

men (Table 1). Furthermore, we found a significant allele–dose effect per copy of haplotype 1 (odds ratio: 1.42; 95% CI: 1.05–1.91, $P=0.02$). The effect of haplotype 1 on anxiety without comorbid depression was even stronger. No relationship was observed with depressive symptoms (Table 1) or DSM-IV depressive disorder ($P=0.73$ for women and $P=0.72$ for men).

Studies of the putative association of ER α haplotypes and variations in behavior have been sparse. ER α polymorphisms have been associated with parent's report of anxiety at the age of 13–14 years.³ Other studies concentrated on personality traits rather than specific mood states.^{4,5} Furthermore, opposite findings were reported in men and women. A gender difference like in our study may be explained by the cessation of gonadal function in postmenopausal women, who have lower estrogen levels than men.⁶ Interestingly, our observations are supported by very detailed studies of behavior in ER α knockout mice.⁷ Female ER α knockout mice were more aggressive, whereas the respective male mice showed hardly any offensive attacks as compared to wild-type mice. At the same time, anxiety and fear levels were increased in female mice but not in male knockout mice. Further support for a role of the ER α in anxiety comes from studies of the receptor distribution in the human brain. ER α mRNA is expressed at relatively higher levels in the amygdala and the hypothalamus, both involved in emotional regulation.⁸

Our results suggest that ER α haplotypes may increase the risk of having an anxiety, but not a depressive, disorder. This is remarkable as anxiety is very frequently accompanied by symptoms of depression. Our observation could be a false-positive finding. However, the population-based design, the large sample, and the allele–dose effect support a true association. Furthermore, ER α haplotypes increased the likelihood of anxiety without significant comorbid depression. This is in line with studies showing that the perimenopausal period is characterized by mild affective symptoms, whereas the incidence of mood disorders does not increase.⁹ Moreover, the core features of the perimenopausal period and the perimenstrual syndrome are irritability, nervousness and tension rather than a depressed effect.¹⁰ Taken together, this suggests that ER α polymorphisms have a causal role in the anxious-irritable phenotype.

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Evidence of association between bipolar disorder and Citron on chromosome 12q24

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SIR – Bipolar disorder (BP) has a predominantly genetic etiology that has been supported by twin, adoption, and family studies. Several genome-wide linkage analyses conducted for BP have identified a variety of susceptibility loci, including chromosome 4p16, 12q24, 18p11.2, 18q22, 21q21, 22q11–13, and Xq26.¹ Many of these loci overlap with those found in schizophrenia (SZ), suggesting common genetic susceptibility factors for both BP and SZ.

In a Scottish family, a hereditary balanced (1;11)(q42.1;q14.3) chromosomal translocation is segregated with major mental illnesses including BP, recurrent major depression, and SZ.² The translocation leads to an interference in the open reading frame of the Disrupted-in-Schizophrenia-1 (DISC1) gene that could produce a C-terminal truncated DISC1 protein. In a previous study, we conducted a yeast two-hybrid screening in order to identify possible protein interactors involved with cellular signaling of DISC1.³ Among more than 30 interacting proteins, we selected *Citron* for further analyses because the gene encoding *Citron* is located on chromosome 12q24, a frequently reported locus linked with BP.^{4–6}

Citron is a synaptic and cytoskeletal protein that can potentially modulate glutamatergic neurotransmission,⁷ a hypothesized mechanism for BP. In dividing cells, *Citron* is required for cytokinesis and is also implicated for neurogenesis.⁸ Considering its chromosomal location, interaction with DISC1, and possible roles in the synapse, we hypothesize that *Citron* may be one of several important candidate genes for BP. Therefore, we conducted a family-based association study of the *Citron* gene and BP.

Table 1 FBAT Results for Citron; 12q24.23

LD	dbSNP-ID	CELERA ID	Location ^a	SNP ^b	MA ^c	Freq. ^c	obs/exp ^c	P-value ^d	Haplotypes over-transmitted to affected offspring					Haplotypes under-transmitted to affected offspring						
■	rs278124	hCV1078831	p-telomere	T/C	C	0.48			229	229	0.044	0.85	0.47	0.40	90	107	74	91	89	80
■	rs2285595	hCV3259859	Intron46	T/C	C	0.47			217	185	0.031	0.47	0.41	101	122	84	101	100	92	
■	rs278109	hCV1078854	Intron31	G/A	A	0.45			339	174	0.065	0.053		165						
■	rs2074052	hCV2547173	Exon26-R/R	G/C	G	0.41			209	0.065	0.053									
■	rs203368	hCV2626794	Intron19	G/A	A	0.16	89/104	0.020	357	222	0.031	0.47	0.40	175	175	175	175	175	175	
■	rs435136	hCV2626820	Intron13	T/C	C	0.19	97/114	0.012	339	209	0.031	0.47	0.40	165	165	165	165	165	165	
■		hCV3259834	Intron9	G/A	A	0.44			229	222	0.031	0.47	0.40	175	175	175	175	175	175	
■			Intron5	G/A	A	0.44			217	174	0.065	0.053								
■			q-telomere						0.47	0.47	0.47	0.47	0.40	0.40	0.16	0.12	0.10	0.12	0.14	0.12
								Obs. Exp. ^d												
								P-value												
								Frequency												

■ $D' > 0.95$.■ $0.85 < D' < 0.95$.^aRelative to GenBank AY681966.^bOn human citron coding strand.^cMinor allele.^dBold type indicates $P < 0.05$.

