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Association analysis of the *PIP4K2A* gene on chromosome 10p12 and schizophrenia in the Irish study of high density schizophrenia families (ISHDSF) and the Irish case-control study of schizophrenia (ICCS)

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Abstract

Molecular studies support pharmacological evidence that phosphoinositide signaling is perturbed in schizophrenia and bipolar disorder. The phosphatidylinositol-4-phosphate-5-kinase type-II alpha (*PIP4K2A*) gene is located on chromosome 10p12. This region has been implicated in both diseases by linkage, and *PIP4K2A* directly by association. Given linkage evidence in the Irish Study of High Density Schizophrenia Families (ISHDSF) to a region including 10p12, we performed an association study between genetic variants at *PIP4K2A* and disease. No association was detected through single-marker or haplotype analysis of the whole sample. However, stratification into families positive and negative for the ISHDSF schizophrenia high-risk haplotype (HRH) in the *DTNBP1* gene and re-analysis for linkage showed reduced amplitude of the 10p12 linkage peak in the *DTNBP1* HRH positive families. Association analysis of the stratified sample showed a trend toward association of *PIP4K2A* SNPs rs1417374 and rs1409395 with schizophrenia in the *DTNBP1* HRH positive families. Despite this apparent paradox, our data may therefore suggest involvement of *PIP4K2A* in schizophrenia in those families for whom genetic variation in *DTNBP1* appears *also* to be a risk factor. This trend appears to arise from under-transmission of common alleles to female cases. Follow-up association analysis in a large Irish schizophrenia case-control sample (ICCS) showed significant association with disease of a haplotype comprising these same SNPs rs1417374-rs1409395, again more so in affected females, and in cases with negative family history of the disease. This study supports a minor role for *PIP4K2A* in schizophrenia etiology in the Irish population.

Keywords

PIP4K2A; schizophrenia; association; family; case-control

Schizophrenia is a complex, debilitating psychiatric disorder with lifetime prevalence ~1% [Jablensky et al., [1992]]. Genetic factors impact substantially upon risk for developing the disease, with heritability ~80% [Sullivan et al., [2003]]. Indeed, >20 genome-wide linkage scans have pinpointed genomic regions harboring schizophrenia susceptibility variants. Association studies in multiply reported linked regions have revealed several candidate genes for the disorder [Riley and Kendler, [2006]].

In the ISHDSF, a genome linkage scan of 265 families with schizophrenia gave strongest signals in 3 regions: 5q21-31, 6p24-22, 8p22-21, all since replicated [Straub et al., [2002]; Pimm et al., [2005]; Riley and Kendler, [2006]]. Elsewhere, D10S674 (10p13) gave one of the highest pairwise heterogeneity lod (H-LOD) scores, 3.2 ($P = 0.0004$), on an 88-family subset [Straub et al., [1998]]. Across all 265 families, strongest linkage was to D10S2443 (10p12.1) with intermediate phenotypic definition (maximum pairwise H-LOD 1.95, $P = 0.005$). Multipoint H-LODS gave a broad peak (maximum 1.91, $P = 0.006$) extending 11 cM from D10S674(10p13)-D10S1426(10p11.23). This locus has been linked to schizophrenia in other genome scans [Faraone et al., [1998]; Schwab et al., [1998], [2000]; Levinson et al., [2000]; DeLisi et al., [2002]; Lewis et al., [2003]; Holliday et al., [2005]].

One gene within the 10p-linked interval became a positional and functional candidate for schizophrenia: phosphatidylinositol-4-phosphate-5-kinase type-II alpha. Formerly *PIP5K2A*, its new gene symbol *PIP4K2A* will be used here (<http://www.genenames.org>). *PIP4K2A* is involved in the synthesis of phosphoinositide-4,5-bisphosphate (PIP_2), precursor to second messengers in the phosphoinositide signal transduction cascade [Doughman et al., [2003]; Weernink et al., [2004]]. Inositol phosphate metabolism is a target of mood stabilizer drugs [e.g., lithium, valproate, carbamazepine; Harwood, [2005]] and there is growing evidence for dysfunction of this pathway in schizophrenia [Kalkman, [2006]]. Linkage of schizophrenia to D10S245, <1 Mb from *PIP4K2A*, was shown by Maziade et al. [2001].

