Replication of association of 3p21.1 with susceptibility to bipolar disorder but not major depression

To the Editor:

McMahon and colleagues[1] recently reported a genome-wide significant association of rs2251219 on chromosome 3p21.1 with mood disorders in a combined sample of individuals with bipolar affective disorder (BP, also known as 'manic depression') and individuals with major depressive disorder (MDD). They meta-analyzed published data from four genome-wide association studies (GWAS) of BP[2–5] and three published GWAS of MDD[6,7], plus an unpublished German BP sample. Their analysis supports the suggestive association with bipolar disorder in this region previously reported by Scott *et al.*[3] in a GWAS meta-analysis of three of the same BP samples (National Institute of Mental Health-BP[4], Wellcome Trust Case Control Consortium[2] and the GlaxoSmithKline-BP samples[3]), but we report here alternative analyses and new data inconsistent with an association in this region with MDD. Thus, rs2251219 appears to be a susceptibility locus for BP alone, and the data do not support a general association of this SNP with mood disorders.

When we examined the data of McMahon *et al.*[1], an alternate and more parsimonious interpretation of the results suggested itself as we could not see compelling evidence that rs2251219 is associated with MDD in their analyses. If we consider the statistics presented in Table 2 of McMahon *et al.*[1], the *P* values reported for bipolar disorder were $P = 0.23$, $P = 0.002$, $P = 0.017$, $P = 0.0002$ and $P = 0.0023$ for the four studies, compared to a less strong $P = 0.026$, $P = 0.32$ and $P = 0.12$ for the major depression studies. Furthermore, the samples were mixed together in two replication stages rather than having the results for each phenotype presented separately and then meta-analyzed. This is important, as a previous analysis of twin data has shown that, although there is an overlap, most of the genetic variance in liability to BP is not shared with MDD[8]. To address this, we re-analyzed the data using the same method as McMahon *et al.*[1] by combining the *P* values and the direction of association using the Stouffer's *Z*-score method (as implemented within METAL (see URLs)), but we also stratified the data by phenotype. We also conducted a random-effect meta-analysis of the data to address issues of heterogeneity of effects between phenotypes and samples (for a review of meta-analyses issues in clinical studies, see ref. 9). For the National Institute of Mental Health-BP and the Systematic Treatment Enhancement Program for Bipolar Disorder samples, we took the *P* value and odds ratio for rs2251219 from a combined analysis that excluded the overlapping individuals shared between each study ($P = 0.0421$; see **Supplementary Table 3** in ref. 1). We summarize the data and our analyses in our **Table 1**.

Using the METAL fixed effects model, there was a clear genome-wide significant association with BP in the samples used by McMahon *et al.*[1] ($P = 5.24 \times 10^{-9}$), with no evidence for heterogeneity ($Q = 3.582$, 3 degrees of freedom, and the *P* value was greater than the traditional significance threshold for this test, 0.1; see ref. 9). When we analyzed their BP and MDD samples together, the genome-wide significant result was essentially unchanged ($P = 4.74 \times 10^{-9}$). However, the evidence for heterogeneity was significant ($Q = 11.38$, 6 degrees of freedom, $P = 0.092$), indicating that a random effects model is needed to analyze this data. When we then conducted a random effects meta-analysis in STATA (StataCorp LP), the *P* value for BP and MDD together reduced to a non-genome–wide significant $P = 9.38 \times 10^{-5}$. We consider that this heterogeneity, and the lack of genome-wide significance when this heterogeneity is taken into account, arises from the fact that there is only marginal evidence for association in the MDD samples (uncorrected meta-analysis *P* value for MDD = 0.048). Multiple testing in this analysis was also an issue for MDD, as five SNPs were analyzed in all the MDD samples and a genome-wide SNP analysis was conducted in the Genetic Association Information Network (GAIN)-MDD sample within the analyses of McMahon *et al.*[1]. We attempted to strike a middle ground and correct for five tests (rather than correcting genome-wide), which gave a Sidak-corrected $P = 0.22$ for MDD in the samples presented by McMahon *et al.*[1]. We also corrected for five tests if we used the data from these samples in our other analyses.

