

Association analysis of CHMP1.5 genetic variation and bipolar disorder

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Objectives The 18p11.2 region surrounding the G-olf gene has been linked in several independent studies to bipolar disorder and schizophrenia, yet association studies between G-olf genetic variations and bipolar disorder have been negative. We hypothesized that the linkage in this region might be due to a gene in close physical proximity to G-olf, and we examined variations in the CHMP1.5 gene within intron 5 of G-olf for association with bipolar disorder.

Methods Two single-nucleotide polymorphisms, rs1786581 and rs1249624, were analyzed for association with bipolar disorder in 402 unrelated bipolar individuals and 181 unrelated controls. Genotyping was performed via pyrosequencing and restriction fragment length polymorphism analysis; results were compared by χ^2 contingency analysis.

Results No evidence was found for association of either allele at rs1249624 with bipolar disorder ($\chi^2 = 1.25$, degrees of freedom = 1, $P = 0.26$); however, a trend towards association with the 'T' allele at rs1786581 and with the 'T/T' 1786581/1249624 haplotype was observed. The χ^2 for the haplotype was 7.16, (degrees of freedom = 3, $P = 0.067$) and

for rs1786581 $\chi^2 = 3.56$, degrees of freedom = 1, $P = 0.060$; these differences are not statistically significant.

Conclusions Variation in the CHMP1.5 gene does not appear to be associated with bipolar disorder. A systematic assessment of genetic variation in the region using association studies will be necessary. *Psychiatr Genet* 15:211–214 © 2005 Lippincott Williams & Wilkins.

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Introduction

Bipolar disorder is a severe psychiatric disorder affecting approximately 1% of the population worldwide (Robins *et al.*, 1984). Despite extensive investigation, the exact causes of bipolar disorder remain unknown. Segregation analyses and twin studies suggest a strong genetic component (Goldin *et al.*, 1983; Taylor *et al.*, 2002; McGuffin *et al.*, 2003). Susceptibility regions have been identified by several genome-wide scans; suggestive linkage to many chromosomal regions has been reported, but the results are often inconsistent between studies (Ewald *et al.*, 2002; Dick *et al.*, 2003; Potash *et al.*, 2003; Segurado *et al.*, 2003). One of the most well-replicated areas of linkage to bipolar disorder is the 18p11.21 region (Berrettini, 2003). Several groups found suggestive evidence for linkage of bipolar disorder to the 18p11.2 region using both parametric logarithm of odds (LOD) score methods and non-parametric linkage analysis (Berrettini *et al.*, 1994; Stine *et al.*, 1995; Detera-Wadleigh *et al.*, 1999; Nothen *et al.*, 1999). Several negative studies are also found in this region; this is probably because of the smaller sample size in several of these studies, and

the fact that bipolar disorder is probably genetically heterogeneous and caused by different interacting genes in different populations (Knowles *et al.*, 1998; Baron and Knowles, 2000; Nancarrow *et al.*, 2000). Linkage and linkage disequilibrium (LD) of schizophrenia to this region have also been reported; a study by Schwab *et al.* (1998) found evidence of linkage disequilibrium between schizophrenia and a microsatellite CA repeat marker within intron 5 of the G-olfactory gene, G-olf. G-olf is the α subunit of a G protein that is highly homologous to G α S, a stimulatory G-protein whose GTPase activity is inhibited by lithium, the standard treatment for bipolar disorder (Wang and Friedman, 1999). G-olf also couples to D1 dopamine receptors in the striatum (Zhuang *et al.*, 2000). For these reasons, G-olf was examined for association with bipolar disorder by several groups (Tsiouris *et al.*, 1996; Berrettini *et al.*, 1998; Zill *et al.*, 2003). No coding single-nucleotide polymorphisms (SNPs) of G-olf were identified, and two intronic SNPs did not appear to be associated with bipolar disorder in several pedigrees (Tsiouris *et al.*, 1996; Berrettini *et al.*, 1998; Zill *et al.*, 2003). G-olf intron 5 was examined for

the presence of novel transcripts via direct sequencing and cDNA selection (Rojas *et al.*, 2000) and several transcripts were discovered, including clone 1C8, which matched gene CHMP1.5 when compared with the National Institute of Biotechnology Information (NCBI) non-redundant database using the basic local alignment search tool (BLAST) algorithm. CHMP1.5 is a small, intronless gene encoding a transcript of 199 amino acids within intron 5 of the G-olf gene (Vuoristo *et al.*, 2001). It is a member of the CHMP1 family of membrane proteins, and appears to be involved in vesicle trafficking and cell cycle progression (Stauffer *et al.*, 2001). Because of its close proximity to the G-olf CA repeat marker, (~9 kb upstream) we decided to investigate the possible role of CHMP1.5 in bipolar disorder by conducting a case-control association study.