Stopkova et al. [2003] first reported association between *PIP4K2A* and both schizophrenia and bipolar disorder (BPD). Schwab et al. [2006] investigated *PIP4K2A* following significant association with D10S211 (900 kb from *PIP4K2A*) in their 10p-linked sample. After initial results [Sewekow et al., [2003]] 31 polymorphisms were tested covering *PIP4K2A* plus ~100 kb flanking regions. Fifteen variants spanning 73.6 kb (*PIP4K2A* intron 4 → 3' UTR) were significantly associated with schizophrenia, in a region of high intermarker linkage disequilibrium (LD). Major alleles were over-transmitted to affected individuals and as a 12-SNP haplotype over 43 kb (intron 6 → 3' UTR, $P = 0.037$ after correction). For non-synonymous SNP rs10828317 (Ser251Asp; exon 7) the associated major allele encoding Ser ($P = 0.001$) was associated with schizophrenia in a case-control study [Bakker et al., [2007]; $P = 0.0004$]. Schwab et al. [2006] also detected association with rs1417374, ~125 kb upstream of *PIP4K2A* ($P = 0.0009$).

Jamra et al. [2006] attempted to replicate Sewekow et al. [2003], analyzing 5 *PIP4K2A* SNPs in a sample of 268 schizophrenia patients, 260 BPD patients and 325 controls. Despite including rs1417374 and one SNP from the high-LD region, they found no association to either disease. Because of its positional candidacy, and association with schizophrenia in another 10p-linked Caucasian sample [which, like the ISHDSF, showed association with *DTNBP1*; Schwab et al., [2006]], we decided to investigate association with *PIP4K2A* in the ISHDSF. Follow-up analysis was performed on The Irish Case-Control Study Sample (ICCSS).

The ISHDSF comprises 265 families with 1,408 individuals for genotyping, divided into four concentric diagnostic categories: core schizophrenia (d2), narrow spectrum (“intermediate phenotype” d5), broad (d8) and very broad spectrum psychiatric disease [d9; Kendler et al., [1996]]. The ICCSS has 1021 cases with schizophrenia or poor-outcome schizoaffective disorder (DSM-III-R) from psychiatric facilities in the Republic of Ireland and Northern Ireland. For the 627 controls inclusion criteria was no history of psychotic illness. Family history (FH) was assessed by clinical interview using FH-RDC criteria [Endicott et al., [1975]]. Inclusion in the study required all grandparents to be born in Ireland/UK and appropriate informed consent.

We assessed five *PIP4K2A* SNPs in the ISHDSF, all significantly associated in Schwab et al. [2006] (rs1417374, rs1409395, rs10828317, rs746203, rs2364115) and one microsatellite [Stopkova et al., [2003]], named PIK53_54 [Schwab et al., [2006]] or D10S0969i [Tamiya et al., [2005]]. PCR and single-base extension primers were designed manually for rs1417374 and rs2364115 (available on request). Other primer sequences were from Schwab et al. [2006]. SNP genotyping was performed by template-directed dye-terminator incorporation with fluorescence polarization detection (FP-TDI) using AcycloPrime FP SNP detection kits (PerkinElmer Life Sciences, Boston, MA) and manufacturer’s instructions, with an automated allele scoring platform [Van den Oord et al., [2003]]. PIK53_54 was typed via standard PCR using one fluorescent dye-labeled primer (6-FAM), electrophoresis on an SCE7610 capillary sequencer (Spectrumedix LLC, State College, PA) and allele-calling using Spectrumedix genotyping software. Genotypes were examined for incompatibilities within families by PEDCHECK v1.1 [O’Connell et al., [1993]], unlikely recombinations by MERLIN [Abecasis et al., [2002]], and corrected as necessary. For unambiguously resolvable errors, respective genotypes were deleted for the whole family. For individuals with >50% missing genotypes all data were deleted (n = 94). Average genotyping rate was 95% (max 98%, min 89%).

We assessed 5 *PIP4K2A* SNPs in the ICCSS, using Taqman SNP genotyping assays from ABI (www.appliedbiosystems.com): rs1417374, rs1409395, rs10828317, rs746203, and rs8341, as proxy for rs2364115 (no assay, complete LD). Manufacturer’s instructions were followed, with slight modifications (available on request). For individuals with >50% missing genotypes all data were deleted (n = 66). Average genotype rate was 98% (max 99%, min 96%). Genotyping error rate was estimated from 205 duplicate genotypes. Of these, 94% were successfully collected and none were discordant, yielding an error rate <0.005%.

