Lack of association between variations in the melanocortin 5 receptor gene and bipolar disorder

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Objective **The melanocortin 5 receptor gene maps to the bipolar susceptibility locus on chromosome 18p11.2. Given the biological role of melanocortins and their influence on the hypothalamic–pituitary–adrenal axis, the melanocortin 5 receptor gene is a plausible candidate gene for bipolar disorder. We tested the hypothesis that the potential functional variation Phe209Leu confers susceptibility to bipolar disorder in a case–control study.**

Methods **Genotypes for two variations in the coding region and one variation approximately 7 kb upstream from the coding region were obtained from 345 unrelated bipolar I patients and 275 control samples. Genotypes and allele frequencies were compared between groups using χ^2 contingency analysis.**

Results **Allele frequencies of the Phe209Leu polymorphism did not differ significantly between bipolar patients and controls ($P = 0.679$). Allele frequencies of the C744T and the intergenic A/G polymorphism did not differ significantly between bipolar patients and controls. All variations were in strong linkage disequilibrium.**

Conclusion **Variations in the melanocortin 5 receptor gene are unlikely to confer susceptibility to bipolar disorder in this sample. Further studies are required to elucidate the susceptibility locus for bipolar disorder on chromosome 18p11.** *Psychiatr Genet* **15:255–258** © **2005 Lippincott Williams & Wilkins.**

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Introduction

Family, adoption and twin studies show that bipolar disorder (BPD) has a strong genetic component (Craddock and Jones, 1999). Genetic causes have been difficult to elucidate, however, because of the complex mode of inheritance and genetic heterogeneity. Linkage studies have repeatedly suggested a susceptibility locus for BPD in the pericentromeric region of chromosome 18 (Berrettini *et al.*, 1994; Stine *et al.*, 1995; Nothen *et al.*, 1999; Bennett *et al.*, 2002). Several candidate genes in that region have been previously investigated but no associated functional variation has so far been identified (Berrettini *et al.*, 1998; Rojas *et al.*, 2000; Yoshikawa *et al.*, 2000; Reyes *et al.*, 2002).

The melanocortin 5 receptor (MC5R) gene maps to the bipolar susceptibility locus on chromosome 18p11.2 (Chowdhary *et al.*, 1995) and encodes a G-protein-coupled receptor widely expressed in the brain (Fathi *et al.*, 1995). The melanocortin system stimulates the hypothalamic–pituitary–adrenal axis (Kim *et al.*, 2000; Dhillo *et al.*, 2002). Dysregulations of this axis are associated with several psychiatric disorders, including mania and depression as seen in BPD (Kathol *et al.*, 1989; Swann *et al.*, 1992; Plotsky *et al.*, 1998; Ehlert *et al.*, 2001). The MC5R gene is an intronless gene of 978 base pairs and encodes 325 amino acids (Fig. 1). A missense mutation at position C627G induces an amino acid substitution Phe209Leu (Haga *et al.*, 2002; dbSNP #2236700). In this study, we tested the hypothesis that the potential functional variation Phe209Leu confers susceptibility to BPD.

Materials and methods

Participants

Three hundred and forty-five unrelated bipolar I patients participated in this study. Patients were collected at centers participating in the National Institute of Mental Health (NIMH) Genetics Initiative on Bipolar Disorder and carried a diagnosis of bipolar I disorder defined by Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) criteria (American Psychiatric Association, 1994). The key criterion for admission of a family to the study was a working diagnosis of bipolar I disorder in two or more siblings. Background and detailed methodology for the NIMH Genetics Initiative are described elsewhere (NIMH Genetics Initiative Bipolar Group, 1997). No evidence was found for linkage of BPD to the 18p region in this sample. All study participants were assessed with the Diagnostic Instrument for

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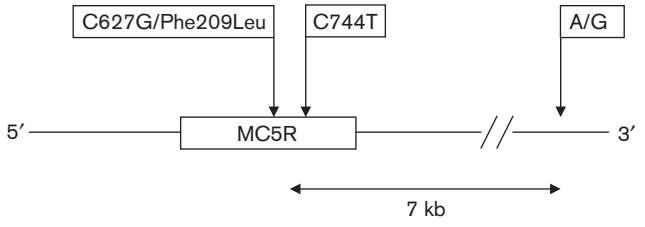
Fig. 1



Variations in the melanocortin 5 receptor (MC5R) gene.

Genetic Studies (Nurnberger *et al.*, 1994). Family history information was obtained through the Family Interview for Genetic Studies and medical records were requested. A final best-estimate diagnosis was made using all available information including medical records, information from relatives, and the Diagnostic Instrument for Genetic Studies interview, by two independent senior diagnosticians, adhering to DSM-IV criteria. Two hundred and seventy-five control samples were obtained from healthy individuals with no history of psychiatric or chronic neurological disease. All cases and controls were of European descent.

Proper informed consent was obtained from all individuals in accordance with Institutional Review Board procedures. Peripheral blood samples were obtained and genomic DNA was extracted from peripheral leukocytes by standard procedures.

DNA analyses

Review of the public database, the Celera database and the literature revealed three single-nucleotide polymorphisms (SNPs) in the coding region of the MC5R gene (accession number NM 005913). Genotyping of two SNPs in the coding region of the MC5R gene, and one additional SNP approximately 7 kb upstream from the missense mutation, was performed using the Applied Biosystems (Foster City, California, USA) 'Assays-on-demand' SNP genotyping assay, as per the manufacturer's protocol (C627G/Phe209Leu: assay ID: C__15954927_10/dbSNP: rs2236700; C744T: C__15954928_10/rs2236701; A/G intergenic: C__7490731_10/rs1787861).

