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Strong genetic evidence for a selective influence of $GABA_A$ receptors on a component of the bipolar disorder phenotype

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Abstract

Despite compelling evidence for a major genetic contribution to risk of bipolar mood disorder, conclusive evidence implicating specific genes or pathophysiological systems has proved elusive. In part this is likely to be related to the unknown validity of current phenotype definitions and consequent aetiological heterogeneity of samples. In the recent Wellcome Trust Case Control Consortium (WTCCC) genome-wide association analysis of bipolar disorder (1868 cases, 2938 controls) one of the most strongly associated polymorphisms lay within the gene encoding the $GABA_A$ receptor β1 subunit, *GABRB1*. Aiming to increase biological homogeneity, we sought the diagnostic subset that showed the strongest signal at this polymorphism and used this to test for independent evidence of association with other members of the $GABA_A$ receptor gene family. The index signal was significantly enriched in the 279 cases meeting Research Diagnostic Criteria for schizoaffective disorder, bipolar type ($p=3.8\times10^{-6}$). Independently, these cases showed strong evidence that variation in $GABA_A$ receptor genes influences risk for this phenotype (independent

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system-wide $p=6.6\times10^{-5}$) with association signals also at *GABRA4*, *GABRB3*, *GABRA5* and *GABRR1*. Our findings have the potential to inform understanding of presentation, pathogenesis and nosology of bipolar disorders. Our method of phenotype refinement may be useful in studies of other complex psychiatric and non-psychiatric disorders.

Introduction

Bipolar disorder (BD; manic depressive illness) (1) refers to an episodic recurrent pathological disturbance in mood (affect) ranging from extreme elation, or mania, to severe depression usually accompanied by disturbances in thinking and behavior. Psychotic features (delusions and hallucinations) often occur. Pathogenesis is poorly understood and despite compelling evidence for a substantial genetic contribution to risk (heritability ~80-90%) (2,3), conclusive evidence implicating specific genes or systems has proved elusive (4,5). In part this is likely to be related to the unknown biological validity of current phenotype definitions which are based solely on clinical features and there is increasing evidence of genetic overlaps in susceptibility across the major mood and psychotic disorders (6-9). Varying approaches have been used to investigate whether taking account of occurrence of psychosis may help to reduce heterogeneity in genetic studies of bipolar disorder (eg. 10,11) and to study samples of bipolar disorder and schizophrenia together in the hope of identifying shared susceptibility loci (eg. 12). Molecular genetic approaches can be expected to contribute to an improvement in diagnostic classification through identification of the biological systems that underpin the clinical syndromes (13).

The recently published Wellcome Trust Case Control Consortium (WTCCC) study (14) described a genome-wide association analysis of 1868 bipolar cases and 2938 controls in which the 92nd most strongly associated polymorphism lay within the gamma amino butyric acid A ($GABA_A$) receptor gene, *GABRB1* (Table 1; rs7680321; allelic odds ratio, OR=1.36 (95% confidence intervals, CI 1.16-1.54); $\chi^2 = 16.03$, 1df, $p=6.2\times10^{-5}$). $GABA_A$ receptors transduce the major CNS inhibitory neurotransmitter GABA. This genetic finding is of substantial interest because $GABA_A$ receptors have been hypothesized to be involved in the pathogenesis of mood (15) and psychotic (16) illnesses, have been implicated in anxiety (17) and alcohol disorders (18), and are known to be involved in the actions of several psychoactive agents (19-21). $GABA_A$ receptors are hetero-pentameric chloride channels constructed from various permutations of the products of multiple genes (α1-6; β1-4; γ1-3; δ, ε, θ, π, ρ1-3). It is not completely known which permutations of subunits combine in nature, but native receptors usually contain 2 α, 2 β and one γ subunits, the precise combination being a critical determinant of the physiological and pharmacological properties of the assembled receptor. Most of the genes encoding $GABA_A$ receptors are arranged genomically within clusters (22). For example, the cluster on chromosome 4p12 includes *GABRB1*, *GABRA4*, *GABRA2* and *GABRG1* within a stretch of 1.4 megabases of DNA.

