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Genome-wide association analysis of age at onset and psychotic symptoms in bipolar disorder

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Electronic Resources

HapMap (http://hapmap.ncbi.nlm.nih.gov/)
Heinz Nixdorf Recall Study(http://www.recall-studie.uni-essen.de)
KORA(http://www.gsf.de/KORA)
MACH(http://www.sph.umich.edu/csg/abecasis/MACH/)
PLINK(http://pngu.mgh.harvard.edu/purcell/plink/)
PopGen (http://www.popgen.de)

Abstract

Genome-wide association studies (GWAS) have identified several susceptibility loci for bipolar disorder (BP), most notably *ANK3*. However, most of the inherited risk for BP remains unexplained. One reason for the limited success may be the genetic heterogeneity of BP. Clinical sub-phenotypes of BP may identify more etiologically homogeneous subsets of patients, which can be studied with increased power to detect genetic variation. Here, we report on a mega-analysis of two widely studied sub-phenotypes of BP, age at onset and psychotic symptoms, which are familial and clinically significant. We combined data from three GWAS: NIMH Bipolar Disorder Genetic Association Information Network (GAIN-BP), NIMH Bipolar Disorder Genome Study(BiGS), and a German sample. The combined sample consisted of 2836 BP cases with information on sub-phenotypes and 2744 controls. Imputation was performed, resulting in 2.3 million SNPs available for analysis. No SNP reached genome-wide significance for either sub-phenotype. In addition, no SNP reached genome-wide significance in a meta-analysis with an independent replication sample. We had 80% power to detect associations with a common SNP at an OR of 1.6 for psychotic symptoms and a mean difference of 1.8 years in age at onset. Age at onset and psychotic symptoms in BP may be influenced by many genes of smaller effect sizes or other variants not measured well by SNP arrays, such as rare alleles.

Introduction

Family, twin and adoption studies provide convincing evidence that bipolar disorder (BP) has a strong genetic component. Recently, several genome-wide association studies (GWAS) have been conducted to identify genes involved in BP susceptibility. Unfortunately, there has been little consistency in the top results reported by individual studies. The Wellcome Trust Case Control Consortium (WTCCC) (2007)reported an association on chromosome 16p12 ($p=6.3\times10^{-8}$). Baum et al (2008) found evidence that *DGKH* was associated with BP ($p=1.5\times10^{-8}$). Sklar et al (2008) identified *MYO5B* as being associated with BP. Smith et al (2009) found that the strongest BP signal was located in the region Xq27.1 in a European American sample ($p=1.6\times10^{-6}$) and in *DPY19L3* in an African American sample ($p=1.5\times10^{-6}$).

The lack in consistency of top results from different studies has been a general property of GWAS, including for non-psychiatric disorders, and may be due to the modest power of individual studies. Consequently, several meta-and mega-analyses have been performed in the hopes that increasing the sample size will lead to stronger results. Scott et al (2009) reported a signal in *ITIH1* in a meta-analysis of three samples from the NIMH/Pritzker, GlaxoSmithKline Research & Development, and WTCCC ($p=1.8\times10^{-7}$). A mega-analysis of the GWAS from the WTCCC, STEP-UCL and ED-DUB-STEP2 studies identified *ANK3* and *CACNA1C* as genes reaching genome-wide statistical significance (Ferreira et al., 2008). Finally, in a far-reaching collaborative effort, the Psychiatric GWAS Consortium(PGC)has now performed a mega-analysis of 11 individual BP GWAS and found compelling evidence that *ANK3* and *SYNE1* are involved in BP (Kelsoe, 2009).

Despite these efforts, the majority of the heritability of BP remains unexplained. One reason for the lack of greater success in elucidating the inherited risk may be due to genetic heterogeneity of the disorder. We have hypothesized that clinical sub-phenotypes of BP identify more homogeneous subsets of patients that can be studied with increased power to detect genetic variation(Potash et al., 2007). This strategy was helpful with Alzheimer's disease (Scott et al., 2000)and breast cancer (King, 1991), where age at onset was used to identify genes contributing to etiology.

