nature genetics

Collaborative genome-wide association analysis supports a role for *ANK3* and *CACNA1C* in bipolar disorder

Manuel A R Ferreira[1–6], Michael C O'Donovan[7], Yan A Meng[1–5], Ian R Jones[7], Douglas M Ruderfer[1,3–5], Lisa Jones[8], Jinbo Fan[1,3–5], George Kirov[7], Roy H Perlis[1–5], Elaine K Green[7], Jordan W Smoller[1–5], Detelina Grozeva[7], Jennifer Stone[1–5], Ivan Nikolov[7,9], Kimberly Chambert[4,5], Marian L Hamshere[7,9], Vishwajit L Nimgaonkar[10], Valentina Moskvina[7,9], Michael E Thase[11,12], Sian Caesar[8], Gary S Sachs[1,2], Jennifer Franklin[5], Katherine Gordon-Smith[7,8], Kristin G Ardlie[5], Stacey B Gabriel[5], Christine Fraser[7], Brendan Blumenstiel[5], Matthew Defelice[5], Gerome Breen[13,14], Michael Gill[15], Derek W Morris[15], Amanda Elkin[14], Walter J Muir[16], Kevin A McGhee[16], Richard Williamson[14], Donald J MacIntyre[16], Alan W MacLean[16], David St Clair[13], Michelle Robinson[17], Margaret Van Beck[16], Ana C P Pereira[17], Radhika Kandaswamy[17], Andrew McQuillin[17], David A Collier[14], Nicholas J Bass[17], Allan H Young[18,19], Jacob Lawrence[17], I Nicol Ferrier[18], Adebayo Anjorin[17], Anne Farmer[14], David Curtis[17], Edward M Scolnick[4,5,20], Peter McGuffin[14], Mark J Daly[5,21–23], Aiden P Corvin[15], Peter A Holmans[7,9], Douglas H Blackwood[16], Wellcome Trust Case Control Consortium[24], Hugh M Gurling[17], Michael J Owen[7], Shaun M Purcell[1–5,25], Pamela Sklar[1–5,20,25] & Nick Craddock[7,25]

To identify susceptibility loci for bipolar disorder, we tested 1.8 million variants in 4,387 cases and 6,209 controls and identified a region of strong association (rs10994336, $P = 9.1 \times 10^{-9}$) in *ANK3* (ankyrin G). We also found further support for the previously reported *CACNA1C* (alpha 1C subunit of the L-type voltage-gated calcium channel; combined $P = 7.0 \times 10^{-8}$, rs1006737). Our results suggest that ion channelopathies may be involved in the pathogenesis of bipolar disorder.

Recent genome-wide association studies (GWAS) have identified genetic variants that show consistent association with common, complex diseases, such as type 2 diabetes[1], prostate cancer[2] and Crohn's disease[3]. In many cases, a critical component for success involved combining results and data across multiple, smaller studies to provide adequate power to detect common variants of modest relative risk. Given that a single study will often not be sufficient, here we present the results of combining two previously published[4,5] and one new ($N = 2{,}365$) GWAS of bipolar disorder.

Three groups have performed independent GWAS of bipolar disorder, with little agreement among the most associated regions. Baum *et al.*[6], using DNA pooling, identified a SNP located in *DGKH* (diacylglycerol kinase eta) that was associated with bipolar disorder in 1,233 cases and 1,439 controls with a P value of 1.5×10^{-8}. The Wellcome Trust Case Control Consortium (WTCCC)[4] analyzed 1,868 cases and 2,938 controls and identified a locus in a gene-rich region of high linkage disequilibrium (LD) on chromosome 16p12 with $P = 6.3 \times 10^{-8}$. Recently, Sklar *et al.*[5] performed a GWAS of 1,461 cases and 2,008 controls (the STEP-UCL study), finding the strongest single SNP result in *MYO5B* (myosin 5B), with $P = 1.7 \times 10^{-7}$. Although there was no obvious correspondence among the studies' top few hits, a broader comparison of the WTCCC and STEP-UCL studies identified *CACNA1C* as showing the strongest consistent signal[5].

