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Replication of bipolar disorder susceptibility alleles and identification of 2 novel genome-wide significant associations in a new bipolar disorder case-control sample

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Abstract

We have conducted a genotyping study using a custom Illumina Infinium HD genotyping array, the ImmunoChip, in a new UK sample of 1,218 bipolar disorder cases and 2,913 controls that have not been used in any studies previously reported independently or in meta-analyses. The ImmunoChip was designed prior to the publication of the Psychiatric GWAS Consortium Bipolar Disorder Working Group (PGC-BD) meta-analysis data. As such 3106 SNPs with a P value less than 1×10^{-3} from the bipolar disorder meta-analysis by Ferreira *et al*., 2008 were genotyped. We report support for two of the three most strongly associated chromosomal regions in the Ferreira study, *CACNA1C* (rs1006737, $p=4.09\times10^{-4}$) and 15q14 (rs2172835, p=0.043) but not *ANK3* (rs10994336, p=0.912). We have combined our ImmunoChip data (569 quasi-independent SNPs from the 3016 SNPs genotyped) with the recently published PGC-BD meta-analysis data, using either the PGC-BD combined discovery and replication data where available or just the discovery data where the SNP was not typed in a replication sample in PGC-BD. Our data provide support for two regions, at *ODZ4* and *CACNA1C*, with prior evidence for genome-wide significant association in PGC-BD meta-analysis. In addition, the combined analysis shows two novel genome-wide significant associations. First, rs7296288 ($P = 8.97 \times 10^{-9}$, OR = 0.9), an intergenic polymorphism on chromosome 12 located between *RHEBL1* and *DHH*. Secondly, rs3818253 ($P = 3.88 \times 10^{-8}$, OR = 1.16), an intronic SNP on chromosome 20q11.2 in the gene *TRPC4AP* which lies in a high linkage disequilibrium region along with the genes *GSS* and *MYH7B*.

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The list of members of WTCCC can be found in the supplementary online material.

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Keywords

Bipolar disorder; genome-wide significant association; ImmunoChip; PGC-BD; rs7296288; rs3818253

Introduction

There is robust evidence that genes influence susceptibility to bipolar disorder (BD)[1,2]. Genome-wide association studies (GWAS) have identified a number of loci which show strong support for their association with BD including common polymorphisms within *CACNA1C, ANK3, NCAN*[3,4] (Ferreira *et al*., 2008, Cichon *et al.*, 2011). Recently, the Psychiatric Genome-wide Association Study Consortium Bipolar Disorder (PGC-BD) group performed a combined analysis of GWAS data from 7,481 BD individuals and 9,250 controls. A replication study tested 34 SNPs that were associated at P-value < 5×10^{-5} in the discovery sample in 4,496 independent cases with bipolar disorder and 42,422 independent controls. The combined analysis of the discovery and replication sample confirmed genome-wide significant evidence of association for *CACNA1C* and identified a new genome-wide significant intronic variant in *ODZ4*[5].

In the current study we have examined previously reported susceptibility loci by genotyping BD cases and controls on the custom content Illumina array, the ImmunoChip. The genotyping was undertaken through our participation in the Wellcome Trust Case Control Consortium Extension study (WTCCC) and included some samples from our earlier GWAS[6] and a new set of cases and controls that have not been reported previously, the latter being the focus of this report. The ImmunoChip was designed to study SNPs and genes covering autoimmune disorders for example Coeliac disease[7], extensive SNP data from the MHC region and regions of interest for follow up and fine mapping arising from previous GWAS studies of a number of clinical phenotypes studied in the Wellcome Trust Case Control Consortium studies, including bipolar disorder. The SNP content relevant to BD was chosen from the BD meta-analysis by Ferreira *et al*., 2008[3], taking SNPs with a P-value less than 10^{-3}. As the ImmunoChip was designed prior to the publication of the large PGC-BD meta-analysis[5] the most significantly associated SNPs from this meta-analysis were not specifically included, although some were on the chip because they were also strongly associated within the meta-analysis for Ferreira *et al.*, 2008[3] (which included samples that were part of the PGC-BD analysis).

In this study we report the results from SNPs both chosen from the Ferreira *et al*., (2008) BD meta-analysis[3] and 21 (directly genotyped or proxies) of the 34 most significantly associated SNPs from the PGC-BD study[5]. We also describe fine mapping data for *CACNA1C* and *ANK3*.