Age at onset and psychotic symptoms are clinical features of BP that are heritable and thus may be especially useful markers for identifying BP related genes. Age at onset has been shown to aggregate in BP families (Lin et al., 2006)and has been incorporated into linkage analyses to identify regions which may contain genes that increase susceptibility for early or late-onset BP (Lin et al., 2005;Zandi et al., 2007). In addition, several candidate gene association studies have suggested that genes such as *BDNF* (Tang et al., 2008)and serotonergic genes including *HTR2C* (Massat et al., 2007), HTR2A (Manchia et al., 2010), and SLC6A4 (Manchia et al., 2010)might be associated with early onset BP. Psychotic symptoms have also been shown to aggregate in BP families (Potash et al., 2001). Linkage studies have identified several regions that may harbor susceptibility genes specific to psychotic BP(Kerner et al., 2007;Potash et al., 2003), and candidate gene association studies have implicated genes such as *NRG1* (Green et al., 2005;Goes et al., 2009) and *DAOA* (Schulze et al., 2005). However, these results are inconclusive as replication is generally lacking.

Here, we report the results of a mega-analysis of combined data from three GWAS in which we incorporate information on age at onset and psychotic symptoms. The three datasets include: NIMH Bipolar Disorder Genetic Association Information Network (GAIN-BP), NIMH Bipolar Disorder Genome Study(BiGS), and a German sample.

Materials and Methods

Subjects

NIMH-BP Samples—The ascertainment and assessment procedures for the NIMH-BP sample are described elsewhere (Dick et al., 2003;Kassem et al., 2006). Briefly, the sample was collected in 5 waves. In waves 1–4 families who had a proband with bipolar I disorder and at least one other sibling with bipolar I or schizoaffective disorder, bipolar type were sought. In wave 5 unrelated bipolar I cases were recruited. All subjects were assessed with the Diagnostic Interview for Genetic Studies (DIGS) and this was combined with family informant data and medical records to assign diagnoses based on DSM-III-R or DSM-IV criteria (Nurnberger, Jr. et al., 1994). Unrelated cases were genotyped in two separate efforts described in detail elsewhere, the Genetic Association Information Network Bipolar Sample (GAIN-BP)(Smith et al., 2009)and the Bipolar Genome Study(BiGS)(manuscript in progress). Genotyping in both efforts was performed using the Affymetrix 6.0 array.

Controls for both the GAIN-BP and BiGS samples were ascertained throughout the United States and genotyped on the Affymetrix 6.0 array as part of the Molecular Genetics of Schizophrenia II (MGS2) Collaboration (Sanders et al., 2008). All control subjects completed a psychiatric questionnaire and those endorsing a history of BP, psychosis or major depression were excluded. Controls were matched by ethnicity, age and sex to the BP cases.

We included the final cleaned dataset from the primary case-control analysis of each sample. The GAIN-BP sample consisted of 1001 BP cases and 1033 controls with genotype data on 724067 SNPs. The BiGS dataset included 1190 BP cases and 401 controls with genotype data on 728187 SNPs.

German Sample—The collection and genotyping the German sample have been described elsewhere (Fangerau et al., 2004;McMahon et al., 2009). In brief, probands with bipolar I disorder were recruited through consecutive hospital admissions and assessed using a structured interview. Best estimate diagnoses were assigned according to DSM-IV criteria. A population-based control sample screened for psychiatric disorders was assembled from the PopGen (www.popgen.de), KORA (www.gsf.de/KORA) and Heinz Nixdorf Recall

Study (www.recall-studie.uni-essen.de). Unrelated cases with a best-estimate diagnosis of bipolar I disorder were genotyped on the Illumina HumanHap550 array. We utilized the final cleaned dataset, which included 645 cases and 1310 controls with genotype data on 516024 SNPs.

Written informed consent was obtained from all subjects and the work described here was approved by the institutional review boards of each collection site.

Phenotype

Age at onset was defined as the year of age at which the earliest onset of an episode of depression or mania occurred. Psychotic symptoms were defined as a lifetime history of delusions and/or hallucinations with duration of at least 1 day. Where insufficient information was available to determine lifetime presence of psychotic symptoms, or where the symptom duration was judged to have lasted less than one day, cases were coded as unknown. Similarly, in cases where the interview indicated that psychotic symptoms occurred solely in the presence of drug use, psychotic status was considered unknown.