We genotyped a new sample of 1,098 individuals with bipolar disorder and 1,267 controls on a similar platform as used by the WTCCC and STEP-UCL studies (**Supplementary Table 1** online), with 331,786 SNPs passing quality control (**Supplementary Methods** online). This dataset, referred to as ED-DUB-STEP2, included additional samples from the STEP-BD study[5] as well as cases and controls from the University of Edinburgh and Trinity College Dublin. Approval for each study was obtained from the appropriate review boards of the participating institutions and informed consent was

[1]Department of Psychiatry, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [2]Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, USA. [3]Psychiatric and Neurodevelopmental Genetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [4]Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142, USA. [5]Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142, USA. [6]Genetic Epidemiology, Queensland Institute of Medical Research, QLD 4029, Australia. [7]Department of Psychological Medicine, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK. [8]Department of Psychiatry, Division of Neuroscience, Birmingham University, Birmingham B15 2QZ, UK. [9]Biostatistics and Bioinformatics Unit, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK. [10]University of Pittsburgh, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania 15213, USA. [11]University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA. [12]University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213, USA. [13]University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, UK. [14]SGDP, The Institute of Psychiatry, King's College London, De Crespigny Park, Denmark Hill, London SE5 8AF, UK. [15]Trinity Centre for Health Sciences, St James's Hospital, Trinity College Dublin, Dublin 8, Republic of Ireland. [16]Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh EH10 5HF, UK. [17]Molecular Psychiatry Laboratory, Department of Mental Health Sciences, Windeyer Institute of Medical Sciences, University College London, 46 Cleveland Street, London W1T 4JF, UK. [18]School of Neurology, Neurobiology and Psychiatry, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK. [19]UBC Institute of Mental Health, 2255 Wesbrook Mall, Detwiller Pavilion Vancouver, British Columbia, Canada V6T 2A1. Departments of [20]Genetics and [21]Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA. [22]Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [23]Center for Human Genetic Research, Massachusetts General Hospital, Boston, Massachusetts 02114, USA. [24]A full list of members is provided in the **Supplementary Note** online. [25]These authors contributed equally to this work. Correspondence should be addressed to P.S. (sklar@chgr.mgh.harvard.edu) or N.C. (craddockn@Cardiff.ac.uk).

Received 22 February; accepted 13 June; published online 17 August 2008; doi:10.1038/ng.209