Our aim in the current study was to identify a subset of bipolar cases showing an enriched signal at the index polymorphism, rs7680321, in the expectation that those cases would represent a group with greater biological homogeneity than the BD group as a whole. Under the hypothesis that this group might also exhibit relative aetiological homogeneity at other functionally related loci, we then sought to use this subset of cases to test for independent evidence for association with other polymorphisms in the $GABA_A$ receptor gene family.

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Materials and methods

Our analyses used a subset of the SNPs and cases reported in the bipolar disorder component of the Wellcome Trust Case Control Consortium (WTCCC) genome-wide association study of 7 common familial diseases (14). All individuals were white and resident in the UK.

Bipolar disorder cases

The WTCCC bipolar dataset comprised 1868 bipolar disorder cases who were all over the age of 16 years, living in mainland UK and of European descent. Recruitment was undertaken throughout the UK by teams based in Aberdeen (8% of cases), Birmingham (35% cases), Cardiff (33% cases), London (15% cases) and Newcastle (9% cases). Individuals who had been in contact with mental health services were recruited if they suffered with a major mood disorder in which clinically significant episodes of elevated mood had occurred. This was defined as a lifetime diagnosis of a bipolar mood disorder according to Research Diagnostic Criteria (23) and included: bipolar I disorder (71% cases), schizoaffective disorder bipolar type (15% cases), bipolar II disorder (9% cases) and manic disorder (5% cases). After providing written informed consent, all subjects were interviewed by a trained psychologist or psychiatrist using a semi-structured lifetime diagnostic psychiatric interview (in most cases the Schedules for Clinical Assessment in Neuropsychiatry (24) and available psychiatric medical records were reviewed). Using all available data, best-estimate ratings were made for a set of key phenotypic measures on the basis of the OPCRIT checklist (25) (which covers both psychopathology and course of illness) and lifetime psychiatric diagnoses were assigned according to the Research Diagnostic Criteria (23). Further details of clinical methodology can be found elsewhere (26,27).

The characteristics of the subset of 279 cases meeting Research Diagnostic Criteria for schizoaffective disorder, bipolar type were: 42% male; mean age at interview: 43.3 (SD 12.1) years; all individuals had experienced psychotic symptoms (delusions or hallucinations); mean age at onset of impairment due to mood disorder: 23.2 (SD 7.9) years; lifetime occurrence of rapid cycling (ie. 4 or more episodes of mood disorder within a 12 month period): 10%; lifetime occurrence of a post-natal episode of mania within 6 weeks of parturition (ie. “post-natal” or “puerperal psychosis”): 8%; lifetime occurrence of a definite suicide attempt: 17%.

Controls

There were 2938 controls, who were not screened to exclude presence of psychiatric illness, and came from two sources.

1958 Birth Cohort Controls—1,458 controls came from the 1958 Birth Cohort (also known as the National Child Development Study) which includes all births in England, Wales and Scotland, during one week in 1958. From an original sample of over 17,000 births, survivors were followed up at ages 7, 11, 16, 23, 33 and 42 yr (http://www.cls.ioe.ac.uk/studies.asp?section5000100020003)135. In a biomedical examination at 44-45 years of age (http://www.b58cgene.sgul.ac.uk/followup.php), 9,377 cohort members were visited at home providing 7,692 blood samples with consent for future Epstein–Barr virus (EBV)-transformed cell lines. DNA samples extracted from 1,500 cell lines of self-reported white ethnicity and representative of gender and each geographical region were selected for use as controls. 50% were male.

UK Blood Services Controls—The second set of controls was made up of 1,480 individuals selected from a sample of blood donors recruited as part of the current project.

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WTCCC in collaboration with the UK Blood Services (NHSBT in England, SNBTS in Scotland and WBS in Wales) set up a UK national repository of anonymized samples of DNA and viable mononuclear cells from 3,622 consenting blood donors, age range 18–69 yr (ethical approval 05/Q0106/74). A set of 1,564 samples was selected from the 3622 samples recruited based on sex and geographical region (to reproduce the distribution of the samples of the 1958 Birth Cohort) for use as common controls in the WTCCC study. 48% were male.