Population Stratification

We used principal components analysis to correct for population stratification in the combined sample. There were 137892 SNPs common to all three datasets (GAIN-BP, BiGS and German) that were used in this analysis. The EIGENSTRAT method in the program EIGENSOFT 3.0 was used to calculate principal components (Price et al., 2006). Based on the scree plot, we selected the top five principal components to include as covariates in our association analysis

Imputation

Imputation was performed to facilitate the mega-analysis of the three datasets, which were genotyped on two different platforms. Each dataset was imputed separately, using phased haplotype data from HapMap I & II release 24 (http://hapmap.ncbi.nlm.nih.gov/) as the reference panel. We used the program BEAGLE to ensure the orientation of our data was in the positive strand and impute allelic dosages for autosomal SNPs in the cases and controls (Browning and Yu, 2009). We applied a common quality control filter across all samples reflecting the most exclusive criteria from the individual studies. This involved excluding any SNP that had a missing data rate >5% in any of the original individual datasets, a minor allele frequency <2%, a HWE p-value $<1\times10^{-4}$, and an imputation $R^2 < 0.3$.

Statistical Analysis

We combined the imputed data from all three datasets for a mega-analysis. The final combined dataset contained 2836 cases and 2744 controls with genotype data on 2373895 SNPs. Tests of association for the continuous sub-phenotype of age at onset were performed among BP cases only using the program mach2qtl (Li et al., 2009), with allelic dosages in a linear regression model. We performed tests of association for psychotic symptoms in a case-only analysis, comparing BP cases with psychotic symptoms to BP cases without psychotic symptoms. Tests of association were performed using the program mach2dat (Li et al., 2009), with allelic dosages in a logistic regression model. We included terms in each model to adjust for the top 5 principal components and a dummy-coded variable indexing the three datasets. Following convention, we considered SNPs with p-values $< 5\times10^{-8}$ as genome-wide significant(Hoggart et al., 2008).

In addition to the primary analysis outlined above, we performed several exploratory analyses. First, we expanded on our case-only comparison in the primary analysis, and performed a comparison of BP cases with psychotic symptoms versus unaffected controls.

This analysis was performed using mach2dat with logistic regression as described above. Second, we investigated the involvement of biological pathways using the method implemented in the program Aligator (Holmans et al., 2009). This method tests for overrepresentation of pathways using the results from GWAS and corrects for linkage disequilibrium between SNPs, gene size and multiple testing. We used a liberal cutoff p-value < 0.01 in these analyses.

Replication

To validate our top findings from the GWAS of age at onset and psychotic symptoms in BP, we attempted to replicate our findings in an independent BP sample(Ferreira et al., 2008). Briefly, this replication sample consisted of 3916 BP cases with information about sub-phenotypes and 5112 controls in a combined sample from the WTCCC, STEP-BD and University College London. Age at onset and psychotic symptoms were defined as described by Ferreira et al (2008). Imputation to HapMap I & II was performed using MACH (Li and Abecasis, 2006). We prioritized results with p<0.001 in our original sample for replication. Association analysis in the replication sample was carried out under an allelic model in PLINK(Purcell et al., 2007). We also meta-analyzed the results from our sample and the replication sample using the program METAL (Willer et al., 2010). While there was some overlap in the controls included in the GAIN-BP and STEP-BD samples for the comparison of BP with psychotic symptoms versus controls, we did not account for the potential over-estimation of significance in the meta-analysis as the results were essentially null.

Results

Demographic and clinical characteristics of the three samples (GAIN-BP, BiGS, and German) are presented in Table 1. In all three studies, participants were of European Caucasian ancestry. While the GAIN-BP and German samples had similar proportions of males, the BiGS sample was predominantly female (χ^2=65.6, d.f.=2, p<0.001).

