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Association Study of Wnt Signaling Pathway Genes in Bipolar Disorder

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Abstract

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

Objective—We tested for the association of candidate genes in the Wnt signaling pathways with disease susceptibility in a family-based BP study

Design—227 tagSNPs 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—554 BP offspring and their parents from 317 families.

Main Outcome Measures—We tested for family-based association using FBAT and HBAT. Exploratory analyses testing for interactions of *PPARD* SNPs with clinical covariates and with other Wnt genes were conducted with GENASSOC.

Results—In the initial analysis, the most significantly associated SNP was rs2267665 in *PPARD* (nominal $p=0.0003$). This remained significant at $p=0.05$ by permutation after accounting for all SNPs tested. Additional genotyping in *PPARD* yielded four SNPs in one haplotype block that were significantly associated with BP at $p<0.01$, the most significant being rs9462082 ($p=0.0001$). Exploratory analyses revealed significant evidence ($p<0.01$) for interactions of rs9462082 with poor functioning on the Global Assessment Scale (OR = 3.36, 95% 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).

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Conclusions—We found evidence for association of BP 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 BP and developing more effective treatments.

Introduction

Wnt proteins are a family of molecules that locally activate cell signaling pathways, which regulate cell fate and play an important role in development [for a review see (Nusse, 2005)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 these pathways may also be involved in the etiology of bipolar disorder (BP) [for a review see (Gould & Manji, 2002)²].

First, Wnt signaling pathways influence neuroplasticity, cell survival, and adult neurogenesis, and recent studies have suggested that BP may involve impairments in these functions. For example, *Wnt7a* was found to be critical to axon and growth cone remodeling in the cerebellum³, mice with inactivated *Wnt1* genes failed to develop large portions of their brain⁴, and *Wnt3* increased neurogenesis in adult rat hippocampus⁵.

Second, mood stabilizing drugs and antipsychotic medications used to treat BP are known to affect Wnt signaling pathways, particularly through glycogen synthase kinase 3 β (GSK3 β), a key enzyme in these pathways. Lithium inhibits this enzyme leading to activation of Wnt signaling⁶, and haploinsufficiency of GSK3 β mimics lithium effects in a mouse model⁷. Clozapine⁸ and haloperidol⁹ have also been found to inhibit GSK3 β , and one study showed that valproic acid similarly activates Wnt signaling¹⁰. Further, another study showed that dopamine increases GSK3 β activity and that this increase is reversed by dopamine D2 receptor blockade and by lithium¹¹.

Third, it has been shown that monozygotic twins who are discordant for BP have differential expression of genes in Wnt signaling pathways¹². Among 292 genes found to be differentially expressed, eight 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 BP 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 BP.

Finally, in an association study of BP with 22 genes on chromosome 22¹³, evidence for association was found with *HMG2LI*, a gene which 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 BP in a family-based study.

Methods

Study Sample

The families in the current study were ascertained through one of three projects: the NIMH Genetics Initiative Bipolar Disorder Consortium involving nine different sites across the United States (including 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); a separate collaboration involving the University of Chicago, Johns Hopkins University, and the NIMH Intramural Program (here referred to as CHIP); or the Clinical Neurogenetics (CNG) collection. In all three samples, the families were recruited opportunistically through probands with bipolar I disorder (BPI) and one 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 (SADS), and diagnoses were made using similar best estimate procedures based on Research Diagnostic Criteria. We have previously reported high diagnostic reliability for major mood episodes and diagnoses using both the SADS (kappas 0.72–1.0) (Simpson et al, 2002) and the DIGS (kappas 0.78–0.96) (Nurnberger, Jr. et al, 1994). We have also found, in a small sample of 33 subjects originally diagnosed with BPI by RDC criteria after assessment with the SADS, that on re-interview an average of 10 years later using the DIGS, that 31 of them were re-diagnosed with BPI and two with BPII, both by RDC and DSM-IV criteria (unpublished data). We used all independent quads (two parents and two affected offspring) and trios (two parents and one affected offspring) from these families with DNA available for genotyping. This yielded 1,118 subjects in 237 quads and 80 trios from 317 families. Among the affected offspring, there were 491 with BPI, 39 with bipolar II disorder (BPII), and 24 with schizoaffective manic disorder or schizoaffective disorder, bipolar type (SAM or SABP). Of these, 60.5% were female, the average age of onset was 18, and the average age at interview was 41. Ninety-six percent of subjects had been treated. Of the 634 parents in our samples, 613 (97%) were of European ancestry (defined as $\geq 90\%$ European) as assessed by a STRUCTURE analysis in which genotype data from 250 SNPs in our samples were analyzed along with data from HapMap CEPH, Yoruban, Chinese, and Japanese samples. This broke down by study sample as follows: NIMH: 458/470 (97% with European ancestry); CHIP: 147/154 (95%); CNG: 10/10 (100%). The 21 other parents came from 14 families and included five with African ancestry (defined as $\geq 90\%$ African), two with Chinese ancestry (defined $\geq 90\%$ Chinese) and 14 with mixed ancestry.. All subjects provided informed consent at the institutions where they were seen, and all protocols were approved by the local IRBs.

