Lack of support for a genetic association of the *XBP1* promoter polymorphism with bipolar disorder in probands of European origin

To the editor:

Kakiuchi and colleagues[1] reported that variation in the gene *XBP1* contributes to susceptibility to bipolar affective disorder (BPAD). They identified a functional promoter polymorphism of *XBP1* (–116C→G) and reported association of the G allele with BPAD in 197 Japanese probands and 451 Japanese controls and in 88 trios from the National Institutes of Health (NIMH) Genetics Initiative collection of (mainly) European American families identified through a sibling pair with BPAD. They also reported evidence that constructs carrying the G allele resulted in an *in vitro* cellular phenotype that was reversed by valproate, an effective treatment for BPAD.

We examined the –116C→G polymorphism in four family-based BPAD samples comprising 586 families: (i) 147 families from the NIMH Genetics Initiative Waves 1 and 2, including the 88 trios studied by Kakiuchi *et al.*[1]; (ii) 176 families from the NIMH Wave 3, identified in a similar manner as the earlier waves; (iii) 173 Bulgarian trios, identified through a proband having DSMIV (ref. 2) bipolar I disorder (BPI); and (iv) 90 similarly identified UK parent-proband trios with DSMIV (ref. 2) BPI.

Our results do not support a significant association of –116C→G with BPAD in any of these four samples (**Table 1**). We reproduced the finding of Kakiuchi *et al.* in the trios that they studied but not in the complete NIMH Waves 1 and 2 sample from which those 88 trios were drawn. The subset of trios studied by Kakiuchi *et al.* has a significantly earlier age at onset of illness than the other Wave 1 and 2 samples. To account for this, we tested for an interaction between genotype and age at onset but found none. Consistent with the NIMH trio results, there was also no evidence for an interaction between genotype and age at onset in the Bulgarian or UK trios.

We also found no evidence for association of the G allele with BPAD in three case-control samples, comprising 1,181 individuals with DSMIV BPI and 1,717 nonpsychiatric ethnically matched controls (UK, 580 affected individuals and 617 controls; Germany, 300 affected individuals and 789 controls; Poland, 301 affected individuals and 311 controls). Data were consistent with Hardy-Weinberg equilibrium and distributions of genotypes and alleles were similar between cases and controls (**Supplementary Table 1** online).

Kakiuchi *et al.* examined eight markers to test for population stratification as a potential cause of their case-control findings. Similarly, we examined six SNPs and one STR marker, taken from unlinked chromosomal regions, with STRUCTURE[3]. We found no evidence for population stratification that might mask an association of *XBP1* in our data. Recent studies suggest that a larger number of markers is usually necessary to rule out population stratification[4,5].

The results in our trio samples from different European and European American populations are mutually consistent and not supportive of the finding of association by Kakiuchi *et al.*[1]. Inadequate power is an unlikely explanation of our results. Our combined family sample is approximately seven times larger than that used by Kakiuchi *et al.*, providing at least 80% power to detect at $P < 0.05$ a heterozygote relative risk of 1.32 (ref. 6; smaller than that estimated by Kakiuchi *et al.*[1]), if present in any one sample. Our findings are supported by our case-control sample, which is approximately six times larger than that used by Kakiuchi *et al.* Each sample had power >0.99 to replicate the effects reported by Kakiuchi *et al.* (calculated by the Genetic Power Calculator[6]).

