Sequence Analysis of Drug Target Genes with Suicidal Behavior in Bipolar Disorder Patients

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Keywords

Suicide attempt · Sequencing · Bipolar disorder · Genetics · Drug target genes

Abstract

Background: A number of genes have been implicated in recent genome-wide association studies of suicide attempt in bipolar disorder. More focused investigation of genes coding for protein targets of existing drugs may lead to drug repurposing for the treatment and/or prevention of suicide. **Methods:** We analyzed 2,457 DNA variants across 197 genes of interest to GlaxoSmithKline across the pipeline in our sample of European patients suffering from bipolar disorder ($N = 219$). We analyzed these variants for a possible association with the suicide severity score (ranging from suicidal ideation/plan to serious suicide attempt) from the Schedule for Clinical Assessment in Neuropsychiatry. We conducted tests of individual variants and gene-based tests. **Results:**

We found a number of DNA variants in the transforming growth factor beta receptor 1 gene (*TGFBR1*) to be suggestively associated with suicide severity scores ($p < 0.005$). The gene-based tests also pointed to *TGFBR1* to be associated with suicide severity ($p = 0.0001$). However, these findings were not replicated in an independent bipolar disorder sample. **Conclusions:** We report no significant association between DNA sequences of drug target genes and suicidal behavior. Additional larger sequencing studies could further interrogate associations between variants in drug target genes and suicidal behavior.

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Introduction

Over 800,000 lives are lost to suicide each year worldwide, and for each completed suicide, there are 20 suicide attempts, making it an important public health issue

(http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/). Psychiatric disorders are present in over 90% of all suicide victims, including bipolar disorder [1], where as many as 8% of the bipolar disorder patients followed for up to 4 decades died by suicide [2, 3].

Family and adoption studies support a genetic component in the susceptibility of suicidal behavior [4, 5, reviewed in 6]. A review of twin studies estimated the heritability of suicidal behavior to be up to 50% [7]. Hypothesis-driven studies have identified a number of potential candidate genes. These include the brain-derived neurotrophic factor (*BDNF*) [8], neurotrophic tyrosine receptor kinase 2 (*NTRK2*) [9], serotonin 2A receptor (*HTR2A*) [10], and tryptophan hydroxylase (*TPH2*) [11]. However, as the effect sizes of markers in these genes have been small, it is likely that suicidal behavior is influenced by many still unidentified genetic factors.

There have been a number of hypothesis-generating genome-wide association studies (GWAS) that investigated the role of common DNA variants in suicidal behavior. Five GWAS of suicide attempt have been reported [12–16]. The acid phosphatase (*ACP1*) gene reported in a GWAS of suicide attempt in bipolar disorder has recently been replicated in a sample of schizophrenia patients [17]. GWASs of suicidal behavior severity [6, 18], suicidal ideation/attempt [19], and suicide attempt/death [20] have also been published. These hypothesis-free studies have yielded a number of intriguing findings that need further validation. GWASs have also implicated neurodevelopment and micro-RNA to play a role in suicidal ideation/behavior [14, 19]. A recent GWAS of suicide attempt in US soldiers indicated a genetic overlap between suicide attempt and bipolar disorder [16]. These GWAS findings will need further validations to confirm.

In 2012, a study reported on sequencing of 202 Glaxo-SmithKline (GSK) drug target genes in over 14,000 participants with various medical and psychiatric conditions, including unipolar depression and bipolar disorder, in order to identify therapeutic switching opportunities [21]. While no study-wide significant variants were identified for bipolar disorder, analyzing bipolar disorder-related phenotypes such as suicidal behavior, which is more clinically relevant because of safety risks, may provide a novel avenue for drug repurposing. In the present study, we aimed to investigate the possible association between potential drug target genes and suicidal behavior severity in bipolar disorder patients. We also aimed to test the contribution of all variants as well as only rare variants of each gene to the variance in suicidal behavior severity.