Age at Onset

The mean age of onset differed among the three samples, with the German sample having an older age at onset than the other two (see Table 1; F=124.26, d.f.=2, p<0.001). Supplementary Figure 1 shows the Q-Q plot from the results of the primary analysis with age at onset. The corresponding inflation factor for this analysis was λ=1.04. Figure 1 shows the Manhattan plot of these results. None of the SNPs tested reached genome-wide significance. The most significant result was for the intronic SNP rs455219 in the gene *FAT1* on chromosome 4 (β=0.70, p=3.04×10^{-7}). We carried forward for replication 3464 SNPs with p<0.001, of which the direction of effect was consistent for 1599. The top ten results in our sample, the corresponding replication results and the combined meta-analytic results are shown in Table 2. Interestingly, the SNP rs2623968 in *SYNE1* was among the top ten findings in this analysis (β=0.52, p=3.77×10^{-6}). *SYNE1* is one of two genes that was highlighted in the analysis by the PGC(Kelsoe, 2009)and is located in the suggestive 6q25 linkage region for early onset of mania identified by Faraone et al (2006). However, this finding did not replicate (β=0.08, p=0.7571). None of the SNPs tested in the replication sample reached genome-wide significance. In addition, none of the SNPs reached genome-wide significance in the meta-analysis of the GAIN-BP+BiGS+German and replication samples. The best meta-analytic p-value was for the SNP rs2934442 on chromosome 15(meta-analytic p=1.16×10^{-6}; GAIN-BiGS-German: β=−0.561, p=5.18×10^{-6}; Replication sample: β=−1.022, p=3.62×10^{-3}). This SNP is an intronic SNP in the gene *CGNL1*, which codes for a cingulin-like protein that may be involved in anchoring the apical junctional complex to actin-based cytoskeletons.

We also tested whether any biological pathways were overrepresented among our top results in the analysis of age at onset. None of the pathways tested reached study-wide significance (p>0.508; see Supplementary Table 1). The top nominally overrepresented GO categories largely implicate oxygen binding and catabolic processes.

Psychotic Symptoms

In the combined sample, 1775 BP cases met criteria for psychotic symptoms and 717 were classified as not having psychotic symptoms. The German sample had a slightly lower rate of psychotic symptoms than the other two samples (see Table 1; χ^2=12.19, d.f.=2, p=0.002). Supplementary Figure 2 shows the Q-Q plot from the results of the primary analysis with psychotic symptoms. The corresponding inflation factor for this analysis was λ=1.05. Figure 2 shows the Manhattan plot of these results. None of the SNPs tested in a case-only analysis of psychotic symptoms reached genome-wide significance. The best result was for the intergenic SNP rs1010376 on chromosome 5 (see Table 3; OR=0.73, p=3.75×10^{-6}). We carried forward for replication 2306 SNPs with p<0.001, of which 1058 showed a consistent direction of effect in the replication sample. However, none of the SNPs tested reached genome-wide significance in the replication sample or in the meta-analysis. The SNP rs7795096 on chromosome 7 had the best meta-analytic p-value at 1.94×10^{-6} (GAIN-BiGS-German: OR=0.79, p=2.41×10^{-4}; Replication: OR=0.83, p=1.55×10^{-3}). This SNP is an intronic SNP in the gene *PRKAG2*, a member of the AMPK gamma subunit family that modulates cellular functions by turning off enzymes involved in regulating the biosynthesis of cholesterol and fatty acid.

In an exploratory analysis, we tested for associations in BP cases with psychotic symptoms versus unaffected controls. None of the SNPs reached genome-wide significance in our sample, the replication sample, or in the meta-analysis of the two samples(See Supplementary Figure 1 and Supplementary Table 2). The best result in our sample was for the intergenic SNP rs6578831 on chromosome 11, with p=7.69×10^{-8} (OR=1.33). However, this SNP showed no association in the replication sample (OR=0.99, p=0.8245).

In our examination of biological pathways, none were significantly overrepresented among our top results at a study-wide level (study-wide p>0.362; see Supplementary Table 1). Among the top ten nominally overrepresented pathways, myeloid cell differentiation and acetylcholine processes were predominant. Acetylcholine is a neurotransmitter that is involved in aspects of synaptic plasticity, arousal and reward.