Exploratory phase of phenotype optimization

The exploratory phase of the analysis involved identifying a subset of the bipolar cases in which there was enrichment of the association signal at the index SNP, rs783021. The following subsets of bipolar cases were considered: (a) RDC Bipolar I disorder or Manic Disorder (N=1418); (b) RDC Bipolar II disorder (N=171), (c) RDC schizoaffective disorder, bipolar type (N=279); (d) DSMIV (28) Bipolar I disorder (N=1594), (e) DSMIV Bipolar II disorder (N=134), (f) DSMIV schizoaffective disorder, bipolar type (N=98), (g) Age of onset of impairment due to affective illness < 20 years (N=484), (h) lifetime occurrence of rapid cycling of mood episodes (ie. 4 or more episodes per year) (N=231), (i) lifetime occurrence of psychotic symptoms (N=1225), (j) lifetime occurrence of psychotic symptoms in at least 50% of episodes of mood disorder (N=316), (k) lifetime occurrence of predominantly mood-incongruent psychotic features (defined as >19 on the Bipolar Affective Disorder Dimension Scale (BADDS) incongruence scale (29)) (N=496). We identified the subset showing optimal signal enhancement using forward step-wise logistic regression as implemented in SPSS version 12.0.1, as follows. We considered only the BD cases and created a genotype-phenotype dataset representing the individual alleles at rs783021 (ie. the dataset contained two entries for each individual with the first entry being for one of the two alleles at the index SNP and the second entry being for the second allele). The phenotype sub-sets were represented using binary variables (case being member of subset was designated by “1”, otherwise case was assigned “0”). All binary phenotype variables were considered within a forward step-wise logistic regression model to predict the risk allele. Only one subset (RDC SABP) was retained in the logistic model as being a significant predictor of the risk allele. Note that this exploratory phase was undertaken prior to, and was independent of, the subsequent hypothesis testing.

Polymorphisms used in analyses

The Wellcome Trust Case Control Consortium dataset comprised 469,557 single nucleotide polymorphisms (SNPs) distributed across the genome. For the hypothesis testing analyses reported here we selected SNPs for analysis that (a) had excellent genotyping quality, and (b) tagged common genetic variation at or near genes encoding $GABA_A$ receptors.

a) Selection of SNPs with high genotyping quality—For the current analyses we selected only SNPs that had a minor allele frequency of at least 5% in our total sample and met stringent levels of genotyping quality. We used the following quality filter for inclusion of SNPs: (a) Call rate >99.5% in WTCCC BD cases and controls, (b) Hardy-Weinberg p value >0.001 in cases, (c) Hardy-Weinberg p value >0.01 in controls, (d) good clustering of genotypes on visual inspection of cluster plots (as described in WTCCC paper). We have demonstrated that SNPs meeting these criteria showed a very high level of genotype agreement with genotypes scored independently in our laboratory using the Sequenom platform (of over 67,000 genotypes typed for 140 SNPs we found 99.95% agreement; data not shown).

b) Selection of SNPs tagging common variation in region of genes encoding $GABA_A$ receptors—The locations of SNPs and the reference sequence of genes encoding

$GABA_A$ receptors were determined using the Golden Path (NCBI build 35: http://genome.ucsc.edu/). There are 19 known $GABA_A$ receptor genes which are arranged in 8 distinct chromosomal locations, with several of the genes being arranged in clusters. For each of these chromosomal locations we selected for analysis typed SNPs meeting our quality filters that were located within the reference sequence of a $GABA_A$ receptor gene or that lay within 20kb (upstream or downstream) of a $GABA_A$ receptor gene. In order to reduce the redundancy of information we selected a set of SNPs that captured the common genetic variation within our sample. This reduced the number of SNPs examined which has the beneficial effect of reducing multiple testing (of most relevance for the logistic regression analyses). The reduced redundancy is also desirable for set-based analyses (30) (see below). We selected this subset using the Tagger option of HaploView v3.32 (31) with the requirement for variation with minor allele frequency > 5% to be captured at $r^2>0.95$. For one of the genes (*GABRD*) there were no typed SNPs in the dataset. In the reduced set of markers we used in the analysis, there was a total of 223 SNPs distributed across 18 genes as follows: (1) 4p12-13 cluster : *GABRB1* (24 SNPs), *GABRA4* (9 SNPs), *GABRA2* (8 SNPs), *GABRG1* (5 SNPs); 5q31-q25 cluster: *GABRB2* (25 SNPs), *GABRA6* (6 SNPs), *GABRA1* (8 SNPs), *GABRG2* (11 SNPs); 15q11-q13 cluster: *GABRB3* (27 SNPs), *GABRA5* (10 SNPs), *GABRG3* (32 SNPs); Xq28 cluster: *GABRQ* (3 SNPs), *GABRA3* (9 SNPs), *GABRE* (5 SNPs); 6q15 *GABRR1* (12 SNPs), *GABRR2* (9 SNPs); 5q35.1: *GABRP* (10 SNPs); 3q11.2: *GABRR3* (12 SNPs).