Materials and Methods

Bipolar I disorder cases in our discovery sample ($N = 219$) were recruited from the CAMH, Toronto, ON, Canada, through advertisements in clinics, family doctor offices, hospitals, and patient support groups [22]. They were at least 18 years of age (average 42.76 ± 12.37 , 91 males, and 128 females), euthymic at the time of the interview with the Schedules for Clinical Assessment in Neuropsychiatry, and self-reported to be of European ethnicity. Bipolar disorder diagnoses were ascertained based on DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, ed 4) or ICD-10 (International Statistical Classification of Diseases and Related Health Problems, 10th revision) criteria, using the computerized algorithm (CATEGO) for the Schedules for Clinical Assessment in Neuropsychiatry 2.1 interview (WHO) through a semi-structured clinical interview. Exclusion criteria were a diagnosis of intravenous drug dependency, reported intravenous drug use, mood-incongruent psychotic symptoms, or manic episodes occurring only in conjunction with or as a result of alcohol, substance abuse, substance dependence, medical illnesses, or medications. Suicidal behavior severity was assessed for the lifetime worst depressive episode by the interviewer using the SCAN Suicide Severity item, with 0 meaning nonsuicidal ($n = 71$), 1 denoting suicidal ideation/plan ($n = 78$), 2 denoting suicide attempt without serious harm ($n = 37$), 3 indicating suicide attempt with serious harm ($n = 11$), and 4 indicating suicide attempt aiming to end life ($n = 22$). The self-reported European ancestry of the sample was validated with GWAS data from a previous study [23] and is briefly described for the replication sample below.

For targeted sequencing on these 219 bipolar disorder patients, 202 genes were selected from drug target genes of interest to GSK across the pipeline, 70 genes coding for targets under preclinical development, 76 genes encoding targets of drugs in phases I–III, 12 genes encoding targets of marketed drugs (phase IV), and 44 genes coding for targets of drugs terminated after administration to humans [21]. Only genes for which drugs have only 1 known target were selected for sequencing. The names of the selected genes are presented in online supplementary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000488029). Approximately 351 kb coding and 323 kb untranslated exon regions plus additional 50 nucleotides of flanking sequence, totaling 863,883 nucleotides, were enriched. The nonoverlapping target regions are provided in online supplementary Table 1. Paired-end DNA sequencing was performed for 48-sample barcoded pools on Illumina Genome Analyzer X2 lanes (San Diego, CA, USA), as previously described [21]. At least half of the samples had >93% of the target bases successfully sequenced, with a median sequencing depth of $\times 27$. The sequencing reads were aligned with SOAP (Short Oligonucleotide Analysis Package) [24], and variants were called with SOAPsnp [25]. For initial quality control, variants with singleton heterozygote reads of <10, a genotype rate smaller than 50%, or duplicate genotype discordance of >2% were excluded. Samples with an average sequencing read depth of <10 and a rate of discordant genotypes between sequencing run and available genome-wide panels of >15% were excluded.

The replication bipolar disorder sample was collected from the Institute of Psychiatry, London, UK, with the same protocol as our discovery sample [22, 23]. The replication sample consisted of 362 patients of European ancestry (average age 47.03 ± 11.10 years; 119 males and 243 females). Suicidal behavior sever-

Table 1. Linear regression analysis of all single-nucleotide variants using PLINK

CHR	B37 POS	A1	rs ID	Beta	p value	Ave. quality	Ave. depth	A1 freq.	Gene	Gene feature
1	154550724	T	rs6680410	0.074	9.87E-04	92.20	25.12	0.085	<i>CHRNA2</i>	3'UTR
10	33209266	G	rs2230396	-0.063	1.34E-03	70.96	28.21	0.105	<i>ITGB1</i>	Gly392Gly
1	101704532	T	rs3737577	-0.042	1.47E-03	87.67	33.95	0.359	<i>SIPR1</i>	5'UTR
9	101916165	G	rs1590	0.042	1.49E-03	90.34	27.08	0.286	<i>TGFB1</i>	3'UTR
21	27423547	T	rs41276550	0.180	1.78E-03	97.55	27.88	0.011	<i>APP</i>	Intron
9	101908915	A	rs334354	0.043	2.05E-03	93.22	30.02	0.222	<i>TGFB1</i>	Intron
2	50574485	G	rs13422484	0.047	3.60E-03	84.00	20.91	0.170	<i>NRXN1</i>	5'UTR
12	121681745	A	rs75456247	0.261	4.03E-03	95.51	31.82	0.005	<i>CAMKK2</i>	3'UTR
10	33200782	T	rs4587680	0.057	4.72E-03	94.20	31.52	0.098	<i>ITGB1</i>	Intron
17	3495391	C	rs55916885	-0.078	5.77E-03	90.21	26.71	0.048	<i>TRPV1</i>	Gln85Arg

The top 10 variants, with the variant ID (rs ID), chromosome (CHR), position in build 37 (B37 POS), location in gene (gene feature), and effect size (Beta) for the test allele (A1), and the frequency of the test allele (A1 freq.).

