Molecular Psychiatry (2012) 17, 433–444

www.nature.com/mp

ORIGINAL ARTICLE

# A genome-wide association study of attempted suicide

VL Willour[1], F Seifuddin[1], PB Mahon[1], D Jancic[1], M Pirooznia[1], J Steele[2], B Schweizer[1], FS Goes[1], FM Mondimore[1], DF MacKinnon[1], The Bipolar Genome Study (BiGS) Consortium, RH Perlis[3], PH Lee[3], J Huang[3], JR Kelsoe[4], PD Shilling[4], M Rietschel[5,6], M Nöthen[7], S Cichon[8,9], H Gurling[10], S Purcell[3], JW Smoller[3], N Craddock[11], JR DePaulo Jr[1], TG Schulze[2,12], FJ McMahon[2], PP Zandi[1] and JB Potash[1]

[1]*Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, USA;* [2]*Genetic Basis of Mood and Anxiety Disorders Unit, National Institute of Mental Health Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD, USA;* [3]*Department of Psychiatry and Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA;* [4]*Department of Psychiatry, University of California, San Diego, La Jolla, CA, USA;* [5]*Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany;* [6]*Department of Psychiatry, University of Bonn, Bonn, Germany;* [7]*Department of Genomics, Life & Brain Center and Institute of Human Genetics, University of Bonn, Bonn, Germany;* [8]*Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany;* [9]*Institute of Neuroscience and Medicine (INM-1), Research Center Juelich, Juelich, Germany;* [10]*Department of Mental Health Sciences, University College London, London, UK;* [11]*Department of Psychological Medicine, School of Medicine, Cardiff University, Cardiff, UK and* [12]*Section on Psychiatric Genetics, Department of Psychiatry and Psychotherapy, Georg-August-Universität, Göttingen, Germany*

**The heritable component to attempted and completed suicide is partly related to psychiatric disorders and also partly independent of them. Although attempted suicide linkage regions have been identified on 2p11-12 and 6q25-26, there are likely many more such loci, the discovery of which will require a much higher resolution approach, such as the genome-wide association study (GWAS). With this in mind, we conducted an attempted suicide GWAS that compared the single-nucleotide polymorphism (SNP) genotypes of 1201 bipolar (BP) subjects with a history of suicide attempts to the genotypes of 1497 BP subjects without a history of suicide attempts. In all, 2507 SNPs with evidence for association at $P<0.001$ were identified. These associated SNPs were subsequently tested for association in a large and independent BP sample set. None of these SNPs were significantly associated in the replication sample after correcting for multiple testing, but the combined analysis of the two sample sets produced an association signal on 2p25 (rs300774) at the threshold of genome-wide significance ($P=5.07\times10^{-8}$). The associated SNPs on 2p25 fall in a large linkage disequilibrium block containing the *ACP1* (*acid phosphatase 1*) gene, a gene whose expression is significantly elevated in BP subjects who have completed suicide. Furthermore, the ACP1 protein is a tyrosine phosphatase that influences Wnt signaling, a pathway regulated by lithium, making *ACP1* a functional candidate for involvement in the phenotype. Larger GWAS sample sets will be required to confirm the signal on 2p25 and to identify additional genetic risk factors increasing susceptibility for attempted suicide.**
*Molecular Psychiatry* (2012) **17,** 433–444; doi:10.1038/mp.2011.4; published online 22 March 2011

**Keywords:** ACP1; LRRTM4; 2p25; Wnt; lithium

## Introduction

Suicidal behavior is a complex phenotype that includes both attempted and completed suicide. Genetic epidemiological studies provide strong evidence for a heritable component to suicidal behavior,[1] and the heritability for serious suicide attempts is estimated at 55%.[2] The heritability for suicidal behavior appears to be partly dependent on the presence of psychiatric disorders such as bipolar disorder (BP), depression and alcoholism. Importantly, the heritability also appears to be partly independent of them. This phenomenon is well illustrated by the study of Brent *et al.*,[3] where the authors assessed suicidal behavior in the offspring of subjects with mood disorders and a history of suicidal behavior and of subjects with mood disorders but no such history.[3] The high-risk offspring had greatly elevated rates of attempted suicide (12 versus 2%, odds ratio (OR) 6.2) despite having similar rates of

Correspondence: Dr VL Willour, Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, 600 North Wolfe Street, Meyer 4-132, Baltimore, MD 21287, USA.
E-mail: willour@jhmi.edu
Received 4 August 2010; revised 27 September 2010; accepted 14 December 2010; published online 22 March 2011

psychiatric and personality disorders. The twin studies of Statham *et al.*[2] and Glowinski *et al.*[4] also reported similar results, with elevated rates of suicidal behavior in MZ:DZ twins after controlling for psychiatric disorders, suggesting that independent genetic factors may be important.

Several studies of suicidal behavior provide evidence supporting the hypothesis that this independent factor may influence the tendency toward impulsive aggression,[5–7] with individuals having both a psychiatric disorder and a tendency toward impulsive aggression having the greatest risk for suicidal behavior.[1] In addition, environmental risk factors, such as parental abuse and early parental loss, may interact with genetic risk factors and thereby increase the risk for suicidal behavior.

Neurobiological studies of suicidal behavior have focused mostly on the serotonergic system, because of several lines of evidence implicating this neurotransmitter pathway, including the finding of decreased serotonin metabolite 5-hydroxyindolacetic acid levels in the cerebrospinal fluid from patients who attempted suicide.[8] Genetic association studies of suicidal behavior have also focused on the serotonergic system, with the tryptophan hydroxylase genes (*TPH1* and *TPH2*) and the serotonin transporter gene receiving considerable attention. However, association studies with these genes and the attempted and completed suicide phenotypes have been mixed.[9]

Four attempted suicide genome-wide linkage studies have been completed using pedigrees with alcoholism, BP and major depression. Three of these studies provided compelling evidence implicating a genetic risk factor for suicidal behavior in the 2p11-12 candidate region, whereas two of them showed additional, more modest, evidence for a locus on 6q25-26.[10–13]

Microarray expression studies have also identified candidate genes for suicidal behavior[14–18] with results implicating genes such as spermine/spermidine $N^1$-acetyltransferase (*SSAT*), which encodes the rate-limiting enzyme in polyamine catabolism.[14]

Although the four attempted suicide linkage studies support the presence of at least two suicidal behavior loci, there are likely many more such loci, the discovery of which will require a much higher resolution approach, such as the genome-wide association study (GWAS). Recent GWAS analyses in schizophrenia[19] and bipolar disorder[20] have identified a number of association signals meeting genome-wide significance, providing potentially groundbreaking advances in these fields.

We are interested in identifying genetic risk factors for suicidal behavior in BP, where ~36.3% of subjects with bipolar I disorder attempt suicide.[21] Toward that end, we have completed an attempted suicide GWAS using 1201 BP subjects with a history of suicide attempts and 1497 BP subjects with no history of suicide attempts. In this study, we present the results of this attempted suicide GWAS and our efforts to replicate the most promising findings.

## Subjects and methods

### *Subjects*

*NIMH-BP samples.* The details of the ascertainment and assessment methods for the National Institute of Mental Health–Bipolar Disorder samples can be found in the original study reports.[22,23] Briefly, the NIMH-BP subjects were collected in five waves. Families were recruited in the first four waves of the study if the proband met criteria for bipolar I disorder and had at least one additional first-degree relative with bipolar I or schizoaffective disorder, bipolar type. In the fifth wave of the study, only unrelated bipolar I subjects were recruited, and no family history of BP was required. Subjects were assessed using the Diagnostic Interview for Genetic Studies (DIGS), and diagnoses were made using DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised) and DSM-IV.[24] Unrelated subjects with self-reported European-American ancestry and a diagnosis of bipolar I disorder or schizoaffective disorder-bipolar type from all five waves were selected for genotyping. All subjects signed written informed consent forms before enrolling into the NIMH-BP study.

