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FBXL21 Association With Schizophrenia in Irish Family and Case–Control Samples

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Abstract

FBXL21 gene encodes an F-box containing protein functioning in the SCF ubiquitin ligase complex. The role of the F-box protein is to recruit proteins designated for degradation to the ligase complex so they would be ubiquitinated. Using both family and case–control samples, we found consistent associations in and around *FBXL21* gene. In the family sample (Irish study of high density schizophrenia families, ISHDSF, 1,350 subjects from 273 families), a minimal PDT *P*-value of 0.0011 was observed at rs31555. In the case–control sample (Irish case–control study of schizophrenia, ICCSS, 814 cases and 625 controls), significant associations were observed at two markers (rs1859427 $P = 0.0197$, and rs6861170 $P = 0.0197$). In haplotype analyses, haplotype 1-1 (C-T) of rs1859427–rs6861170 was overtransmitted in the ISHDSF ($P = 0.0437$) and was over-represented in the ICCSS ($P = 0.0177$). For both samples, the associated alleles and haplotypes were identical. These data suggested that *FBXL21* may be associated with schizophrenia in the Irish samples.

Keywords

pedigree disequilibrium test; association; ubiquitination; schizophrenia

INTRODUCTION

It has long been established by family, twin and adoption studies that schizophrenia is strongly influenced by genetic risk factors. To understand its genetic architecture, many linkage and association studies have been conducted [Badner and Gershon, 2002; Lewis et al., 2003]. In the last several years, many promising candidates have been identified [Chowdari et al., 2002; Stefansson et al., 2002; Straub et al., 2002a; Brzustowicz et al., 2004; Duan et al., 2004; Pimm et al., 2005]. There are multiple linkage studies implicating the long arm of chromosome 5 as harboring susceptibility genes for schizophrenia [Schwab et al., 1997; Gurling et al., 2001; Paunio et al., 2001; Straub et al., 2002b; Sklar et al., 2004], and the 5q linkage region covers a large genomic distance of about 100 million basepairs. Several candidates [Sklar et al., 2004; Pimm et al., 2005] have been reported in this region,

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including our recent report of the *SPEC2/PDZ-GEF2/ACSL6* locus [Chen et al., 2006b] and the interleukin 3 (*IL3*) gene [Chen et al., 2006a]. Since there is a cluster of interleukin genes located in this region, our study of *IL3* leads us to examine other interleukins in this region more closely. Interleukin 9 (*IL9*) is one of the interleukins in the 5q region, and there was a report of linkage of a microsatellite within this gene [Schwab et al., 1997]. In the first batch of SNPs typed, we did not find evidence of association within *IL9*. However, we found some markers close to the *FBXL21* gene showed association signals. We then followed up with more SNPs in an interval of 60 kb that contains both the *IL9* and *FBXL21* genes. In this article, we report our study of this region.

The *FBXL21* gene encodes an F-box containing protein, which is a component of the SCF (SKP1-cullin-F-box) ubiquitin protein ligase complex. The SCF complex is involved in the phosphorylation-dependent ubiquitination of targeted proteins, leading to the degradation of the targeted proteins [Cardozo and Pagano, 2004; Ho et al., 2006]. The function of the F-box protein in the SCF complex is to target specific proteins. Several neuron specific F-box proteins have been identified, including *FBL2* and Parkin that are involved in NMDA receptor degradation and Parkinson's disease respectively [Liao et al., 2004; Nateri et al., 2004; Kato et al., 2005; Noda et al., 2005]. Gene expression studies of schizophrenia have also shown altered expression of several ubiquitin related genes [Middleton et al., 2002; Vawter et al., 2002; Altar et al., 2005]. These results suggest that *FBXL21* may be a plausible candidate gene for schizophrenia.