ity was assessed using the Schedules for Clinical Assessment in Neuropsychiatry suicide severity item, with 0 meaning nonsuicidal ($n = 112$), 1 denoting suicidal ideation/plan ($n = 136$), 2 denoting suicide attempt without serious harm ($n = 60$), 3 indicating suicide attempt with serious harm ($n = 13$), and 4 indicating suicide attempt aiming to end life ($n = 41$). These patients were genotyped on the Illumina HumanHap550 Beadchip at Illumina as described previously [22]. Genotype quality control strategies were described in our GWAS of suicidal behavior severity [23]. Briefly, we applied quality control measures using PLINK (a whole-genome association studies tool set) [26] and R (R Development Core Team, 2008). Briefly, individuals with at least 95% of the markers genotyped were kept, and markers that were at least 95% genotyped or had a minor allele frequency of at least 5% were included. We removed 1 individual of each pair of related individuals (defined as pairs with $PI^{HAT} > 0.05$). Sex was matched with genetic data. Outliers on mean heterozygosity were removed. Markers with genotypes that deviated significantly from the Hardy-Weinberg equilibrium ($p < 0.0001$) were excluded from subsequent analyses. We ran a multidimensional scaling analysis of the genotypes to ascertain the ethnicity of the samples, and we removed the discrete cluster that corresponded to the self-reported Jewish ancestry for all 4 grandparents. After the quality control steps described above, we conducted whole-genome imputation using IMPUTE2 [27] in 5-Mb segments after prephasing in SHAPEIT2 [28], then converted the output to PLINK format using GTOOL (Genetics Software Suite, © 2007; The University of Oxford) with a minimum imputation score of 0.9. Additional quality control (alleles observed, genotyping rate per SNP of at least 95% and per individual of at least 98%) was performed in PLINK, resulting in 2,457 single markers from 197 genes.

Statistical Analysis

Linear regression analyses of 2,457 single markers (with an r^2 threshold of 0.8) with log-transformed SCAN suicide severity scores were conducted on 219 bipolar disorder patients (with available phenotype and covariate data) using PLINK. Covariates included a large number (at least 40) of depressive episodes [29,

30], age, sex, lifetime alcohol (ever drank at least 5 alcoholic drinks every day) [31–33], and the first 2 principal components from the population structure analysis described previously [23]. We used gene- or region-based analysis of variants of intermediate and low frequency to conduct gene-based analysis [34], first involving all variants in each gene region and then involving only variants with a minor allele frequency of $< 5\%$. We have set the two-tailed significance threshold to 0.05/2457 or $2.035e-5$ for 2,457 single-marker tests, and to 0.05/197 or $2.54e-4$ for 197 gene-based tests.

Results

The 2,457 single-marker tests did not yield statistically significant findings (Table 1). Two of the top 10 variants were located in the transforming growth factor beta receptor 1 (*TGFB1*) gene ($p < 0.005$).

Gene-based analysis using all variants yielded a significant finding with the *TGFB1* gene (Table 2; $p = 0.0001$). We attempted to replicate the *TGFB1* findings from this study using available GWAS data from a replication bipolar disorder sample that was collected from the Institute of Psychiatry in London, UK [22, 23]. The *TGFB1* gene was not significant in the gene-based analysis in the replication sample.

Discussion

We report here DNA sequence analysis of drug target genes with the phenotype of suicidal behavior severity. While we found the *TGFB1* gene and individual markers to be significant in the discovery sample, we did not

Table 2. Analysis of variants with all single-nucleotide variants, and variants with minor allele frequencies of <5% using gene- or region-based analysis of variants of intermediate and low frequency