*German sample*

The details of the ascertainment and assessment methods for the German sample have been described elsewhere.[25] Briefly, bipolar I disorder probands were recruited via consecutive hospital admissions, assessed by a structured interview and diagnosed according to the DSM-IV criteria. Written informed consent was obtained from all study participants, and unrelated subjects with a diagnosis of bipolar I disorder were selected for genotyping.

### *Genotyping*

Genotyping of the NIMH-BP samples was conducted in two separate efforts, referred to as the Genetic Association Information Network Bipolar Sample (GAIN-BP)[23] and the Translational Genomics Research Institute (TGEN) sample.[23] Genotyping in both efforts was performed using the Affymetrix 6.0 array (Santa Clara, CA, USA), and standardized quality control measures were applied to both BP data sets and matched normal controls as described in the study's primary manuscript.[23] Briefly, subjects with missing data rates $\geqslant 5\%$ were dropped from the analysis, and single-nucleotide polymorphisms (SNPs) were dropped from the analysis if they had minor allele frequencies $<1\%$, missing data rates $\geqslant 5\%$ or Hardy–Weinberg equilibrium $P$-values $<10^{-6}$. The final cleaned GAIN-BP sample consists of 1001 BP subjects (42.7% suicide attempters) and 724 067 SNPs, and the final cleaned TGEN data set includes 1190 BP subjects (46.7% suicide attempters) and 728 187 SNPs. We included all subjects from both of these canonical data sets in our association study.

Genotyping of the German sample was completed using the Illumina HumanHap550 array (San Diego,

CA, USA). Subjects with missing data rates >5% were dropped from the analysis, and SNPs were dropped if they had missing data rates >2%, minor allele frequencies <2% or Hardy–Weinberg equilibrium *P*-values <0.0001. We included the final cleaned data set, including 645 BP subjects (33.8% suicide attempters) and 516 024 SNPs, in our association study.

*Phenotype definition*
Our goal was to conduct an analysis of these genotype data using attempted suicide as the phenotype. Our analytic strategy involved comparing the genotypes of BP subjects with a self-reported history of attempted suicide (attempters) to the genotypes of BP subjects without a self-reported history of attempted suicide (nonattempters), thereby eliminating loci that are associated with BP in general. The attempted suicide GWAS analysis included 1201 attempters, 1497 nonattempters and 138 subjects with an unknown attempted suicide status.

*Population stratification*
We used the principal components method as implemented by EIGENSTRAT in the program EIGENSOFT 3.0 (Price *et al*.[26]) to correct for population stratification in our GWAS. We conducted the analysis using the 137 892 genotyped SNPs that were in common to all three data sets (GAIN-BP, TGEN and German). We selected the top five principal components based on an examination of the scree plot of eigenvalues to include as covariates in our association analysis (Supplementary Figure 1).

*Imputation*
Our three data sets (GAIN-BP, TGEN and German) were genotyped on two different platforms, requiring us to impute them before conducting a mega-analysis. We imputed each data set separately using the phased haplotype data from HapMap phase I and II release 24 (http://hapmap.ncbi.nlm.nih.gov/) as the reference panel. The program BEAGLE (http://faculty.washington.edu/browning/beagle/beagle.html) was used to orient all SNPs of our three data sets to the positive strand consistent with the reference panel data and to generate imputed allelic dosages for autosomal SNPs.[27] Following this procedure, we removed all low confidence imputed SNPs (minor allele frequency $<0.01$, $r^2 \leqslant 0.3$, Hardy–Weinberg equilibrium *P*-value $<1\times10^{-6}$).

*Statistical analysis*
The three imputed data sets were then combined into one consisting of a total of 2 408 051 SNPs for a mega-analysis. We ran our association analyses using the program mach2dat,[28] which incorporates estimated allelic dosages into a logistic regression model for tests of association between genotype and phenotype. We included terms in our logistic regression model adjusting for the top five principal components and also included a dummy-coded variable that indexed the three data sets to control for any possible confounding because of heterogeneity across samples. Supplementary Figure 2 shows the resulting Q–Q plot. The $\lambda$ was estimated as 1.01. We reported the likelihood ratio *P*-values and used the conventional *P*-value ($P<5\times10^{-8}$)[29,30] as the threshold for declaring a finding genome-wide significant.

In addition to the primary analysis outlined above, we also performed two exploratory analyses. We stratified the samples according to sex or to the presence of substance abuse/dependence and repeated the attempted suicide association analysis within each stratum. This analysis was performed using mach2dat with logistic regression and the imputed allelic dosages as described above. The female sample had 779 attempters and 779 nonattempters, whereas the male sample had 422 attempters and 718 nonattempters. Substance abuse/dependence information was not available for the German sample, and hence this secondary analysis was conducted using only the GAIN-BP and TGEN samples. Thus, the substance-free sample had 388 attempters and 610 nonattempters. The substance abuse/dependence sample had 595 attempters (190 with only alcohol abuse/dependence, 54 with only substance abuse/dependence and 351 with both alcohol and substance abuse/dependence) and 532 nonattempters (226 with only alcohol abuse/dependence, 55 with only substance abuse/dependence and 251 with both alcohol and substance abuse/dependence).

*Replication study details*
We attempted to replicate our top GWAS findings from the primary attempted suicide analysis in an independent BP sample consisting of 3117 BP subjects with information about attempted suicide (1295 attempters and 1822 nonattempters) taken from a combined and imputed sample from the Wellcome Trust Case Control Consortium, STEP-BD and University College London (WTCCC/STEP-BD/UCL).[20] SNPs with *P*-values <0.001 in our primary analysis were tested for evidence of association in the replication sample, which was conducted using allelic dosages in PLINK.[31] We used the program METAL (http://www.sph.umich.edu/csg/abecasis/Metal/) to conduct a fixed-effects meta-analysis of the results from our sample and the replication sample to determine the combined evidence of association.

*Gene expression in post-mortem brain*
We reanalyzed Stanley Medical Research Institute (SMRI) gene expression microarray data from studies of post-mortem brains derived from subjects with BP. There were 34 BP subjects (14 with suicide and 20 without suicide) for whom data were available to assess expression of *ACP1* (*acid phosphatase 1*) among those who died by suicide compared with those who died by other means. Reanalysis was performed because the meta-analytic results for this comparison on the SMRI website (www.stanleygenomics.org) mix data from different brain regions and

different platforms. We reanalyzed the three data sets that focus on Brodmann area 46 and use the Affymetrix hgu133a platform.

Normalization was performed using MAS 5.0 (Affymetrix, Santa Clara, CA, USA). Expression values were scaled so that the median expression value for each study was equal to 100 on the linear scale. Quality control was carried out using hierarchical clustering and principal component analysis, and genome-wide outliers for each microarray study were detected and removed (Study 1: A-19, A-28, A-29, A-31, A-41, A-50, A-59, A-87; Study 3: none; Study 7: IDs: A-50, A-59). Intensity values from the three *ACP1* probes present in the hgu133a platform (201629_s_at, 201630_s_at and 215227_x_at) were extracted. Data from the three studies were averaged to yield one expression value per probe. We used linear regression to determine whether the BP subjects who died by suicide showed higher intensity levels at these probes than did BP subjects who died by other means, after controlling for these covariates: age, sex, brain pH and post-mortem interval.