MATERIALS AND METHODS

The ISHDSF Sample

The ISHDSF was collected in the Republic of Ireland and Northern Ireland. Phenotypes were assessed using DSM-III-R. The diagnoses were formed into a hierarchy of 10 categories reflecting the probable genetic relationship of these syndromes to classic schizophrenia. This hierarchy consisted of three definitions of affection: (i) Narrow—categories D1 and D2, or "core schizophrenia"—schizophrenia, poor-outcome schizoaffective disorder and simple schizophrenia; (ii) Intermediate—categories D1 through D5, or a narrow definition of the schizophrenia spectrum, adding to the narrow definition schizotypal personality disorder, schizophreniform disorder, delusional disorder, atypical psychosis and good-outcome schizoaffective disorder; (iii) Broad—categories D1–D8, including all disorders which significantly aggregated in relatives of schizophrenic probands in the Roscommon Family Study [Kendler et al., 1993] and adding to the intermediate definition mood incongruent and mood congruent psychotic affective illness, and paranoid, avoidant and schizoid personality disorder. The final inclusion criteria for pedigrees in the ISHDSF sample required two or more first, second or third degree relatives with a diagnosis of D1–D5, one or more of whom had a D1–D2 diagnosis. The sample contained 273 pedigrees and about 1,350 subjects had DNA sample for genotyping. Of them, 515 were diagnosed with the narrow definition (351 males and 164 females), 634 were diagnosed with the intermediate definition and 686 were diagnosed with the broad definition. Detail descriptions of the sample were published previously [Kendler et al., 2000]. To be consistent with the case–control sample, we used only the narrow disease definition in our statistic analyses in this study.

The ICCSS Sample

The Irish case–control study of schizophrenia (ICCSS) sample was collected in the same geographic regions as that of the ISHDSF sample. In this study, we used 814 affected subjects (553 males and 261 females) and 625 controls (353 males, 269 females, and 3 unknown) from the ICCSS sample. The affected subjects were selected from in-patient and

out-patient psychiatric facilities in the Republic of Ireland and Northern Ireland. Subjects were eligible for inclusion if they had a diagnosis of schizophrenia or schizoaffective disorder by DSM-III-R criteria, which was confirmed by a blind expert diagnostic review. Controls, selected from several sources, including blood donation centers, were included if they denied a lifetime history of schizophrenia. Both cases and controls were included only if they reported all four grandparents as being born in Ireland or the United Kingdom. The sex of subjects was determined experimentally by genotyping 3 X-specific (rs320991, rs321029, and rs6647617) and 3 Y-specific (rs1558843, rs2032598, and rs2032652) SNPs because most of the controls were obtained from blood donation centers and the sex of the donors was not available to us. Subjects with XXY (9 subjects, 4 cases, and 5 controls) and XYY (1 case) genotypes were excluded from the analyses.

Marker Selection and Genotyping

We used the HapMap data and the available assays developed by Applied BioSystems to assist in our selection of markers. We elected to use SNPs that tag major haplotypes (with frequency >1%) in the region. We used the TaqMan method for SNP genotyping [Livak, 1999]. SNPs typed by this method were either validated assays or custom designed assays developed by Applied BioSystems Incorporated (Foster City, CA). All genotypes were scored using a Euclidean clustering procedure developed in our lab. All markers typed were checked for deviation from the Hardy–Weinberg equilibrium (HWE) and Mendelian errors by the PEDSTATS program [Wigginton et al., 2005].

Statistical Analyses

We used the pedigree disequilibrium test (PDT) [Martin et al., 2000] as implemented in the UNPHASED [Dudbridge, 2003] program (PDTPHASE module, version 2.04) to analyze the ISHDSF sample. For each typed SNP, the PDT program was run for the narrow disease definition. In these analyses, both vertical and horizontal transmissions were included. The *P* values reported were based on weighting all families equally (the ave option in the program). In multi-locus haplotype analyses, we used 10 restarts for the expectation-maximization (EM) algorithm [Excoffier and Slatkin, 1995] and used 1% as the cutoff for minor haplotypes. For the case–control sample, a new version of the UNPHASED program (version 3.06) was used to analyze both individual markers and multi-marker haplotype associations. In this version of the UNPHASED program, a retrospective regression model was implemented, which allows covariates to be evaluated simultaneously with the main effect. In the analyses of the ICCSS sample, we included subject sex as modifier in the association analyses. We used the HAPLOVIEW program [Barrett et al., 2004] to estimate pairwise LD and to illustrate haplotype blocks. The haplotype blocks were partitioned by the confidence interval algorithm [Gabriel et al., 2002]. To correct for multiple testing, we used the SNPSpD [Nyholt, 2004] method to estimate the number of independent tests for genotyped SNPs, and Bonferroni correction was applied. This method used pairwise LD information to estimate the number of independent test.