Methods and Materials

Samples

All of the participants in these studies were unrelated, white, living in the British Isles and were of European descent. The protocols and procedures were approved by the relevant ethics review panels where patients were recruited.

BD cases

The individuals were recruited if they suffered with a major mood disorder in which clinically significant episodes of elevated mood had occurred. Bipolar cases were excluded

if they: (i) had experienced mood or psychotic illness only as a result of alcohol or substance dependence; (ii) had experienced mood or psychotic illness only secondary to medical illness or medication; or (iii) were biologically related to another study participant. The following methodology was used for assessment of bipolar cases: a semi-structured lifetime ever psychiatric interview (Schedules for Clinical Assessment in Neuropsychiatry)[8], a review of the available case notes and completion of the Operational Criteria (OPCRIT) checklist of items of psychopathology[9] which has been shown to be valid in studies of mood disorders[10], followed by clinical ratings and a best-estimate lifetime diagnosis according Research Diagnostic criteria[11]. In cases where there was doubt as to the best-estimate lifetime diagnosis, diagnostic and clinical ratings were made by at least two members of the research team blind to each other's ratings.

Independent new BD cases

The independent cases consisted of 1,218 individuals of which 29% were male. The mean age of recruitment was 46 (s.d. 12) years, with a mean age at first impairment due to bipolar disorder of 22 (s.d. 9) years. A lifetime diagnosis was made according to Research Diagnostic Criteria[11] and the 1,218 individuals were categorised as follows: bipolar I disorder/mania: 63 % cases, bipolar II disorder/hypomania: 29 % cases, schizoaffective disorder, bipolar type: 8 % cases. Of those individuals for whom we were able to make a definite rating 65% of the cases had a lifetime experience of psychotic symptoms (defined as a score over 9 on the Bipolar Affective Disorder Dimension Scale (BADDS)[12] and 25% had a lifetime experience of predominantly mood-incongruent psychotic symptoms (defined as a score over 29 on the Bipolar Affective Disorder Dimension Scale (BADDS)[12] mood incongruence scale).

Additional BD cases

The 1,368 additional BD cases had been previously genotyped as part of the BD sample for the WTCCC GWAS[6]. In combination with the independent BD cases described above these additional cases were used for the fine mapping analyses in this report.

Of these 1,368 cases, 37% were male, with a mean age at recruitment of 48 (s.d. 13 years) and a mean age at first impairment due to bipolar disorder of 25 (s.d. 10) years. A lifetime diagnosis according to RDC[11] was made as follows: bipolar I disorder/mania: 78 % cases, bipolar II disorder/Hypomania: 6 % cases, schizoaffective disorder, bipolar type: 16 % cases. Of those individuals for whom we were able to make a definite rating 70% of the cases had a lifetime experience of psychotic symptoms (defined as a score over 9 on the Bipolar Affective Disorder Dimension Scale (BADDS)[12] and 30% had a lifetime experience of predominantly mood-incongruent psychotic symptoms (defined as a score over 29 on the Bipolar Affective Disorder Dimension Scale (BADDS)[12] psychosis and mood incongruence scale).

Controls

The controls, which were not screened to exclude presence of psychiatric illness, came from two sources: the 1958 Birth Cohort (58C) Controls and the UK blood service (UKBS) controls. The characteristics and recruitment of both are described in WTCCC, 2007[6].

Independent new controls

There were 2,913 controls in the independent sample, of which 47% were male.

Additional controls

The additional controls consisted of 1,409 samples of which 48% were males. A total of 5 of these samples were part of the 58C and 1,404 from UKBS control samples which had been genotyped in the earlier GWAS[6].

Genotyping

ImmunoChip design was undertaken centrally at the Sanger Institute and the genotyping of the BD samples using this chip was performed at the Wellcome Trust Sanger Institute within the context of our participation in WTCCC.

The ImmunoChip is a consortium based, custom Illumina Infinium HD genotyping array. The array was designed to study SNPs and genes covering 12 autoimmune disorders, extensive SNP data from the MHC region and regions of interest for follow up arising from previous GWAS studies of a number of clinical phenotypes studied in the Wellcome Trust Case Control Consortium, including bipolar disorder. To supplement the fine mapping SNPs, 1000 Genomes Project pilot CEU population variants (Sept 2009 release) were included. Finally, investigator-specific "wildcard" SNPs were also submitted and it is this content (see section below) that forms the basis for this report

Bipolar disorder (investigator-specific) SNPs selection for replication

We selected SNPs for replication from the Ferreira *et al*., (2008)[3] meta-analysis of the STEP-UCL, WTCCC, and ED-DUB-STEP2 studies which included a total of 4,387 cases and 6,209 controls. All SNPs from this study with a P-value less than or equal to 1×10^{-3} were submitted to be included on the ImmunoChip.