## Results

*Attempted suicide GWAS*

The Manhattan plot illustrating the results for the primary attempted suicide association analysis is shown in Figure 1, with 2507 SNPs (0.1%) showing evidence for association at $P<0.001$ (Supplementary Table 1). Table 1 highlights the top associated regions from this analysis. The strongest signal was at rs300774 ($P=1.09\times10^{-6}$; OR 1.42) in an intergenic region on 2p25 (Figure 2 and Supplementary Table 2). There was a significant difference in the distribution of males and females among the attempters and nonattempters ($P$-value $=2.68\times10^{-11}$), but there was no significant difference for age at interview ($P=0.99$). Thus, we repeated the association analysis controlling for sex. The top association signals remained essentially the same after controlling for this variable (for example rs300774 unadjusted $P=1.09\times10^{-6}$, adjusted $P=1.24\times10^{-6}$), indicating that the evidence for association was not because of the sex distribution differences in the attempters and nonattempters.

We also performed two hypothesis-generating secondary analyses on the GWAS data set. First, we stratified the sample set according to sex, with the goal of increasing homogeneity and identifying sex-specific risk loci, and then repeated the attempted suicide association analysis. There was no overlap between the top male and female SNP lists ($P<0.001$; Supplementary Tables 3 and 4). The most significant association signal in males was at rs5752388 on chromosome 22q12 in the *AK026502* gene that generates a noncoding RNA ($P=1.07\times10^{-6}$; OR 1.67; Figure 3a), and the most significant association signal in females was at rs10170138 on chromosome 2p12 in the *LRRTM4* gene ($P=9.27\times10^{-7}$; OR 0.60; Figure 3b), which codes for a nervous system transmembrane protein. The stratification analysis also indicated that the evidence for association with rs300774 was stronger in males ($P=4.55\times10^{-6}$; OR 1.69) than in females ($P=0.01077$; OR 1.27).

Second, we stratified the sample set according to the presence or absence of substance abuse/depen-

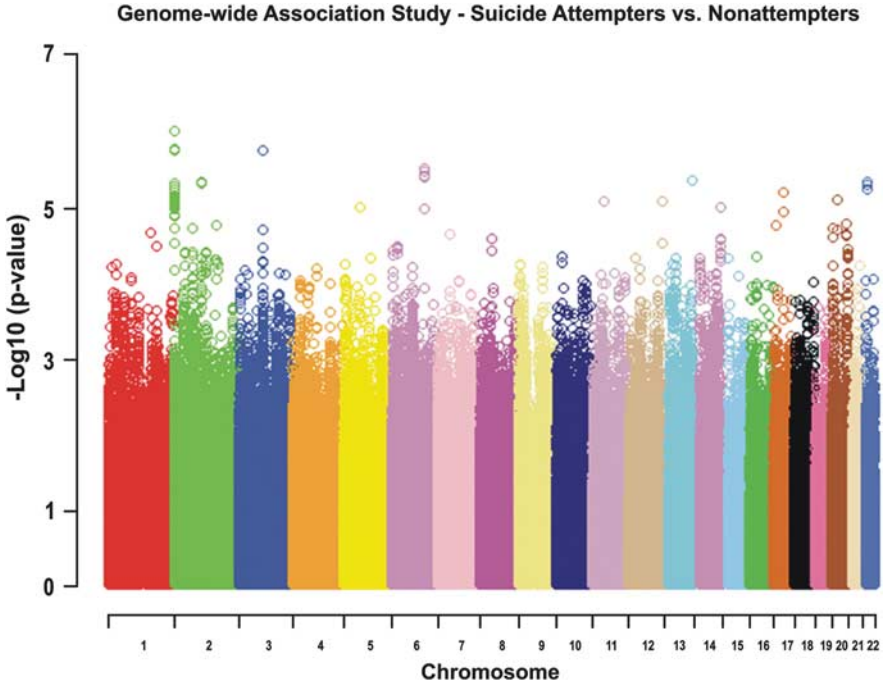


**Figure 1** The Manhattan plot for the primary attempted suicide analysis. The chromosomes are presented in order (pter to qter) and color coded for ease of identification. The individual single-nucleotide polymorphisms (SNPs) are represented by open circles and their corresponding *P*-values are graphed according to the –log10 (*P*-value).

**Table 1** Top associated regions ($P < 1 \times 10^{-5}$) from the primary attempted suicide GWAS

| *Chromosome region* | *Best SNP in region* | *Chromosome location (bp)* | *Minor/major allele*[a] | *Minor allele frequency*[b] | *RefSeq gene*[c] | *Odds ratio*[d] | P-*value* |
|---|---|---|---|---|---|---|---|
| 2p25 | rs300774 | 102496 | A/C | 0.18 | Intergenic | 1.42 | $1.09 \times 10^{-6}$ |
| 2q12 | rs10189155 | 104102684 | G/C | 0.04 | Intergenic | 2.01 | $5.03 \times 10^{-6}$ |
| 3p12 | rs2175671 | 86346973 | T/C | 0.27 | Intergenic | 1.38 | $1.94 \times 10^{-6}$ |
| 6q22 | rs9320964 | 124237506 | T/A | 0.34 | *NKAIN2* | 0.76 | $3.35 \times 10^{-6}$ |
| 11p11 | rs12801214 | 44449493 | T/C | 0.13 | Intergenic | 0.68 | $9.02 \times 10^{-6}$ |
| 12q24 | rs7296262 | 127661025 | T/C | 0.51 | *TMEM132C* | 1.43 | $9.08 \times 10^{-6}$ |
| 13q33 | rs1543002 | 107301357 | C/T | 0.32 | *FAM155A* | 0.76 | $4.80 \times 10^{-6}$ |
| 17q21 | rs11650719 | 39007411 | A/G | 0.15 | Intergenic | 1.42 | $7.10 \times 10^{-6}$ |
| 20p11 | rs6076080 | 23439778 | T/C | 0.12 | Intergenic | 1.46 | $8.81 \times 10^{-6}$ |
| 22q12 | rs5752388 | 25439475 | C/T | 0.23 | Intergenic | 1.35 | $5.12 \times 10^{-6}$ |

Abbreviations: GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.
[a]The minor allele as based on the HapMap phase I and II release 24 (http://hapmap.ncbi.nlm.nih.gov/) data is listed first.
[b]The frequency of the listed minor allele is calculated based on our combined three data sets; as a result, the minor allele frequency (MAF) may be $>0.50$.
[c]Indicates whether the SNP localizes to a known RefSeq gene or to an intergenic region (University of California Santa Cruz (UCSC) Genome Browser, March 2006).
[d]The odds ratio is provided for the listed minor allele from a logistic regression analysis under an additive model.