Statistical analyses

Testing association at a single SNP—The Armitage trend test was used to assess association at each SNP, by comparing genotype distributions in cases vs. controls using the association model option within the analysis package, PLINK version 0.99 (32). We also calculated allelic odds ratios (ORs) and their 95% confidence intervals (CIs) from the 2×2 contingency tables of allele counts.

Testing for additional evidence of association at genes in the 4p12 cluster whilst allowing for the specific association signal at the index SNP, rs7683021—At the 4p12 cluster, logistic regression was used to confirm the presence of additional independent evidence of association over and above that resulting from association with the index SNP rs7683021. For logistic regression analyses we used the forward stepwise option of SPSS version 12.0.1 and compared logistic models that included only rs7683021 with models that included rs7683021 and an additional SNP at the 4p12 cluster. Correction of the significance of model improvement was made by Bonferroni adjustment (ie. By multiplying the p value by the total number of SNPs, excluding rs7683021, at the gene examined).

Testing for gene-wide significance for SNP association at genes outside the 4p12 cluster—An empirical p value was determined for the best SNP association in each gene, allowing for all SNPs tested, by using the set-based analysis option within PLINK (32) with the set-max option set to 1 and with 100,000 permutations. (We discussed the analyses with Dr Purcell, developer of PLINK, to ensure that the version of PLINK used correctly implemented the set-based analyses). At the 15q cluster, logistic regression was used in a manner consistent with its use for the 4p12 cluster to allow for the effect of the *GABRB3* SNP, rs890319, and test the independent significance of rs17561681 in *GABRA5*.

Testing overall statistical significance of association at $GABA_A$ receptor gene SNPs excluding the haplotype block with index SNP—In order to test the support in our dataset for association over the set of SNPs examined we used set-based analysis (30) as implemented in PLINK (32) using the default options and with 1,000,000 permutations. We omitted from this analysis the 5 SNPs that are within the haplotype block (as determined

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from our dataset using HaploView version 3.32 (31)) that includes the index SNP, rs7683021.

Population attributable fraction—We estimated population attributable fraction, AF, using the formula, AF= f(1-OR)/[1 + (1-OR)] where f is the population frequency of the risk allele and OR is the estimated allelic odds ratio between cases and controls for the risk allele (33). The confidence intervals for AF were estimated assuming that population allele frequency was equal to that in the controls.

Canonical correlation analysis—We used canonical correlation analysis (34) as implemented in SAS 8.02 (PROC CANCOR) to attempt to further refine the genotypephenotype relationship within the RDC SABP sample (N=279). The genetic variables in the analysis were the genotypes at each of the 5 SNPs rs3934674, rs6414684, rs890319, rs17561681, rs854579. The phenotype variables used were binary (present/ not present) measures of the following variables (further details of definitions used for ratings are available on request from the corresponding author): male sex; lifetime occurrence of mood instability; lifetime occurrence of marked mood fluctuation; lifetime occurrence of alcohol abuse; family history of major mood disorder in first or second degree relative; family history of psychotic illness in first or second degree relative; onset of illness <20 years age; onset of illness > 30 years age; presence of psychotic features in at least 50% of mood episodes; definite lifetime mood incongruence of psychotic features; definite incapacitating manic episode; definite major depressive episode; definite incapacitating major depressive episode; lifetime occurrence of postnatal mania; lifetime occurrence of rapid cycling; lifetime occurrence of suicidal ideation; lifetime occurrence of suicide attempt; objective good response to lithium; episodic course of illness; chronic course of illness; lifetime occurrence of features of disorganization; lifetime occurrence of persecutory delusions; sudden onset of first episode of illness; lifetime occurrence of auditory hallucinations; lifetime occurrence of panic episodes.

