants with a MAF > 0.05 do not follow the expected distribution, as our sample size was underpowered to detect significant associations for rare variants. The top individual-variant test result was rs75981117, a nonsynonymous exonic variant in the *GRIN3A* gene (Table 2; $p = 0.0017$, odds ratio (OR) = 19.21). Because past research has demonstrated sex-specific differences in suicidal behavior, we also performed both male- and female-specific individual-variant tests. In the male-specific analysis, 23 variants reached nominal significance (Table S4). The top male-specific variant was rs45537432, an intronic variant in *GRIN3A* ($p = 0.0014$, OR = 3.56). In the female-specific analysis, 25 variants reached nominal significance (Table S5). The top female-specific variant was rs141340037, an intronic variant in *NRXN2* ($p = 0.0059$, OR = 0.063).

We also examined the NMDA receptor, *NRXN*, and *NLGN* gene families using a gene-level approach. The top result for the gene-level tests was the *GRIN3A* gene (Table 3, Tables S6-7). This top finding came from the SKAT test of broad coding variants and produced a p-value of 0.0081. As was done for the individual-variant tests, we performed male- and female-specific gene-level tests. The top result for the male-specific gene-level tests came from the SKAT test of broad coding variants in the *NLGN1* gene (Table 3, Tables S8-9; $p = 0.0044$). For the female-specific tests, the top result was from the SKAT test of broad coding variants in *GRIN3A* (Table 3, Tables S10-11; $p = 0.0049$). No gene-level results remained significant following correction for multiple testing.

As a final test, we ran a pathway analysis to determine whether this set of 16 genes was more enriched for variants than would be expected by chance. However, this pathway analysis provided no evidence for an enrichment of variants within our gene set (Table S12).

Discussion

Glutamate is the main excitatory neurotransmitter in the brain. When released from the presynaptic neuron, glutamate binds to receptors like NMDA receptors to propagate the excitatory signal to the postsynaptic neuron. NMDA receptors are a class of ionotropic glutamate receptors that have been implicated in synaptic plasticity, learning, and memory [Meldrum, 2000; Volianskis et al., 2015]. They are heterotetrameric proteins typically composed of two compulsory *GRIN1* subunits linked to a combination of *GRIN2* (A-D) or *GRIN3* (A-B) subunits [Paoletti, 2011], potentially alongside a glutamate-binding protein, *GRINA* [Chen et al., 1988]. NMDA receptors have been implicated in impulsivity and aggression [Chang et al., 2015; Finlay et al., 2015], and three NMDA receptor genes (*GRIN1*, *GRIN2C*, *GRINA*) have altered expression levels in postmortem brains of suicide completers [Higgs et al., 2006]. These features make NMDA receptors and their interacting partners, such as the *NRXN* and *NLGN* gene families, prime candidates for involvement in

the impulsive aggression phenotype and suicidal behavior. Interestingly, two NLGN genes (*NLGN1*, *NLGN4Y*) and two NRXN genes (*NRXN1*, *NRXN3*) have altered expression levels in postmortem brains of suicide completers, suggesting that these genes may play a role in suicide etiology [Higgs et al., 2006].

Based on the roles of the NRXN, NLGN, and NMDA receptor proteins in glutamatergic synapse maintenance [Craig and Kang, 2007] and their implication in multiple psychiatric disorders [Jamain et al., 2003; Gauthier et al., 2011; Vinas-Jornet et al., 2014], as well as in suicidal behavior [Willour et al., 2012; Gray et al., 2015], we decided to perform a targeted sequencing study to look for variants in these genes that might be associated with suicidal behavior. However, no results from this study achieved study-wide statistical significance following correction for multiple testing. One potential reason for the lack of significant results is that we did not examine all genes involved in glutamatergic signaling. We chose to look at the NRXN, NLGN, and NMDA receptor gene families due to their interactions with one another and previous evidence implicating them in psychiatric disorders. However, there are additional families of glutamatergic genes that were not examined through this approach. These additional gene families include other glutamate receptors such as AMPA receptors, metabotropic receptors, and kainate receptors, and several genes encoding these types of receptors are differentially expressed [Gray et al., 2015] or differentially methylated [Nagy et al., 2015] in suicidal behavior. Future studies should examine sequence variation in these other glutamatergic gene families to fully understand the relationship between glutamatergic signaling and suicidal behavior. These studies should also investigate how genetic variation in glutamatergic genes alters the effects of ketamine and lithium on suicidal behavior.