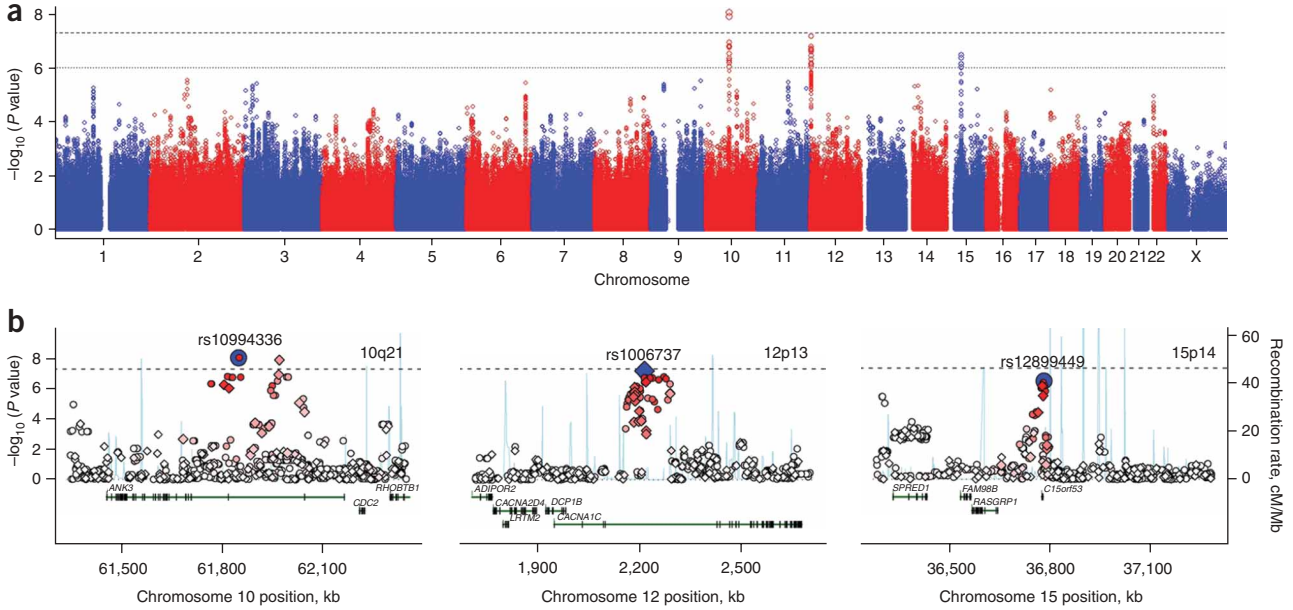

Figure 1 Association results for the combined analysis of the WTCCC, STEP-UCL and ED-DUB-STEP2 studies. (**a**) Genome-wide results ($-\log_{10}P$) are shown in chromosomal order for directly genotyped ($N = 325{,}690$) and imputed ($N = 1{,}444{,}258$) SNPs that were tested for association in the overall sample of 4,387 bipolar cases and 6,209 controls. Horizontal lines indicate a P value of 5×10^{-8} (dashed) and 1×10^{-6} (dotted). (**b**) Plots for the three regions of strongest association. Results ($-\log_{10}P$) are shown for directly genotyped (diamonds) and imputed (circles) SNPs. The most associated SNP for each region is shown in blue, and the color of the remaining markers reflects the linkage disequilibrium (r^2) with the top SNP in each panel (increasing red hue associated with increasing r^2). The recombination rate (second y axis) is plotted in light blue and is based on the CEU HapMap population. The dashed horizontal line indicates a $P = 5 \times 10^{-8}$. Exons for each gene are represented by vertical bars, based on all isoforms available from the Mar 2006 UCSC genome browser assembly.

obtained from all subjects. SNPs were tested for association using logistic regression co-varying for sample collection site and quantitative indices of ancestry based on a multi-dimensional scaling analysis; the genomic inflation factor (see **Supplementary Methods**) was $\lambda_{1,000} = 1.056$ (**Supplementary Fig. 1** online).

Analysis of the ED-DUB-STEP2 samples identified 14 chromosomal regions associated at $P < 5 \times 10^{-5}$ (**Supplementary Table 2** online). No region exceeded our threshold for genome-wide significance of 5×10^{-8}, correcting for an effective number of one million independent, common SNPs in the genome[1,7]. However, one of these regions spanned the *CACNA1C* association identified previously in the WTCCC and STEP-UCL studies[5] (**Supplementary Fig. 2** online), thus providing further independent support that this gene is associated with risk of bipolar disorder. In this region, the best single SNP across the WTCCC and STEP-UCL studies showed a consistent association in ED-DUB-STEP2 (rs1006737, $P = 0.011$, allele A, OR = 1.21). Furthermore, the SNP with strongest association in ED-DUB-STEP2 (rs10774037, $P = 4.6 \times 10^{-5}$, allele G, OR = 1.43) also had a consistent effect ($P = 0.0008$, OR = 1.14) in the WTCCC and STEP-UCL samples and was in LD with rs1006737 ($r^2 = 0.39$).

Next, we combined individual genotyping data from the WTCCC, STEP-UCL and ED-DUB-STEP2 studies, resulting in an overall sample of 4,387 cases and 6,209 controls genotyped on 325,690 overlapping SNPs, which substantially improves power to detect risk alleles of modest effect (**Supplementary Table 1**). The case sample comprised the following diagnoses: 81% bipolar 1, 16% bipolar 2, 2% schizoaffective manic and 1% bipolar NOS (**Supplementary Table 3** online).

Genomic coverage was increased by imputing the genotypes for 1,444,258 additional HapMap SNPs using PLINK[8] (**Supplementary Methods**), resulting in a total set of 1,769,948 SNPs. Applying a leave-one-out procedure for every genotyped SNP, we estimated concordance between imputed and true genotypes as 0.987 (**Supplementary Table 4** online). Similar results were obtained using MACH1 (ref. 9; data not shown).

Our primary analysis was a logistic regression of disease state on single SNP allelic dosage, either directly genotyped or imputed, controlling for study site and two quantitative indices of ancestry (**Supplementary Methods**). The genomic inflation factor was $\lambda_{1,000} = 1.024$ when considering all SNPs, and $\lambda_{1,000} = 1.021$ for directly genotyped SNPs (**Supplementary Fig. 1** and **Supplementary Fig. 3** online).

Results for the primary analysis are shown in **Figure 1a** and available for download (http://pngu.mgh.harvard.edu/purcell/bpwgas2/). Thirty-nine SNPs showed a $P < 10^{-6}$ and were located in three distinct chromosomal regions (**Table 1**). An additional 18 regions had at least one SNP with a $P < 10^{-5}$ (**Supplementary Fig. 4** and **Supplementary Table 5** online). Cluster plots for these SNPs are shown in **Supplementary Figure 5** online.