In this study, we tested for association in the National Institutes of Mental Health (NIMH) Genetics Initiative wave 3 and wave 4 pedigrees, previously described.^{9,10} The sample set available to us consisted of 307 nuclear families consisting of 1012 individuals, including probands diagnosed with BPI, BP, schizoaffective disorder, or unipolar depression. We chose seven common single nucleotide polymorphisms (SNPs) roughly equidistant across the 195 kb *Citron* gene. These included rs278124, rs2285595, rs278109, rs2074052, rs203368, rs435136 (NCBI), and hCV3259834 (Celera). Taqman Assays-on-Demand (Applied Biosystems) were used to genotype all samples. Raw genotype data were checked for errors using MERLIN (<http://www.sph.umich.edu/csg/abecasis/Merlin/index.html>). Individuals with unlikely recombination events and families with Mendelian errors were excluded from our analysis. Marker-to-marker linkage disequilibrium (LD) and Hardy-Weinberg equilibrium were determined using Haploview (<http://www.broad.mit.edu/mpg/haploview/index.php>). Pedigrees were analyzed for the presence of association using the Family-Based Association Test (FBAT), a version of a transmission disequilibrium test (TDT) proposed by Rabinowitz and Laird (2000), to detect preferential transmission of single-markers and haplotypes to affected offspring. Monte-Carlo P -values were obtained in FBAT for single markers and haplotypes using 10 000 simulations. We analyzed haplotypes outlined by Haploview based on D' measures of LD. We found evidence for transmission distortion for two individual SNPs, rs203368 ($P = 0.020$) and rs435136 ($P = 0.012$). Strong LD was observed between markers rs2285595, rs278109, rs203368, rs435136, and hCV3259834. We tested haplotypes composed of these markers and found that our single marker associations were reinforced with preferential transmissions to affected offspring in two- to five-marker haplotypes, which include rs203368 and rs435136 ($0.011 < P < 0.065$) (Table 1). No haplotypes without at least one of these markers were found to be significant.

To our knowledge, this study provides the first evidence of a candidate gene for BP on chromosome 12q24, a linkage locus for BP common to many studies. Our evidence suggests a possible association of the *Citron* gene and BP in a family-based sample set. Of particular interest are two SNPs (rs203368 and rs435136) in the proximity of exons that code for the DISC1 binding domain on *Citron*. The rs203368 and rs435136 polymorphisms may have a key role in biology, but it is more likely that these are markers of causal genetic variation. If *Citron* plays a role in the pathophysiology of BP, we can predict at least two scenarios. First, the variation in the *Citron* gene may lead to abnormal expression, and result in altered *Citron* function. Second, we consider that the two polymorphisms are in LD with their surrounding region, which corresponds to the coding region for the DISC1 binding domain on *Citron* (between amino acids 499 and 680

(unpublished observation: AK and AS). These SNPs may either directly influence the binding of DISC1 and *Citron* proteins, or they may be markers of polymorphism(s) responsible for modulating binding. Alternatively, we found the SNPs to have an association with BP in the study may reflect causal genetic variation in an adjacent gene. It is conceivable that *Citron* is one of a cluster of susceptibility genes for BP in the chromosomal region of 12q23–24. Together, our studies warrant further study of *Citron* and adjacent genes in larger samples and in other ethnic groups in the investigation of a possible etiology for major mood disorders.

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The *BDNF* val66met polymorphism is not associated with late onset Alzheimer's disease in three case-control samples

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SIR—Brain-derived neurotrophic factor (BDNF) has been implicated in hippocampal anatomy and plasticity, learning and memory. *BDNF*, located on chromosome 11p13, contains a common, functional single nucleotide polymorphism (SNP), rs6265, at codon 66 (val66met) of the pro-region of the protein, which appears to exert an effect on intracellular trafficking and activity-dependent secretion of BDNF.¹ Individuals carrying the methionine allele have impaired hippocampal function and volume and poor episodic memory.¹ Memory deterioration and hippocampal pathology are prominent features of late-onset Alzheimer's disease (LOAD), and this has prompted several groups to seek association between *BDNF* polymorphisms and Alzheimer's disease (AD). Association between the val66met polymorphism in *BDNF* and LOAD was claimed,² and positive associations of the 270C/T polymorphism, in the 5'-noncoding region of the *BDNF* gene have also been reported in two different sample sets.^{3,4} Other studies have not found evidence for association of either the val66met SNP^{5–8} or the 5'-noncoding 270C/T SNP with AD.^{8,9} The studies with lack of association were based on samples of very different ethnic backgrounds including sample sets from Brazil, China, Italy, and Spain. This and the fact that samples of relatively small size were employed in these studies make it difficult to definitively assess the potential association of the *BDNF* SNPs with LOAD. As a result of its suggested functional significance in AD and other psychiatric disorders such as schizophrenia, bipolar disorder, and anxiety, we chose to genotype the val66met SNP in three independently collected LOAD case control series of Caucasian descent, totaling 2041 individuals (935 cases vs 1106 controls) and examined whether the SNP is associated with LOAD.

The three case-control series were the WU series, collected through the Washington University Alzheimer's Disease Research Center (ADRC) patient registry, the UK series, collected as part of the MRC LOAD Genetic Resource by Cardiff University, Wales College of Medicine and King's College London, and the UCSD series, collected through the ADRC of the University of California, San Diego. Cases in these series have a clinical diagnosis of dementia of the Alzheimer's type according to NINCDS-ADRDA or similar criteria with a minimum age of onset of 65 years. Nondemented controls have a full neuropsychological and clinical

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