SNPs selection for fine mapping of the genes: CACNA1C and ANK3

SNAP (SNP Annotation and Proxy Search) (http://www.broadinstitute.org/mpg/snap/) was used to define recombination points around the SNPs of interest using the genotype data from the original BD meta-analysis[3]. Chromosomal regions around the index SNPs rs1006737 (*CACNA1C*) and rs10994366 (*ANK3*) that are flanked by high recombination rates (≥ 20cM/Mb) were noted. Hapmap 3 SNPs and 1K genome Project pilot CEU population variants (Sept 2009 release) within the following chromosomal locations (according to the March 2006 human reference sequence, NCBI Build 36.1) were genotyped: CACNA1C, chromosome 12: 2,151,068 to 2,299,686. ANK3, chromosome 10: 61,676,267 to 62,232,861.

Statistical analysis

Central analysis group (Sanger) Quality control of the data—Unless stated otherwise, all data management and quality control assessment was performed with PLINK (v1.07)[13] and a series of shell scripts. All genotypes were called with the GenoSNP software[14] and any genotypes with a call probability <85% were scored as missing data. Following preliminary quality control assessment by the Sanger central data pipeline, data on 2,893 BD and 4,539 control individuals were made available to us, genotyped for 192,402 autosomal SNPs.

Association analyses of the independent sample—The genomic inflation factor (λ), calculated as the ratio between the observed and expected median χ^2 statistic using the 43K SNPs in relative linkage equilibrium was 1.017 (λ_{1000}=1.01) and was used to correct for the degree of inflation. To explore potential effects of population stratification within the sample, combinations of the first 10 principal components from Eigenstrat were included as covariates in a logistic regression analysis (additive model). The first principal component

had a noticeable effect on the genomic control lambda statistics. Logistic regression of disease state was preformed with one covariate (the first principal component from Eigenstrat analysis) using PLINK[13]. Post QC quantile-quantile (Q-Q) plots are shown in supplementary Figure 1.

Meta-analyses—Results from the current analysis were combined with data from published analyses by fixed-effects meta-analysis using PLINK 1.07[13] to estimate a common odds ratio weighted by individual study's standard errors (SE). The sample sets used were; our Independent ImmunoChip data; the PGC-BD discovery data GC adjusted based on a lambda of 1.148 (ie. the lambda calculated for the PGC-BD dataset), or the PGC combined GWAS and replication data which had been previously adjusted by a lambda of 1.176[5] (if the SNP or a proxy of the SNP was one of the 34 SNPs in the PGC-BD replication study). If a SNP was not present in our ImmunoChip data a proxy SNP in strong LD ($D'=1$, $r^2 \geq 0.8$) was substituted and the SE weighted to account for the lack of information: SEW = SE/sqrt(r^2).

Fine mapping using independent and additional sample—For the fine mapping analyses, logistic regression was used to investigate the possibility of more than one independent signal in *CACNA1C* after accounting for the effects of the most strongly associated SNP.

Results

We genotyped in an independent sample (1,218 BD individuals and 2,913 controls) the most significant SNPs in a BD meta-analysis[3] including those that had surpassed a P-value of less than 1×10^{-3} (n=3,016, list in Table S4) (See Supplementary Table 2 for the association results for all SNPs with a P-value < 10^{-2}). The 3016 SNPs were reduced to a subset of 569 quasi-independent SNPs by removing one SNP from each pair of SNPs with $r^2 > 0.5$. The SNP that was removed in each case was the one with the least significant p-value in the Ferreira *et al*., (2008)[3] study. A sign test for consistency of direction of effect between the meta-analysis study and our independent ImmunoChip study for the 569 SNPs was highly significant (sign test P-value, 5.32×10^{-7}). The same direction of effect was observed in 343 of the 569 (60%) SNPs. By reducing the level of dependence between the SNPs and applying a r^2 value of greater than 0.2 and performing the same analysis we also noted a highly significant sign test ($p=3.36 \times 10^{-6}$) for the consistency of direction of effect, 288 of the 477 (60%) SNPs had the same direction of effect.