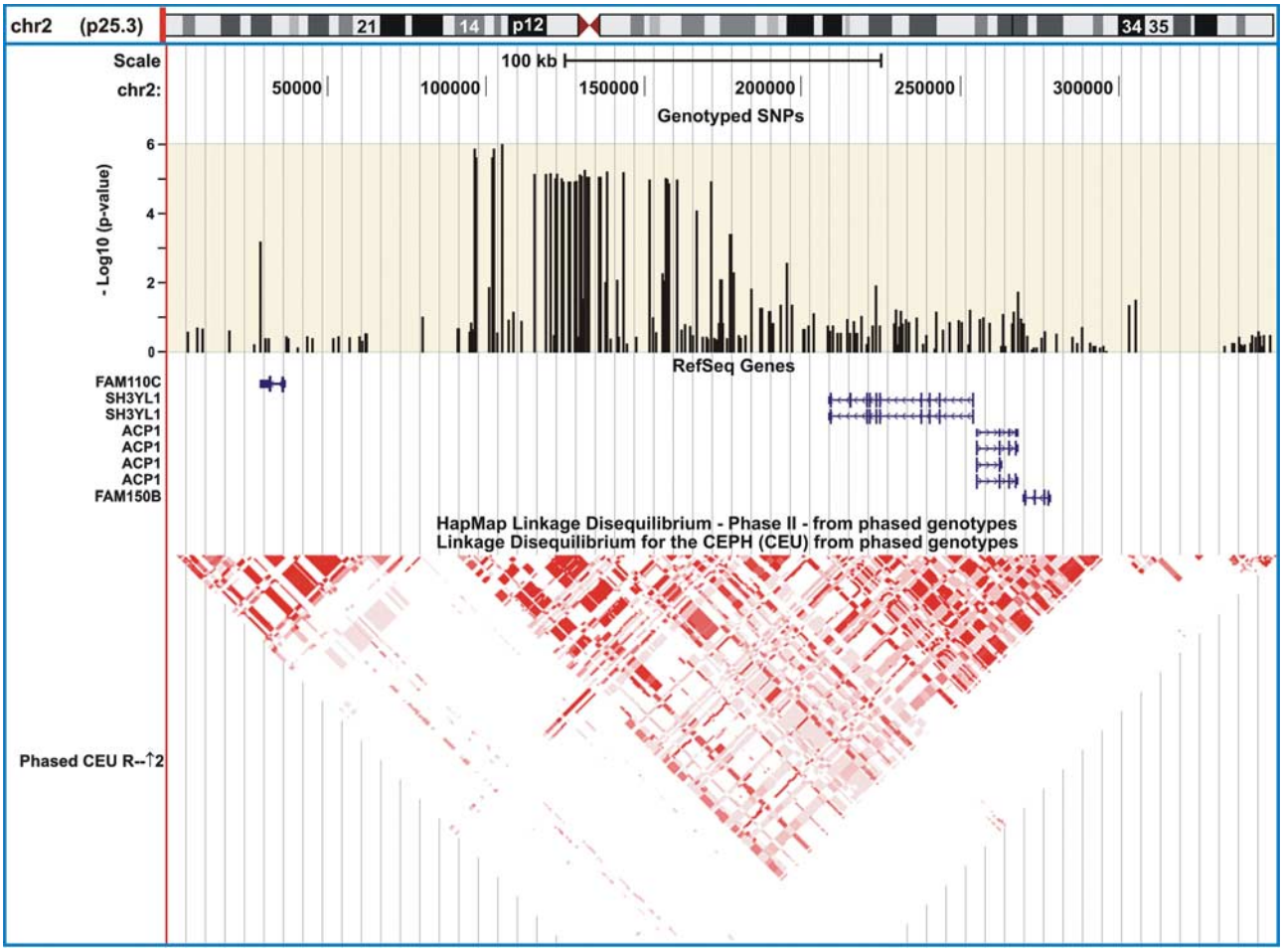


**Figure 2** The 2p25 candidate region—shown are the chromosomal location, genotyped single-nucleotide polymorphisms (SNPs) from the primary analysis, gene structure for each of the candidate genes in the region and linkage disequilibrium structure for the region based on the HapMap CEU population (University of California Santa Cruz (UCSC) Genome Browser; http://genome.ucsc.edu/, March 2006).