Discussion

Here we present the results from a GWAS of two clinically relevant sub-phenotypes of BP, age at onset and psychotic symptoms. No SNP reached genome-wide significance for either sub-phenotype. In addition, no SNP reached genome-wide significance in a meta-analysis with a replication sample. The full results of the primary analysis with age at onset and psychotic symptoms are available upon request.

We hypothesized a priori that the use of sub-phenotypes would facilitate the identification of loci relevant to bipolar disorder and its clinical manifestation. Although we did not identify any genome-wide significant findings, the study had several limitations that should be considered. One limitation is the loss of power inherent in the analysis of sub-phenotypes. By examining sub-phenotypes of BP, we focused on a portion of our total sample in each analysis, decreasing the power to detect associations. We hoped to overcome this limitation by combining the data from three large GWAS. As a result, our analysis of sub-phenotypes in BP is the largest to date. However, the power of the combined sample may still not have been sufficient. We estimated that the combined sample had 80% power to detect loci of

genome-wide significance with a mean difference of 1.8 years for age at onset and an odds ratio of 1.6 for psychotic symptoms. By focusing on certain sub-phenotypes we sought to identify more homogeneous sub-groups of patients in which loci of such effect sizes might operate. However, our results do not support this hypothesis in relation to age at onset or psychotic symptoms in BP. It may be these sub-phenotypes are no less genetically complex than BP and that larger sample sizes of the sort being assembled by the PGC are needed to identify relevant loci. For example, we estimated that a sample of 15,775 BP cases with psychosis and 6,752 without psychosis would be needed to detect a genome-wide significant association with 80% power at an odds ratio of 1.15. It is also possible there are loci of larger effect sizes influencing these sub-phenotypes, but they are of low frequency and as a result are not adequately captured by the genotyping panels currently used in GWAS. We imputed to the HapMap phase I & II panel, which is estimated to cover 94% of the genome (2005). Thus, there could also be common loci influencing the sub-phenotypes that were not captured in our study.

Another limitation of this study is the possibility of measurement error in the sub-phenotypes. Previous studies have suggested that age at onset and psychotic symptoms can be measured reliably. One study showed that the intraclass correlation varied between 0.68 and 0.97 for different measures of age at onset(Egeland et al., 1987), and another study showed that the rater agreement for diagnosing hallucinations and delusions yielded a coefficient of reliability of 0.91(Endicott and Spitzer, 1978). However, to the extent that these sub-phenotypes are measured with error, results will likely be biased towards the null. Related to this is the fact that the samples used different diagnostic instruments to measure these sub-phenotypes. While the GAIN-BP and BiGS studies used the DIGS, the German sample used the Structured Clinical Interview for DSM-IV (SCID). Such differences may introduce error into the characterization of the sub-phenotypes across the samples, despite our efforts to harmonize their definitions. Indeed, we observed significant differences in the ages at onset and rates of psychotic symptoms in the German sample compared to GAIN-BP and BiGS. The observed differences may reflect sampling variation, differences in the measurement of the sub-phenotypes, or true differences in the patient populations across the samples. Future work aimed at matching the definition and clinical assessment of sub-phenotypes across studies may facilitate efforts to examine genotype-phenotype correlations(Potash et al., 2007). In addition, alternative approaches to defining the clinical sub-phenotypes, such as using admixture analysis to define the boundaries of age at onset classes(Bellivier et al., 2001;Bellivier et al., 2003;Lin et al., 2006), may provide further insights. Finally, we only assessed two sub-phenotypes in this study. It is possible that other clinical variables, such as response to treatment with lithium, or clusters of variables, may more robustly correlate with common genetic variation than do age at onset or psychotic symptoms. Sample sizes comparable to ours may be sufficient to detect loci relevant to these other sub-phenotypes.