RESULTS

LD Structure in the Two Samples

In this study, we typed a total of 14 SNPs in the ISHDSF. These SNPs cover both *IL9* and *FBXL21* genes. *IL9* and *FBXL21* genes are arranged in head-head orientation, sharing a common promoter region (Fig. 1A). The LD structure of these 14 SNPs in the ISHDSF sample was shown in Figure 1B. From these SNPs, we selected 6 that showed associations to be typed in the ICCSS sample. These 6 SNPs represented the third and fourth LD blocks of the ISHDSF sample (comparing Fig. 1B,C). LD blocks 1, 2, and 5 of the ISHDSF sample did not show association signals. For all markers typed in both samples, Mendelian

consistency and Hardy–Weinberg Equilibrium were verified. Of these markers only one, rs31519, showed a mild Hardy–Weinberg deviation. The details of this and other markers' characteristics were listed in Table I.

The six SNPs typed in both ISHDSF and ICCSS samples covered about 25 kb genomic DNA (see Fig. 1). We estimated pairwise LD with the HAPLOVIEW program for these markers. Similar patterns were seen in the two samples: the six markers were partitioned into two LD blocks and the pairwise LDs were very comparable in the two samples. We noticed that although rs31555 had high LDs with both rs31553 and rs31549, they were not in the same LD block.

Association Analyses of the ISHDSF Sample

For the 14 markers typed in the ISHDSF, we analyzed them with PDT using the narrow disease definition. As shown in Table II, markers 1–5 and 12–14 did not show any association. Markers 6-11 showed modest associations. These 6 markers covered the promoter and coding sequence of the *FBXL21* gene. Markers 6–9 had high pairwise LD, and markers 10 and 11 also had high LD with each other, they formed the third and fourth LD blocks of the ISHDSF sample respectively (Fig. 1B and C). Of the markers typed, rs31555 was the most significant ($P = 0.0011$). The transmitted to not-transmitted (T/NT) ratios for the associated alleles were modest (1.05–1.20).

Based on the results from single marker analyses, we carried out haplotype analyses for the ISHDSF sample. Several multi-marker combinations were significant, the most significant was combination 8-9, with global *P* value of 0.0032 (Table III). Another combination, 6–7, had marginally significant global and haplotype *P* values.

We used the SNPSpD method to estimate the number of independent tests for the 14 SNPs typed in the ISHDSF sample. The SNPSpD program produced 9 independent tests for these 14 SNPs. With these 9 tests and a few haplotype tests, rs31555 and the haplotypes in combination 8–9 remained significant after Bonferroni correction.

Association Analyses of the ICCSS Samples

Based on our results from the ISHDSF, we followed up the six significant markers in an independent case–control sample collected from the same geographical region and ethnic group. Of the six markers typed for the ICCSS sample, rs1859427 and rs6861170 showed significant main effect and sex effect. Another marker, rs185069, showed significant sex effect (Table IV). The significant sex effect observed in these markers implied that the male and female subjects had different risks. To understand how the male and female subjects differed in risk, we separated the subjects by sex and examined the frequencies in males and females separately. We found that rs1859427, rs6861170, and rs185069 all showed significant sexual dimorphism in allele frequencies between cases and controls. For example, for rs1859427, the major allele frequencies for male subjects for cases and controls were 74.7% and 69.6%; for the females, the frequencies were 69.0% and 76.2% respectively for cases and controls. These data indicated that the risk alleles for the male subjects were protective in the females.

