ORIGINAL ARTICLE

Association Study of Wnt Signaling Pathway Genes in Bipolar Disorder

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Context: The Wnt signaling pathways promote cell growth and are best known for their role in embryogenesis and cancer. Several lines of evidence suggest that these pathways might also be involved in bipolar disorder.

Objective: To test for an association between candidate genes in the Wnt signaling pathways and disease susceptibility in a family-based bipolar disorder study.

Design: Two hundred twenty-seven tagging single-nucleotide polymorphisms (SNPs) from 34 genes were successfully genotyped. Initial results led us to focus on the gene *PPARD*, in which we genotyped an additional 13 SNPs for follow-up.

Setting: Nine academic medical centers in the United States.

Participants: Five hundred fifty-four offspring with bipolar disorder and their parents from 317 families.

Main Outcome Measures: Family-based association using FBAT and HBAT (http://www.biostat.harvard.edu/~fbat/default.html; Harvard School of Public Health, Boston, Massachusetts). Exploratory analyses testing for interactions of *PPARD* SNPs with clinical covariates and with other Wnt genes were conducted with GENASSOC (Stata Corp, College Station, Texas).

Results: In the initial analysis, the most significantly associated SNP was rs2267665 in *PPARD* (nominal $P<.001$). This remained significant at $P=.05$ by permutation after accounting for all SNPs tested. Additional genotyping in *PPARD* yielded 4 SNPs in 1 haplotype block that were significantly associated with bipolar disorder ($P<.01$), the most significant being rs9462082 ($P<.001$). Exploratory analyses revealed significant evidence ($P<.01$) for interactions of rs9462082 with poor functioning on the Global Assessment Scale (odds ratio [OR], 3.36; 95% confidence interval [CI], 1.85-6.08) and with SNPs in *WNT2B* (rs3790606: OR, 2.56; 95% CI, 1.67-4.00) and *WNT7A* (rs4685048: OR, 1.79; 95% CI, 1.23-2.63).

Conclusions: We found evidence for association of bipolar disorder with *PPARD*, a gene in the Wnt signaling pathway. The consistency of this result with one from the Wellcome Trust Case-Control Consortium encourages further study. If the finding can be confirmed in additional samples, it may illuminate a new avenue for understanding the pathogenesis of severe bipolar disorder and developing more effective treatments.

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WNT PROTEINS ARE A family of molecules that locally activate cell signaling pathways, which regulate cell fate and play an important role in development.[1] Aberrations in these pathways have been implicated in a number of chronic diseases, such as cancer. There are several lines of evidence to suggest that these pathways may also be involved in the etiology of bipolar disorder.[2] First, Wnt signaling pathways influence neuroplasticity, cell survival, and adult neurogenesis, and recent studies have suggested that bipolar disorder may involve impairments in these functions. For example, *Wnt7a* was found to be critical to axon and growth cone remodeling in the cerebellum[3]; mice with inactivated *Wnt1* genes failed to develop large portions of their brain[4]; and *Wnt3* increased neurogenesis in the adult rat hippocampus.[5]

Second, mood-stabilizing drugs and antipsychotic medications used to treat bipolar disorder are known to affect Wnt signaling pathways, particularly through glycogen synthase kinase 3β (GSK3β), a key enzyme in these pathways. Lithium inhibits this enzyme, leading to activation of

Wnt signaling,[6] and haploinsufficiency of GSK3β mimics lithium effects in a mouse model.[7] Clozapine[8] and haloperidol[9] have also been found to inhibit GSK3β, and 1 study showed that valproic acid similarly activates Wnt signaling.[10] Furthermore, another study showed that dopamine increases GSK3β activity and that this increase is reversed by dopamine receptor D_2 blockade and by lithium.[11]

Third, it has been shown that monozygotic twins who are discordant for bipolar disorder have differential expression of genes in Wnt signaling pathways.[12] Among 292 genes found to be differentially expressed, 8 were in the Wnt pathway. One of these was *TCF7*, a transcription factor activated by β-catenin. This supports a role for the Wnt pathway in bipolar disorder pathophysiology. At the level of etiology, this study directly suggests a role for environmental and epigenetic factors given that monozygotic twins are genetically identical, though it also indirectly suggests that variations in genes that alter Wnt signaling could be etiologic factors in bipolar disorder.

Finally, in an association study of bipolar disorder with 22 genes on chromosome 22,[13] evidence for association was found with *HMG2L1*, a gene that influences Wnt signaling by interacting with *NLK*, a negative regulator of *TCF7*.[14] Motivated by these considerations, we sought to systematically test whether variation in candidate genes of the Wnt signaling pathways was associated with susceptibility for bipolar disorder in a family-based study.