The strongest association was in *ANK3* on chromosome 10q21 for the imputed SNP rs10994336, with $P = 9.1 \times 10^{-9}$ (**Fig. 1b**). This result was supported by the following observations. First, multiple SNPs were associated across a 195-kb region. Second, three genotyped SNPs showed P values similar to rs10994336 (for example, rs1938526, $P = 1.3 \times 10^{-8}$, $r^2 = 0.40$ with rs10994336). Third, haplotype analysis of genotyped SNPs supported the single SNP results (most associated haplotype $P = 2.0 \times 10^{-9}$, **Supplementary Table 6** online). Fourth, the association remained (rs10994336, $P = 5.2 \times 10^{-9}$) after including the six additional WTCCC disease panels as extra controls for the WTCCC dataset ('expanded reference group' analysis). Finally, individual Sequenom genotyping confirmed that rs10994336 was imputed with high accuracy (concordance = 0.983, $N = 3{,}293$).

Table 1 Chromosomal regions with at least one SNP associated with bipolar disorder at $P < 10^{-6}$ in the combined analysis of WTCCC, STEP-UCL and ED-DUB-STEP2 datasets

		Combined analysis				WTCCC			STEP-UCL			ED-DUB-STEP2		
				MA frequency			MA frequency			MA frequency			MA frequency	
Type	SNP, minor allele	*P*	OR	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls	*P*	Cases	Controls
Chromosome 10q21 (*ANK3*)														
Imputed	rs10994336,T	9.1×10^{-9}	1.450	0.070	0.053	0.0006	0.070	0.054	0.0004	0.073	0.056	0.0002	0.070	0.049
Genotyped	rs1938526,G	1.3×10^{-8}	1.395	0.075	0.056	0.0013	0.074	0.058	0.0008	0.076	0.059	0.0002	0.073	0.047
Chromosome 12p13 (*CACNA1C*)														
Genotyped	rs1006737,A	7.0×10^{-8}	1.181	0.356	0.324	0.0015	0.357	0.324	0.0003	0.357	0.315	0.0108	0.353	0.337
Imputed	rs1024582,A	1.7×10^{-7}	1.180	0.382	0.351	0.0019	0.381	0.349	0.0012	0.384	0.348	0.0056	0.379	0.359
Chromosome 15q14														
Imputed	rs12899449,G	3.5×10^{-7}	0.836	0.246	0.276	0.0140	0.253	0.277	0.0005	0.237	0.276	0.0013	0.247	0.273
Genotyped	rs2172835,T	7.5×10^{-7}	0.853	0.281	0.312	0.0314	0.292	0.315	0.0004	0.267	0.309	0.0011	0.281	0.312

The most associated directly genotyped and imputed SNPs are shown for each region. MA, minor allele.

The second strongest region of association was located in the third intron of *CACNA1C* on chromosome 12p13 (rs1006737, $P = 7.0 \times 10^{-8}$). The third region was 3.3 kb away from an uncharacterized gene (*C15orf53*) on chromosome 15q14 (rs12899449, $P = 3.5 \times 10^{-7}$), in the same LD block with the brain-expressed *RASGRP1* (RAS guanyl releasing protein 1) gene. The association with both regions remained in the expanded reference group analysis (rs1006737, $P = 4.0 \times 10^{-8}$; rs12899449, $P = 9.3 \times 10^{-6}$).

Genotypic and heterogeneity analyses for these three regions suggested that the effect at each locus was consistent with an additive model (**Supplementary Table 7** online) and of similar magnitude across studies (**Supplementary Table 8** online). There was no clear indication of differential association across bipolar subtypes, psychosis, age-at-onset and sex (**Supplementary Table 9** online). No significant treatment response effects were observed for these three loci (data not shown).

Finally, we carried out a two-locus genome-wide search to identify epistatically acting loci of moderate-to-large effect that single-locus analysis may have missed[10,11]. From over 4×10^{10} pairwise tests, we identified 61 interactions with a $P < 5 \times 10^{-10}$, representing seven distinct pairs of regions (**Supplementary Table 10** online). No single interaction exceeded a Bonferroni corrected–threshold of 1.2×10^{-12}, in spite of reasonable power to detect large interaction effects (**Supplementary Methods**).

In summary, we present evidence that variation in *ANK3* confers risk of bipolar disorder in three independent datasets. ANK3 is an adaptor protein found at axon initial segments that has been shown to regulate the assembly of voltage-gated sodium channels[12,13]. We also provide independent support that *CACNA1C* is associated with bipolar disorder. In addition, we have recently shown that both *ANK3* and subunits of the calcium channel are downregulated in the mouse brain in response to lithium[14], one the most effective bipolar pharmacotherapies. Taken together, these results point to the possibility that bipolar disorder is in part an ion channelopathy[15].