We carried out haplotype analyses for combination 6–7, markers showing associations in individual analyses (Table V). In this haplotype analysis, the global *P* value showed a trend, and the two major haplotypes were statistically significant. Both the protective and risk haplotypes identified were the same as that identified in the family sample. The ORs were also comparable. As observed in the individual markers, we also found highly significant sex effect for the two haplotypes. These results were consistent with those observed in the individual markers.

In order to correct for multiple comparisons, we used the SNPSpD [Nyholt, 2004] method to estimate the number of independent tests for the six SNPs we typed in the ICCSS sample. The SNPSpD program suggested that the six typed SNPs had four equivalent independent tests. Adding one haplotype test, we needed to correct for five tests. Since the risk alleles and haplotype were the same as that observed in ISHDSF sample, one tailed test criterion, that is, 10% significance level could be applied. Under this condition, the corrected threshold for significance would be 0.02, and rs1859427, rs6861170 and the 1-1 haplotype also remained significant.

CONCLUSION AND DISCUSSION

Chromosome 5q is a region having broad linkage findings in multiple samples. These linkages cover a genomic distance of more than 100 million basepairs, suggesting that multiple susceptibility genes may lie there. Following on our recent finding of association of *IL3* and its receptor gene CSF2RB [Chen et al., 2007], we initiated our studies of other interleukin genes in the 5q region. *IL9* is one of the interleukin genes in this region. In this search, we typed a total of 14 SNPs in a 60 kb interval containing the *IL9* and *FBXL21* genes. We did not find association signals in the *IL9* gene, but 6 consecutive SNPs in and around the *FBXL21* gene showed significant associations. To follow up on these findings, we genotyped the six associated markers in an independent case–control sample and we found that two of them were associated with schizophrenia. Single marker and haplotype analyses suggested that the associated alleles and haplotype were the same between the two samples. The effect sizes in the two samples were also comparable. Based on these consistent data, we concluded that *FBXL21* may be associated with schizophrenia.

It is worth mentioning that the *FBXL21* gene is not directly under the linkage peak found in the ISHDSF sample. The peak of the linkage is located at about 128 million basepairs of chromosome 5. The MEGF10 and *SPEC2/PDZ-GEF2/ACSL6*, which we reported in our fine-mapping of the linkage peak, are at 126.5 and 131.5 million basepairs respectively. While the *FBXL21* location may be slightly off the linkage peak of the ISHDSF, it is under the linkage peak of other samples [Schwab et al., 1997]. Since the broad linkage region in 5q covers a large genomic distance (about 100 million basepairs) [Schwab et al., 1997; Hovatta et al., 1998; Levinson et al., 2000; Gurling et al., 2001; Paunio et al., 2001; DeLisi et al., 2002; Straub et al., 2002b; Sklar et al., 2004], it is not surprising that multiple susceptibility genes may be located this region.

The associations we observed in both of the ISHDSF and ICCSS samples are of modest strength. While it is possible that our findings may be false positives, several lines of evidence indicate that this likelihood is low. First, the associations observed in the ISHDSF are verified by the FBAT program [Horvath et al., 2001; Laird et al., 2000] with the use of empirical estimate of sibship sharing (the −e option). This option is intended to exclude linkage from association. The results obtained with this option are comparable with that obtained by the PDT program. For example, for markers rs185069, rs31555 and rs31553, the *P* values obtained by the FBAT −e option are 0.005286, 0.003194, and 0.005242 respectively for the narrow disease definition (data not shown). These results suggest that the associations from the PDT program are not likely due to linkage. Second, the associations found in both the ISHDSF and ICCSS are essentially the same. In both samples, the risk alleles and haplotypes are identical. Of particular interest is that the associations are largely restricted to the narrow disease definition. This matches well with the diagnosis of the ICCSS sample. Third, while the associations in the ICCSS sample are of modest strength, both single markers and haplotype survive multiple testing correction. These, in part, meet the criteria proposed for replication of association studies [Neale and Sham, 2004; Sullivan, 2007] in the recent literature.