Genotyping

We genotyped, in these subjects, 34 candidate genes from the Wnt signaling pathways (see Table 1 for details). We compiled a list of genes from the tables and diagrams 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: a) expressed in the brain; and b) located in a chromosomal region that had been implicated by previous linkage studies of bipolar disorder and/or schizophrenia. Evidence for linkage included a significant result from one of two meta-analyses^{16,17}, genome-wide significance in at least one family study, or consistent replication of a “suggestive” finding¹⁸ across more than one independent family study. Two Wnt signaling genes were included although they did not meet all of the above criteria. These were *GSK3 β* because of its well-established inhibition by lithium⁶ and *NLK* because it interacts with *HMG2L1*, which was previously associated with psychotic bipolar disorder^{13,14}.

We selected for genotyping non-synonymous coding SNPs identified from NCBI (<http://www.ncbi.nlm.nih.gov>) as well as a set of tag SNPs chosen to capture the known common genetic variation (minor allele frequency [MAF] > 0.1) across each gene ± 10 kb at an $r^2 \geq 0.8$. The tag SNPs were selected using LDSelect¹⁹ with HapMap Phase I data (<http://www.hapmap.org>) which was available at the time of the design of the study. These SNPs were genotyped by the Illumina Integrated BeadArray System at Illumina. A total of 265 SNPs were originally selected for genotyping. Of these, 34 SNPs could not be genotyped by Illumina. 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 (estimated among the founders) at $p < 0.05$ ($n=8$), leaving 227 SNPs for the initial analyses.

For follow-up, we sought to do denser genotyping in our most significant gene, *PPARD* (see below). We used the web-based tool QuickSNP²⁰ to select an additional 18 tag SNPs from HapMap Phase II data that captured the known common variation in the gene with a MAF > 0.05 at an $r^2 > 0.9$. We used the TaqMan-5' nuclease assay to genotype these SNPs. Two of the assays failed and three SNPs were excluded because of missing data > 5.0%, leaving 13 additional SNPs for analyses.

Statistical Analyses

We carried out two main analyses. First, we conducted a single-locus analysis in which we individually tested each SNP for association with BP using FBAT. FBAT is a flexible program for testing allelic associations with family data. Under certain conditions, it reduces to the commonly used transmission/disequilibrium test (TDT). However, FBAT is more general and allows tests of association that are robust to population confounds in the case where parental data is missing and/or other offspring are included in the analysis²¹. We used the biallelic mode and examined the additive model for counting alleles. We specified the option to calculate the variance empirically in order to provide valid tests of association in the presence of linkage²², because each of the candidate genes were 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 was transmitted to the offspring, we re-calculated the FBAT tests for each SNP and counted the number of replicates out of 10,000 in which there was a more significant finding than what we observed.

Second, we conducted a multi-locus analysis in which we tested haplotypes of adjacent SNPs for association with BP using HBAT²³. It has been shown that in some situations (such as when $r^2=1$ between the risk variant and a particular multi-SNP haplotype) haplotypes may provide more information for association than corresponding single-locus tests²⁴. HBAT is an elaboration of FBAT that allows for family-based association tests of haplotypes, even when 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 <1 kb separated SNPs in the two genes. This happened three times for the following gene pairs: *WNT9A-WNT3A*, *WNT2A-RHOA*, *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 sub-group 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 9.0 (<http://www-gene.cimr.cam.ac.uk/clayton/software/stata/genassoc/>) which allows for tests of interactions. Here, the family dataset is converted into cases and matched pseudocontrols and analyzed for genotypic associations with a 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 without the interaction terms using likelihood ratio tests (LRT). For the analysis looking at clinical features, we assumed the cases 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 sub-group differences in the association with a 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 or younger), ever attempted suicide, ever hospitalized, history of psychosis, history of mood incongruent psychosis, poor functioning in the past month (as assessed by the Global Assessment Scale [GAS], which is a measure of general functioning ranging from 0–100 with a score lower than the mean of 68 representing poor functioning). For the analyses looking at two-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 2, 3, and 4 SNP haplotype analyses are shown in Figure 1. The most significant single SNP was rs2267665 in *PPARD* (nominal $p=0.0003$). The association with this SNP was significant at $p=0.05$ by permutation after accounting for all the tests carried out with the SNPs. The most significant haplotype association was with a two SNP haplotype that included rs2267665 ($p=0.0009$). No other single SNP or haplotype was associated with BP at the nominal $p<0.01$.