Gene	Marker count	Sample count	Rare variant sum	Total MAF	Average MAF	Beta	SE	Z	p
All single-nucleotide variants									
<i>TGFBR1</i>	17	219	262	0.6906	0.0406	0.5331	0.1380	3.8627	0.0001
<i>ITGAV</i>	9	219	275	0.7370	0.0819	0.3607	0.1119	3.2223	0.0015
<i>EVI5</i>	27	219	431	1.1227	0.0416	-0.4256	0.1431	-2.9751	0.0033
<i>PDE4A</i>	12	219	99	0.2489	0.0207	0.5244	0.1778	2.9499	0.0035
<i>DPP4</i>	14	219	326	0.8878	0.0634	0.3915	0.1370	2.8581	0.0047
<i>CCKAR</i>	12	219	292	0.7397	0.0616	0.2230	0.0905	2.4641	0.0145
<i>CXCL3</i>	3	219	3	0.0069	0.0023	0.5001	0.2224	2.2487	0.0256
<i>P4HA1</i>	10	219	35	0.0847	0.0085	0.4488	0.2025	2.2164	0.0277
<i>METAP2</i>	15	219	158	0.3725	0.0248	-0.3650	0.1761	-2.0724	0.0394
<i>KIAA1967</i>	22	219	453	1.2497	0.0568	0.4025	0.1987	2.0258	0.0440
Variants with minor allele frequencies of <5%									
<i>LRK2</i>	18	219	67	0.1564	0.0087	-0.8602	0.3028	-2.8411	0.0049
<i>DPP4</i>	12	219	49	0.1143	0.0095	0.6598	0.2371	2.7829	0.0059
<i>MMP12</i>	5	219	7	0.0161	0.0032	0.6395	0.2447	2.6141	0.0096
<i>ITGAV</i>	6	219	6	0.0138	0.0023	0.7902	0.3158	2.5022	0.0131
<i>CXCL3</i>	3	219	3	0.0069	0.0023	0.5001	0.2224	2.2487	0.0256
<i>TACR3</i>	7	219	8	0.0183	0.0026	0.7245	0.3237	2.2381	0.0263
<i>SLC5A1</i>	11	219	14	0.0320	0.0029	0.8066	0.3933	2.0506	0.0415
<i>CHRNA5</i>	7	219	13	0.0299	0.0043	-0.5240	0.2562	-2.0456	0.0420
<i>CDH2</i>	10	219	57	0.1303	0.0130	0.2815	0.1393	2.0208	0.0446

Shown are the top genes with p values of <0.05, with the number of markers for each gene (marker count), effect sizes (Beta), test statistics (Z), and p values. Rare variant sum, count of rare alleles found in individuals; sample count, number of individuals; total MAF, sum of the minor allele frequencies of all used markers in a given gene region; average MAF, total MAF/marker count.

replicate these findings in an independent sample. The lack of replication could be due to the possibility that the original findings were spurious. It could also be a result of subtle differences between the discovery and replication sample sets. Even though the suicidal behavior severity score distribution did not differ significantly between the samples ($p > 0.1$), compared to the replication sample, the discovery sample had more males (42 vs. 33%; $p < 0.05$), a younger average age (42.76 vs. 47.03 years; $p < 0.05$), and a nonsignificantly higher rate of heavy alcohol use (39 vs. 32%; $p < 0.1$). Other variables, including environmental stressors, might also have contributed to the mixed findings, though these variables were not available for the present study. It is important to note that we did not have complete data for all genes in the replication sample. For example, half of the top 10 variants, and variants with minor allele frequencies of <5%, were not available in the imputed GWAS data.

The role of *TGFBR1* signaling in the central nervous system is unclear. Together with BDNF, TGFBR1 may

regulate DNA methylation in the hippocampus [35]. SB-431542 is a small-molecular inhibitor of *TGFBR1* developed by GSK; it has been investigated for antitumor activities, though the effects of this molecule on mood and behavior will need to be explored in preclinical models [36].

The findings from this study should be interpreted with caution due to a number of considerations. First, the sequence data are available only for our moderately sized discovery sample set. Our sample of 219 bipolar disorder patients had over 80% power to detect an r^2 of 0.036 (average minor allele frequency for the 2,457 tested variants being 0.077, additive model, two-tailed alpha 0.05 [37]). Second, we did not have complete data available for the replication data set. Furthermore, our discovery and replication samples may differ in the proportion of patients with bipolar I disorder versus bipolar II disorder. The recently reported incomplete genetic correlation between bipolar I and bipolar II disorders and the potentially different risk factors of suicidal be-

havior between bipolar I and bipolar II disorders could explain the lack of substantial replication for the majority of our findings [38, 39]. Furthermore, we did not have detailed information on alcohol use, which might have contributed to the mixed findings. Nonetheless, while our overall findings are negative, additional studies on these and other drug target genes in larger samples are warranted.

Statement of Ethics

All participants gave informed consent for this study, and all procedures contributing to this work received institutional ethics approval.

Disclosure Statement

C.C.Z., A.K.T., and J.L.K. applied for patent applications. J.L.K. received honoraria from Roche, Novartis, and Lilly. C.C.Z. received honoraria from WebMD. G.C.Z., V.L., S.A.S., N.K., J.S., J.B.V. have no conflicts of interest to disclose.

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