We should point out that the results presented in this article do not necessarily exclude the association of the *IL9* gene. While the two markers located within *IL9* did not show association, markers in the shared promoter interval did show association signals. Since these two genes are transcribed in opposite directions (Fig. 1), the interval between these genes could serve as promoters for both genes. Because some of the markers showing associations are located in the *FBXL21* gene, we incline to suggest that the associations are with *FBXL21*.

FBXL21 is a relatively small gene. It spans about 6 kb genomic sequences and has 4 exons, Figure 1A. Based on UniGene database (http://www.ncbi.nlm.nih.gov/unigene/estprofileviewer.cgi?uglist=hs.591275), it is only expressed in adult (17 years and older) brain and kidney. It encodes a protein that contains the F-Box motif and is a member of protein family functioning in the ubiquitin ligase complex. Ubiquitin ligase complex is a molecular machine that conjugates ubiqutins to protein designated for destruction and is a critical regulator of protein turnover [Cardozo and Pagano, 2004; Ho et al., 2006]. This machine is made of 3 proteins, 2 of them (SKP1 and Cullin) are fixed members and are responsible for the assembly of the complex and catalytic activities. The 3rd member, an F-box containing protein, is responsible for recruiting target protein. It appears that there is a battery of F-box proteins in the human genome and each of them is responsible for recognizing different proteins. In other words, which protein is to be degraded rests solely on the F-box protein. F-box proteins interact with the SKP1-Collin complex through the F-box motif, a common structure of F-box proteins, and other motifs of F-box proteins associate with targeted proteins when they are phosphorylated. It has been shown that this targeted protein destruction plays a critical role in many biological processes, including control of cell division [Craig and Tyers, 1999], regulation of neuronal receptor density [Liao et al., 2004; Kato et al., 2005], apoptosis and signal transduction [Cardozo and Pagano, 2004; Nateri et al., 2004]. There is evidence that aberrations in ubiquitination are involved in Parkinson's disease [Staropoli et al., 2003; Noda et al., 2005] and Alzheimer's disease [Niikura et al., 2003; von Rotz et al., 2005]. There is a report of association with schizophrenia in an ubiquitin fusion protein [De Luca et al., 2001]. In microarray studies of postmortem human brains and animal model of schizophrenia, genes involved in the ubiquitin system have altered expression [Middleton et al., 2002; Vawter et al., 2002; Altar et al., 2005]. These results suggest that *FBXL21* is a plausible candidate for schizophrenia. Interestingly, *FBXL21* is located within the interval of a recently identified copy number variation [Redon et al., 2006]. This may be a lead for future studies.