To corroborate the finding with *PPARD*, we consulted the publicly available results from the Wellcome Trust Case-Control Consortium (WTCCC) Genome-Wide Association Study (GWAS) with BP. The SNP rs2267665 was not genotyped in this study, but a SNP (rs9470015) that was in perfect linkage disequilibrium with it ($r^2=1.0$) was, and this SNP was associated with BP at $p=0.02$ 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 the WTCCC GWAS, the common allele was over-represented in BP.

Encouraged by these findings, we sought to genotype a denser panel of SNPs in *PPARD* in order 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 two intronic SNPs (including rs2267665 described above) that captured approximately 44% of the known variation in the gene according to the HapMap phase II data with a $MAF>0.05$ at an $r^2>0.80$. With the successful genotyping of 13 additional tagSNPs we increased the coverage to capture 86% of the known variation with a $MAF>0.01$ at an $r^2>0.90$.

The results of the single SNP and 2, 3, and 4 SNP haplotype analyses with all 15 SNPs in *PPARD* are graphically depicted in Figure 2 and the details shown in Table 2. Of the 13 new tagSNPs, four were significantly associated with BP at $p<0.01$. The most significant of these (rs9462082) was associated at $p=0.0001$. The most significant haplotype was a two SNP haplotype that included rs9462082 ($p=0.0003$). All of the SNPs associated at $p<0.01$ resided in a single haplotype block that spanned exons 3 to 7 of the gene. The two most significant SNPs overall rs2267665 and rs9462082 were highly correlated at $r^2=0.84$.

In an exploratory analysis, we tested whether the association with the two best SNPs in the *PPARD* gene varied depending upon certain covariates related to the clinical presentation of BP. Of the different clinical covariates examined, only poor functioning as measured by the Global Assessment Scale (GAS) showed a significant interaction (LRT $p < 0.01$) with these two SNPs. The SNP rs9462082 was significantly associated with BP among all subjects with an odds ratio (OR) of 1.46 (95% CI = 1.16, 1.85) for those who were homozygous for the common allele compared to others. However, among subjects with poor functioning (GAS < 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 BP 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 two-way interactions between the two best SNPs in *PPARD* and the best SNP from each of the other Wnt signaling pathway genes. Evidence for significant interactions (LRT $p < 0.01$) was detected with *WNT2B* and *WNT7A*. In particular, rs9462082 was significantly associated with BP 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 BP 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).

Discussion

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 BP. We sought to test whether variation in genes of the Wnt signaling pathways is associated with susceptibility to BP using a family-based design. We observed an association with a SNP in the *PPARD* gene that remained significant after correcting for the multiple tests carried out. Interestingly, another SNP that was in perfect LD with this SNP was genotyped in the WTCCC GWAS and was also nominally associated with BP. Further genotyping in our own sample suggested 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 BP subjects with the poorest functioning and among those carrying a rare allele at rs3790606 in *WNT2B* or at rs4685048 in *WNT7A*. These findings merit further investigation in order to identify the putative causal variant(s) within this region of the gene and to confirm their effects on poor functioning BP in particular.

The *PPARD* gene is located on chromosome 6p21. Chromosome 6p has not directly been implicated by linkage studies of BP, although one study found evidence of interaction between loci on 6q and 6p²⁵. 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 scans²⁶ identified two broad loci on 6p, one stretching from 6pter-6p22.3 and another from 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 (*DTNBPI*) that has been associated with both schizophrenia and psychotic BP^{27,28}. *PPARD* itself has not previously been reported to be associated with either BP 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 [for a review see (Burger & Moller)²⁹]. They

act by dimerizing with the retinoid X receptor and upon binding with various ligands serve as transcription factors for a number of different target genes that have peroxisome proliferation response elements (PPREs) within their promoters. *PPARD* is expressed in a wide range of tissues, but most notably the brain, adipose and skin³⁰. 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 neostriatum²⁹. Interestingly, *PPARD* appears to be expressed at its highest levels in embryonic brain, suggesting it may help regulate differentiation of cells during neurodevelopment³⁰. Consistent with this, several studies have shown that *PPARD* agonists augment differentiation and myelogenesis of cultured murine oligodendrocytes^{31,32}, and *PPARD* null mice have diminished myelination levels of the corpus callosum and other neurodevelopmental abnormalities³¹. These findings suggest that if *PPARD* influences susceptibility to BP, it could be through neurodevelopmental processes. Further, agonists of *PPARD* were neuroprotective in rat models of stroke and of neurodegenerative disease³³.