Using sub-phenotypes to help resolve the complex genetic architecture of common psychiatric disorders is attractive. We sought to use this strategy to identify susceptibility genes related to age at onset and psychotic symptoms in BP, features with strong evidence of familiality and thus high likelihood of being genetically relevant. Although we were not able to definitively implicate genes in this analysis, the strategy may still have merit. It is possible that age at onset and psychotic symptoms in BP may reflect the action of genes of smaller effect sizes only detectable with larger samples. Alternatively, uncommon variants not measured well by current SNP arrays may be relevant for these sub-phenotypes and strategies such as deep sequencing may be necessary to identify them. Finally, other sub-phenotypes may be better markers of the underlying genetic heterogeneity of BP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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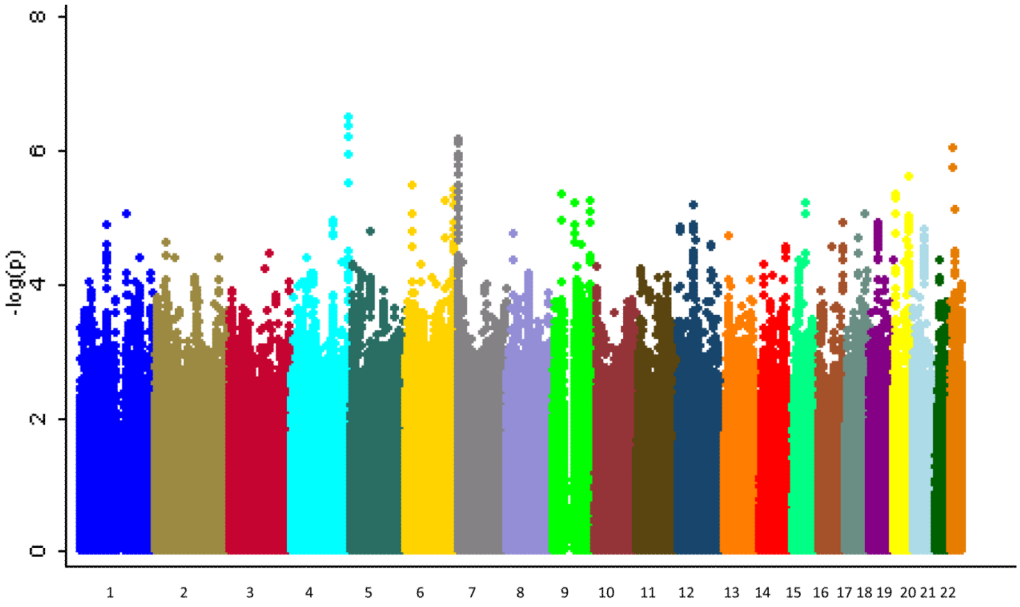

Figure 1.
Manhattan plot for GWA of age at onset of BP. Genome-wide association results for observed and imputed allelic dosages using linear regression.

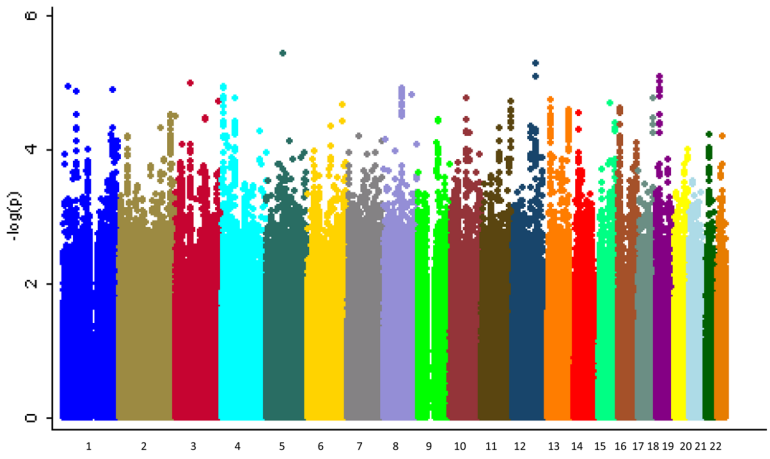

Figure 2.

Manhattan plot for GWA of psychotic symptoms in BP. Genome-wide association results for observed and imputed allelic dosages using logistic regression in a case-only analysis. Results are for the analysis comparing BP cases with psychotic symptoms to BP cases without psychotic symptoms.