While we did not examine all possible glutamatergic genes, several of the genes we did sequence had previous evidence for involvement in suicidal behavior that we were unable to replicate in this study. A GWAS conducted by our lab had previously implicated *NRXN3* in attempted suicide among females [Willour et al., 2012]. While several of our top female-specific variants occurred in *NRXN3* (Table S5), none of these variants survived correction for multiple testing nor did *NRXN3* occur among our top female-specific gene-level results. GWAS examines common variants, while this study focused specifically on rare variants (MAF = 0.05). Thus, the genetic contribution of *NRXN3* to the risk of attempted suicide in the female population could be due mainly to common variation. Studies in larger sample sets examining both common and rare variation are needed to determine the role of *NRXN3* in attempted suicide.

Previous evidence also suggested an association between *GRIN2B* and suicidal behavior. Sokolowski et al. (2013) performed a family-based study of candidate SNPs and found a novel association between two *GRIN2B* SNPs and suicidal behavior [Sokolowski et al., 2013]. They also discovered a specific four-SNP *GRIN2B* haplotype that was associated with a higher risk for suicide. In our study, *GRIN2B* was the second best overall gene-level finding ($p = 0.011$, OR = 6.43) but did not appear among any of our top individual-variant results. Again, while Sokolowski et al. looked at common SNPs, our study focused on rare variants. It is of interest, however, that the variants that make up the risk haplotype identified by Sokolowski et al. localize to the same linkage disequilibrium block as the majority of the variants contributing to our gene-level *GRIN2B* finding. Future studies should aim to

examine both common and rare *GRIN2B* SNPs in independent sample sets. The rationale for these future studies is also enhanced by the recent finding that expression of *GRIN2B* is increased in suicide attempters with major depressive disorder, particularly in females [Gray et al., 2015].

Our analytic strategy also allowed us to test for evidence of rare-burden association across the entire collection of candidate genes. To do this, we conducted a pathway analysis that tested for rare-variant gene-burden in attempters versus non-attempters. Pathway analyses are especially valuable for complex phenotypes like suicidal behavior, because risk variants may be spread across multiple interacting genes rather than localized within a single candidate gene. The best result from our pathway analysis came from the SKAT test of disruptive coding variants ($p = 0.085$). While this result showed a trend towards significance, larger sample sizes are needed to determine whether this trend represents a true increase in the risk for suicidal behavior conferred by rare variation within our candidate genes.

There are several limitations in our study. The first is that our sample size, while substantial, was only large enough to detect a genotypic relative risk 2.6 with 80% power and a MAF of 0.05 at study-wide significance for the individual-variant tests (calculated using Quanto v1.2.4; <http://biostats.usc.edu/Quanto.html>). For the gene-level tests, we could detect a genotypic relative risk 2.2 with 80% power and a MAF of 0.05 at study-wide significance. This power was likely further reduced, because not all variants in a given locus affect the risk for suicide. We note that samples with greater than 2,000 subjects will likely be needed to detect the kind of modest effects (genotype relative risk 1.5) likely to be present in rare suicidal behavior risk genotypes. Another inherent limitation with our study design is that we used a targeted sequencing approach. We designed this study with the goal of sequencing regions that were most likely to affect gene function, including both coding exons and potential regulatory regions. The way in which we defined these regions, however, may have resulted in the exclusion of additional regulatory genomic regions. The third limitation of our study design is that we took a candidate gene approach. This means that we may have missed variation in other glutamatergic genes that significantly contributes to the phenotype of suicidal behavior. A final limitation of our study is that individuals classified as non-attempters at the time of interview could potentially attempt suicide later in life. Our study design favors those who attempt earlier in life, potentially biasing our study towards early-onset attempters.

Here we have presented a targeted sequencing study of the NMDA receptor, *NRXN*, and *NLGN* gene families in suicide attempters and non-attempters. While none of our results reached study-wide statistical significance, further sequencing in expanded gene sets and datasets will be necessary to ultimately determine whether genetic variation in glutamatergic signaling influences suicidal behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Effect of drug treatments on glutamatergic signaling. Both chronic lithium treatment and acute ketamine treatment inhibit NMDA receptors (shown in purple). These NMDA receptors interact with postsynaptic NLGN proteins (shown in green) through scaffolding protein complexes. These NLGN proteins interact with presynaptic NRXN proteins (shown in blue) to stabilize the synapse and determine synapse specificity.

Table 1
Sample Set Demographics

		Suicide Attempters	Non-Attempters
Sex:	Male (%)	224 (47.06%)	222 (46.93%)
	Female (%)	252 (52.94%)	251 (53.07%)
Age:	Average	42.99 (19-82)	42.72 (18-88) ^a
	(Min-Max)		
Ethnicity:	European-American^b	476	473
Diagnosis:	BP I	475	473
	SABP	1	0
Alcohol Dependence:		194 (40.76%) ^c	148 (31.29%)
Duration of Illness:		25.6 years ^d	22.6 years
Total:		476	473

BP I, bipolar I; SABP, schizoaffective disorder, bipolar type

All subjects are unrelated.