METHODS

STUDY SAMPLE

The families in the current study were ascertained through 1 of 3 projects: (1) the National Institute of Mental Health (NIMH) Genetics Initiative Bipolar Disorder Consortium involving 9 different sites across the United States (Johns Hopkins University, Indiana University, Washington University in St Louis, University of California–San Diego, University of Iowa, University of Pennsylvania, University of Chicago, Rush-Presbyterian Medical Center, University of California–Irvine, and the NIMH Intramural Program); (2) a separate collaboration involving the University of Chicago, Johns Hopkins University, and the NIMH Intramural Program (here referred to as CHIP); or (3) the Clinical Neurogenetics (CNG) collection. In all 3 samples, the families were recruited opportunistically through probands with bipolar disorder type I and 1 or more relatives affected with a mood disorder. The NIMH Genetics Initiative families were assessed with the Diagnostic Interview for Genetic Studies (DIGS),[15] and diagnoses were assigned using best-estimate procedures based on *DSM-III-R* or *DSM-IV* criteria. The CHIP and CNG families were assessed with the Schedule for Affective Disorders and Schizophrenia, Lifetime Version (SAD-L), and diagnoses were made using similar best-estimate procedures based on Research Diagnostic Criteria (RDC). We have previously reported high diagnostic reliability for major mood episodes and diagnoses using both SAD-L ($\kappa = 0.72$-1.0)[16] and DIGS ($\kappa = 0.78$-0.96).[15] In a small sample of 33 individuals with original diagnoses of bipolar disorder type I by RDC after assessment with the SAD-L, we found that on reinterview a mean 10 years later using DIGS, 31 of them were rediagnosed with bipolar disorder type I and 2 of them were rediagnosed with bipolar disorder type II, both by RDC and *DSM-IV* criteria (P.P.Z, unpublished data, 2002). We used all independent quads (2 parents and 2 affected offspring) and trios (2 parents and 1 affected offspring) from these families with DNA available for genotyping. This yielded 1118 participants in 237 quads and 80 trios from 317 families. Among the affected offspring, there were 491 with bipolar disorder type I, 39 with bipolar disorder type II, and 24 with schizoaffective manic disorder or schizoaffective disorder, bipolar type. Of these, 60.5% were female, the mean age of onset was 18 years, and the mean age at interview was 41 years. Ninety-six percent of participants had been treated. Of the 634 parents in our samples, 613 (97%) were of European ancestry (defined as >90% European), as assessed by a STRUCTURE analysis in which genotype data from 250 single-nucleotide polymorphisms (SNPs) in our samples were analyzed along with data from HapMap Centre d'Etude du Polymorphisme Humain (CEPH), Yoruban, Chinese, and Japanese samples. This broke down by study sample as follows: NIMH, 458 of 470 (97% with European ancestry); CHIP, 147 of 154 (95%); and CNG, 10 of 10. The other 21 parents came from 14 families and included 5 with African ancestry (defined as >90% African), 2 with Chinese ancestry (defined >90% Chinese), and 14 with mixed ancestry. All participants provided informed consent at the institutions where they were seen, and all protocols were approved by the local institutional review boards.

GENOTYPING

In these individuals, we genotyped 34 candidate genes from the Wnt signaling pathways (**Table 1**). We compiled a list of genes reflecting various aspects of Wnt signaling, including Wnt ligands, receptors, interactors, and targets, that were present on the Wnt Homepage (http://www.stanford.edu/~rnusse/wntwindow.html) as of June 2005. We localized 131 of these genes to chromosomal locations using the University of California–Santa Cruz Genome Browser. We selected candidates from these based on whether they were (1) expressed in the brain and (2) located in a chromosomal region that had been implicated by previous linkage studies of bipolar disorder and/or schizophrenia. Evidence for linkage was a significant result from 1 of 2 meta-analyses,[17,18] genome-wide significance in at least 1 family study, or consistent replication of a suggestive finding[19] across more than 1 independent family study. Two Wnt signaling genes were included, though they did not meet all of these criteria. These were *GSK3B*, because of its well-established inhibition by lithium,[6] and *NLK*, because it interacts with *HMG2L1*, which was previously associated with psychotic bipolar disorder.[13,14]

We selected for genotyping nonsynonymous coding SNPs identified from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) as well as a set of tagging SNPs chosen to capture the known common genetic variation (minor allele frequency [MAF] >0.1) across each gene ±10 kilobases (kb) ($r^2 \geq 0.8$). The tagging SNPs were selected using LDSelect[20] with HapMap Phase I data (http://www.hapmap.org), which were available at the time of the study's design. These SNPs were genotyped by the Illumina Integrated BeadArray System (Illumina, San Diego, California). A total of 265 SNPs were originally selected for genotyping. Of these, 34 SNPs could not be genotyped using the Illumina system. Another 6 replacement SNPs were identified, resulting in 237 successfully genotyped SNPs. Of these, 10 SNPs were excluded because they were either monomorphic (n=2) or not in Hardy-Weinberg equilibrium ($P < .05$) among the founders (n=8), leaving 227 SNPs for the initial analyses.

For follow-up, we sought to do denser genotyping in our most significant gene, *PPARD* (OMIM 600409). We used the Web-based tool QuickSNP[21] to select an additional 18 tagging SNPs from HapMap Phase II data, which captured the known common variation in the gene (MAF >0.05; $r^2 > 0.9$). We used the TaqMan-5′ nuclease assay (Applied Biosystems, Foster City, California) to genotype these SNPs. Two of the assays failed and 3 SNPs were excluded because more than 5.0% of data were missing, leaving 13 additional SNPs for analyses.