For these 569 independent SNPs we combined the PGC-BD primary dataset (7,481 BD cases and 9,250 controls) with our Independent ImmunoChip dataset. In Table 1 we report 6 SNPs which do not overlap with the 34 SNPs genotyped in the PGC-BD[5] replication sample, and that reached significance levels of P-value $<10^{-5}$. One SNP, rs3818253, on chromosome 20q11 met genome-wide significance (GWS) ($P = 3.88 \times 10^{-8}$, OR = 1.164). Within this new GWS LD region are the genes *GSS*, *MYH7B* and *TRPC4AP*.

Within the independent ImmunoChip sample, data were available for 21 of the 34 top SNPs in the PGC-BD replication study. Thirteen SNPs had been directly genotyped and proxies in high LD with an index SNP ($D'=1$, $r^2 \geq 0.8$) were available for a further 8 SNPs. Of these 21 SNPs the same direction of effect between our independent ImmunoChip study and the PGC-BD data was seen for 15 SNPs (71%), with a significant sign test for consistency of effect (sign test P-value = 0.039). Supplementary Table 3 indicates the proxy SNP used and the LD between the proxy and the original PGC-BD SNP. A combined analysis had been performed by the PGC analyzing the primary and replication samples for all 34 SNPs and it is this P-value we used. In the combined datasets (PGC-BD and Independent ImmunoChip),

the 2 previously reported SNPs in *CACNA1C* and *ODZ4* showed increased evidence of GWS association, rs125766774 in *ODZ4* (P-value = 6.20×10^{-9}, OR = 0.90) and rs4765913 located in *CACNA1C* (P-value = 9.78×10^{-10}, OR= 1.14) (Table 2). In addition, genome-wide significant evidence for association was established with rs7296288 on chromosome 12 (P = 8.97×10^{-9}, OR = 0.902).

The 3 regions of strongest association reported in the Ferreira *et al.*, study[3] were *ANK3*, *CACAN1C* and chromosome 15q14. Comparison of the independent BD cases with the independent controls showed an association with rs1006737 (P = 4.09×10^{-4}) and rs1024582 (P = 6.28×10^{-4}) in *CACNA1C*, with the same risk alleles. We also noted support for the previously identified SNPs on chromosome 15q14 (rs12899449 & rs2172835, P-value, 0.066 and 0.044, respectively). We saw no replication for the 2 SNPs that previously showed strongest association in *ANK3*, rs10994336 and rs1938526 (Table 3).

In addition, we performed fine mapping of recombination regions around the most significantly associated SNPs in *CACNA1C* and *ANK3*. The additional cases and controls genotyped in our earlier GWAS[6] were included in these analyses (total of 2,586 BD cases and 4,322 controls). Both Hapmap and 1K genome (Sept 2009 release) SNPs were genotyped. For *ANK3* 1075 SNPs in a recombination region of approximately 498Kb around rs10994336 were genotyped. The most associated SNP was rs10821708 with a P-value of 0.0017 (OR, 1.21). For *CACNA1C*, fine mapping was performed around rs1006737 encompassing approximately 159Kb including 293 SNPs. The most significantly associated SNP was rs882194 (P-value, 5.53×10^{-5}, OR, 1.22) (Supplementary Figure 2). There was no evidence of more than one independent signal after conditioning on rs882194.

Discussion

We report replication of previous BD GWAS findings in an independent BD and control sample set from the UK. Combining our data with the largest BD meta-analysis to date, the PGC-BD[5], we observed 2 novel genome wide significant associations on chromosome 20 at *TRPC4AP* and on chromosome 12 between *RHEBL1* and *DHH*. In addition, the 2 independent SNPs reported as showing genome-wide significance by the PGC-BD[5], rs4765913 and rs12576775, lying in *CACNA1C* and *ODZ4* respectively, also showed increased evidence of association by approximately a level of magnitude for both loci.