Genotype deviation from Hardy-Weinberg equilibrium (HWE) and pairwise intermarker LD were calculated using Haploview v4.0 [Barrett et al., [2005]] from unrelated founders (ISHDSF) or controls (ICCSS). We used the Tagger function of Haploview to estimate the proportion of common regional variation ($MAF > 0.20$) identified in HapMap Phase II data that was tagged at $r^2 > 0.8$ by the SNPs (including proxies for PIK53_54) genotyped in this study.

We performed association analysis using PDTPHASE v4.0 [Martin et al., [2000]; Dudbridge, [2003]] for the ISHDSF and COCAPHASE [Dudbridge, [2003]] for the ICCSS. For haplotype analyses, we included only those with frequency $>2\%$ to avoid statistical problems from rare alleles/haplotypes. To investigate epistasis between known susceptibility variants in *DTNBP1* and other linked loci in the ISHDSF, we stratified the sample on the *DTNBP1* high-risk haplotype (HRH; up to and including d8 affection), re-analyzing for linkage to schizophrenia using GENEHUNTER [v1.3; Kruglyak et al., [2006]].

For FH-conditioned analyses (ICCSS), all FH- cases were set to unknown affection status. For sex-stratified analysis, only male or female subjects (ICCSS) or affected offspring (ISHDSF) were used. We further explored influence of subject sex on association between *PIP4K2A* and schizophrenia in the ICCSS via logistic regression, parameterizing each genotype (assuming an additive model), including sex and testing for their interaction. Gender-specific LD contrast analysis between cases and controls was also performed, using correlation and D measures of LD.

Accounting for all comparisons, including *DTNBP1* HRH-stratified and sex-specific analyses, we used false discovery rate (FDR) methodology to control false positive rate. The procedure applied controls FDR for correlated data [“nested” diagnostic categories d2-d9 in the ISHDSF and SNPs in LD; Benjamini and Hochberg, [1995]; Storey, [2003]]. We also used weighted FDR to allow inclusion of prior information [Genovese et al., [2006]; Roeder et al., [2006]] via the R package wFDR (<http://wpicr.wpic.pitt.edu/WPICCompGen/fdr/>) that is, *P*-values of published single marker tests of *PIP4K2A* association with schizophrenia as prior weights. For multiply reported tests we combined *P*-values using the approach of Fisher [1932]. Previously untested marker haplotype combinations were assigned a prior *P* = 0.5. For the ICCSS, permutation testing was performed in Haploview [Barrett et al., [2005]].

All SNP genotype frequencies conformed to HWE (data not shown). Regional LD structure in the ISHDSF and ICCSS is consistent with prior reports [HapMap CEU release 21, Schwab et al., [2006]] that is, a high-LD block spanning *PIP4K2A* exon 7 $\rightarrow$ 3' UTR in modest LD with rs1409395 (intron 1; Table I). 5' Upstream SNP rs1417374 shows modest LD with rs1409395 ($D' \approx 0.5$), lower with the high-LD block ($D' \approx 0.4$; Table I). SNP allele frequencies were similar in the ISHDSF, ICCSS, and dbSNP (CEU) (data not shown).

For the ISHDSF, we saw no association with single markers in any diagnostic group, in the whole sample (Table SI) or restricted to the 10p-linked subset (data not shown). For susceptibility loci of small effect, positive association may only be detected with haplotypes [Akey et al., [2001]]. However, key haplotypes from Schwab et al. [2006] showed no greater evidence for association here, perhaps owing to high regional LD (data not shown).

Re-analysis of the ISHDSF genome scan data [Straub et al., [2002]] conditioned on the *DTNBP1* HRH (B.T. Webb, unpublished work) showed an increased 10p linkage signal in the HRH– families (n = 176) and negative linkage in the HRH+ families (n = 73; Fig. 1) that is, *DTNBP1* HRH+ families do not appear to harbor the 10p risk locus (by linkage), or, they attenuate the 10p linkage signal from *DTNBP1* HRH– families when analyzed altogether. Somewhat counter intuitively, we saw uncorrected evidence for association to *PIP4K2A* SNPs rs1417374 and rs1409395 in the *DTNBP1* HRH+ families (Table II). We also saw nominal association of haplotypes comprising rs1417374 with *PIP4K2A* LD-block SNPs (Table II) in this subset, a significant *under*-transmission of the common alleles ($P = 0.012$; Table II; Table SII). We saw no association between *PIP4K2A* and schizophrenia in *DTNBP1* HRH– families (data not shown).