Table 1. Candidate Genes Involved in Wnt Signaling Pathways

Chromosome	Gene	Location	No. of Exons	No. of SNPs	Size, kb[a]	1 SNP/N, kb	% of Genes Captured[b]	Mean D′	Mean r^2	Blocks[c]	Function
1	*WNT2B*	1p13	5	10	73.87	7.39	0.65	0.42	0.08	4	Wnt protein
1	*BCL9*	1q21	10	8	104.83	13.10	0.34	0.55	0.15	4	Bind β-Catenin, promote transcription
1	*BGLAP*	1q25-q31	4	6	38.84	6.47	0.78	0.62	0.20	2	TG cell adhesion
1	*WNT9A*	1q42	4	2	46.90	23.45	0.33	0.34	0.09	2	Wnt protein
1	*WNT3A*	1q42	4	5	74.21	14.84	0.89	0.95	0.30	1	Wnt protein
1	*RHOU*	1q42.11-q42.3	3	3	31.54	10.51	0.65	0.99	0.47	1	Mediates Wnt1 signaling
3	*WNT7A*	3p25	4	9	81.54	9.06	0.43	0.51	0.12	5	Wnt protein
3	*CTNNB1*	3p21	15	2	61.00	30.50	0.94	0.99	0.25	1	β-Catenin
3	*GSK3B*	3q13.3	11	6	286.97	47.83	0.56	0.91	0.21	2	GSK3β receptor
4	*CTBP1*	4p16	9	5	57.68	11.54	0.60	0.82	0.22	2	Phosphorylation
5	*NEUROG1*	5q23-q31	1	1	21.66	21.66	0.57	NA	NA	1	TG neuronal differentiation
5	*WNT8A*	5q31	8	5	27.43	5.49	1.00	1.00	0.22	1	Wnt protein
5	*PTTG1*	5q35.1	6	6	26.88	4.48	0.58	0.70	0.19	3	TG chromosome stability, DNA repair
6	*PPARD*	6p21.2-p21.1	8	2	105.62	52.81	0.41	1.00	0.59	1	TG neurodevelopment
6	*VEGFA*	6p12	8	7	36.27	5.18	0.58	0.53	0.12	3	TG binds neurophilin
6	*WISP3*	6q21	5	5	35.61	7.12	0.96	0.97	0.19	1	TG skeletal growth
6	*GJA1*	6q21-q23.2	2	4	34.13	8.53	0.46	0.57	0.16	3	TG transmembrane channels
8	*FZD3*	8p21	8	5	90.19	18.04	0.96	0.57	0.12	3	Frizzled receptor
8	*ENPP2*	8q24.1	25	9	101.79	11.31	0.68	0.62	0.20	4	TG lysophosphatidic acid
8	*MYC*	8q24.12-q24.13	3	3	25.36	8.45	0.36	0.55	0.16	2	TG transcription of growth gene
8	*WISP1*	8q24.1-q24.3	5	18	58.26	3.24	0.64	0.41	0.11	8	TG cell proliferation
10	*DKK1*	10q11.2	4	3	23.38	7.79	0.75	0.87	0.17	1	Inhibits Wnt signaling; feedback TG
10	*NODAL*	10q22.1	3	4	29.35	7.34	0.79	0.80	0.36	2	TG forms mesoderm, axial organization
11	*MMP7*	11q21-q22	6	5	30.24	6.05	0.44	0.62	0.08	3	TG matrix metalloproteinase
11	*MMP3*	11q22.3	10	5	27.81	5.56	0.76	0.88	0.30	1	TG matrix metalloproteinase
12	*WNT10B*	12q13	5	2	26.42	13.21	0.50	1.00	0.76	1	Wnt protein
12	*IGF1*	12q22-q23	4	9	104.65	11.63	0.40	0.65	0.16	4	TG mediates growth hormone
12	*FZD10*	12q24.33	1	5	23.25	4.65	0.29	0.70	0.16	3	Frizzled receptor
13	*EFNB2*	13q33	5	13	65.24	5.02	0.67	0.49	0.10	3	TG mediates developmental events
16	*AXIN1*	16p13.3	10	10	85.02	8.50	0.40	0.63	0.20	7	Tumor suppressor
17	*NLK*	17q11.2	11	5	173.72	34.74	0.68	0.96	0.25	1	Chromosome separation; feedback TG
18	*SMAD4*	18q21.1	13	2	69.53	34.77	0.89	1.00	0.52	1	Mediates TGFβ1
18	*TCF4*	18q21.1	20	42	386.30	9.20	0.82	0.42	0.14	10	Transcription factor 4
22	*CBY1*	22q12	5	1	37.20	37.20	0.90	NA	NA	1	Inhibits Wnt signaling

Abbreviations: GSK3β, glycogen synthase kinase 3β; kb, kilobase; NA, not applicable; SNP, single-nucleotide polymorphism; TG, target gene; TGFβ1, transforming growth factor β1.

[a] Reflects the size of the most inclusive National Center for Biotechnology Information Reference Sequence gene definition ±10 kb. For *BGLAP*, 1 SNP lay outside of the most inclusive National Center for Biotechnology Information Reference Sequence gene definition ±10 kb and the distance to this SNP was added to the total size.

[b] Percentage of known common variations in the gene ±10 kb according to HapMap Phase II captured by the SNPs genotyped in the gene ±10 kb.

[c] Number of linkage disequilibrium blocks in the gene ±10 kb based on the genotyped SNPs and defined by a solid spine D′ of 0.8 or greater.