^aOne subject did not provide age information.

^bA principal component analysis demonstrated that all subjects were consistent with having a European-American background.

^cAttempters had significantly higher incidence of alcohol dependence ($p = 0.0029$).

^dAttempters had significantly longer duration of illness ($p = 0.00049$).

Table 2

Top Individual-Variant Results

Gene	Chromosome Location ^{a,b}	Position	dbSNP142 ^c	P-value ^d	Odds Ratio ^e	Attempter MAF	Non-Attempter MAF
GRIN3A	chr9:104433048 (T/C)	exonic	rs75981117	0.0017	19.21 (2.43-2480.1)	0.0095	0
GRIN2A	chr16:9850531 (A/T)	ncRNA_exonic	rs78377517	0.0028	7.92 (1.88-73.26)	0.012	0.0011
NRXN3	chr14:78708141 (CT/C)	upstream	NULL	0.0031	17.46 (2.16-2260.6)	0.0086	0
NLGNA4X	chrX:5950784 (G/C)	intronic	rs181486066	0.0047	0.13 (0.014-0.58)	0.0014	0.014
NRXN3	chr14:80095895 (G/C)	intronic	rs190741106	0.0061	0.064 (0.00049-0.53)	0	0.0074
NRXN3	chr14:79432311 (G/T)	intronic	rs189072590	0.0064	0.065 (0.00050-0.54)	0	0.0074
NRXN3	chr14:79434875 (C/T)	intronic	rs140275772	0.010	2.24 (1.21-4.34)	0.033	0.015
NRXN3	chr14:80128259 (C/A)	intronic	rs139082260	0.011	6.44 (1.48-60.21)	0.0095	0.0011
NLGNI	chr3:173323481 (A/G)	intronic	rs140298604	0.012	0.073 (0.00056-0.62)	0	0.0063
NLGNI	chr3:173117349 (A/G)	intronic	rs6762349	0.012	6.27 (1.44-58.59)	0.0095	0.0011

MAF, minor allele frequency; ncRNA, non-coding RNA

Results are shown for our sample set of 476 bipolar suicide attempters and 473 bipolar non-attempters.

^aChromosome location was determined using the UCSC Genome Browser (hg19).^bVariants are listed as (major allele/minor allele).^c“NULL” designates a novel variant.^dUncorrected P-values are shown. No P-values survived correction for multiple testing.^eOdds ratios shown are for the minor allele. Numbers in parentheses indicate the 95% confidence interval for the odds ratio.

Table 3

Gene-Level Results ($p < 0.05$)

Gene	Sample Group	Test	Variant Type	Genomic Region	Number of Variants Assessed ^a	P-Value ^b	Odds Ratio ^c	Attempter Frequency ^d	Non-Attempter Frequency ^d
GRIN3A	All	SKAT	Broad	Coding	26	0.0081	NULL	0.11	0.13
GRIN2B	All	Gene-burden	Broad	Coding	9	0.011	6.43 (1.47-60.17)	0.019	0.0021
GRIN1	All	SKAT	Disruptive	Regulatory	72	0.014	NULL	0.20	0.19
GRIN3B	All	Gene-burden	Disruptive	Coding	3	0.028	10.97 (1.24-1442.4)	0.011	0
NLGN1	Male	SKAT	Broad	Coding	8	0.0044	NULL	0.013	0.031
GRIN3A	Male	SKAT	Broad	Regulatory	153	0.025	NULL	0.57	0.55
NLGN1	Male	Gene-burden	Disruptive	Regulatory	82	0.043	0.64 (0.42-0.99)	0.21	0.30
NRXN1	Male	SKAT	Broad	Regulatory	529	0.047	NULL	0.88	0.89
GRIN3A	Female	SKAT	Broad	Coding	26	0.0049	NULL	0.13	0.14

SKAT, sequence kernel association test

Results are shown for our sample set of 476 bipolar suicide attempters (224 males and 252 females) and 473 bipolar non-attempters (222 males and 251 females).

^a Denotes the number of unique variant sites included in the assessment for a given result.^b Uncorrected P-values are shown. No P-values survived correction for multiple testing.^c SKAT analyses do not generate odds ratios. Numbers in parentheses indicate the 95% confidence interval for the odds ratio.^d Indicates how often variants occurred within each gene in attempters and non-attempters.