We also independently tested the role of rs2251219 in major depression by testing it in three further samples: the RADIANT (Recurrent Depressive dIsorder ANd Treatment study) sample[10] of 1,636 cases with recurrent MDD and 1,594 screened controls of UK ancestry; a sample from deCODE Genetics[11] of 322 cases with severe MDD and 25,460 controls of Icelandic ancestry (given the very much larger control group, this sample had an equivalent power as a sample of 700 cases and 700 controls); and the MDD2000+ community sample of North European ancestry[12], which, after exclusion of individuals related to those who gave samples in McMahon *et al.*[1], contributed 2,419 MDD cases and 3,462 screened controls. The allele frequencies and results from these three studies are presented in **Table 1**. Our replication samples had a combined power, assuming unscreened controls, of 86.7% to detect

Table 1 Results of association tests for rs2251219

Sample groups	Meta-analysis	Sample	Frequency (C allele) (cases)	Frequency (C allele) (controls)	Allelic *P*	OR	95% CI	N	Meta-analysis *P*, uncorrected (fixed effects unless otherwise stated)	Heterogeneity (*Q*) statistic *P*
Bipolar disorder samples included in McMahon *et al.* (2010)		NIMH-BP+ STEP-BD	Not available		0.421	0.91	0.83–1.00	3,495		
		German BD	0.38	0.44	0.002	0.81	0.70–0.93	1,955		
		WTCCC	0.36	0.40	2×10^{-4}	0.85	0.78–0.93	4,797		
		GSK BD	Not available		2×10^{-3}	0.78	0.67–0.92	1,536		
	McMahon BD meta-analysis								5.24×10^{-9}	
Major depression samples from McMahon *et al.* (2010)		GSK Lausanne	Not available		0.3219	1.09	0.92–1.28	1,349		
		GSK Munich	Not available		0.1227	0.9	0.79–1.03	1,792		
	McMahon MDD meta-analysis								0.0482	0.11
	McMahon MDD + BP								4.74×10^{-9}	0.09
	McMahon MDD + BP (random effects)								9.38×10^{-5}	
New major depression samples		RADIANT	0.39	0.41	0.1948	0.94	0.85–1.03	3,226		
		deCODE MDD	0.40	0.41	0.5948	0.96	0.89–1.23	25,636 (weighted to 1,400)		
		MDD2000+	0.39	0.40	0.4638	0.97	0.90–1.05	5,888		
	New MDD samples								0.14	0.83
	All MDD samples								0.018	0.39
	All MDD + BP samples								2.8×10^{-8}	0.05
	ALL MDD + BP (random effects)								9.82×10^{-5}	

Results are presented for each bipolar disorder and depression case-control cohort alongside results of fixed and, where indicated, random effects meta-analyses of each phenotype group.

an odds ratio (OR) of 0.92 (the upper 95% confidence limit reported by McMahon *et al.*[1]). The results of our meta-analysis of these independent MDD samples shows a convincing lack of association ($P = 0.14$).

We then carried out an additional meta-analysis across both our MDD samples and the McMahon *et al.*[1] MDD samples, which gave a non-significant $P = 0.018$ (with a Sidak-corrected $P = 0.087$). This suggests that there could be a trend for a weaker effect in major depression than in bipolar disorder, with OR = 0.96 and 95% CI 0.93–0.99, but we note that the 95% CI is nonoverlapping with the confidence interval for bipolar disorder and that small effects of this nature require huge sample sizes (that is, >100,000) for well-powered detection and replication.

However, further fixed- and random-effects meta-analyses do not support a homogeneity of effect for this locus between bipolar disorder and major depression. Although the addition of these three new MDD studies to those of BP and MDD reported in McMahon *et al.*[1] still gave genome-wide significant associations (defined as $P < 5 \times 10^{-8}$) under a fixed effects model ($P = 2.8 \times 10^{-8}$), there was increased and significant evidence for heterogeneity (Q statistic $P = 0.05$), indicating that a random effects analysis was required. Thus, we again used STATA to carry out a random effects meta-analysis and found that it gave $P = 9.82 \times 10^{-5}$ when we included all mood disorder samples. The results of all the meta-analyses are summarized in **Table 1**.

In summary, our heterogeneity analyses and random-effect meta-analysis showed clearly that McMahon *et al.*[1] were correct to claim rs2251219 as a genome-wide significant locus for BP, but our analyses and new data do not support its role in MDD and thus not mood disorders in general. Our attempt to independently replicate their finding in three MDD cohorts gave a non-significant $P = 0.14$ and had >80% power to replicate an effect within the 95% CI for rs2251219 for the mood disorders reported by McMahon *et al.*[1]. This demonstrates that there is a need for a more structured design of meta-analyses in the context of GWAS, first looking within disorders for evidence of association before meta-analyzing across disorders, as is currently being carried out by the Psychiatric Genetics Consortium. If this methodology had been adopted by McMahon *et al.*[1], it seems unlikely that they would have reported 3p21 as a locus for mood disorders in general rather than a genome-wide significant locus for bipolar disorder, which is what is strongly supported by their data.