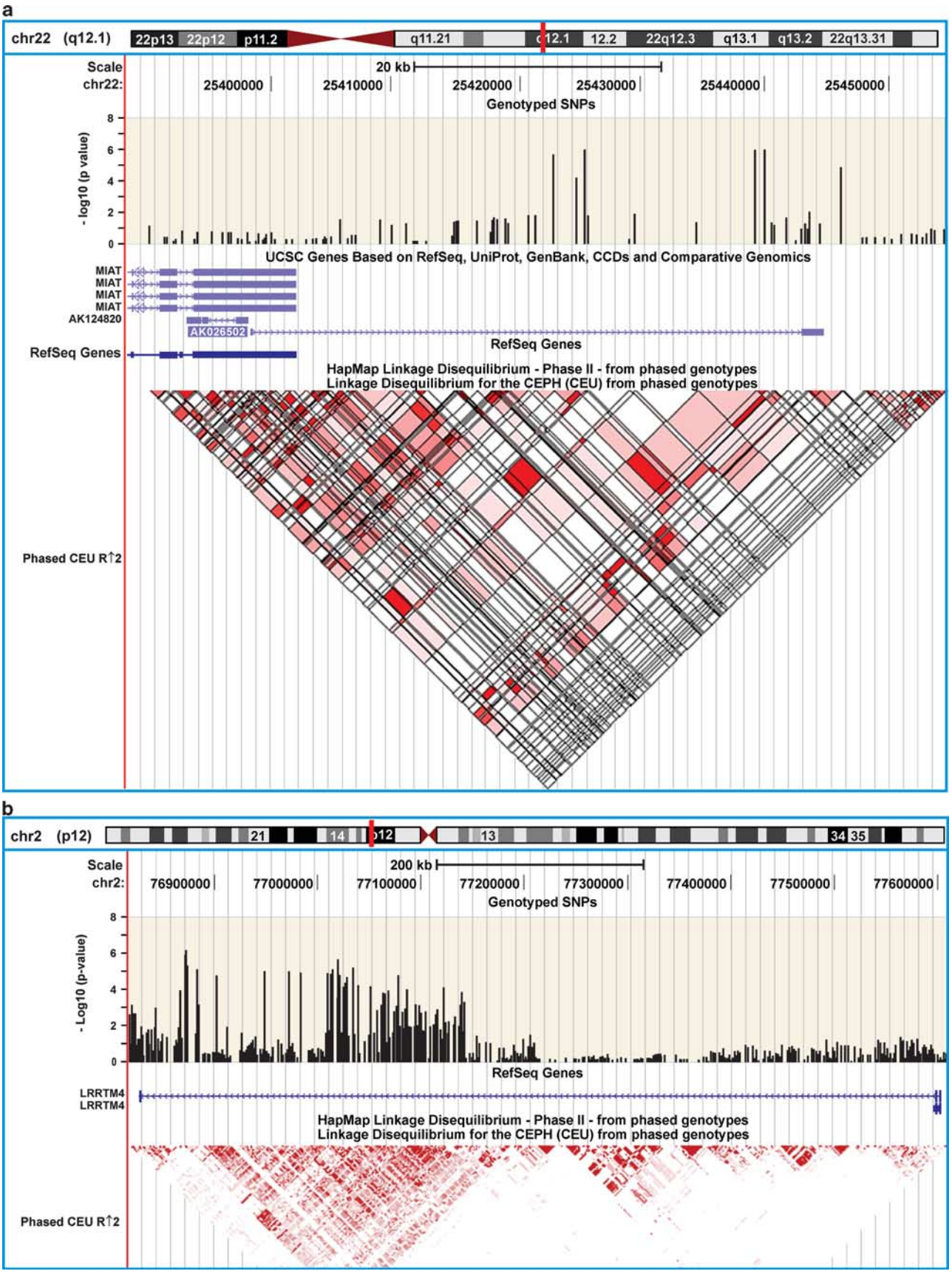


**Figure 3** For legend see next page.

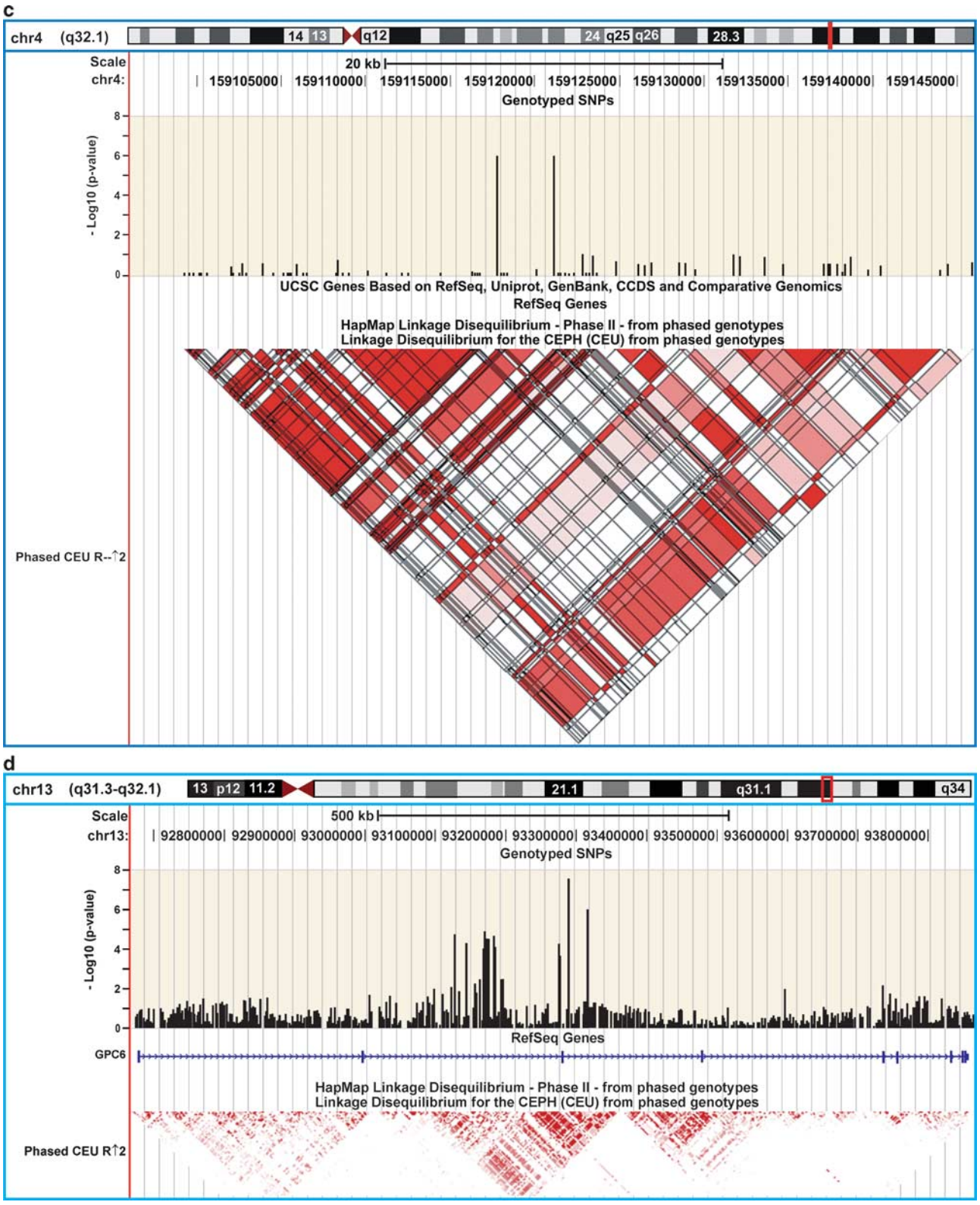


**Figure 3** Top association signals for the secondary analyses—shown are the chromosomal locations, genotyped single-nucleotide polymorphisms (SNPs), gene structures and linkage disequilibrium structures for each of the top association signals (University of California Santa Cruz (UCSC) Genome Browser, March 2006). (**a**) Male-specific results; (**b**) female-specific results; (**c**) substance abuse/dependence-specific results; and (**d**) substance-free results.

**Table 2** Attempted suicide replication and meta-analysis results

| *Chromosome region* | *Best SNP in region* | *RefSeq gene*[a] | *RefSeq genes in LD* | *Initial odds ratio*[b] | *Initial* P-*value* | *Replication odds ratio*[b] | *Replication* P-*value* | *Combined* P-*value* |
|---|---|---|---|---|---|---|---|---|
| 2p25 | rs300774 | Intergenic | *SH3YL1, ACP1, FAM150B* | 1.42 | $1.09 \times 10^{-6}$ | 1.22 | 0.0036 | $5.07 \times 10^{-8}$ |
| 11p13 | rs10437629 | *C11orf41* | None | 1.62 | $8.56 \times 10^{-5}$ | 1.34 | 0.0078 | $3.77 \times 10^{-6}$ |
| 12q24 | rs7296262 | *TMEM132C* | None | 1.43 | $9.08 \times 10^{-6}$ | 1.22 | 0.011 | $1.09 \times 10^{-6}$ |

Abbreviations: LD, linkage disequilibrium; SNP, single-nucleotide polymorphism.
[a]Indicates whether the SNP localizes to a known RefSeq gene or to an intergenic region (University of California Santa Cruz (UCSC) Genome Browser, March 2006).
[b]The odds ratio is provided for the minor allele from a logistic regression analysis under an additive model.

dence, with the goal of identifying loci with evidence for an interaction with this important clinical comorbidity. Only two SNPs (rs2900032 and rs2175671) appeared on both top SNP lists with a $P<0.001$ (Supplementary Tables 5 and 6). The most significant association signal with substance abuse/dependence was at rs4072169 in an intergenic region on 4q32 ($P=1.61 \times 10^{-6}$; OR 1.69; Figure 3c), whereas the most significant association signal in the substance-free sample was at rs2150127 on 13q31 in the *GPC6* gene ($P=6.37 \times 10^{-8}$; OR 0.12; Figure 3d), which codes for a heparan sulfate proteoglycan belonging to a family of proteins that have a role in signal transduction including the stimulation of Wnt signaling.[32]

*Attempted suicide replication*

We were also interested in determining whether the associated SNPs from the primary analyses would replicate in an independent sample set. We tested the 2507 observed and imputed SNPs that met a threshold of $P<0.001$ in the primary attempted suicide analysis for evidence of association in the combined WTCCC/STEP-BD/UCL sample,[20] which was also imputed to HapMap I and II, allowing us to test directly for evidence of replication. None of the 2507 SNPs were significant in the replication sample alone after Bonferroni correction for multiple comparisons ($P=0.05 \div 2507$ SNPs $=2 \times 10^{-5}$; Supplementary Table 1). However, we did identify a number of SNPs with evidence for association in both sample sets. Thus, we conducted a meta-analysis using the attempted suicide results from both the primary and replication samples and identified one marker on 2p25, rs300774, with a *P*-value of $5.07 \times 10^{-8}$, which was on the threshold of genome-wide significance ($P<5 \times 10^{-8}$; Table 2). Two additional chromosomal regions (12q24 and 11p13) had *P*-values $<1 \times 10^{-5}$ in the combined sample set.