STATISTICAL ANALYSIS

We carried out 2 main analyses. First, we conducted a single-locus analysis in which we individually tested each SNP for association with bipolar disorder using FBAT (http://www.biostat.harvard.edu/~fbat/default.html; Harvard School of Public Health, Boston, Massachusetts), a flexible program for testing allelic associations with family data. Under certain conditions, it reduces to the commonly used transmission disequilibrium test. However, FBAT is more general and allows tests of association that are robust to population confounds in the case in which parental data are missing and/or other offspring are included in the analysis.[22] We used the biallelic mode and examined the additive model for counting alleles. We specified the option to calculate the variance empirically to provide valid tests of association in the presence of linkage,[23] because each of the candidate genes was selected based on prior evidence of linkage. We used permutations to estimate the significance of our best finding after accounting for all the tests carried out with FBAT. After randomly shuffling the haplotypes across each of the genes that were transmitted to the offspring, we recalculated the FBATs for each SNP and counted the number of replicates out of 10 000

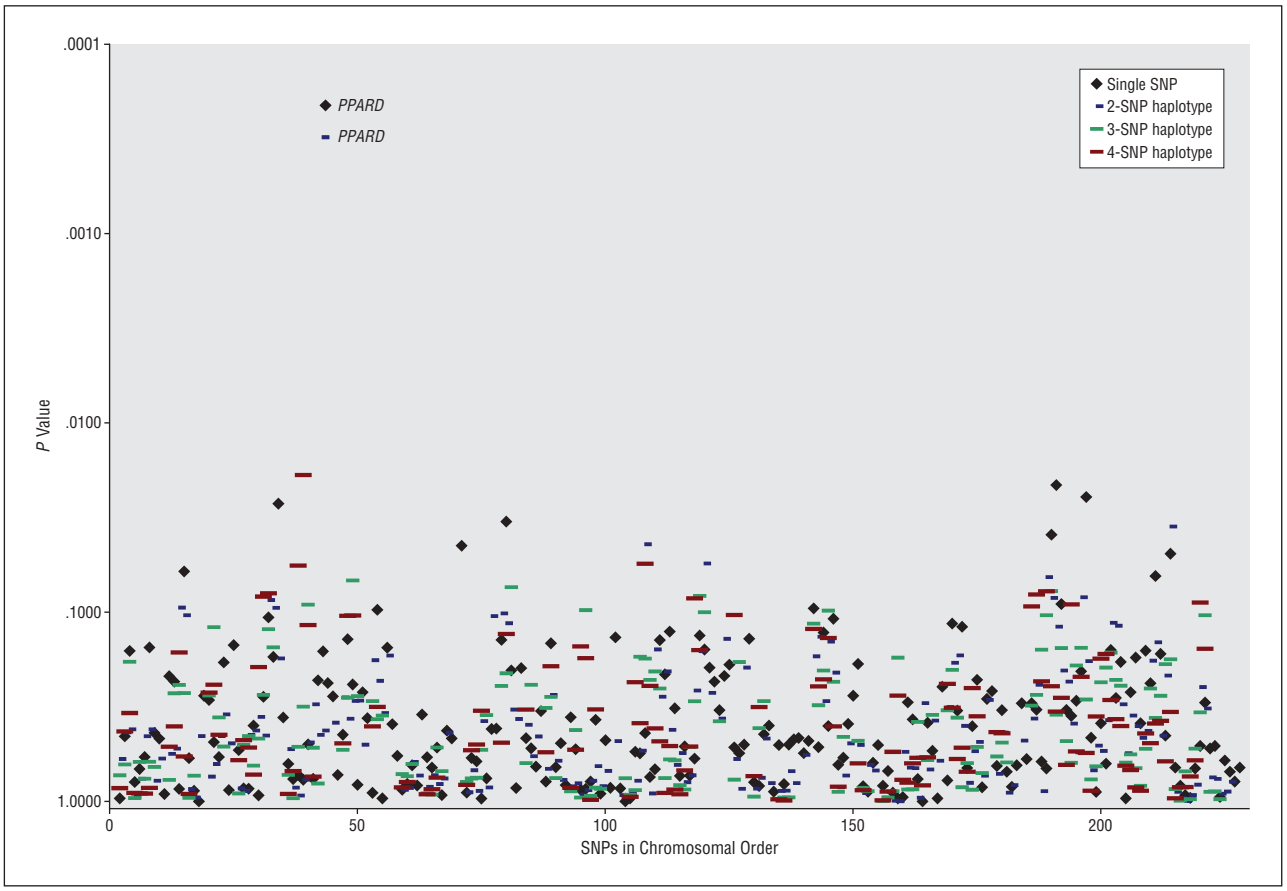

Figure 1. Association of 227 single-nucleotide polymorphisms (SNPs) in Wnt signaling pathway genes in patients with bipolar disorder. Results with nominal $P<.001$ are labeled with the gene name.

in which there was a more significant finding than what we observed.