URLs. METAL, http://www.sph.umich.edu/csg/abecasis/metal/; genetic power calculator, http://pngu.mgh.harvard.edu/~purcell/cgi-bin/gpc/; psychiatric genetics consortium, https://pgc.unc.edu/.

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AUTHOR CONTRIBUTIONS
G.B., C.M.L., I.P. and E.V. performed the data analysis. G.B., D.C. and P.M. wrote the manuscript. M.L.P., D.H.R.B., B.P., D.I.B. and P.F.S. provided additional replication data and comments.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Gerome Breen[1,2], Cathryn M Lewis[1,3], Evangelos Vassos[1], Michele L Pergadia[4], Douglas H R Blackwood[5], Dorret I Boomsma[6], Brenda Penninx[7], Patrick F Sullivan [8], Inti Pedroso[1,2], David Collier[1] & Peter McGuffin[1,2]

[1]Medical Research Council Social Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, King's College London, London, UK. [2]National Institute for Health Research Biomedical Research Centre, South London and Maudsley National Health Service Trust and Institute of Psychiatry, King's College London, London, UK. [3]Medical and Molecular Genetics, King's College London, London, UK. [4]Department of Psychiatry, Washington University School of Medicine, St. Louis, Missouri, USA. [5]Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburg, UK. [6]Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. [7]Department of Psychiatry, Vrije University Amsterdam, Amsterdam, The Netherlands. [8]Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA. Correspondence should be addressed to G.B. (g.breen@iop.kcl.ac.uk).

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McMahon *et al.* reply:

Breen *et al.* report that their re-analysis of our published data[1] supports association of rs2251219 with bipolar disorder (BP) at the $P < 10^{-8}$ level. However, in the independent samples they examined, this SNP did not show much evidence of association with major depressive disorder (MDD).

Neither result is surprising. Although we are confident in the analyses we performed using the data we had available, MDD is likely to be even more heterogeneous than BP[2], making a negative association result in individual samples very difficult to interpret. In our paper[1], we already stated that the association signal on 3p21.1 is more robust in BP than in MDD. If the genetic effect size is actually different in BP and MDD, as we suggested, then the power to replicate in each of the two disorders alone must be calculated separately. For their power calculation, Breen *et al.* use the upper confidence interval we reported for our combined analysis. This is almost certainly too high for MDD and would lead to an overestimation of the power to replicate in their sample. Therefore it is likely that their study is not adequately powered to support their strong conclusion.

Much larger sample sizes will ultimately be needed to reliably detect most loci having modest effects on risk[3]. This is one reason why it makes sense to group similar disorders as we did, especially when they so often run together in families. Additional kinds of data, such as gene expression and functional variants, should also be considered before reaching final conclusions[4,5]. We do agree with Breen *et al.* that large consortium efforts may ultimately offer a clearer picture, not because they are 'more structured' than our analysis, but simply because large consortia can muster even larger sample sizes.

It is becoming clear that SNP associations arising from large meta-analyses will often cross traditional diagnostic boundaries. Genes do not encode diseases, even when those diseases are much better validated than the clinical syndromes with which we work in psychiatry. The next big challenge for psychiatric genetics lies in the identification of higher risk alleles that may possess some diagnostic specificity. This will not be achieved in genome-wide association studies of common alleles but rather will require innovative approaches[6].

We thank Breen *et al.* for pointing out the strong evidence of association with bipolar disorder that emerges from our study and for pulling together the major depression data they present, which certainly have some value. They should press on until they have the necessary statistical power to draw truly convincing conclusions about replication or non-replication. Anything less misjudges the complexity of the problem.

Francis J McMahon[1], Nirmala Akula[1], Sven Cichon[2,3], Sevilla D Detera-Wadleigh[1], Howard Edenberg[4], Florian Holsboer[5], Markus M Nöthen[2,3], John I Nurnberger[4], James Potash[6], Martin Preisig[7], Marcella Rietschel[8] & Thomas G Schulze[1,8,9]

[1]Genetic Basis of Mood and Anxiety Disorders Section, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA. [2]Department of Genomics, Life and Brain Center, University of Bonn, Bonn, Germany. [3]Institute of Human Genetics, University of Bonn, Bonn, Germany. [4]Indiana University-Purdue University, Indianapolis, Indiana, USA. [5]Max-Planck Institute of Psychiatry, Munich, Germany. [6]Department of Psychiatry, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [7]University Hospital Center and University of Lausanne, Department of Psychiatry, Lausanne, Switzerland. [8]Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany. [9]Department of Psychiatry and Psychotherapy, University of Goettingen, Goettingen, Germany. Correspondence should be addressed to F.J.M. (mcmahonf@mail.nih.gov).

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