Sex-stratified association analysis revealed the positive trend for markers rs1417374-rs1409395 to derive from over- (or under)transmission of the minor (or major) alleles to affected females (Table IIIA). This reached nominal significance for rs1409395 at d8 ($P = 0.042$) and in *DTNBP1* HRH+ families, for rs1417374 and rs1409395 at d2-d9 (Table IIIB). We found no significant difference in the transmission counts according to sex alone (analyzed as 2×2 tables), refuting the notion of transmission ratio distortion on 10p [data not shown; Paterson and Petronis, [1999]]. However, only one single marker test (rs1409395 in female *DTNBP1* HRH+ d8 cases) was significant at FDR = 20% under either weighting scheme.

Follow-up analysis in the ICCSS showed significant yet modest association between rs1417374 ($P = 0.023$), rs1409395 ($P = 0.026$), and the rs1417374-rs1409395 common allele haplotype ($P = 0.003$) with schizophrenia (Table IV). This results from under-representation of common alleles in cases, echoing the sex-specific association in the ISHDSF *DTNBP1* HRH+ families (Table IIIB). Although the associations with rs1417374 and rs1409395 in the ICCSS did not survive 100,000 permutations (perm $P = 0.121$ and $P = 0.139$), their common haplotype remained significant (perm $P = 0.015$).

Like the ISHDSF, association with rs1417374 in the ICCSS stems primarily from allele frequency differences in female cases versus controls ($P = 0.028$; Table V) but does not survive 100,000 permutations (perm $P = 0.145$). Significant association with common allele haplotype rs1417374-rs1409395 in female cases ($P = 0.009$, global $P = 0.049$; Table II) survived permutation (perm $P = 0.045$). After accounting for gender in the logistic regression model, the association between rs1417374 and schizophrenia (unadjusted $P = 0.031$, OR (95% CI) = 1.197 (1.017–1.408)) and between rs1409395 and schizophrenia (unadjusted $P = 0.022$, OR (95% CI) = 1.207 (1.027–1.418)) were not significant after correction (data not shown). No other markers were significant in the model and we found no significant differences in LD pattern between cases and controls for correlation or D' -based composite LD comparisons (data not shown).

There was no greater evidence in the ICCSS for association in FH+ cases (n = 256) compared to controls, or significant difference in allele frequencies between FH+ and FH– (n = 467) cases (data not shown). However, nominally significant associations were observed between several *PIP4K2A* SNPs and schizophrenia in FH– cases versus controls

(Table VI). Only rs1417374 ($P = 0.004$) and haplotype rs1417374-rs1409395 ($P = 0.0008$) remained significant after 100,000 permutations (perm $P = 0.023$, perm $P = 0.004$ respectively).

This study attempted to replicate association of *PIP4K2A* with schizophrenia in the ISHDSF. We found no evidence for this in the whole sample. Markers rs1417374 (5' upstream) and rs1409395 (intron 1) were nominally associated with disease in the *DTNBP1* HRH+ families, via under-transmission of common alleles preferentially to affected females. In follow-up analysis of the ICCSS, rs1417374 and rs1409395 were significantly associated with schizophrenia, similarly sex-specific (under-representation in female cases of common-allele haplotype) and in FH- individuals.

Previously, major alleles of rs1417374 and rs1409395 were over-transmitted to cases regardless of gender [Schwab et al., [2006]]. We observe their under-representation in female cases from both ICCSS and *DTNBP1* HRH+ families (ISHDSF). This negative association of previously positively associated alleles is one structure of the “flip-flop” phenomenon [Lin et al., [2007]]. The other structure (positive association of opposite alleles between two samples) was seen with *PIP4K2A* and schizophrenia by He et al. [2007]. The latter pattern is intuitively consistent with multiple risk alleles in the gene. The former is consistent with the schizophrenia family data of Hennah et al. [2005], where under-transmission to affected females of haplotype HEP3 in the *DISC1* gene suggested a sex-specific protective variant. However, their sample had over-representation of HEP3, upwardly biasing the expected transmissions so that the observed under-transmission to affected females was more likely an over-transmission to affected males [Hennah et al., [2005]]. Closer inspection of our data for rs1409395 indicates a potentially similar epiphenomenon here (data not shown). Moreover, Schwab et al. [2006] detected *PIP4K2A* association in their 10p-linked families. The fact that most significant (albeit negative) association occurred in ISHDSF families unlinked to 10p, and FH- cases of the ICCSS, may suggest that *PIP4K2A* variants associated in our Irish samples are acting as protective alleles.