Our original aims were to replicate findings from the BD meta-analysis of Ferreira *et al.*, (2008)[3] and to refine previously reported association signals at *CACNA1C* and *ANK3*. We found independent support for the associations at *CACNA1C* and chromosome 15q14, but not at *ANK3*. In fine mapping of *CACNA1C*, an alternative SNP (rs882914) was more significantly associated than the original variant that had showed strongest association (rs1006737). The SNPs rs1006737 and rs882194 are both intronic SNPs with no known functional role, and are in moderate LD ($D' = 0.998$ and $r^2 = 0.64$). We did not observe a second independent association signal within the region studied.

Within *ANK3* neither of the 2 previously associated meta-analysis SNPs[3] were associated in our independent sample. This is unlikely to be a type II error as our sample was large and provided power of 97% ($\alpha = 0.05$) to detects effects of the size observed by Ferreira *et al.*, (2008)[3]. The most strongly associated SNP in our independent sample analysis was rs1821708 with a P-value of 0.0017. This signal is of borderline statistical significance after correcting for multiple testing: it would be nominally significant at $p<0.05$ following Bonferroni multiple testing correction for the 124 independent SNPs ($r^2 > 0.5$) genotyped, but not significant after correcting for all 1075 SNPs examined. We note that, in similar vein to our current finding, in the PGC-BD meta-analysis study[5] the primary association reached

genome-wide significance but there was no association at *ANK3* in the replication sample. This observation was interpreted as being consistent with an overestimation of the original odds ratios, rather than disproving the association. To resolve this and make a conclusive decision regarding the involvement of ANK3 may involve the genotyping of a large number of additional samples.

Combining our ImmunoChip independent data with the PGC-BD meta-analysis data provided support for the genome-wide significant associations at *CACNA1C* and *ODZ4*, plus 2 novel genome-wide significant associations. The first novel region is located on chromosome 20 at rs3818253 for which combining the independent ImmunoChip data with the primary PGC-BD data gave a P-value of 3.88×10^{-8} (OR=1.16). SNPS in this region were not genotyped in the replication sample in the PGC study[5], but were included in the current study because the P-value was less than 1×10^{-3} in the Ferreira *et al*., meta-analysis (rs3818253 P-value, 5.3×10^{-4})[3]. Interestingly, rs3818253 was the most significantly associated SNP in our independent sample on the ImmunoChip. Three genes lie in the LD block implicated, *GSS*, *MYH7B* and *TRPC4AP*, encoding glutathione synthetase, myosin-7B, and transient receptor potential cation channel, subfamily C, member 4 associated protein, respectively. rs3818253 lies in the intron of *TRPC4AP*. Further detailed analysis of this chromosomal region in additional samples for replication and refinement of the association signal is needed.

Chromosome 12q13.1 at rs7296288 is the second chromosomal region showing genome-wide significant association. rs7296288 lies intergenic between *RHEBL1* and *DHH*, 16.2Kb and 3.2 Kb up and downstream of the SNP respectively. The genes encode Ras homolog enriched in brain like 1, and desert hedgehog protein. Association at this SNP did not quite reach the level of genome-wide significance in the PGC-BD combined analysis on its own (P-value, 5.4×10^{-8}, OR, 0.90).

There has been substantial debate about the most appropriate way to correct for multiple testing in GWAS, including cogent arguments about the relative benefits of frequentist and Bayesian approaches to data evaluation (see for example Box 1 in WTCCC, Nature, 2007[6]). Within the context of the frequentist approach to genome-wide experiments, the gold standard for statistical significance is genome-wide significance using all available data: in other words, correcting for all independent tests across the genome and putting together by meta-analysis all available, relevant datasets. Our reports of novel genome-wide significant signals at rs7296288 on chromosome 12 and rs3818253 on chromosome 20q11.2 meet this stringent test. When we consider a less stringent test of support for association within our sample, for example looking at whether we find association support for the top 3 associations in the Ferreira *et al*., meta-analysis[3], a simple Bonferroni correction for 3 independent tests is appropriate. With that correction, we find support for one of the 3 loci: *CACNA1C*.

The gender ratio for the new independent BD sample (71% female: 29% male) is broadly in line with previous bipolar samples recruited and reported by us (eg. WTCCC [6]). However, this female predominance is different from the ratio in our controls (53% female: 47% male) and from the usually quoted equal gender prevalence of bipolar disorder in the population. This raises the possibility of a gender-specific effect influencing our results. There are two observations that make this extremely unlikely. The first is that strongly associated loci reported to date, and replicated within the current study, have not been shown to have any gender-specific effect of association. Thus, a priori, we do not expect gender-specific effects. Second, an analysis within our current sample shows no evidence of differences in allele frequency distributions between male and female bipolar cases. Hence, we can be confident that differences in gender distribution in our case and control samples did not

cause or influence the findings. The predominance of females in our sample also raises the possibility that our findings may not generalize to samples with more equal gender ratio. Whilst we have no reason to think this will be the case, it is a possibility and points to the potential benefits of taking account of a wide range of clinical variables (not just gender) when analysing data across multiple clinical samples[15].