*Gene expression in post-mortem brain*

Our results on 2p25 led us to examine microarray data from the SMRI website, which includes an assessment of expression levels of *SH3YL1* and *ACP1* in the brains of BP subjects who died by suicide compared with those BP subjects who died by other means. The *SH3YL1* transcript is not reported as significantly altered in those who committed suicide. *ACP1* expression, however, is reported as significantly elevated in the brains of those who committed suicide. Because of the potential for heterogeneity and confounding in these results, we downloaded and reanalyzed the *ACP1* data, focusing on three of the 20 SMRI studies that used the same platform, and looked at the same brain region, Brodmann area 46, in the prefrontal cortex. We found that there were significant effects on *ACP1* expression for sex and for brain pH, but even after controlling for them, we continued to see significantly increased expression in those who died by suicide, for one of the three probes (201629_s_at; $P<0.043$), although the effects were slightly more modest for the other two ($P<0.10$; Supplementary Figure 3).

## Discussion

In this report, we describe the results of a GWAS of attempted suicide. Our primary analysis, using 1201 attempters and 1497 nonattempters, identified 2507 SNPs with nominal evidence for association ($P<0.001$), including 10 candidate regions with evidence for association at $P<1 \times 10^{-5}$. Follow-up in a sample that combined our original one plus an independent replication data set identified one candidate region on 2p25 with $P=5.07 \times 10^{-8}$, a finding that is on the threshold of genome-wide significance. In addition, secondary analyses aimed at identifying sex-specific loci and loci associated with substance abuse/dependence identified distinct lists of SNPs with more modest evidence for association.

The 2p25 association signal lies in the intergenic region between the *SH3YL1* and *FAM110C* genes. Although both of these genes are expressed in the brain, little is known about their potential functional contribution in the brain. Work to date indicates that *SH3YL1* may have a role in hair follicle development[33] and that *FAM110C* may have a role in cell spreading and migration.[34] Our top association signals on 2p25 lie in a large linkage disequilibrium block that includes *SH3YL1* and two other genes, *ACP1* and *FAM150B*. *ACP1* codes for a protein tyrosine phosphatase that is expressed in the brain,[35]

whereas *FAM150B* is an uncharacterized protein-coding transcript.

The ability of lithium to reduce the risk of suicidal behavior is well established, and it is hypothesized to exert its therapeutic effect by inhibiting glycogen synthase kinase-3 and activating the Wnt signaling pathway.[36] Activated glycogen synthase kinase-3 phosphorylates the β-catenin protein, resulting in its degradation and in reduced Wnt signaling, an event that is thought to be inhibited by lithium. Long-term lithium treatment results in increased cytoplasmic protein levels of β-catenin in the rat brain.[37] In addition, elevation of β-catenin positively regulates neurogenesis just as lithium does.[38] Interestingly, the *ACP1* gene product also regulates β-catenin. Overexpression of *ACP1*, as seen in the SMRI BP suicide completers, results in decreased cytoplasmic β-catenin,[39] which is the opposite effect of lithium administration, thus making *ACP1* a functional candidate for involvement in suicidal behavior.

Our sex-specific secondary analyses identified primarily female-specific loci (90 female SNPs versus 16 male SNPs with *P*-values $< 1 \times 10^{-5}$), which is an intriguing finding given that women attempt suicide at a rate two to three times higher than men.[40,41] The most significant female-specific signal was seen with marker rs10170138, located in intron 3 of the *LRRTM4* gene on 2p12. Interestingly, the *LRRTM4* gene sits under the 2p11-12 attempted suicide linkage peak, which was initially identified using mood disorder pedigree sets that were predominantly female.[11,12] The LRRTM4 gene encodes a transmembrane protein known to trigger the formation of excitatory synapses.[42]

We identified these two association signals on 2p25 and 2p12 by comparing the genotypes of BP subjects who have attempted suicide to those of BP subjects without attempts. This 'case-only' approach was designed to identify genetic variants conferring risk to attempted suicide specifically and not to BP in general, and the current association findings with the attempted suicide phenotype provide support for the existence of gene(s) influencing risk for suicidal behavior on the short arm of chromosome 2, an observation that is consistent with the linkage and association[43,44] findings using pedigrees with BP, major depression and alcoholism.

Neurobiological and genetic studies of suicidality have implicated genes from both the serotonergic system and the hypothalamic–pituitary–adrenal axis in the development of suicidal behavior.[45] However, our attempted suicide GWAS analysis failed to identify significant associations with genes in either pathway or with other important suicidal behavior candidate genes, such as *BDNF*, *NTRK2* and *SSAT* (Supplementary Table 1). One possible explanation for our inability to detect association in these genes is that our 'case-only' approach may have missed loci that influence suicidal ideation, as both our attempters and nonattempters have a high rate of this phenotype. A second possible explanation for this lack of association is that our study focused on association with SNPs, whereas many of these previous studies identified association with insertion/deletion polymorphisms and variable number of tandem repeats, which may not be tagged efficiently by our SNP genotyping panel. A third possible explanation is that one or more of these loci influence suicidal behavior in a phenotypic subset of these samples (such as subjects with highly lethal attempts), and this heterogeneity has reduced our ability to detect evidence for association at these loci.

Our findings should be interpreted in light of several limitations. First, our study was limited by the loss of power inherent in the analysis of subphenotypes. We estimated that our combined sample had 80% power to detect a locus of genome-wide significance with an OR of 1.5. Second, our study was designed to identify common variants conferring risk for the attempted suicide phenotype, and hence we did not assess the impact of rare variants or copy number variants. Third, we did not include environmental risk factors, such as parental abuse and early parental loss, which may interact with genetic factors and increase the risk for suicidal behavior. Fourth, it is possible that some subjects—who were classified as nonattempters at the time of ascertainment—went on to attempt suicide.[46] However, this would mean that our attempter sample had been biased toward those with early-onset suicide attempts, which may have increased sample homogeneity. Finally, the *ACP1* gene expression study was limited by the fact that the BP subjects were heterogeneous with respect to their medication use. Additional studies using larger sample sets and more detailed medication histories will be required to determine the impact (if any) of each medication and medication combination on *ACP1* gene expression.

Our primary and replication analyses from this GWAS of attempted suicide identified an association signal on 2p25 at the threshold of genome-wide significance, providing support for the presence of common genetic variation influencing the risk for suicidal behavior. Analysis of the attempted suicide phenotype in additional GWAS samples, such as the ones being assembled by the PGC (Psychiatric GWAS Consortium), will be required to confirm the signal on 2p25 and will allow for the identification of additional loci influencing suicidal behavior in BP and related psychiatric phenotypes.

## Conflict of interest

The authors declare no conflict of interest. Dr Perlis has received consulting fees from Concordant Rater Systems, Proteus Biomedical, and RIDVentures, and royalties/patent fees from Concordant Rater Systems.

