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Is the Histidine Triad Nucleotide-binding Protein 1 (*HINT1*) Gene a Candidate for Schizophrenia?

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Abstract

Background—The histidine triad nucleotide-binding protein 1, *HINT1*, hydrolyzes adenosine 5'-monophosphoramidate substrates such as AMP-morpholidate. The human *HINT1* gene is located on chromosome 5q31.2, a region implicated in linkage studies of schizophrenia. *HINT1* had been shown to have different expression in postmortem brains between schizophrenia patients and unaffected controls. It was also found to be associated with the dysregulation of postsynaptic dopamine transmission, thus suggesting a potential role in several neuropsychiatric diseases.

Methods—In this work, we studied 8 SNPs around the *HINT1* gene region using the Irish study of high density schizophrenia families (ISHDSF, 1350 subjects and 273 pedigrees) and the Irish case-control study of schizophrenia (ICCSS, 655 affected subjects and 626 controls). The expression level of *HINT1* was compared between the postmortem brain cDNAs from schizophrenic patients and unaffected controls provided by the Stanley Medical Research Institute.

Results—We found nominally significant differences in allele frequencies in several SNPs for both ISHDSF and ICCSS samples in sex-stratified analyses. However, the sex effect differed between the two samples. In expression studies, no significant difference in expression was observed between patients and controls. However, significant interactions amongst sex, diagnosis and rs3864283 genotypes were observed.

Conclusion—Data from both association and expression studies suggested that variants at *HINT1* may be associated with schizophrenia and the associations may be sex-specific. However, the markers showing associations were in high LD to the *SPEC2/PDZ-GEF2/ACSL6* locus reported previously in the same samples. This made it difficult to separate the association signals amongst these genes. Other independent studies may be necessary to distinguish these candidate genes.

Keywords

Linkage disequilibrium test; association; Histidine Triad Nucleotide-binding protein 1; schizophrenia; Irish families; case-control study; gene expression

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2 Introduction

Schizophrenia is a severe psychiatric disorder characterized by a complex mode of inheritance. Family linkage studies and studies of chromosomal abnormalities associated with schizophrenia have identified a number of schizophrenia susceptibility loci (Lewis et al. 2003b). Our group has found promising linkages at 5q21-31, 6p25-24, 6p23, 8p22-21 and 10p15-p11 in the Irish Study of High Density Schizophrenia Families (ISHDSF) (Straub et al. 2002). These linkage regions had also been reported by several other groups (Lewis et al. 2003a; Schwab et al. 2000). In our previous fine-mapping of the linkage peak at 5q21-31 with the ISHDSF sample, we found that a 758 kb interval coding for the *SPEC2/PDZ-GEF2/ACSL6* gene was associated with schizophrenia (Chen et al. 2006). Markers in this area show sex-specific associations with schizophrenia. Telomeric to this area, we also found that interleukin 3 (*IL3*) gene, which is in a head-to-head orientation to *ACSL6*, is associated with schizophrenia in our Irish family and case control samples (Chen et al. 2007a). The histidine triad nucleotide-binding protein 1 (*HINT1*) gene is located immediately centromeric (about 194 kb) to the *SPEC2/PDZ-GEF2/ACSL6* locus. In the fine-mapping study, we observed that several markers in the vicinity of *HINT1* showed nominal significance (Chen et al. 2006).

The *HINT1* gene is a ubiquitous member of the histidine triad (HIT) protein family characterized by the presence of a conserved HIT sequence motif (Klein et al. 1998). The human *HINT1* gene is about 6 kb in length, containing 3 exons (Figure 1). *HINT1* has tumor suppressor function through inhibiting transcription factor AP-1 activity by binding to a POSH-JNK2 complex, thus inhibiting the phosphorylation of c-Jun (Wang et al. 2007), or by interacting with Pontin and Reptin and inhibiting TCF-beta-catenin-mediated transcription (Welske and Huber 2005). *HINT1* protein is widely expressed in mammalian brains including the mesocorticolimbic and mesostriatal regions. Weitzdoerfer and colleagues reported significant reduction of *HINT1* protein in fetal brains of Down Syndrome patients (Weitzdoerfer et al. 2001). In microarray expression studies, Vawter et al reported decreased mRNA expressions of *HINT1* in the dorsolateral prefrontal cortex of schizophrenia patients (Vawter et al. 2002). These differences were confirmed by real-time quantitative polymerase chain reaction (Q-PCR) and in situ hybridization (Vawter et al. 2004). In that study, male subjects with schizophrenia had lower expression than male controls. Using *HINT1* knockout mice, Wang et al. reported that systemic administration of apomorphine, a dopamine receptor agonist, significantly increased locomotor activity in *HINT1* knockout mice, suggesting that postsynaptic dopamine function may be altered in these animals (Wang et al. 2006). Furthermore, the authors indicated that the absence of *HINT1* protein may be associated with the dysregulation of postsynaptic dopamine transmission. In view of the documented link between dopamine transmission and schizophrenia, they suggested the possible involvement of *HINT1* in the pathophysiology of the disease. All these studies suggest that *HINT1* is a plausible candidate gene for schizophrenia. To define the centromeric boundary of the association signals observed in the *SPEC2/PDZ-GEF2/ACSL6* region we performed association and expression studies of the *HINT1* gene.