Materials and methods

Study participants

The bipolar sample consisted of 402 unrelated European-American individuals from around the United States gathered by the National Institute of Mental Health (NIMH) as part of the NIMH Genetics Initiative on Bipolar Disorder. The mean age of the males was 46.2 ± 15.3 years ($n = 178$), and the mean age of the females was 48.1 ± 10.2 years ($n = 224$). All bipolar individuals had a diagnosis of bipolar I according to Diagnostic and Statistical Manual of Mental Disorders IV criteria. All individuals also had at least one sibling with bipolar I or schizo-affective disorder. Control DNA samples were purchased from the Coriell Institute as part of the Human Variation Caucasian Panel 200. The mean age of the men was 45.4 ± 10.6 years ($n = 88$), and the mean age of the women was 44.4 ± 12.2 years ($n = 93$). This study was performed in compliance with standards set forth by the institutional review board at the University of Pennsylvania, and all samples were used with informed consent.

Single-nucleotide polymorphism identification

Two SNPs were identified using the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>): rs1786581, a C→T (5'-3') transition at bp 735 in the 3' untranslated region of CHMP1.5, and rs1249624, a T→C (3'-5') transition in G-olf intron 5.

DNA amplification

Polymerase chain reaction (PCR) was performed with 100 ng of DNA in a total volume of 50 μ l containing 100 ng of each primer, 5 μ l 5M betaine, 10 mM dNTPs, and 5 μ l reaction buffer containing MgCl₂. Thirty-five cycles were run at 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and an initial hot start step at 94°C for 5 min and a final extension step at 72°C for 7 min were also performed. The following primers were used to amplify a 624 bp product containing SNP rs1786581: forward primer, 5'-GATGCGACATTGAAGACC-3'

and reverse primer, 5'-CTGATCATTCTGGGCAGG-3'. Primers 5'-GGAACCCAGATGATACTGTGT-3' (forward) and 5'-TGGAGTTTCGCTCTT GTTGCC-3' (reverse) were used to amplify a 441 bp product containing G-olf intron 5 SNP rs1249624.

Genotyping

SNP rs1249624 was genotyped by restriction fragment length polymorphism analysis; the 'C' allele destroyed a *BsMB* I site. G-olf intron 5 PCR products were incubated with *BsMB* I at 55°C overnight. Restriction enzyme digest products were analyzed by electrophoresis on a 5% non-denaturing polyacrilamide gel, stained with ethidium bromide, and photographed. SNP rs1786581 was assayed via pyrosequencing. The CHMP 1.5 PCR was performed using a biotinylated version of the reverse primer at a concentration of 65 ng/ μ l. The biotinylated PCR products were then incubated with binding buffer and sepharose beads, denatured, annealed to a sequencing primer (5'-CTCTGTGTGTTAGAGAG-3') 3 bp from the SNP, and analyzed as per the manufacturer's instructions.

Statistical analysis

Chi-squared analysis was performed using the online χ^2 calculator at <http://www.unc.edu/~preacher/>. Haplotype frequencies were estimated using the Estimating Haplotypes program (Curtis and Sham, 1995) and linkage disequilibrium was calculated using 2LD (Zhao, 2004). Power analysis was performed using Quanto Version 0.5 (Gauderman, 2002).

Results

Bipolar individuals ($n = 402$) and control individuals ($n = 181$) were analyzed in a case-control study using one SNP from the 3' UTR of CHMP1.5 (rs1786581) and one SNP separated by 3.5 kb in G-olf intron 5 (rs1249624). These SNPs were estimated to be in tight linkage disequilibrium with one another ($D' = 0.94$, $SD = 0.038$) and were analyzed individually as well as together for a haplotype study. Genotype and allele frequencies for SNPs rs1786581 and rs1249624 were compared in bipolar and control individuals using χ^2 analysis, and these results are presented in Table 1. A significant difference in genotype or allele frequencies between cases and controls was not observed for either SNP, nor for the haplotype. For SNP rs1786581, the χ^2 was 5.2 ($df = 2$, $P = 0.07$) for genotype frequencies and 3.56 ($df = 1$, $P = 0.06$) for allele frequencies. For SNP rs1249624, the χ^2 was 2.46 ($df = 2$, $P = 0.29$) for genotype frequencies and 1.25 ($df = 1$, $P = 0.26$) for allele frequencies. All genotypes were found to be in Hardy-Weinberg equilibrium. The rs1786581-rs1249624 haplotype frequencies calculated by the Estimating Haplotypes program are summarized in Table 2. The χ^2 for the haplotypes was 7.16 ($df = 3$, $P = 0.07$). A power analysis estimating power to detect association in