The Applied Biosystems 'Assays-on-demand' SNP genotyping system is based on a combination of the TaqMan technology and the ABI PRISM 7700 (Applied Biosystems) real-time sequence detection system. The use of two primers and an internal labeled TaqMan probe combined with the 5′–3′ nuclease activity of *Taq* DNA polymerase allows direct quantitation of the polymerase chain reaction product accumulation by the detection of a fluorescent reporter released during the exponential amplification phase of the polymerase chain reaction. As increase of the fluorescence intensity of the reporter dye is achieved only when probe hybridization and amplification of the target sequence have occurred, the TaqMan assay offers a sensitive method to determine the presence or absence of specific sequences.

Statistical analyses

Genotypes and allele frequencies were compared between groups using χ^2 contingency analysis. A two-tailed type I error rate of 5% was chosen for the analysis. No correction for multiple testing was made because of the explorative approach to a genetically complex disorder in which a phenotype–genotype relationship has not been established (Perneger, 1998). Our sample size had greater than 81% power to detect a disease association at a *P*-value less than or equal to 0.05, assuming an odds ratio of 1.5 (see later Table 2). Power analysis was performed using the *Quanto* program (Gauderman, 2002). Linkage disequilibrium (LD) was calculated using the *2ld* program (Zapata *et al.*, 2001).

Results

None of the genotype counts deviated significantly from those expected from Hardy–Weinberg equilibrium. Genotype and allele frequencies of the two exonic variations and the 7 kb upstream intergenic A/G variation did not differ significantly between bipolar patients and controls (Table 1). Haplotype analysis showed that no combinations of alleles were associated with illness. The variations in the coding region C627G/Phe209Leu and C744T were in strong LD ($D' = 1.00$) and in strong LD ($D' = 0.93$) with the 7-kb upstream intergenic A/G variation.

Discussion

The MC5R gene is an interesting candidate gene for BPD not only on the basis of its chromosomal location, but also on the basis of its biological function. Disruption of the protein structure of the receptor could lead to a different ligand affinity and malfunction downstream of the melanocortin system, which is involved in the hypothalamic–pituitary–adrenal axis. We investigated the role of an amino acid substitution polymorphism in a population-based case–control association study.

Association studies are a powerful method to detect genes that are involved in the etiology of complex traits like BPD (Lander and Schork, 1994). In particular, association studies are most powerful when a pathophysiologically plausible candidate gene is examined and a functional polymorphism is studied that itself might mediate the predisposing effect. Although no information is available on the biological effect of mutations in the MC5R gene, the Phe209Leu variation might be functional, given its location in the important fifth transmembrane domain of the receptor and the relatively high sequence conservation among species.

Table 1 Genotype and allele frequencies of two exonic single nucleotide polymorphisms (SNPs) and one intergenic SNP in the melanocortin 5 receptor gene

SNP	Sample	*n*	Genotype frequency			Allele frequency	*P**
C627G (Phe209Leu)			C/C	C/G	G/G	*f* (C)	
	Bipolar	345	0.658	0.319	0.023	0.817	0.679
	Controls	268	0.668	0.317	0.015	0.826	
C744T			C/C	C/T	T/T	*f* (C)	
	Bipolar	345	0.661	0.316	0.023	0.819	0.581
	Controls	272	0.676	0.309	0.015	0.831	
7 kb upstream intergenic A/G			G/G	G/A	A/A	*f* (G)	
	Bipolar	342	0.561	0.380	0.059	0.751	0.384
	Controls	275	0.600	0.345	0.055	0.773	

* Type I error rates for comparison of allele frequencies between bipolar patients and controls.

LD is the central concept of association studies. LD between SNPs is unlikely to extend beyond an average distance of approximately 10 kb (Patil *et al.*, 2001). The MC5R gene is an intronless gene of 978 base pairs and the Phe209Leu polymorphism is located approximately in the middle of the gene. The Phe209Leu SNP is in strong LD with the C744T SNP and the 7 kb upstream located A/G SNP. Given the assumption that LD extends just about 5 kb upstream and downstream from the investigated SNP, we would expect to see an increase in the Phe209Leu variation in patients, even when that mutation is not the causative variation. We observed no difference in allele frequencies of the two coding variations between bipolar patients and controls, and demonstrated strong LD throughout the gene extending about 7 kb upstream, thus reducing the likelihood that variants in the promoter region or 3′-untranslated region are associated with illness; however, direct search for sequence variations in bipolar cases might be necessary in order to detect rare variations affecting illness.

Although the sample size may not be sufficient to detect small or rare allelic effects, there was no indication for a trend towards statistical significance and we had good power to detect moderate genetic effects (Table 2). Larger sample sizes in the thousands are required for future studies of genes with small effects. Population stratification is unlikely to play a relevant confounding role in the population; however, a more accurate control would be the use of family-based association design that matches the genotype of an affected offspring with those parental alleles not inherited by the offspring (Spielman and Ewens, 1996).

Even with narrow defined entry criteria for the study, BPD is a spectrum disorder with probable multiple genes involved, each contributing only a small fraction to the overall risk. Further dissection of the patient group using endophenotypes would greatly increase the yield for specific candidate genes but would also require a much larger sample size.

Table 2 Power analysis for the case–control study with a two-tailed significance level of 0.05

Sample size	Minor allele frequency	R_G	Recessive	Dominant	Log additive
300	0.10	1.5	0.08	0.53	0.61
300	0.18	1.5	0.16	0.66	0.81
300	0.30	1.5	0.33	0.68	0.90
300	0.18	2.0	0.41	0.98	0.99

In summary, we showed that variations in the MC5R gene are not associated with BPD in this group of patients and controls. Additional studies are required to elucidate the susceptibility locus for BPD on chromosome 18p11.

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