How are we to reconcile our findings with those of the previous study? One possibility is that Kakiuchi *et al.* unwittingly chose probands with more severe illness, characterized by earlier onset, and that only in this subset is *XBP1* etiologically relevant. This is unlikely, because we found no interaction between genotype and age at onset and Kakiuchi *et al.* apparently used unselected cases for both their family-based and case-control studies. It is also possible

Table 1 Family-based association analysis of *XBP1* –116C→G

Sample	Number of trios or pedigrees	Transmission ratio (TDT)	Test-statistic[a,b]	*P* value[b]
Subset of NIMH Waves 1 and 2, used by Kakiuchi *et al.*[1]	88 trios	1.56	3.98	0.046
NIMH Waves 1 and 2 complete	147 pedigrees	–	1.61	0.2
NIMH Wave 3	176 pedigrees	–	0.07	0.79
Bulgarian trios	173 trios	0.97	0.03	0.86
UK trios	90 trios	0.86	0.45	0.5

[a]χ^2 with 1 degree of freedom. NIMH Waves 1, 2 and 3 were analyzed by PDT[7], as not all trios were complete and multiple siblings per family were used to extract the maximal information. All other samples consist of complete trios and were analyzed by TDT[8]. [b]For PDT tests, test statistics and *P* values using the 'average' option are shown. Results did not differ significantly with the 'sum' option.

that, although the finding of association by Kakiuchi *et al.* was in a sample of European ancestry, the –116 C→G polymorphism contributes to BPAD mainly in populations of Asian origin. Alternatively, it is possible that the polymorphism is in linkage disequilibrium with the true functional polymorphism and differential linkage disequilibrium patterns obscure the association. Although this is possible, the functional data that comprise much of the evidence presented by Kakiuchi *et al.* depend on –116C→G itself being the susceptibility variant.

The biological data in the original report remain interesting, but we believe our data indicate that the reported genetic association represents a type I error resulting from random variation in small samples. It is possible that there is a small, population-specific effect of *XBP1* on the development of BPAD. This would best be tested in a large, independent sample of Japanese origin.

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

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Sven Cichon[1,2], Silvia Buervenich[3], George Kirov[4], Nirmala Akula[3], Albena Dimitrova[4], Elaine Green[4], Johannes Schumacher[5], Norman Klopp[6], Tim Becker[7], Stephanie Ohlraun[8,9], Thomas G Schulze[8,9], Monja Tullius[9], Magdalena M Gross[9], Lisa Jones[10], Stefan Krastev[11], Ivan Nikolov[12], Marian Hamshere[4], Ian Jones[4], Piotr M Czerski[13], Anna Leszczynska-Rodziewicz[13], Pawel Kapelski[13], Ann Van Den Bogaert[2], Thomas Illig[6], Joanna Hauser[13], Wolfgang Maier[9], Wade Berrettini[14], William Byerley[15], William Coryell[16], Elliot S Gershon[17], John R Kelsoe[18], Melvin G McInnis[19], Dennis L Murphy[20], John I Nurnberger Jr[21], Theodore Reich[22], William Scheftner[23], Michael C O'Donovan[4], Peter Propping[5], Michael J Owen[4], Marcella Rietschel[8,9], Markus M Nöthen[1,2], Francis J McMahon[3] & Nick Craddock[4]

[1]Life & Brain Center, University of Bonn, Bonn, Germany. [2]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium. [3]Genetic Basis of Mood and Anxiety Disorders, Mood and Anxiety Program, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA. [4]Neuropsychiatric Genetics Unit, Department of Psychological Medicine, University of Wales College of Medicine Heath Park, Cardiff, UK. [5]Institute of Human Genetics, University of Bonn, Bonn, Germany. [6]Institute of Epidemiology, GSF-National Research Center for Environment and Health, Neuherberg, Germany. [7]Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany. [8]Division of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany. [9]Department of Psychiatry, University of Bonn, Bonn, Germany. [10]Department of Medical Genetics, Medical University of Birmingham, Queen Elizabeth Psychiatric Hospital, Birmingham, UK. [11]Department of Psychiatry, Medical University Sofia, Sofia, Bulgaria. [12] Biostatistics Bioinformatics Unit, Department of Psychological Medicine, University of Wales College of Medicine Heath Park, Cardiff, UK. [13]Department of Adult Psychiatry, University of Medical Sciences Poznan, Poznan, Poland. [14]Department of Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [15]University of California, Irvine, California, USA. [16]Department of Psychiatry, University of Iowa, Iowa City, Iowa, USA. [17]Department of Psychiatry, University of Chicago, Chicago, Illinois, USA. [18]Department of Psychiatry, University of California, San Diego, California, USA. [19]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University, Baltimore, Maryland, USA. [20]Laboratory of Clinical Science, Mood and Anxiety Program, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA. [21]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, Indiana, USA. [22]Department of Psychiatry, Washington University, St. Louis, Missouri, USA. [23]Department of Psychiatry, Rush University Medical Center, Chicago, Illinois, USA. Correspondence should be addressed to S.C. (sven.cichon@uni-bonn.de).