Second, we conducted a multilocus analysis in which we tested haplotypes of adjacent SNPs for association with bipolar disorder using HBAT.[24] In some conditions (such as when $r^2=1$ between the risk variant and a particular multi-SNP haplotype), it has been shown that haplotypes may provide more information for association than corresponding single-locus tests[25]; HBAT is an elaboration of FBAT that allows for family-based association tests of haplotypes, even when the phase is ambiguous. We used a sliding window approach to test haplotypes of 2, 3, and 4 adjacent SNPs. Haplotypes were only allowed to cross intergenic regions when less than 1 kb separated SNPs in the 2 genes. This happened 3 times for the following gene pairs: *WNT9A*/*WNT3A*, *WNT2A*/*RHOU*, and *MMP7*/*MMP3*. Significance values were obtained for tests of each specific haplotype.

In exploratory analyses of our most significant findings in the *PPARD* gene, we separately tested for subgroup differences in the evidence of association by certain clinical features or for interactions with SNPs from other genes in the Wnt signaling pathways. For both analyses, we used the method implemented by the program GENASSOC in Stata, version 9.0 (Stata Corp, College Station, Texas) (http://www-gene.cimr.cam.ac.uk/clayton/software/stata/genassoc/), which allows for tests of interactions. Here, the family data set is converted into cases and matched pseudocontrols and analyzed for genotypic associations with an SNP of interest using conditional logistic regression models. These models may then be expanded to incorporate interactions between the SNP and other covariates. The significance of the interactions is determined by comparing models with and models without the interaction terms using likelihood ratio tests (LRTs). For the analysis looking at clinical features, we assumed the patients and pseudocontrols were matched on the feature under investigation. This is artificial because the pseudocontrols do not have clinical features, but it allows us to test for subgroup differences in the association with an SNP among the cases by including interaction terms between the SNP and a covariate for the feature. The different features tested in this way, selected to capture various aspects of severity, included the following: number of depressive episodes, number of manic episodes, age at onset (dichotomized at ≤21 years), having ever attempted suicide, having ever been hospitalized, history of psychosis, history of mood incongruent psychosis, and poor functioning in the past month (as assessed by the Global Assessment Scale [GAS], which is a measure of general functioning with a range of 0-100; a score lower than the mean of 68 represents poor functioning). For the analyses looking at 2-way interactions between *PPARD* and the other genes examined in the Wnt signaling pathway, we individually tested interaction terms for the best SNPs in *PPARD* with the single best SNP from each of the other genes. In all models for both analyses, we used a dominant coding for the SNPs being tested.

RESULTS

The results of the single SNP and the 2-, 3-, and 4-SNP haplotype analyses are shown in **Figure 1**. The most significant single SNP was rs2267665 in *PPARD* (nominal $P<.001$). The association with this SNP was significant ($P=.05$) by permutation after accounting for all the tests carried out with the SNPs. The most significant haplotype

association was with a 2-SNP haplotype that included rs2267665 ($P<.001$). No other single SNP or haplotype was associated with bipolar disorder at the nominal $P<.01$.

To corroborate the finding with *PPARD*, we consulted the publicly available results from the Wellcome Trust Case-Control Consortium Genome-Wide Association Study (WTCCC GWAS). The SNP rs2267665 was not genotyped in this study, but an SNP (rs9470015) that was in perfect linkage disequilibrium with it was ($r^2=1.0$), and this SNP was associated with bipolar disorder ($P=.02$) in the analysis in which all available controls were used to maximize the power of the sample. In both the SNP from our study and from WTCCC GWAS, the common allele was over-represented in bipolar disorder.

Encouraged by these findings, we sought to genotype a denser panel of SNPs in *PPARD* to provide better coverage of the gene and refine the boundaries of the apparent association. The *PPARD* gene, which is located on chromosome 6p21.2-p21.1, has 8 exons and spans approximately 85.6 kb (http://www.ncbi.nlm.nih.gov/RefSeq/). In the initial genotyping effort, we successfully genotyped only 2 intronic SNPs (including rs2267665, as described) that captured approximately 44% of the known variation in the gene according to the HapMap Phase II data (MAF >0.05, $r^2>0.80$). With the successful genotyping of 13 additional tagging SNPs, we increased the coverage to capture 86% of the known variation (MAF >0.01, $r^2>0.90$).

The results of the single SNP and 2-, 3-, and 4-SNP haplotype analyses with all 15 SNPs in *PPARD* are shown in **Figure 2** and **Table 2**. Of the 13 new tagging SNPs, 4 were significantly associated with bipolar disorder ($P<.01$). The most significant of these (rs9462082) was associated with bipolar disorder at $P<.001$. The most significant haplotype was a 2-SNP haplotype that included rs9462082 ($P<.001$). All of the SNPs associated at $P<.01$ resided in a single haplotype block that spanned exons 3 to 7 of the gene. The 2 most significant SNPs overall, rs2267665 and rs9462082, were highly correlated ($r^2=0.84$).

In an exploratory analysis, we tested whether the association with the 2 best SNPs in the *PPARD* gene varied depending on certain covariates related to the clinical presentation of bipolar disorder. Of the different clinical covariates examined, only poor functioning as measured by the GAS showed a significant interaction (LRT, $P<.01$) with these 2 SNPs. The SNP rs9462082 was significantly associated with bipolar disorder among all participants with an odds ratio (OR) of 1.46 (95% confidence interval [CI], 1.16-1.85) for those who were homozygous for the common allele compared with others. However, among participants with poor functioning (GAS score <68), this risk increased to an OR of 3.36 (95% CI, 1.85-6.08). There was no apparent association of this SNP with bipolar disorder among those who were high functioning (OR, 1.28; 95% CI, 0.85-1.93). The results were nearly identical for rs2267665.