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 prior to the interview. We have previously shown that social functioning was the most highly familial feature of BP, among 40 variables tested, and that loss of employment was also familial³⁴. We have previously hypothesized³⁵ that psychotic features in BP represent clinical manifestations of etiologic overlap between BP 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 BP patients are, by this metric, the most similar to schizophrenia patients, who are typically chronically impaired.

Several prior studies have implicated Wnt signaling genes in BP as well as in schizophrenia. For schizophrenia, the frizzled 3 gene (*FZD3*), which encodes a receptor for Wnt ligands, has been associated in three different samples³⁶⁻³⁸, and Proitsi et al.³⁹, employing a Wnt pathway approach similar to ours, found evidence for association with *DKK4*. We did not find any support for the former gene, and did not study the latter. For BP, one study reported a positive association with *GSK3B*⁴⁰, but we did not observe a similar finding with this gene in our sample. There are three potential explanations for our failure to find signals in *FZD3* and *GSK3B*. First, these genes may not be related to BP etiology. Second, these genes may be involved in BP, but we may have failed to assay the relevant SNPs. This possibility is particularly relevant for *GSK3B* for which we assayed only 56% of known common variation. Third, we could have assayed SNPs that are etiologically relevant to BP (or are in high LD with relevant SNPs), but our sample may have had insufficient power to detect the potentially very modest effect sizes the risk alleles confer (see below). More recently, a GWAS using pooled samples of BP subjects⁴¹, 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. The Baum et al. (2007) study did not detect significant associations with *PPARD*, *WNT7A*, or *WNT2B*. There are several potential reasons for this. Despite some overlap in the BP subjects included in the two studies, there were substantial differences in sample composition which may have introduced heterogeneity. Additionally, Baum et al.⁴¹ used a pooling approach for genotyping which likely reduced their power to detect genetic variants with modest effect sizes.

We also found evidence for two-way 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 beta-catenin Wnt pathway. There are three Wnt

signaling pathways: the beta-catenin pathway (the canonical pathway), the planar cell polarity pathway and the Wnt/Ca²⁺ pathway; *PPARD* is a target gene in the beta-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 beta-catenin pathway are needed in order to substantially influence BP 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 BP. We estimated that this sample had 80% power to detect association with a locus exerting a genotypic relative risk (GRR) of approximately 1.6, assuming an additive model, a disease prevalence of 1%, and a conservative $\alpha = 0.00022$ that is Bonferroni adjusted for the 227 SNPs tested). However, BP appears to be a very genetically complex disease, and it is possible the GRRs of BP susceptibility genes may be smaller than 1.6. Second, when we designed the experiment the HapMap Phase II data was not yet available, and as a result our selection of tag SNPs provided incomplete coverage of the currently known common variation in the candidate genes under study. 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 BP and/or schizophrenia linkage regions. Because of the limited robustness of linkage studies, we may have omitted genes with an etiologic role in BP. This problem should soon be overcome as the results of several large whole genome association studies in BP and schizophrenia will soon be available. Combining these datasets, as is 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 maximal 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 with a gene that is a downstream target of Wnt signaling, *PPARD*. If the finding of association can be confirmed in additional samples, it may illuminate a new avenue for understanding the pathogenesis of severe BP and developing more effective treatments.

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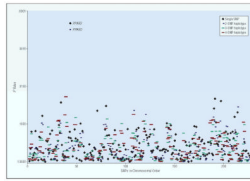


Figure 1. Association of 227 SNPs in Wnt signaling pathway genes with bipolar disorder. P-values for allelic single SNP analyses (black diamonds) and haplotype analyses (blue, green and red lines) on the log scale. Results with nominal $p < 0.01$ are labeled with the gene name.