Two reviews have demonstrated a male/female risk ratio of 1.4 for developing schizophrenia [Aleman et al., [2003]; McGrath et al., [2004]]. A recent genome-wide association study of schizophrenia identified female-specific association to a SNP in the *RELN* gene ($P = 2.9 \times 10^{-5}$), with significant gene-sex interaction ($P = 4.2 \times 10^{-3}$) and replicated in additional populations [Shifman et al., [2008]]. Sex hormones were postulated to mediate this effect via modulation of *RELN* expression, impacting cortical structure and susceptibility to psychosis. Although we detected no straightforward genotype-sex interaction for *PIP4K2A* via logistic regression, sex hormones may affect *PIP4K2A* expression, for example, estrogen-response elements are predicted in the promoter via MatInspector (www.genomatix.de, data not shown).

Although only five markers over 315 kb were used to cover *PIP4K2A* and the 5' region highlighted by Schwab et al. [2006], this effectively tagged ~50% of regional SNPs, capturing haplotypic diversity reasonably well in our study. However, for rs1417374, the most significant SNP of Schwab et al. [2006] and significantly associated in the ICCSS, the

ISHDSF maybe underpowered to detect disease association. We estimated power using rs1417374 and the Genetic Power Calculator [Purcell et al., [2003]] under “TDT for discrete traits.” Reducing the ISHDSF to 265 trios, with high risk (associated) allele frequency 0.69 and relative risk set at 1.1, 1.3, and 1.5 for the heterozygous genotype and multiplicative model, the ISHDSF had power 11%, 48%, and 82% respectively to detect association at $\alpha = 0.05$. This is a conservative estimate, considering the multigenerational nature of ISHDSF pedigrees. Moreover, Straub et al. [1998] noted the 10p susceptibility locus segregated in only 5–15% families, so we might not expect strong association signals in the whole sample.

In summary, this study shows that genetic variation in *PIP4K2A* plays a minor role in schizophrenia in the Irish population. Together with associations in these samples between *AKT1* and disease [Thiselton et al., [2008]; in preparation], these data provide additional evidence that schizophrenia, at least in part, may result from defective phosphoinositide signaling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Linkage signal on chromosome 10p in the ISHDSF, in the whole sample at the d5 (intermediate phenotype) diagnostic category, and stratified on the presence/absence of *DTNBP1* high-risk haplotype. [Normal View 29K | Magnified View 51K]

Table 1

Allele Frequencies and Intermarker Pairwise LD (D' and r^2 Values) for *PIP4K2A* Gene SNPs Used in This Study

dbSNP name	SNP alias	Genome location (Hg17)	Inter SNP Distance (kb)	MAF	<i>PIP4K2A</i> location	rs1417374	rs1409395	rs10828317	rs746203	rs2364115
(a) ISHDSF sample										
rs1417374	TSC615653	23168481		0.31	Intergenic 5'	-	0.32	0.18	0.14	0.18
rs1409395	TSC599587	22972497	196	0.32	Intron 1	0.56	-	0.48	0.45	0.6
rs10828317	PIK153_154	22879634	93	0.31	Exon 7	0.42	0.7	-	0.62	0.65
rs746203	TSC123902	22870547	9	0.35	Intron 8	0.31	0.65	0.87	-	0.71
rs2364115	TSC1513749	22857725	11.2	0.34	Intergenic 3'	0.4	0.76	0.86	0.86	-
(b) ICCSS sample										
dbSNP name	SNP alias	Genome location (Hg17)	Inter SNP Distance (kb)	MAF	<i>PIP4K2A</i> location	rs1417374	rs1409395	rs10828317	rs746203	rs8341
rs1417374	TSC615653	23168481		0.31	Intergenic 5'	-	0.21	0.52	0.52	0.09
rs1409395	TSC599587	22972497	196	0.34	Intron 1	0.49	-	0.14	0.49	0.52
rs10828317	PIK153_154	22879634	93	0.34	Exon 7	0.4	0.73	-	0.7	0.69
rs746203	TSC123902	22870547	9	0.39	Intron 8	0.36	0.77	0.94	-	0.91
rs8341	TSC1513749	22857725	11.2	0.37	Intergenic 3'	0.36	0.77	0.9	0.99	-

N.B. D' below the diagonal, r^2 above the diagonal. Shading indicates degree of LD.