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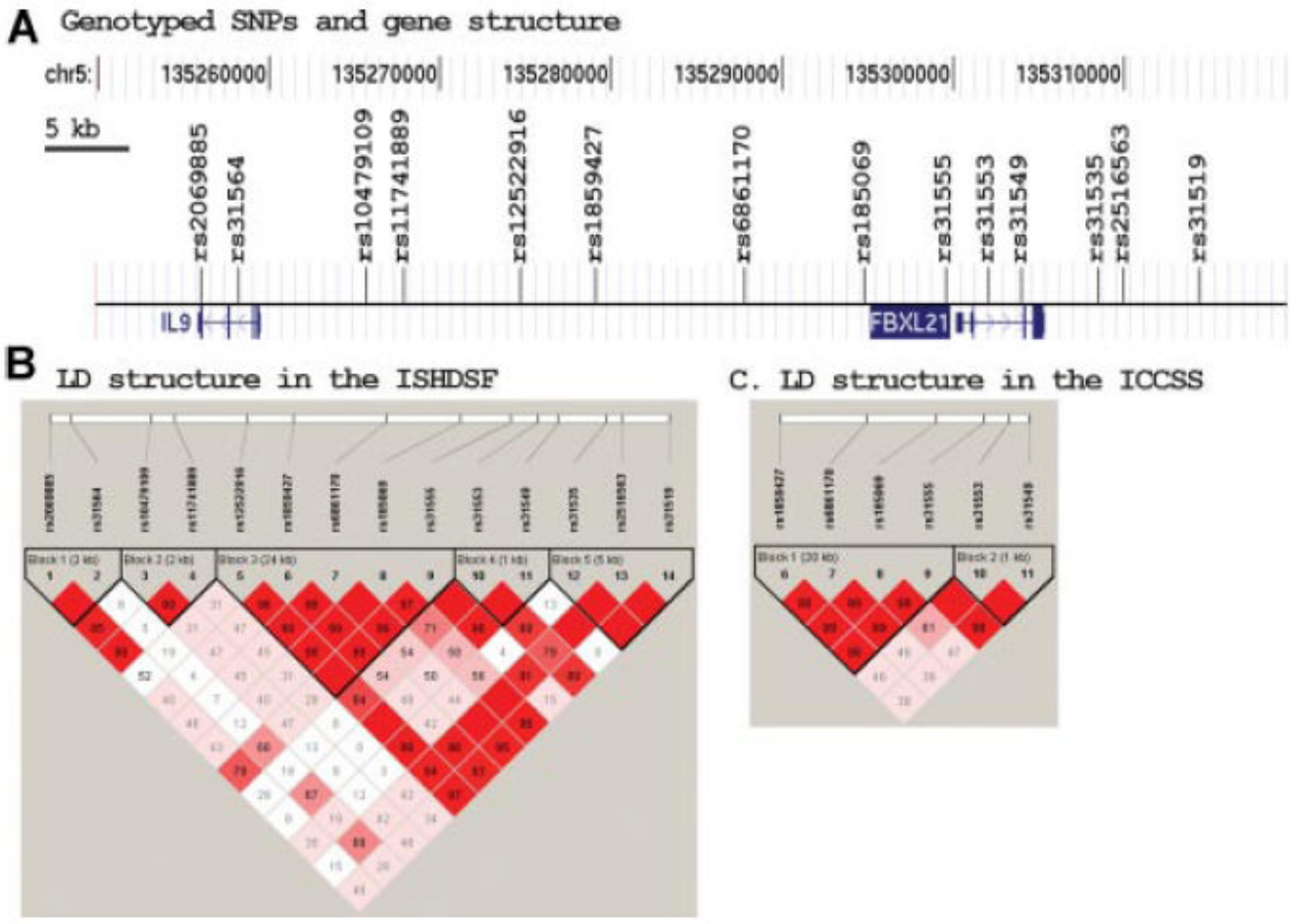

Fig. 1.

The structure of *IL9* and *FBXL21* genes (**A**). *IL9* is transcribed from left to right and FBXL is transcribed from right to left. These two genes share a 40 kb promoter region. The LD structure of the ISHDSF (**B**) and ICCSS (**C**) samples. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I

Marker Characteristics

				ISHDSF		ICCSS	
Marker name	**Marker number**	**Position (may2004)**	**Polymorphism (minor allele)**	**MAF**	**HWE *P* value**	**MAF**	**HWE *P* value**
rs2069885	1	135256064	G/A(A)	0.118	0.2579		
rs31564	2	135258125	G/T(G)	0.396	0.1101		
rs10479109	3	135265608	C/A(A)	0.296	0.4006		
rs11741889	4	135267861	A/T(T)	0.335	0.7585		
rs12522916	5	135274689	A/G(A)	0.201	0.9788		
rs1859427	6	135279071	T/C(T)	0.265	0.9708	0.273	0.9397
rs6861170	7	135287745	G/T(G)	0.264	0.8930	0.273	0.7281
rs185069	8	135294758	T/C(T)	0.331	0.1995	0.338	0.2709
rs31555	9	135299520	C/A(C)	0.376	0.0574	0.350	0.9004
rs31553	10	135302047	T/C(C)	0.315	0.1176	0.316	0.2719
rs31549	11	135303948	G/A(A)	0.462	0.3722	0.439	0.4312
rs31535	12	135308451	G/A(G)	0.478	0.1976		
rs2516563	13	135309866	G/A(A)	0.219	0.2980		
rs31519	14	135314336	G/A(G)	0.419	**0.0305**		

All *P* values less than 0.05 are in bold.