Results

In our initial exploratory analysis using logistic regression we found that, of 11 clinical phenotypic sub-sets considered, the subset of 279 bipolar cases that met Research Diagnostic Criteria (23) for schizoaffective disorder, bipolar type (SABP) showed the strongest evidence for association with the risk allele at the index SNP, rs7680321 (Table 1; OR=1.80 (1.37-2.37); χ^2 = 21.34, 1 df, p=3.8×10^{-6}). As can be seen in table 1b, the association signal is substantially less strong in the subset of cases meeting criteria for DSMIV SABP or the larger subset of cases with predominantly mood incongruent psychotic features. The RDC SABP subset differed significantly from the remaining 1589 BD cases (χ^2 = 7.38, 1df, p=0.0066) in which the association signal at this polymorphism was attenuated (OR=1.26 (CI 1.08-1.47); χ^2 = 9.06 1 df, p=2.6 ×10^{-3}). It is important to stress that this exploratory phase of phenotype refinement was undertaken prior to, and independently of, the subsequent hypothesis testing.

We next sought additional evidence for association at $GABA_A$ receptor genes in these 279 SABP cases. In order to make this test independent of the index SNP (and therefore independent of the associated prior multiple testing), we excluded from our analysis all SNPs within the *GABRB1* haplotype block containing rs7680321 because they are highly correlated with the index SNP.

Five of the 18 genes tested showed evidence independent of the index signal for association at gene-wide levels of statistical significance: *GABRB1* (p=0.0039), *GABRA5* (p=0.0024), *GABRB3* (p=0.0107), *GABRA4* (p=0.013), *GABRR3* (p=0.0439) (table 2). For each gene the

significance level has been corrected for all SNPs examined within that gene. We also obtained an experiment-wide empirical significance for association across the total set of 220 SNPs (excluding the index block) across the $GABA_A$ receptor genes using a permutation based analysis (empirical $p=6.6\times10^{-5}$, with 1,000,000 permutations). This significance level takes account of the multiple SNPs tested across all genes. We observed no evidence for statistical interaction between the risk alleles although it is important to recognize that our sample is not well powered to detect interactions.

We failed to find association at $GABA_A$ receptor genes when the control sample was compared to the 1589 bipolar cases not meeting criteria for RDC SABP (table 3). Furthermore, we found no evidence of association when, using the same methodology and genotyping platform, we examined the set of SNPs at these genes within our sample of 476 white UK cases meeting DSMIV (28) criteria for schizophrenia (table 3).

Canonical correlation analysis did not reveal any significant relationship between genotype and phenotype variables or subgroups of these variables (ie. first canonical correlation coefficient not significant).

Discussion

Our data provide strong statistical support for the involvement of $GABA_A$ receptor genes in susceptibility to a component of the bipolar mood phenotype and point to a relatively specific effect on a form of bipolar spectrum illness meeting RDC criteria for schizoaffective disorder, bipolar type. Such cases, in addition to clear-cut episodes of mania, display psychotic symptoms (delusions and/or hallucinations) that are not easily understood as being the result of extreme mood change and that are often seen also in individuals diagnosed with schizophrenia. We note that the category of RDC schizoaffective disorder, bipolar type, is itself a clinically heterogeneous category (albeit substantially less heterogeneous than the BD sample as a whole). However, we did not find any further clinical subdivision of this category that usefully refined the genetic signal in our dataset.

The exploratory, phenotype refinement phase of our analysis identified the RDC SABP diagnostic subset of the BD cases as being of particular interest in the context of association at the index polymorphism in *GABRB1*. It is important to recognize that this phase of analysis was data-driven and considered a range of phenotypic subsets. It was not based upon a specific prior hypothesis about the genetic relationship between mood and psychotic disorders. This contrasts with the hypothesis-based approaches of researchers who have used occurrence of psychosis in an attempt to reduce heterogeneity in bipolar disorder (eg. 10,11) and to seek loci that overlap between bipolar disorder and schizophrenia (eg. 12).