Table II

Uncorrected *P*-Values From Association Analysis With PDTPHASE of *PIP4K2A* Markers in *DTNBPI* High-Risk Haplotype Positive Families of the ISHDSF Sample

Markers (see haplotype*)	d2	d5	d8	d9
rs1417374 (1)	0.068	0.040	0.041	0.028
rs1409375 (2)	0.049	0.090	0.140	0.104
rs10828317 (3)	0.281	0.301	0.309	0.256
rs746203 (4)	0.963	0.943	0.677	0.490
PIK53_54 (5)	0.983	0.801	0.565	0.594
rs2364115 (6)	0.185	0.228	0.286	0.345

*Haplotype	d2	d5	d8	d9
1-2	0.055	0.046	0.069	0.026
1-3	0.144	0.071	0.087	0.038
1-4	0.217	0.065	0.041	0.040
1-6	0.077	0.033	0.047	0.045
1-2-6	0.120	0.098	0.151	0.090
1-3-4	0.243	0.049	0.074	0.030
1-4-6	0.134	0.034	0.031	0.038

Significant values are highlighted in bold.

Table IIIA
Association Analysis by PDTPHASE Between *PIP4K2A* Markers and Schizophrenia According to Offspring Sex in the ISHDSF Sample

Single markers	P-values (male affecteds)				P-values (female affecteds)			
	d2	d5	d8	d9	d2	d5	d8	d9
rs1417374	0.641	0.640	0.721	0.883	0.140	0.066	0.114	0.098
rs1409375	0.677	0.738	0.721	0.795	0.076	0.060	0.042	0.061
rs10828317	0.965	0.677	0.816	0.505	0.978	0.634	0.650	0.710
rs746203	0.808	0.670	0.605	0.398	0.634	0.619	0.340	0.884
PIK53_54	0.689	0.864	0.743	0.874	0.788	0.519	0.352	0.311
rs2364115	0.957	0.409	0.441	0.438	0.927	0.509	0.409	0.617
Haplotype								
rs1417374-rs1409375	0.316	0.440	0.509	0.713	0.164	0.120	0.137	0.148

Detailed output for rs1409375 to show under-transmission of common allele to affected females									
d8									
All offspring									
Allele	Z	P	Trio-T	Trio-NT	AffSib	UnafSib	Freq		
1	-0.9715	0.3313	857	859.7	943	956	0.6798		
2	0.9715	0.3313	411	408.3	393	380	0.3202		
Global test: chi-sq 0.9438, P 0.3313									

d8									
Male affected offspring									
Allele	Z	P	Trio-T	Trio-NT	AffSib	UnafSib	Freq		
1	0.3569	0.7212	566	548.8	301	293	0.6867		
2	-0.3569	0.7212	246	263.2	109	117	0.3133		
Global test: chi-sq 0.1274, P 0.7212									

d8									
Female affected offspring									
Allele	Z	P	Trio-T	Trio-NT	AffSib	UnafSib	Freq		
1	-2.038	0.04154	291	314	155	173	0.6767		

d8

Female affected offspring						
Allele	Z	P	Trio-T	Trio-NT	AffSib	UnafSib
2	2.038	0.04154	165	142	99	81
Global test: chi-sq 4.154, P 0.04154						
Significant (uncorrected) P-values are shown in bold, those showing a trend in gray.						

Table IIIB
Association Analysis by PDTPHASE Between *PIP4K2A* Markers and Schizophrenia According to Offspring Sex in the *DTNBP1* HRH+ Families