1. Kakiuchi, C. *et al. Nat. Genet.* **35**, 171–175 (2003).
2. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* 4th edn. (American Psychiatric Press, Washington, D.C., 1994).
3. Pritchard, J.K. *et al. Genetics* **155**, 945–959 (2000).
4. Turakulov, R. & Easteal, S. *Hum. Hered.* **55**, 37–45 (2003).
5. Freedman, M.L. *et al. Nat. Genet.* **36**, 388–393 (2004).
6. Purcell, S. *et al. Bioinformatics* **19**,149–150 (2003).
7. Martin, E.R. *et al. Am. J. Hum. Genet.* **67**, 146–154 (2000).
8. Spielman, R.S. *et al. Am. J. Hum. Genet.* **52**, 506–516 (1993).

In reply:

We previously concluded that an impaired XBP1 loop is a genetic risk factor for bipolar disorder[1] based on multiple lines of evidence: (i) downregulation of *XBP1* and *HSPA5* in twins discordant for bipolar disorder; (ii) reduced response of *XBP1* and *HSPA5* to endoplasmic reticulum (ER) stress in cells lines established from individuals with bipolar disorder; (iii) identification of a functional polymorphism, –116C→G, in the promoter of *XBP1*; (iv) association of this polymorphism with bipolar disorder in Japanese case-control samples; (v) confirmation of this association in a small number of European American trios; and (vi) improvement of the functional impairment due to the –116G allele by valproate. The findings of Cichon and colleagues, by genotyping enough samples of European origin to test our finding, indicate that the fifth finding was type I error. We agree that the association in Japanese individuals should be tested in larger number of independent samples.

So far, no genetic associations with bipolar disorder have been consistently replicated. The endophenotypes may be common among ethnicities, but the genetic risk factors responsible for the endophenotypes may be different between populations, as suggested by the difference in allele frequencies of –116C→G between European Americans and Japanese[1]. Thus, other genes in the ER stress response pathway also need to be examined.

We recently investigated the next candidate gene, *HSPA5*. Its expression was downregulated in affected twins, and its response to ER stress was reduced, like *XBP1* (ref. 1).

By screening all exons and the upstream region (1 kb) of *HSPA5* in 24 Japanese individuals with bipolar disorder, we found that the entire *HSPA5* gene was in one haplotype block consisting of four main haplotypes. By genotyping three key polymorphisms (–370C→T (nucleotide position from the

Table 1 ***HSPA5*** **haplotype frequencies in bipolar disorder**

	Japanese case-control samples					NIMH trios (n = 88)		
		All cases (n = 195)		With family history (n = 67)				
Haplotype	Controls (n = 254)	Cases	P	Cases	P	Freq. T	Freq. NT	P
1: C-del-C	0.116	0.135	0.375	0.141	0.426	0.074	0.069	0.835
2: C-del-T	0.076	0.128	0.010	0.201	0.000084	0.0057	0	0.239
3: C-G-C	0.368	0.325	0.185	0.276	0.043	0.38	0.41	0.583
4: T-del-T	0.439	0.410	0.388	0.380	0.222	0.52	0.51	0.914

Other haplotypes estimated from the NIMH samples were less than 2%. Haplotypes consist of three polymorphisms (–307C→T-rs3216733-rs12009). Freq. T or Freq. NT indicates the frequency of the transmitted or nontransmitted haplotype, respectively. *P* values were calculated by COCAPHASE.

transcription start site), rs3216733 (Gdel), and rs12009 (C→A)) in Japanese case-control samples (described previously[1]), we found that haplotype 2 (C-del-T) was significantly associated with bipolar disorder (P = 0.010). This association was stronger in affected individuals with family history (P = 0.000084; Table 1). This risk haplotype was extremely rare in the NIMH trio samples, and no association was found (Table 1).