An important strength of our study is that, because our hypothesis testing was independent of the initial exploratory procedure, the results we have presented do not require correction for examining either multiple phenotypes or multiple biological systems. Neither do our hypothesis-driven analyses require the extremely stringent levels of statistical significance needed to assess the discovery-oriented findings from a genome-wide association study (4,35). Moreover, the significance level should be interpreted against the background knowledge of several lines of evidence implicating $GABA_A$ receptors in psychiatric illness. Thus, the strong statistical support within the context of the substantial prior probability helps to provide confidence in the validity of our findings.

A measure of the population level importance of a risk factor is provided by the attributable fraction (33) which can be interpreted as the proportion of cases in the population that could, in principle, be avoided if the risk of illness for those with risk alleles could be reduced to

that of those without risk alleles. In our sample, estimates of population attributable fraction were in the range 10-20% for several of these risk alleles (table 2). This suggests that variation at $GABA_A$ receptor genes make an important contribution to the burden of this disease phenotype in the population.

None of the associated polymorphisms is predicted to cause a change in the amino acid sequence of an encoded receptor protein, and there are no known coding variants that are sufficiently common to explain the observed association (by linkage disequilibrium). Thus, it is likely that the associations reflect pathologically relevant variants that modify gene expression and, hence, the subunit composition, and thereby the physiological and pharmacological properties, of the $GABA_A$ receptors. It will require other research approaches to confirm which subunit(s) are most relevant to bipolar illness and to identify the mechanisms involved.

Our findings have several implications. First, within our sample and at least with respect to $GABA_A$ receptor (dys)function, the cases meeting criteria for RDC SABP are more biologically homogeneous than our total sample of bipolar cases. This is consistent with emerging molecular genetic evidence for the existence of relatively specific genetic susceptibility for a form of major psychiatric illness that has features of both bipolar disorder and schizophrenia (27,36,37). The RDC and other modern diagnostic criteria in psychiatry were developed on largely descriptive grounds and we consider it most unlikely that the SABP category will map directly onto the underlying biology. We do not believe that “schizoaffective disorder” in general, or RDC schizoaffective disorder in particular, is a neatly defined, discrete, biological diagnostic entity. Our findings do, however, show that it can be useful for the purposes of research (and probably also clinical practice) to identify and classify together sets of cases with such clinical features. Whether, in the long run, this is best achieved by using categories, dimensions or some mixture of the two will require future study. Such further work aimed at refining the relationship between clinical phenotype and genetic risk factors has the potential to help psychiatry move towards a system of classification that relates more closely to underlying pathogenesis.

Second, our findings may help to explain some of the common and clinically important co-morbidity between bipolar disorder and alcohol abuse, anxiety states, and panic episodes (38) as these are disorders in which GABAergic transmission has been robustly implicated (17,18). Indeed, it may soon be possible to start developing diagnostic classifications that group disorders together according to underlying pathogenesis (13). Such a move is likely to be beneficial for aetiological research as well as clinical management and would signal a shift from the current situation of a purely descriptive approach.

Finally we note that our findings demonstrate the utility of a data-driven, iterative approach (39) in biological studies of psychiatric, and other complex, disorders in which exploratory phenotype refinement is used to optimize an initial biological signal for further independent testing of specific hypotheses about the involvement of genes, proteins or systems. Here we have described use of this approach to perform independent tests within the same large dataset. Provided close attention is paid to the comparability of phenotypic measures, the approach can also be used across independent datasets.

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Appendix

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Table 1a

Refinement of index signal: rs7680321

Phenotype	Frequency of risk allele	OR (95% CI)	Cases v. controls p value (Armitage trend test)
Controls (N=2938)	0.0793	N/A	N/A
Total BD (N=1868)	0.1032	1.36 (1.16-1.54)	6.2×10^{-5}
RDC SABP (N=279)	0.1362	1.80 (1.37-2.37)	3.8×10^{-6}
Non-SABP BD (N=1589)	0.0974	1.26 (1.08-1.47)	2.6×10^{-3} †

N/A: not applicable; SABP: RDC schizoaffective bipolar type; BD: bipolar disorder; Non-SABP BD: All bipolar cases excluding SABP set.

† Comparison of SABP v. Non-SABP BD cases: p=0.0066.