In further exploratory analyses, we tested for 2-way interactions between the 2 best SNPs in *PPARD* and the best SNP from each of the other Wnt signaling pathway genes. Evidence for significant interactions (LRT, $P<.01$) was detected with *WNT2B* and *WNT7A*. In particular, rs9462082 was significantly associated with bipolar disorder only among those who were carriers of the rare allele at rs3790606 (MAF=0.31) in *WNT2B* (OR, 2.56; 95% CI, 1.67-4.00), but not among those who were homozygous for the common allele (OR, 1.16; 95% CI, 0.79-1.72). The results were nearly identical for rs2267665. Similarly, rs9462082 was significantly associated with bipolar disorder only among those who were carriers of the rare allele at rs4685048 (MAF=0.49) in *WNT7A* (OR, 1.79; 95% CI, 1.23-2.63) but not among those who were homozygous for the common allele (OR, 0.75; 95% CI, 0.42-1.32).

COMMENT

Several converging lines of evidence suggest that Wnt signaling pathways, which play an important role in a number of cellular functions, may also contribute to the etiology of bipolar disorder. We sought to test whether variation in genes of the Wnt signaling pathways is associated with susceptibility to bipolar disorder using a family-based design. We observed an association with an SNP in the *PPARD* gene that remained significant after correcting for the multiple tests carried out. Further genotyping in our sample suggested that the association was delimited within a single haplotype block that spanned exons 3 to 7 of the gene. Moreover, the association appeared to be strongest among patients with bipolar disorder who had the poorest functioning and among those carrying a rare allele at rs3790606 in *WNT2B* or at rs4685048 in *WNT7A*. These findings merit further investigation to identify the putative causal variant(s) within this region of the gene and to confirm their effects on poor functioning in bipolar disorder in particular.

The *PPARD* gene is located on chromosome 6p21. Chromosome 6p has not been implicated directly by linkage studies of bipolar disorder, though 1 study found evidence of interaction between loci on 6q and 6p.[27] By contrast, this region has been implicated in schizophrenia by a number of linkage studies. A rigorous meta-analysis of 20 different genome-wide linkage scans[28] identified 2 broad loci on 6p, one stretching 6pter-6p22.3 and another 6p22.3-21.1, that were genome-wide significant and among the top 10 signals across the entire genome. This region contains several interesting candidate genes, including one (*DTNBP1*) that has been associated with both schizophrenia and psychotic bipolar disorder.[29,30] The *PPARD* gene itself has not previously been reported to be associated with either bipolar disorder or schizophrenia.

The *PPARD* gene encodes a member of the peroxisome proliferator-activated receptor (PPAR) family. The PPARs are nuclear hormone receptors that mediate a wide variety of cellular and biochemical processes, including peroxisomal functioning, lipid oxidation, lipid synthesis, cell proliferation, and inflammation.[31] They act by dimerizing with the retinoid X receptor and, on binding with various ligands, serve as transcription factors for a number of different target genes that have peroxisome proliferation response elements within their promoters. The *PPARD* gene is expressed in a wide range of tissues, most notably the brain, adipose, and skin.[32] Studies have shown that in the murine brain, it is expressed at particularly high levels in the entorhinal cortex, hypothalamus, and hippocampus as well as the corpus callosum and neostriatum.[31] Interestingly, *PPARD* appears to be expressed at its highest levels