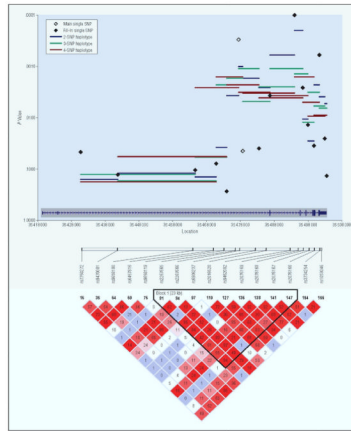


Figure 2. Specific association results for *PPARG*. Plotted are empiric p-values for single-SNP TDT tests (black diamonds) and 2- 3- and 4-SNP haplotype windows (blue, green and red solid lines). Diamonds representing the SNP results for the 2 SNPs in the main analysis are open and diamonds representing the SNP results for the 13 fill in SNP are closed. The exon structure of the gene is included at the bottom of the graph in blue. The LD plot is shown below the graph with r^2 values in the diamonds and the corresponding LD blocks⁴².

Table 1

Candidate genes involved in Wnt signaling pathways

Chr	Gene	Location	Exons	SNPs	Size (kb) ^a	1 SNP/N kb	% Capture ^b	Avg D ^c	Avg r ²	Blocks ^c	Function ^d
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	RHOA	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	CTNNA1	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	GSK3- β 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	N/A	N/A	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 neuropilin
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

Chr	Gene	Location	Exons	SNPs	Size (kb) ^a	1 SNP/N kb	% Capture ^b	Avg D'	Avg r2	Blocks ^c	Function ^d
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	N/A	N/A	1	inhibits Wnt signaling

^aSize reflects the size of the most inclusive RefSeq gene definition \pm 10kb; For BGLAP, one SNP lay outside of the most inclusive RefSeq gene definition \pm 10kb and the distance to this SNP was added to the total size

^bPercent of known common variation in the gene \pm 10 kb according to HapMap Phase II captured by the SNPs typed in the gene \pm 10 kb

^cNumber of LD blocks in the gene \pm 10 kb based on the typed SNPs and defined by a solid spine $D' \geq 0.8$

^dTG = target gene

Table 2

Details of association results with all genotyped SNPs in *PPARD*

SNP	Location	Function	Alleles	MAF ^a	Allelic ^b					Haplotypic ^c			
					Informative Families	S	E(S)	Var(S)	P	2 SNP p	3 SNP p	4 SNP p	
rs7744392	35430741	intron	A/G	0.048	31	22.5	18.25	0.0466	0.1588	0.1271	0.1770		
rs9470001	35441719	intron	G/C	0.079	44	41.5	31.25	0.1284	0.1195	0.1689	0.0010		
rs9658100	35464618	intron	T/G	0.08	44	42.5	34.25	0.1045	0.1421	0.0011	0.0008		
rs6457816	35470826	intron	T/C	0.085	51	50.0	39.00	0.0782	0.0008	0.0004	0.0006		
rs2267665	35477469	intron	G/A	0.155	94	89.5	75.25	0.0003	0.0010	0.0012	0.0036		
rs2267666	35478706	intron	T/A	0.237	110	124.5	94.25	0.0446	0.0016	0.0052	0.0035		
rs6906237	35483504	intron	C/A	0.073	44	40.5	31.25	0.0397	0.0029	0.0026	0.0041		
rs2016520	35486756	5' UTR	A/G	0.184	95	98.0	80.00	0.0037	0.0005	0.0012	0.0041		
rs9462082	35494019	intron	G/A	0.143	86	79.5	67.25	0.0001	0.0003	0.0012	0.0020		
rs2076169	35496457	intron	T/C	0.095	64	62.0	53.50	0.0026	0.0106	0.0132	0.0049		
rs2076168	35497977	intron	A/C/T	0.201	98	102.5	76.25	0.0138	0.0268	0.0032	0.0096		
rs2076167	35499765	synonymous	A/G	0.223	106	112.5	85.75	0.0352	0.0021	0.0062	0.0089		
rs2076166	35501382	intron	C/T	0.137	88	78.0	67.00	0.0006	0.0040	0.0064			
rs3734254	35502988	3' UTR	T/C	0.187	100	88	108.5	0.0255	0.0051				
rs1053046	35503556	3' UTR	G/A	0.06	38	42	34.5	0.1356					

^aMAF = Minor Allele Frequency^bAllelic results from FBAT; statistics for the minor allele shown^cHaplotypic results from HBAT; Results presented for 2-, 3-, and 4-SNP sliding window haplotypes. The p-value for each haplotype is listed in the row for the first SNP in the haplotype.