In summary, we have provided additional support for prior association findings in *CACNA1C*, *ODZ4*, and at chromosome 15q14. In addition combined analysis with the PGC-BD data shows 2 novel genome-wide significant associations. Firstly, in a region of high LD at *TRPC4AP* on chromosome 20q11.2 which has not been highlighted previously and secondly at a region on chromosome 12q13.1 with a SNP located between *RHEBL1* and *DHH*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Combined ImmunoChip bipolar disorder and primary PGC-BD data.

The SNPs listed are those with a ImmunoChip data P-value < 10^{-5} which are not covered by the top 34 SNPs in the PGC-BD primary and replication combined study[5].

					BD Independent Immunochip data		PGC-BD Primary data		ImmunoChip, PGC-BD combined data			
CHR	**SNP**	**Position**	**A1**	**A2**	**P-Value**	**OR**	**P-Value**	**OR**	**P-Value**	**OR**	**Gene & Location**	
3	rs9834970	36831034	T	C	0.0519	0.910	6.19×10^{-6}	0.899	3.04×10^{-6}	0.901	LBA	Intergenic
3	rs3755806	52618725	C	T	0.0222	0.891	8.70×10^{-7}	0.889	4.21×10^{-7}	0.889	PBRM1	Coding, Syn
12	rs1006737	2215556	A	G	4.09×10^{-4}	1.198	1.73×10^{-5}	1.110	2.16×10^{-7}	1.128	CACNA1C	Intronic
12	rs1274726	47787665	C	T	0.0820	1.090	1.51×10^{-6}	1.130	1.48×10^{-6}	1.121	LMBR1L	Intronic
20	rs2273684	32993427	G	T	9.23×10^{-6}	1.245	2.74×10^{-4}	1.088	4.72×10^{-7}	1.119	GSS	Intronic
20	rs3818253	33060537	A	G	2.65×10^{-6}	1.333	6.18×10^{-5}	1.124	**3.88×10^{-8}**	1.164	TRPC4AP	Intronic

CHR, Chromosome, Position, base pair position according to NCBI Build 36.1; A1, allele 1, odds ratio (OR) is predicted towards allele 1; A2, allele 2; P-value in bold indicates genome-wide statistical significance.

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Table 2

Association results for combined ImmunoChip bipolar disorder and combined PGC-BD samples for the 21 overlapping SNPs from the top 34 SNPs in PGCBD study[5].