MAF, Minor allele frequency; HWE, Hardy–Weinberg equilibrium.

TABLE II

PDT Statistics in the ISHDSF Sample (Narrow Definition)

Marker name	Transmitted allele	Transmitted (trio + Sib)	Not transmitted (trio + Sib)	T/NT	*P* value
rs2069885	G	101 + 918	104 + 831	1.09	0.4267
rs31564	T	141 + 1204	138 + 1193	1.01	0.9571
rs10479109	C	103 + 926	106 + 897	1.03	0.7062
rs11741889	T	62 + 470	56 + 492	0.97	0.7681
rs12522916	G	114 + 1074	124 + 1032	1.03	0.2556
rs1859427	C	118 + 1054	122 + 986	1.06	**0.0469**
rs6861170	T	116 + 1015	121 + 953	1.05	**0.0495**
rs185069	C	108 + 969	100 + 899	1.08	**0.0211**
rs31555	C	68 + 578	60 + 478	1.20	**0.0011**
rs31553	T	116 + 969	114 + 891	1.08	**0.0431**
rs31549	A	83 + 655	80 + 549	1.17	**0.0365**
rs31535	A	85 + 680	88 + 684	0.99	0.6952
rs2516563	G	128 + 1072	128 + 1073	1.00	0.5879
rs31519	G	68 + 602	61 + 569	1.06	0.6997

All *P* values less than 0.05 are in bold.

T/NT, transmitted/not transmitted ratio.

TABLE III

Haplotype Analyses of the ISHDSF Sample

Marker Combination	Global *P* value	Haplotype	Transmitted (trio + Sib)	Not transmitted (trio + Sib)	T/NT	Haplotype *P* value
6–7	0.0634	1–1	116 + 1012	120 + 947	1.06	**0.0437**
		2–2	48 + 318	43 + 377	0.87	0.0926
8–9	0.0032	1–2	65 + 562	56 + 462	1.21	**0.0015**
		2–1	47 + 374	53 + 445	0.85	**0.0152**

All *P* values less than 0.05 are in bold.

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TABLE IV

Single Marker Association Analyses for the ICCSS Sample

Marker name	Allele	Case cnts (freq)[a]	Control cnts (freq)[a]	OR[a]	Main effect *P* value	Sex effect *P* value
rs2069885						
rs31564						
rs10479109						
rs11741889						
rs12522916						
rs1859427	C	815 (0.748)	490 (0.696)	1.07	**0.0197**	**0.0006**
rs6861170	T	811 (0.748)	487 (0.698)	1.07	**0.0197**	**0.0013**
rs185069	C	716 (0.673)	438 (0.639)	1.05	0.1647	**0.0047**
rs31555	C	696 (0.641)	437 (0.648)	0.99	0.8622	0.5524
rs31553	T	741 (0.681)	462 (0.672)	1.01	0.5213	0.4743
rs31549	A	591 (0.537)	302 (0.436)	1.23	0.5641	0.6287
rs31535						
rs2516563						
rs31519						

All *P* values less than 0.05 are in bold.

[a]Due to sexual dimorphism, the counts (cnts), frequencies (freq) and odds ratios (OR) listed were from the male subjects.

TABLE V

Haplotype Analyses of the ICCSS Sample

Marker combination	Global *P* value	Haplotype	Case cnt (freq)[a]	Control cnt (freq)[a]	OR[a]	Haplotype main effect *P* value	Haplotype sex effect *P* value
6–7	0.0965	1–1	805 (0.744)	482 (0.691)	1.08	**0.0177**	**0.00085**
		2–2	268 (0.248)	206 (0.295)	0.84	**0.0299**	**0.00085**

All *P* values less than 0.05 are in bold.

[a]Due to sexual dimorphism, the counts (cnts), frequencies (freq) and odds ratios (OR) listed were from the male subjects.