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

Study supported by US National Institute of Mental Health (MH062137, MH067288, P.S.; MH063445, J.W.S.; MH63420, V.L.N.; N01MH80001, G.S.S., M.E.T. and M.S. Bauer), NARSAD (R.H.P., S.P., J.F., P.S.); Johnson & Johnson Pharmaceutical Research & Development, Sylvan C. Herman Foundation, Stanley Medical Research Institute, Dauten Family, Merck Genome Research Institute (E.M.S.); and National Health Medical Research Council (MRC), Australia (M.A.R.F.). Broad Institute Center for Genotyping and Analysis supported by U54 RR020278, National Center for Research Resources. UCL sample collection supported by MDF–The Bipolar Organization, Neuroscience Research Charitable Trust, central London NHS Blood Transfusion Service, Priory Hospitals and UK MRC (G9623693N, G0500791). Dublin sample collection supported by the Health Research Board and Science Foundation Ireland; controls supplied by J. McPartlin, Trinity College Biobank. Edinburgh cohort collection supported by the Wellcome Trust, London, Chief Scientist Office of the Scottish Executive and MRC, London. WTCCC genotype analyses were funded by the Wellcome Trust and undertaken within the context of the WTCCC. WTCCC members are listed in **Supplementary Note**.

AUTHOR CONTRIBUTIONS

Writing group: M.A.R.F., P.S., N.C., S.M.P. *Analytic group*: S.M.P., M.A.R.F., Y.A.M., D.M.R., J. Fan, M.J.D., P.A.H., M.C.O. N.C., P.S. *Project management*: P.S., E.M.S., A.P.C., D.H.B., H.M.G., M.C.O., N.C. *Clinical characterization for STEP-BD*: J.W.S., V.L.N., R.H.P., M.E.T., G.S.S. *Clinical characterization for Trinity College Dublin:* D.W.M., M.G., A.P.C. *Clinical characterization for University of Edinburgh*: W.J.M., K.A.M., D.J.M., A.W.M., M.V.B., D.H.B. *Clinical characterization for University College London*: A. McQuillin, N.J.B., M.R., J.L., A.C.P.P., R.K, A.A., D.C., H.M.G. *Clinical characterization, phenotype assessment and sample management and curation for WTCCC*: G.B., D.S. (Aberdeen); S.C., K.G.-S. L.J. (Birmingham); C.F., E.K.G., D.G., M.L.H., P.A.H., I.R.J., G.K., V.M., I.N., M.C.O., M.J.O., N.C. (Cardiff); D.A.C., A.E., A.F., R.W., P.M. (London); A.H.Y., I.N.F. (Newcastle). *DNA sample QC and genotyping for ED-DUB-STEP2*: K.C., J.S., J. Fan, J. Franklin, K.G.A., S.B.G., B.B., M.D.

Published online at http://www.nature.com/naturegenetics/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Diabetes Genetics Initiative *et al. Science* **316**, 1331–1336 (2007).
2. Gudmundsson, J. *et al. Nat. Genet.* **39**, 631–637 (2007).
3. Rioux, J.D. *et al. Nat. Genet.* **39**, 596–604 (2007).
4. Wellcome Trust Case Control Consortium. *Nature* **447**, 661–678 (2007).
5. Sklar, P. *et al. Mol. Psychiatry* **13**, 558–569 (2008).
6. Baum, A.E. *et al. Mol. Psychiatry* **13**, 197–207 (2008).
7. The International HapMap Consortium. *Nature* **437**, 1299–1320 (2005).
8. Purcell, S. *et al. Am. J. Hum. Genet.* **81**, 559–575 (2007).
9. Li, Y. & Abecasis, G.R. *Am. J. Hum. Genet.* **S79**, 2290 (2006).
10. Evans, D.M., Marchini, J., Morris, A.P. & Cardon, L.R. *PLoS Genet.* **2**, e157 (2006).
11. Marchini, J., Donnelly, P. & Cardon, L.R. *Nat. Genet.* **37**, 413–417 (2005).
12. Kordeli, E., Lambert, S. & Bennett, V. *J. Biol. Chem.* **270**, 2352–2359 (1995).
13. Poliak, S. & Peles, E. *Nat. Rev. Neurosci.* **4**, 968–980 (2003).
14. McQuillin, A., Rizig, M. & Gurling, H.M. *Pharmacogenet. Genomics* **17**, 605–617 (2007).
15. Gargus, J.J. *Biol. Psychiatry* **60**, 177–185 (2006).