Single markers	P-values (male cases)				P-values (female cases)			
	d2	d5	d8	d9	d2	d5	d8	d9
rs1417374	0.693	0.483	0.379	0.287	0.004	0.013	0.017	0.012
rs1409375	0.370	0.460	0.417	0.578	0.001	0.006	0.012	0.005
rs10828317	0.842	0.805	0.644	0.841	0.084	0.065	0.088	0.030
rs746203	0.843	0.833	0.762	0.405	0.647	0.186	0.250	0.581
PIK53_54	0.684	0.956	0.945	0.995	0.297	0.039	0.140	0.075
rs2364115	0.667	0.853	0.678	0.701	0.282	0.123	0.141	0.166
*Haplotype (see below)								
rs1417374-rs1409375	0.518	0.61	0.529	0.469	0.0009	0.005	0.011	0.004
d2 diagnostic category (female cases)								
Haplotype	Z	P	Trio-T	Trio-NT	AFSib	UnafSib	Freq	
1-1	-3.376	0.0007	49.86	67.72	51.11	59.45	0.61	
1-2	0.7885	0.4304	14.5	11.93	13.63	14.83	0.13	
2-1	0.9808	0.3267	8.147	6.747	5.577	2.552	0.09	
2-2	3.003	0.0027	27.49	13.61	27.68	21.17	0.17	
Global test: chi-sq 16.5, P 0.0009								
Significant (uncorrected) global P-values are in bold.								

Table IV

Association of *PIP4K2A* SNPs With Schizophrenia in the ICCSS

SNPs	Case freq	Control freq	Chi-sq	P	Perm P	Odds ratio
rs1417374	0.68	0.72	5.195	0.023	0.121	0.94
rs1409395	0.64	0.68	4.943	0.026	0.139	0.94
rs10828317	0.66	0.68	1.411	0.235	0.784	0.97
rs746203	0.60	0.63	3.965	0.046	0.236	0.94
rs8341	0.62	0.64	2.529	0.112	0.490	0.98
rs1417374-rs1409395						
Haplotype						
1-1	0.54	0.59	8.869	0.003	0.015	0.91
1-2	0.11	0.09	1.497	0.221	0.761	1.13
2-1	0.14	0.13	1.413	0.235	0.784	1.12
2-2	0.22	0.19	3.019	0.082	0.385	1.14

Significant (uncorrected) values are shown in bold.

Table V

Association of *PIP4K2A* SNPs With Schizophrenia in the Sex-Stratified ICCSS

SNPs	Female cases (major allele freq)	Female controls (major allele freq)	Chi-sq	P	Perm P	Odds ratio	Male cases (major allele freq)	Male controls (major allele freq)	Chi-sq	P	Odds ratio
rs1417374	0.68	0.74	4.804	0.028	0.143	0.92	0.68	0.70	0.896	0.343	0.97
rs1409395	0.64	0.68	1.986	0.158	0.623	0.94	0.64	0.68	3.301	0.068	0.96
rs10828317	0.66	0.70	2.021	0.155	0.615	0.94	0.66	0.66	0.035	0.851	0.99
rs746203	0.60	0.65	3.430	0.063	0.306	0.92	0.60	0.62	0.884	0.347	0.94
rs8341	0.62	0.65	1.449	0.228	0.774	0.95	0.62	0.64	0.946	0.330	0.97
rs1417374+rs1409395											
Haplotype											
1-1	0.54	0.62	6.890	0.009	0.043	0.87	0.53	0.57	2.38	0.123	0.93
1-2	0.10	0.07	3.682	0.055	0.268	1.48	0.11	0.11	0.07	0.790	0.96
2-1	0.14	0.12	0.834	0.361	0.926	1.15	0.15	0.13	0.75	0.387	1.11
2-2	0.22	0.19	1.269	0.260	0.820	1.14	0.22	0.19	1.85	0.173	1.14

Significant (uncorrected) *P*-values are in bold.

Table VI

Association Analysis of *PIP4K2A* SNPs in the FH– Cases Compared to Controls

SNP	Case freq	Control freq	Chi-sq	P	Perm P	Odds ratio
rs1417374	0.343	0.282	8.54	0.004	0.024	1.22
rs1409395	0.367	0.317	5.43	0.020	0.158	1.16
rs10828317	0.351	0.322	1.97	0.161	0.807	1.09
rs746203	0.414	0.368	4.68	0.031	0.230	1.13
rs8341	0.401	0.355	4.51	0.034	0.247	1.13
rs1417374-rs1409395						
Haplotype						
1-1	0.54	0.59	8.869	0.0008	0.004	0.88
1-2	0.11	0.09	1.497	0.033	0.590	1.20
2-1	0.14	0.13	1.413	0.366	0.985	1.10
2-2	0.22	0.19	3.019	0.032	0.242	1.24

Significant (uncorrected) *P*-values are in bold.