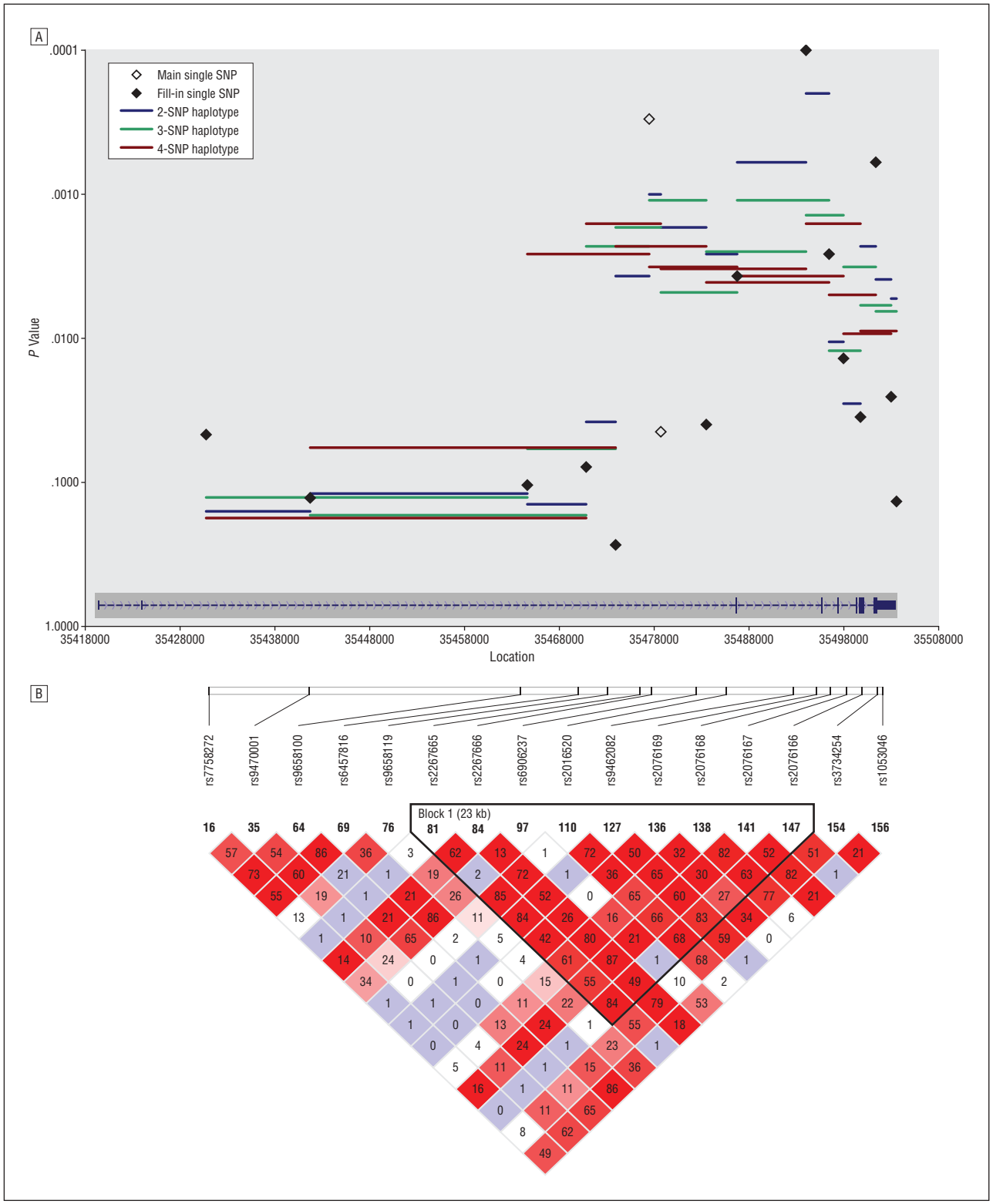

Figure 2. A, Specific association results for *PPARD*. Plotted are empirical *P* values for single-nucleotide polymorphisms (SNPs) transmission disequilibrium tests and 2-, 3-, and 4-SNP haplotype windows. The exon structure of the gene is included at the bottom of the graph in blue. B, The linkage disequilibrium plot is shown with r^2 values in the diamonds and the corresponding linkage disequilibrium blocks.[26] kb Indicates kilobase.

in the embryonic brain, suggesting that it may help regulate differentiation of cells during neurodevelopment.[32] Consistent with this, several studies have shown that PPAR delta (PPARD) agonists augment differentiation and myelogenesis of cultured murine oligodendrocytes[33,34] and that *PPARD*-null mice have diminished myelination levels of the corpus callosum and other neurodevelopmental abnormalities.[33] These findings suggest that if *PPARD* influences susceptibility to bipolar disorder, it could be through neurodevelopmental processes. Furthermore, agonists of

Table 2. Details of Bipolar Disorder Association Results With All Genotyped SNPs in *PPARD*

					Allelic[a]					Haplotypic[b]		
SNP	Location	Function	Alleles	MAF	Informative Families	S	E(S)	Var(S)	*P* Value	2-SNP *P* Value	3-SNP *P* Value	4-SNP *P* Value
rs7744392	35430741	Intron	A/G	0.048	31	31	22.5	18.25	.047	.16	.13	.18
rs9470001	35441719	Intron	G/C	0.079	44	50	41.5	31.25	.13	.12	.17	.001
rs9658100	35464618	Intron	T/G	0.08	44	52	42.5	34.25	.11	.14	.001	<.001
rs6457816	35470826	Intron	T/C	0.085	51	61	50.0	39.00	.08	<.001	<.001	<.001
rs2267665	35477469	Intron	G/A	0.155	94	58	89.5	75.25	<.001	.001	.001	.004
rs2267666	35478706	Intron	T/A	0.237	110	105	124.5	94.25	.045	.002	.005	.004
rs6906237	35483504	Intron	C/A	0.073	44	52	40.5	31.25	.04	.003	.003	.004
rs2016520	35486756	5′ UTR	A/G	0.184	95	72	98.0	80.00	.004	<.001	.001	.004
rs9462082	35494019	Intron	G/A	0.143	86	48	79.5	67.25	<.001	<.001	.001	.002
rs2076169	35496457	Intron	T/C	0.095	64	40	62.0	53.50	.003	.01	.01	.05
rs2076168	35497977	Intron	A/C/T	0.201	98	81	102.5	76.25	.01	.03	.003	.01
rs2076167	35499765	Synonymous	A/G	0.223	106	93	112.5	85.75	.04	.002	.006	.009
rs2076166	35501382	Intron	C/T	0.137	88	50	78.0	67.00	<.001	.004	.006	
rs3734254	35502988	3′ UTR	T/C	0.187	100	88	108.5	84.25	.03	.005		
rs1053046	35503556	3′ UTR	G/A	0.06	38	42	34.5	25.25	.14			

Abbreviations: E(S), expected value of the test statistic; MAF, minor allele frequency; S, test statistic from FBAT reflecting the number of transmissions to the affected offspring; SNP, single-nucleotide polymorphism; UTR, untranslated region; Var(S), variance of the tests.