These findings suggest that genetically determined interindividual variability of ER stress–response does relate to bipolar disorder, that there may be functional polymorphisms in other ER stress–response–related genes, in addition to the –116 polymorphism of *XBP1*, and that genetic risk factors may differ among populations.

The antimalaria drug mefloquine, which often causes an episode of depression or mania in susceptible individuals, was recently reported to cause ER stress in the brain[2]. Further investigations of the ER stress–response signaling system in the pathophysiology of bipolar disorder is warranted.

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Chihiro Kakiuchi[1], Shinichiro Nanko[2], Hiroshi Kunugi[3] & Tadafumi Kato[1]

[1]Laboratory for Molecular Dynamics of Mental Disorders, Brain Science Institute, RIKEN, Wako, Saitama 351-0198, Japan. [2]Department of Psychiatry, Teikyo University School of Medicine, Itabashi, Tokyo 173-8605, Japan. [3]Department of Mental Disorder Research, National Institute of Neuroscience, Kodaira, Tokyo 187-8502, Japan. Correspondence should be addressed to T.K. (kato@brain.riken.jp).

1. Kakiuchi, C. *et al.* Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. *Nat. Genet.* **35**, 171–175 (2003).
2. Dow, G.S., Hudson, T.H., Vahey, M. & Koenig, M.L. The acute neurotoxicity of mefloquine may be mediated through a disruption of calcium homeostasis and ER function in vitro. *Malar. J.* **2**, 14 (2003).

A national DNA bank in The Gambia, West Africa, and genomic research in developing countries

To the editor:

The Gambian National DNA Bank, the first National Bio-Bank developed in Africa, was funded in November 2000 by the Medical Research Council (MRC) as one of 14 DNA collection sites established to study the genetics of complex diseases. One of these sites is housed at the MRC Laboratories in The Gambia and has a special, though not exclusive, focus on malaria, HIV and tuberculosis. Additional projects include analyses of genome diversity in West African populations and a collection of twin-sister pairs to study the genetic basis of dizygotic twins (~2% of live births in the country). So far, more than 30,000 DNA samples have been collected, with many ongoing studies and more planned.

For the first time in a sub-Saharan country, a centralized structure and database for archiving DNA samples has been created, in collaboration with the Jean Dausset Foundation-CEPH. The bank is regulated by guidelines (**Supplementary Note** online) for sample collection, archiving, data storage and privacy protection, which were developed and approved by the MRC, the MRC Laboratories Scientific Coordinating Committee and by the Gambia Government/MRC Joint Ethics Committee. The Guidelines, which are enforced by these Committees, stemmed from the need to adapt to the local reality the many existing recommendations on bio-banking, privacy protection, genetics research and, generally, on medical research in developing countries (http://www3.who.int/whosis/genomics/pdf/genomics08.pdf, http://www.mrc.ac.uk/pdf-devsoc.pdf, http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf, http://www.nuffieldbioethics.org/publications/pp_0000000013.asp).

The Gambian DNA Bank promotes sharing of information and resources with centers around the world, and one of its ultimate goals is health improvement. In the short term, benefits should accrue to the participants in the studies. A recent example is a large project on genetic and environmental factors for susceptibility to tuberculosis, designed as a household association and family-based study and carried out in The Gambia, Guinea-Bissau and Guinea-Conakry[1]. The project focused on the systematic detection of tuberculosis cases in the families of individuals with tuberculosis and controls. Clinical services