## Acknowledgments

This work was supported by grants from the National Institute of Mental Health (MH079240 to VLW) and

the American Foundation for Suicide Prevention (VLW). It was also supported in part by R01 MH079799 (JWS). Drs Willour and Potash were also supported by Margaret Price Investigatorships. DNA samples were prepared and distributed by Rutgers University under a contract from the NIMH. We are grateful to the many interviewers and diagnosticians who contributed to this project, and to the families who devoted their time and effort to the study. Genome-wide SNP genotyping of the NIMH samples was performed through the Genetic Association Information Network under the direction of The Bipolar Genome Study (BiGS) Consortium. The Principal Investigators and Co-Investigators were: University of California San Diego, La Jolla, CA: John R Kelsoe (PI), Tiffany A Greenwood, Thomas B Barrett, Caroline M Nievergelt, Rebecca McKinney, Paul D Shilling; Scripps Research Institute, La Jolla, CA: Nicholas Schork (PI), Erin N Smith, Cinnamon S Bloss; Indiana University, Bloomington, IN: John I Nurnberger, Jr (PI), Howard J Edenberg, Tatiana Foroud, Daniel M Koller; University of Chicago, Chicago, IL: Elliot Gershon (PI), Chunyu Liu, Judith A Badner; Rush University Medical Center, Chicago, IL: William A Scheftner; Howard University, Washington, DC: William B Lawson (PI), Evaristus A Nwulia, Maria Hipolito; University of Iowa, Iowa City, IA: William Coryell (PI); Washington University, St Louis, MO: John Rice (PI); University of California San Francisco, San Francisco, CA: William Byerley (PI); National Institute of Mental Health, Bethesda, MD: Francis McMahon (PI), Thomas G Schulze; University of Pennsylvania, Philadelphia, PA: Wade Berrettini (PI); Johns Hopkins University, Baltimore, MD: James B Potash (PI), Peter P Zandi, Pamela B Mahon; University of Michigan, Ann Arbor, MI: Melvin G McInnis (PI), Sebastian Zöllner, Peng Zhang; The Translational Genomics Research Institute, Phoenix, AZ: David Craig (PI), Szabolics Szelinger. Data and biomaterials were collected in four projects that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 1991 to 1998, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, U01 MH46282: John Nurnberger, Marvin Miller and Elizabeth Bowman; Washington University, St Louis, MO, U01 MH46280: Theodore Reich, Allison Goate and John Rice; Johns Hopkins University, Baltimore, MD U01 MH46274: J Raymond DePaulo, Jr, Sylvia Simpson and Colin Stine; NIMH Intramural Research Program, Clinical Neurogenetics Branch, Bethesda, MD: Elliot Gershon, Diane Kazuba and Elizabeth Maxwell. Data and biomaterials were collected as part of 10 projects that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 1999 to 2003, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, R01 MH59545: John Nurnberger, Marvin J Miller, Elizabeth S Bowman, N Leela Rau, P Ryan Moe, Nalini Samavedy, Rif El-Mallakh (at the University of Louisville), Husseini Manji (at the Wayne State University), Debra A Glitz (at the Wayne State University), Eric T Meyer, Carrie Smiley, Tatiana Foroud, Leah Flury, Danielle M Dick, Howard Edenberg; Washington University, St Louis, MO, R01 MH059534: John Rice, Theodore Reich, Allison Goate, Laura Bierut; Johns Hopkins University, Baltimore, MD, R01 MH59533: Melvin McInnis, J Raymond DePaulo, Jr, Dean F MacKinnon, Francis M Mondimore, James B Potash, Peter P Zandi, Dimitrios Avramopoulos and Jennifer Payne; University of Pennsylvania, PA, R01 MH59553: Wade Berrettini; University of California at Irvine, CA, R01 MH60068: William Byerley and Mark Vawter; University of Iowa, IA, R01 MH059548: William Coryell and Raymond Crowe; University of Chicago, IL, R01 MH59535: Elliot Gershon, Judith Badner, Francis McMahon, Chunyu Liu, Alan Sanders, Maria Caserta, Steven Dinwiddie, Tu Nguyen, Donna Harakal; University of California at San Diego, CA, R01 MH59567: John Kelsoe, Rebecca McKinney; Rush University, IL, R01 MH059556: William Scheftner, Howard M Kravitz, Diana Marta, Annette Vaughn-Brown and Laurie Bederow; NIMH Intramural Research Program, Bethesda, MD, 1Z01MH002810-01: Francis J Mc Mahon, Layla Kassem, Sevilla Detera-Wadleigh, Lisa Austin and Dennis L Murphy. Data and biomaterials were collected as part of 11 projects (Study 40) that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 2003 to 2007, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, R01 MH59545: John Nurnberger, Marvin J Miller, Elizabeth S Bowman, N Leela Rau, P Ryan Moe, Nalini Samavedy, Rif El-Mallakh (at the University of Louisville), Husseini Manji (at Johnson and Johnson), Debra A Glitz (at the Wayne State University), Eric T Meyer (at the Oxford University, UK), Carrie Smiley, Tatiana Foroud, Leah Flury, Danielle M Dick (at the Virginia Commonwealth University), Howard Edenberg; Washington University, St Louis, MO, R01 MH059534: John Rice, Theodore Reich, Allison Goate, Laura Bierut K02 DA21237; Johns Hopkins University, Baltimore, MD, R01 MH59533: Melvin McInnis, J Raymond DePaulo, Jr, Dean F MacKinnon, Francis M Mondimore, James B Potash, Peter P Zandi, Dimitrios Avramopoulos and Jennifer Payne; University of Pennsylvania, PA, R01 MH59553: Wade Berrettini; University of California at San Francisco, CA, R01 MH60068: William Byerley and Sophia Vinogradov; University of Iowa, IA, R01 MH059548: William Coryell, and Raymond Crowe; University of Chicago, IL, R01 MH59535: Elliot Gershon, Judith Badner, Francis McMahon, Chunyu Liu, Alan Sanders, Maria Caserta, Steven Dinwiddie, Tu Nguyen, Donna Harakal; University of California at San Diego, CA, R01 MH59567: John Kelsoe, Rebecca McKinney; Rush University, IL, R01 MH059556: William Scheftner, Howard M Kravitz, Diana Marta, Annette Vaughn-Brown, and Laurie Bederow; NIMH Intramural Research Program, Bethesda, MD, 1Z01MH002810-01: Francis J McMahon, Layla Kassem, Sevilla Detera-

Wadleigh, Lisa Austin, Dennis L Murphy; Howard University: William B Lawson, Evarista Nwulia, and Maria Hipolito.