Table 1

Demographic and Clinical Characteristics of BP Cases in Three Samples

	GAIN-BP	BiGS	German	Combined GAIN-BP+BiGS+German
BP Cases [N]	1001	1190	645	2836
Sex [% Male][1]	50.0	34.1	48.4	43.0
Age at Onset [mean (s.d.)][2]	19.3 (9.3)	18.5 (9.4)	28.6 (11.2)	21.0 (10.6)
Psychotic Symptoms [% positive][3]	71.3	74.2	66.1	71.2

[1] χ^2=65.6, d.f.=2, p<0.001

[2] F=124.26, d.f.=2, p<0.001

[3] χ^2=12.19, d.f.=2, p=0.002

Table 2

Top regions in the analysis of age at onset in the GAIN-BP+BiGS+German sample[1]

				GAIN-BP+BiGS+German		Replication Sample		Meta-Analysis
SNP	**Chr**	**Gene**	**BP Location**	**β[2]**	**p-value**	**β[2]**	**p-value**	**p-value[3]**
rs455219	4	FAT1	187859058	0.70	3.04×10^{-7}	0.16	0.5940	1.47×10^{-4}
rs10267593	7	MAD1L1	1903787	−0.70	6.66×10^{-7}	−0.16	0.6080	2.32×10^{-4}
rs4820556	22	Intergenic	22202936	−0.69	8.94×10^{-7}	−0.62	0.8194	6.02×10^{-4}
rs393760	19	Intergenic	51627052	−0.62	2.51×10^{-6}	0.18	0.5327	N/A
rs16883399	6	MBOAT1	20239014	−0.62	3.30×10^{-6}	−0.34	0.2294	6.75×10^{-5}
rs2623968	6	SYNE1	152879005	0.52	3.77×10^{-6}	0.08	0.7571	9.71×10^{-4}
rs1678869	19	FUT5	5837528	0.76	4.33×10^{-6}	−0.26	0.4704	N/A
rs2060409	9	Intergenic	26704614	−0.55	4.34×10^{-6}	0.20	0.4258	N/A
rs17054536	6	ECHDC1	127692059	1.21	5.62×10^{-6}	0.53	0.3354	1.89×10^{-4}
rs11142517	9	TRPM3	72434000	0.12	5.94×10^{-6}	−0.06	0.8188	N/A

[1] The most significant SNP per region is listed as a representative SNP

[2] β is calculated for the rare allele.

[3] N/A = not applicable when the direction of effect is opposite in the two samples

Table 3

Top regions from the analysis of psychotic symptoms in the GAIN-BP+BiGS+German sample[1]

				GAIN-BP+BiGS+German		Replication Sample		Meta-Analysis
SNP	**Chr**	**Gene**	**BP Location**	**OR[2]**	**p-value**	**OR[2]**	**p-value**	**p-value[3]**
rs1010376	5	Intergenic	71339064	0.727	3.75×10^{-6}	0.96	0.4650	5.87×10^{-4}
rs7132927	12	ANKS1B	98821773	0.745	4.88×10^{-6}	1.06	0.2213	N/A
rs11080384	18	Intergenic	10078962	1.378	5.80×10^{-6}	0.99	0.8066	N/A
rs6446101	3	FHIT	59932023	1.323	8.81×10^{-6}	1.02	0.7630	2.80×10^{-3}
rs607777	1	Intergenic	61171347	1.816	1.14×10^{-5}	1.12	0.2676	4.18×10^{-4}
rs10903047	1	IL28RA	24381661	0.747	1.15×10^{-5}	1.02	0.7900	N/A
rs871938	4	SORCS2	7617449	0.748	1.15×10^{-5}	1.01	0.8645	N/A
rs6472842	8	GDAP1	75441012	0.744	1.21×10^{-5}	1.06	0.2805	N/A
rs714861	4	PDGFRA	54649188	1.329	1.37×10^{-5}	0.90	0.0464	N/A
rs4846647	1	Intergenic	218691910	0.717	1.49×10^{-5}	1.14	0.0344	N/A

[1] The most significant SNP per region is listed as a representative SNP

[2] Oodds ratio is calculated for the rare allele.

[3] N/A = not applicable when the direction of effect is opposite in the two samples