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Table 1b

Refinement of index signal rs7680321: Frequency of risk allele in each of the 11 phenotype subsets of BD sample that were examined

Phenotype set in BD sample	Frequency of risk allele
BD rapid cycling (N=231)	0.0693
RDC BPII (N=171)	0.0763
DSMIV BPII (N=134)	0.0854
DSMIV SABP (N=98)	0.0903
Psychosis with predominant mood incongruence (N=496)	0.0955
RDC BPI or Manic Disorder (N=1418)	0.0984
DSMIV BPI (N=1594)	0.1045
Lifetime psychosis (n=1225)	0.1062
Psychosis in ≥50% of episodes (N=316)	0.1123
Age onset <20 years (N=484)	0.1149
RDC SABP (N=279)	0.1362

RDC : Research Diagnostic Criteria; BPI: Bipolar I Disorder; BPII: Bipolar II Disorder; SABP: schizoaffective disorder, bipolar type. See text for description of the phenotype subsets. Number of samples in each subset is shown in parentheses.

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Table 2

Genes showing gene-wide significance

Gene (cluster)	Best SNP	Controls frequency of risk allele	SABP frequency of risk allele	BD-nonSABP frequency of risk allele	SABP OR (95% CI)	Nominal p value	Gene-wide p value	Population attributable fraction (95% CI)
GABRA4 (4p12)	rs3934674	0.2722	0.3620	0.2888	1.52 (1.23-1.87)	6.87×10^{-6}	0.013[a]	0.124 (0.060-0.191)
GABRB1 (4p12)	rs6414684	0.4910	0.5989	0.5051	1.56 (1.27-1.91)	1.23×10^{-6}	0.0039[a]	0.176 (0.104-0.234)
GABRB3 (15q11-q13)	rs890319	0.3231	0.3961	0.3382	1.38 (1.11-1.71)	0.00046	0.0107[b,c]	0.089 (0.032-0.134)
GABRA5 (15q11-q13)	rs17561681	0.0471	0.0830	0.0612	1.87 (1.24-2.81)	0.00016	0.0024[b,c]	0.022 (0.009-0.030)
GABRR3 (3q11.2)	rs854579	0.6647	0.7240	0.6579	1.32 (1.07-1.64)	0.0044	0.044[b]	0.161 (0.043-0.259)

$GABA_A$ receptor genes in which the association signal for the comparison of SABP against controls has gene-wide statistical significance at $p<0.05$. The genomic cluster in which the gene is located is given in parentheses after the gene name. Note that the significance levels are independent of the association at the index SNP, rs7680321, and thus, provide additional association support for these genes over and above the strong association signal ($p=3.8\times10^{-6}$) at the index SNP.

[a] Gene-wide significance calculated using logistic regression to allow for effect of index SNP with Bonferroni adjustment for the number of SNPs within the gene; logistic regression showed the signals at rs3934674 and rs6414684 to be independently significant (significance of model improvement when adding rs3934674 to a model that already includes rs6414684: p=0.019).

[b] Empirical gene-wide significance determined using set-based analysis implemented in PLINK with 100,000 simulations.

[c] The presence of independent gene-wide significant association signals at the *GABRB3* and *GABRA5* genes in the 15q cluster was confirmed by logistic regression (allowing for the association signal at rs890319 in *GABRB3*, the association at rs17561681 in *GABRA5* is significant at p=0.0068 after correcting for the 10 SNPs tested in *GABRA5*).

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Table 3

Global test of significance for association across all GABR SNPs outside the index haplotype block for comparisons between different phenotypic sample sets

Sample sets compared	Empirical p value for all GABR SNPs
SABP (N=279) v. controls (N=2938)	6.6×10^{-5}
BD – non SABP (N=1589) v. controls (N=2938)	0.46
Schizophrenia (N=476)v. controls (N=2938)	0.22
SABP (N=279) v. BD-non SABP (N=1589)	0.02

SABP: RDC schizoaffective bipolar type; BD: bipolar disorder; Non-SABP BD: All bipolar cases excluding SABP set.

For these analyses all SNPs across the $GABA_A$ receptor genes were used except the 5 SNPs within the haplotype block within *GABRB1* containing the index SNP, rs7680321. Empirical p values were determined using set-based analysis implemented in PLINK using default options with 1,000,000 permutations for SABP v. Controls and 10,000 for the other comparisons.