## References


1 Brent DA, Mann JJ. Family genetic studies, suicide, and suicidal behavior. *Am J Med Genet C Semin Med Genet* 2005; **133C**: 13–24.
2 Statham DJ, Heath AC, Madden PA, Bucholz KK, Bierut L, Dinwiddie SH *et al.* Suicidal behaviour: an epidemiological and genetic study. *Psychol Med* 1998; **28**: 839–855.
3 Brent DA, Oquendo M, Birmaher B, Greenhill L, Kolko D, Stanley B *et al.* Familial pathways to early-onset suicide attempt: risk for suicidal behavior in offspring of mood-disordered suicide attempters. *Arch Gen Psychiatry* 2002; **59**: 801–807.
4 Glowinski AL, Bucholz KK, Nelson EC, Fu Q, Madden PA, Reich W *et al.* Suicide attempts in an adolescent female twin sample. *J Am Acad Child Adolesc Psychiatry* 2001; **40**: 1300–1307.
5 Brent DA, Oquendo M, Birmaher B, Greenhill L, Kolko D, Stanley B *et al.* Peripubertal suicide attempts in offspring of suicide attempters with siblings concordant for suicidal behavior. *Am J Psychiatry* 2003; **160**: 1486–1493.
6 Pfeffer CR, Normandin L, Kakuma T. Suicidal children grow up: suicidal behavior and psychiatric disorders among relatives. *J Am Acad Child Adolesc Psychiatry* 1994; **33**: 1087–1097.
7 Melhem NM, Brent DA, Ziegler M, Iyengar S, Kolko D, Oquendo M *et al.* Familial pathways to early-onset suicidal behavior: familial and individual antecedents of suicidal behavior. *Am J Psychiatry* 2007; **164**: 1364–1370.
8 Asberg M, Traskman L, Thoren P. 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? *Arch Gen Psychiatry* 1976; **33**: 1193–1197.
9 Brezo J, Klempan T, Turecki G. The genetics of suicide: a critical review of molecular studies. *Psychiatr Clin North Am* 2008; **31**: 179–203.
10 Hesselbrock V, Dick D, Hesselbrock M, Foroud T, Schuckit M, Edenberg H *et al.* The search for genetic risk factors associated with suicidal behavior. *Alcohol Clin Exp Res* 2004; **28**: 70S–76S.
11 Zubenko GS, Maher BS, Hughes HB, Zubenko WN, Scott Stiffler J, Marazita ML. Genome-wide linkage survey for genetic loci that affect the risk of suicide attempts in families with recurrent, early-onset, major depression. *Am J Med Genet B Neuropsychiatr Genet* 2004; **129B**: 47–54.
12 Willour VL, Zandi PP, Badner JA, Steele J, Miao K, Lopez V *et al.* Attempted suicide in bipolar disorder pedigrees: evidence for linkage to 2p12. *Biol Psychiatry* 2007; **61**: 725–727.
13 Cheng R, Juo SH, Loth JE, Nee J, Iossifov I, Blumenthal R *et al.* Genome-wide linkage scan in a large bipolar disorder sample from the national institute of mental health genetics initiative suggests putative loci for bipolar disorder, psychosis, suicide, and panic disorder. *Mol Psychiatry* 2006; **11**: 252–260.
14 Sequeira A, Gwadry FG, Ffrench-Mullen JM, Canetti L, Gingras Y, Casero RA *et al.* Implication of SSAT by gene expression and genetic variation in suicide and major depression. *Arch Gen Psychiatry* 2006; **63**: 35–48.
15 Sequeira A, Klempan T, Canetti L, ffrench-Mullen J, Benkelfat C, Rouleau GA *et al.* Patterns of gene expression in the limbic system of suicides with and without major depression. *Mol Psychiatry* 2007; **12**: 640–655.
16 Sequeira A, Mamdani F, Ernst C, Vawter MP, Bunney WE, Lebel V *et al.* Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. *PLoS One* 2009; **4**: e6585.
17 Tochigi M, Iwamoto K, Bundo M, Sasaki T, Kato N, Kato T. Gene expression profiling of major depression and suicide in the prefrontal cortex of postmortem brains. *Neurosci Res* 2008; **60**: 184–191.
18 Kim S, Choi KH, Baykiz AF, Gershenfeld HK. Suicide candidate genes associated with bipolar disorder and schizophrenia: an exploratory gene expression profiling analysis of post-mortem prefrontal cortex. *BMC Genomics* 2007; **8**: 413.
19 Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe I *et al.* Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* 2009; **460**: 753–757.
20 Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L *et al.* Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008; **40**: 1056–1058.
21 Novick DM, Swartz HA, Frank E. Suicide attempts in bipolar I and bipolar II disorder: a review and meta-analysis of the evidence. *Bipolar Disord* 2010; **12**: 1–9.
22 Nurnberger Jr JI, DePaulo JR, Gershon ES, Reich T, Blehar MC, Edenberg HJ *et al.* Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: a preliminary report. *Am J Med Genet* 1997; **74**: 227–237.
23 Smith EN, Bloss CS, Badner JA, Barrett T, Belmonte PL, Berrettini W *et al.* Genome-wide association study of bipolar disorder in European American and African American individuals. *Mol Psychiatry* 2009; **14**: 755–763.
24 Nurnberger JI, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J *et al.* Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH genetics initiative. *Arch Gen Psychiatry* 1994; **51**: 849–859; discussion 863–4.
25 McMahon FJ, Akula N, Schulze TG, Muglia P, Tozzi F, Detera-Wadleigh SD *et al.* Meta-analysis of genome-wide association data identifies a risk locus for major mood disorders on 3p21.1. *Nat Genet* 2010; **42**: 128–131.
26 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; **38**: 904–909.
27 Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet* 2009; **84**: 210–223.
28 Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet* 2009; **10**: 387–406.
29 Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 2008; **32**: 227–234.
30 Ioannidis JP, Thomas G, Daly MJ. Validating, augmenting and refining genome-wide association signals. *Nat Rev Genet* 2009; **10**: 318–329.
31 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
32 Filmus J, Capurro M, Rast J. Glypicans. *Genome Biol* 2008; **9**: 224.
33 Aoki N, Ito K, Ito M. A novel mouse gene, Sh3yl1, is expressed in the anagen hair follicle. *J Invest Dermatol* 2000; **114**: 1050–1056.
34 Hauge H, Fjelland KE, Sioud M, Aasheim HC. Evidence for the involvement of FAM110C protein in cell spreading and migration. *Cell Signal* 2009; **21**: 1866–1873.
35 Souza AC, Azoubel S, Queiroz KC, Peppelenbosch MP, Ferreira CV. From immune response to cancer: a spot on the low molecular weight protein tyrosine phosphatase. *Cell Mol Life Sci* 2009; **66**: 1140–1153.
36 O'Brien WT, Klein PS. Validating GSK3 as an in vivo target of lithium action. *Biochem Soc Trans* 2009; **37**: 1133–1138.
37 Gould TD, Chen G, Manji HK. In vivo evidence in the brain for lithium inhibition of glycogen synthase kinase-3. *Neuropsychopharmacology* 2004; **29**: 32–38.
38 Wexler EM, Geschwind DH, Palmer TD. Lithium regulates adult hippocampal progenitor development through canonical wnt pathway activation. *Mol Psychiatry* 2008; **13**: 285–292.
39 Taddei ML, Chiarugi P, Cirri P, Buricchi F, Fiaschi T, Giannoni E *et al.* Beta-catenin interacts with low-molecular-weight protein tyrosine phosphatase leading to cadherin-mediated cell-cell adhesion increase. *Cancer Res* 2002; **62**: 6489–6499.
40 Baca-Garcia E, Perez-Rodriguez MM, Keyes KM, Oquendo MA, Hasin DS, Grant BF *et al.* Suicidal ideation and suicide attempts in the United States: 1991-1992 and 2001-2002. *Mol Psychiatry* 2010; **15**: 250–259.

41 Hawton K, van Heeringen K. Suicide. *Lancet* 2009; **373**: 1372–1381.
42 Linhoff MW, Laurén J, Cassidy RM, Dobie FA, Takahashi H, Nygaard HB *et al*. An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. *Neuron* 2009; **61**: 734–49.
43 Perlis RH, Huang J, Purcell S, Fava M, Rush AJ, Sullivan PF *et al.*, Wellcome Trust Case Control Consortium Bipolar Disorder Group, Craddock N, Sklar P, Smoller JW. Genome-wide association study of suicide attempts in mood disorder patients. *Am J Psychiatry* 2010; **167**: 1499–507.
44 Dick DM, Meyers J, Aliev F, Nurnberger J Jr, Kramer J, Kuperman S *et al*. Evidence for genes on chromosome 2 contributing to alcohol dependence with conduct disorder and suicide attempts. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 1179–1188.
45 Currier D, Mann JJ. Stress, genes and the biology of suicidal behavior. *Psychiatr Clin North Am* 2008; **31**: 247–269.
46 Angst J, Angst F, Gerber-Werder R, Gamma A. Suicide in 406 mood-disorder patients with and without long-term medication: a 40 to 44 years' follow-up. *Arch Suicide Res* 2005; **9**: 279–300.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)