					BD ImmunoChip data		BD-PGC combined analysis data		ImmunoChip, BD-PGC combined data		
CHR	SNP	Position	A1	A2	P	OR	PGC	OR	P	OR	Genes in LD region
1	rs263906	101,750,922	C	T	0.545	1.034	4.43×10^{-4}	1.08	4.83×10^{-4}	1.074	
1	rs875326	173,556,022	C	T	0.378	0.945	1.11×10^{-4}	1.10	1.05×10^{-3}	1.078	*TNR*
2	rs6746896	96,774,676	A	G	0.011	1.146	2.36×10^{-6}	1.10	1.14×10^{-7}	1.106	*LMAN2L, FER1L5, CNNM4*
2	rs7578035	98,749,324	G	T	0.532	1.031	2.77×10^{-6}	1.09	4.13×10^{-6}	1.083	
2	rs4668059	168,874,528	C	T	0.731	1.026	1.32×10^{-4}	1.12	2.3×10^{-4}	1.107	*STK39*
7	rs4332037	1,917,335	C	T	0.739	1.022	7.25×10^{-6}	0.90	4.29×10^{-5}	0.914	*MAD1L1*
10	rs9804190	61,509,837	C	T	0.682	0.977	1.20×10^{-4}	1.10	7.36×10^{-4}	1.080	
10	rs10994397	61,949,130	C	T	0.923	1.010	3.08×10^{-7}	0.82	2.15×10^{-6}	0.842	*ANK3*
11	rs12576775	78,754,841	A	G	0.047	0.887	4.4×10^{-8}	0.88	6.20×10^{-9}	0.881	*ODZ4*
12	rs7296288	47,504,438	A	C	0.055	0.912	5.41×10^{-8}	0.90	8.97×10^{-9}	0.902	*TUBA1B, TUBA1A, RHEBL1, PPKAG1, MLL2, LMBR1L, DHH, DDN*
15	rs16966413	36,267,191	A	G	0.015	0.824	9.97×10^{-5}	0.88	5.9310^{-6}	0.872	*SPRED1*
15	rs12912251	36,773,660	G	T	0.029	1.125	3.27×10^{-6}	1.10	2.94×10^{-7}	1.103	*C15orf53*
19	rs2287921	53920084	C	T	0.059	1.098	3.08×10^{-6}	1.10	2.85×10^{-7}	1.100	*SPHK2, SEC1, RPL18, RASIP1, NTN5, MAMSTR, IZUMO1, FUT2, FUT1, FGF21, FAM83E, DBP, CA11,*
2	rs3845817**	65628191	C	T	0.309	0.951	1.76×10^{-6}	0.91	1.45×10^{-6}	0.915	
3	rs6550435**	36846511	G	T	0.819	0.988	9.32×10^{-6}	1.09	4.54×10^{-5}	1.077	*LBA1*
3	rs736408**	52808259	C	T	0.0756	1.098	2.19×10^{-6}	1.10	5.03×10^{-7}	1.100	*WDR82, TWF2, TNNC1,TMEM110, TLR9, STAB1,SPCS1, SNORD69, SNORD19, SNORD19B, SFMBT1, SEMA3G, RFT1, PRKCD, PPM1M, PHF7, PBRM1, NT5DC2, NISCH, NEK4, MUSTN1, LOC440957, ITIHI, ITIH3, ITIH4, GNL3, GLYCTK, GLT8D1, DNAH1, BAP1, ALAS1*
6	rs9371601**	152823432	G	T	0.0764	0.916	6.71×10^{-7}	0.91	1.38×10^{-7}	0.911	*SYNE1*
9	rs3968**	4931997	C	g	0.3809	1.055	0.0174	1.04	0.0125	1.041	
12	rs4765913**	2290638	A	T	0.0149	1.153	1.52×10^{-8}	1.14	$9.78\times10^{-1}0$	1.142	*CACNA1C*
19	rs11085829**	13035312	A	G	0.98	1.001	6.96×10^{-5}	0.92	2.49×10^{-4}	0.931	*NFIX*
20	rs6102947**	40652833	C	G	0.2562	1.189	8.45×10^{-5}	1.31	5.13×10^{-5}	1.289	*PTPRT*

rsxx**, indicates a proxy SNP from the ImmunoChip data has been used in the analysis; CHR, chromosome; Position, base pair position according to NCBI Build 36.1; A1/2, allele 1/2, odds ratio (OR) is predicted towards allele 1; P, P-value; PGC, genomic control P value.

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Table 3

Analysis of 3 chromosomal regions which previously showed association with bipolar disorder at $P < 10^{-6}$ in Ferreira et al., (2008) meta-analysis study[3].

		Ferreira et al., 2008				ImmunoChip Independent BD & Controls (Cases = 1218, Controls = 2913			
SNP	**Minor allele**	**P-value**	**OR**	**Cases MAF**	**Controls MAF**	**P-value**	**OR**	**Cases MAF**	**Controls MAF**
ANK3 (chr 10q21)									
rs10994336	T	9.1×10^{-9}	1.45	0.07	0.053	0.912	0.989	0.059	0.059
rs1938526	G	1.3×10^{-8}	1.395	0.075	0.056	0.993	0.993	0.065	0.065
CACNA1C (chr 12p13)									
rs1006737	A	7.0×10^{-8}	1.181	0.356	0.324	4.09×10^{-4}	1.198	0.372	0.332
rs1024582	A	1.7×10^{-7}	1.18	0.382	0.351	6.28×10^{-4}	1.190	0.383	0.345
Chromosome 15q14									
rs12899449	G	3.5×10^{-7}	0.836	0.246	0.276	0.066	0.905	0.276	0.296
rs2172835	T	7.5×10^{-7}	0.853	0.281	0.312	0.044	0.896	0.278	0.301

OR, odds ratio.