Table 1 Genotype and allele frequencies of bipolar patients and controls, rs1786581, rs1249624

SNP	Group	n	Genotypes ^a			Alleles ^b	
			1	2	3	1	2
rs1786581	Bipolar	402	293 (73)	101 (25)	8 (2)	687 (85)	117 (15)
	Control	181	147 (81)	30 (17)	4 (2)	324 (90)	38 (10)
rs1249624	Bipolar	402	221 (55)	154 (38)	27 (7)	596 (74)	208 (26)
	Control	181	95 (52)	67 (37)	19 (11)	257 (71)	105 (29)

Genotype distributions and allele distributions for rs1786581 and rs1249624 presented as counts (%). Genotypes 1, 2, and 3 are wild-type, heterozygote, and homozygote for the minor allele, respectively. Allele 1= major allele, allele 2= minor allele. rs1249624: ^aGenotype distribution (bipolar disorder patients versus controls), $\chi^2=5.2$, degrees of freedom=2, $P=0.07$. ^bAllele distribution (bipolar disorder patients versus controls), $\chi^2=3.56$, degrees of freedom=1, $P=0.06$. rs1249624: ^aGenotype distribution (bipolar disorder patients versus controls), $\chi^2=2.46$, degrees of freedom=2, $P=0.29$. ^bAllele distribution (bipolar disorder patients versus controls), $\chi^2=1.25$, degrees of freedom=1, $P=0.26$.

Table 2 Estimated haplotype frequencies of rs1786561 (C/T) and rs1249624 (T/C) among bipolar patients and controls

Haplotypes	Patients		Controls	
	No association	Association	No association	Association
C,T	0.628	0.590	0.633	0.610
C,C	0.225	0.264	0.262	0.285
T,T	0.108	0.147	0.074	0.098
T,C	0.039	0.000	0.031	0.007

Table 3 Power analysis

Genetic effect (R_G)	Mode of inheritance		
	Recessive	Dominant	Log-additive
1.5	0.10	0.52	0.63
2.0	0.21	0.94	0.98
2.5	0.36	1.00	1.00

Power analysis was conducted with the following parameters: allele frequency (q_a)=0.13, sample size=402, population frequency (K_p)=0.01, significance level=0.05, two-sided.

dominant, recessive and log-additive modes of inheritance was conducted and estimates are summarized in Table 3.

Discussion

18p11.2 is a region of the human genome with replicated linkage to bipolar disorder, yet a genetic variation at this locus that is associated with bipolar illness remains to be identified. CHMP1.5 is a plausible candidate gene for association with bipolar disorder on the basis of its chromosomal location and potential function in neuronal transmission. It is in close proximity to a G-olf intron 5 microsatellite marker previously associated with a broad definition of schizophrenia, and lies between two of the most commonly linked markers to bipolar disorder on 18p, D18S53 and D18S37. Using a case-control design, we found that variations in and flanking CHMP1.5 are not significantly associated with bipolar disorder. This lack of association could be explained by several factors. It is possible that CHMP1.5 is truly not associated with bipolar illness and the predisposing gene in this region

remains to be elucidated. The variations in CHMP1.5 lie far enough from the previously linked bipolar chromosome 18 loci (> 100 kb) so that they are probably not in linkage disequilibrium with those loci and therefore do not deter from those positive linkages. It is possible that the study design had a lack of power to detect association, because of a rare allele or small sample size. A power analysis (Table 3) predicted sufficient power to detect association of an allele with a genetic effect of 2.0 or greater in a dominant or log-additive mode of behavior, but not a recessive mode. Segregation analyses of bipolar disorder favor a model of genetic heterogeneity over Mendelian inheritance; this indicates that an additive mode of inheritance might best explain the genetic transmission of bipolar disorder, but the exact mode of transmission is still unknown. Bearing this in mind, if the genetic effect of a CHMP1.5 allele was 1.5, our study might not have detected it. Yet, it is worth noting that although neither SNP rs1786581, rs1249624, nor the haplotype were significantly associated with bipolar disorder, the rs1786581 'T' allele and the 'T/T' 1786581/1249624 haplotype containing it showed a trend to linkage disequilibrium. Thus, although our findings did not support a role for variations in CHMP1.5 in the etiology of bipolar disorder, further examinations of the 18p11.2 region are warranted.

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