[a] Allelic results from FBAT (http://www.biostat.harvard.edu/~fbat/default.html; Harvard School of Public Health, Boston, Massachusetts); statistics for the minor allele are shown.

[b] Haplotypic results from HBAT (http://www.biostat.harvard.edu/~fbat/default.html; Harvard School of Public Health).

PPARD were neuroprotective in rat models of stroke and of neurodegenerative disease.[35]

The increased evidence of association for *PPARD* among those with poor functioning is consistent with a potential role for Wnt dysfunction in severe bipolar disorder. The GAS score captures both social-occupational and interpersonal functioning in the month before the interview. We have previously shown that social functioning was the most highly familial feature of bipolar disorder among 40 variables tested and that loss of employment was also familial.[36] We have previously hypothesized[37] that psychotic features in bipolar disorder represent clinical manifestations of etiologic overlap between bipolar disorder and schizophrenia; we found no evidence to support that hypothesis in these data. However, functional impairment represents another potential clinical indicator of etiologic overlap, given that the most impaired patients with bipolar disorder are, by this metric, the most similar to schizophrenia patients, who are typically chronically impaired.

Several studies have implicated Wnt signaling genes in bipolar disorder as well as in schizophrenia. For schizophrenia, the frizzled 3 gene (*FZD3*), which encodes a receptor for Wnt ligands, has been associated in 3 different samples,[38-40] and Proitsi et al,[41] employing a Wnt pathway approach similar to ours, found evidence for an association with *DKK4*. We did not find any support for the former gene and did not study the latter. For bipolar disorder, 1 study reported a positive association with *GSK3B*,[42] but we did not observe a similar finding with this gene in our sample. There are 3 potential explanations for our failure to find signals in *FZD3* and *GSK3B*. First, these genes may not be related to bipolar disorder etiology. Second, these genes may be involved in bipolar disorder, but we may have failed to assay the relevant SNPs. This possibility is particularly relevant for *GSK3B*, for which we assayed only 56% of the known common variations. Third, we could have assayed SNPs that are etiologically relevant to bipolar disorder (or are in high linkage disequilibrium with relevant SNPs), but our sample may have had insufficient power to detect the potentially very modest effect sizes the risk alleles confer. More recently, a GWAS using pooled samples of patients with bipolar disorder,[43] some of which were also used in the current study, reported replicated associations with several genes involved with the Wnt signaling pathway, including *NXN*, *A2BP1*, and *DFNB31*. Unfortunately, we did not study these genes. Baum et al[43] did not detect significant associations with *PPARD*, *WNT7A*, or *WNT2B*. There are several potential reasons for this. Despite some overlap in the patients with bipolar disorder included in the 2 studies, there were substantial differences in sample composition, which may have introduced heterogeneity. Additionally, Baum et al[43] used a pooling approach for genotyping, which likely reduced the power to detect genetic variants with modest effect sizes.

We also found evidence for interactions of *PPARD* with *WNT2B* and *WNT7A*. These Wnt ligand genes are at the upstream end of the Wnt signaling pathways, while *PPARD* is on the downstream end of the β-catenin Wnt pathway. There are 3 Wnt signaling pathways: the β-catenin pathway (the canonical pathway), the planar cell polarity pathway, and the Wnt/Ca^{2+} pathway; *PPARD* is a target gene in the β-catenin pathway, which is the one that has been implicated in the mechanism of action of bipolar disorder medications. The current findings may suggest that multiple hits along the Wnt β-catenin pathway are needed to substantially influence bipolar disorder susceptibility. We note, however, that the findings of statistical interactions are exploratory and further confirmation is required.

The current study has several limitations that merit attention. First, the sample size may not have been sufficient to detect associations with loci of smaller effects on bipolar disorder. We estimated that this sample had 80%

power to detect association with a locus exerting a genotypic relative risk of approximately 1.6 (assuming an additive model, a disease prevalence of 1%, and a conservative $\alpha = .00022$ that is Bonferroni adjusted for the 227 SNPs tested). However, bipolar disorder appears to be a very genetically complex disease, and it is possible that the genotypic relative risks of bipolar disorder–susceptibility genes may be smaller than 1.6. Second, when we designed the experiment, the HapMap Phase II data were not yet available and as a result our selection of tagging SNPs provided incomplete coverage of the currently know common variation in the candidate genes studied. Consequently, we may have missed some of the relevant associations in these candidate genes. Third, we only studied 34 of at least 131 known Wnt-related genes, chosen largely because of their location in bipolar disorder and/or schizophrenia linkage regions. Because of the limited robustness of linkage studies, we may have omitted genes with an etiologic role in bipolar disorder. This problem should soon be overcome because the results of several large whole-genome association studies in bipolar disorder and schizophrenia will soon be available. Combining these data sets, as planned, will allow for comprehensive study of all Wnt-related genes.

The study also has several important strengths. Most notably, we used a family-based design with data available on both parents. Thus, we were able to extract the most information from this sample for association testing, and the findings are robust to potential confounding by population stratification.

In summary, we found evidence for association between bipolar disorder and a gene that is a downstream target of Wnt signaling, *PPARD*. If this association can be confirmed in additional samples, it may illuminate a new avenue for understanding the pathogenesis of severe bipolar disorder and developing more effective treatments.

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