3 Materials and Methods

3.1 The ISHDSF sample

The ISHDSF was collected in Northern Ireland, United Kingdom and the Republic of Ireland. The sample contained 273 pedigrees and about 1350 subjects had DNA sample for genotyping. Historically, the ISHDSF sample had multiple disease definitions reflecting the probable genetic relationship of the syndromes to classic schizophrenia. In this study, we used the narrow definition, which includes schizophrenia, poor-outcome schizoaffective disorder and simple schizophrenia according to DSM-III criteria (Kendler et al. 2000), so we could compare the

results with that of the ICCSS sample directly. Using this definition, 522 subjects were classified as affected.

3.2 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. The affected subjects were selected from in-patient and out-patient psychiatric facilities in the Republic of Ireland and Northern Ireland, United Kingdom. Subjects were eligible for inclusion if they had a diagnosis of schizophrenia or schizoaffective disorder by DSM-III-R criteria. 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. In this study, we used 655 (436 males and 219 females) affected subjects and 626 (354 males, 269 females) controls.

3.3 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 selected tagged SNPs that cover haplotypes with frequency > 1%. A total of 8 SNPs were used in this study. rs4696 is a synonymous SNP in exon 1, the other SNPs are in the introns or 5' or 3' flanking regions of *HINT1*. Their distribution in the gene was shown in Figure 1. The selected SNPs were developed by Applied BioSystems Corporation (Foster city, CA). All genotyping was conducted with the TaqMan method (Livak 1999). Genotypes were scored using a semi-automated Excel Template developed in our lab (van den Oord et al. 2003). All typed SNPs were checked for Mendelian consistency and Hardy-Weinberg Equilibrium (Wigginton et al. 2005).

3.4 Expression studies

The expression studies were carried out with postmortem brain cDNAs from the Stanley Medical Research Institute (<http://www.stanleyresearch.org/>). The mRNA isolation and reverse transcription to cDNA were carried out by the Stanley researchers. The Stanley panel consisted of 104 subjects, of them, thirty five individuals were diagnosed as schizophrenia (26 males and 9 females; mean $\pm$ SD age, 42.6 $\pm$ 8.5 years; postmortem interval (PMI), 31.4 $\pm$ 15.5 h; brain pH, 6.5 $\pm$ 0.2), thirty four individuals were diagnosed as bipolar disorder (16 males and 18 females; mean $\pm$ SD age, 45.4 $\pm$ 10.6 years; PMI, 37.9 $\pm$ 18.6 h; brain pH, 6.4 $\pm$ 0.3), and thirty five were unaffected controls (26 males and 9 females; mean $\pm$ SD age, 44.2 $\pm$ 7.6 years; PMI, 29.4 $\pm$ 12.9 h; brain pH, 6.6 $\pm$ 0.3). Diagnoses were made according to DSM-IV criteria. There were no significant demographic differences between the schizophrenia, bipolar disorder and control subjects. All schizophrenic patients were medicated with antipsychotics.

Q-PCRs were conducted with TaqMan expression probe (Hs00602163_m1) for the *HINT1* gene, and human TATA box binding protein (*TBP*) gene was used as internal reference. Specifically, each sample was amplified in triplicates, and for each reaction, 0.25 ng of cDNAs were used in 15 μ L of PCR mixture containing the FAM-labeled *HINT1* probe and VIC-labeled *TBP* probe. PCR was conducted with the iCycler real time PCR machine from Bio-Rad (Hercules, CA). PCR cycling parameters were 95°C for 2 min followed by 55 cycles of 92°C for 15 sec and 60°C for 1 min. The expression level of each reaction was determined by the C_T value (calculated by the iCycler software, version 3.1). The results from three repeat assays were averaged to produce a single mean C_T value for each individual. The relative expression level between the *HINT1* and *TBP* for each individual was calculated by the $2^{-\Delta C_T}$ method, where $\Delta C_T = C_T^{HINT1} - C_T^{TBP}$ (Livak and Schmittgen 2001).

3.5 Statistical analyses

We used the pedigree disequilibrium test (PDT) (Martin et al. 2000) as implemented in the UNPHASED program (version 2.4, PDTPHASE module) (Dudbridge 2003) to analyze the ISHDSF sample. In these analyses, both vertical and horizontal transmissions were included. The p values reported were based on weighing all families equally (the ave option in the program). In multilocus 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, the UNPHASED program (Dudbridge 2008) was used to analyze both single marker and multi-marker haplotype associations. As in the family sample, haplotypes with frequencies less than 1% were aggregated. For all multi-marker combinations, the global and individual haplotype tests were performed simultaneously and p values obtained were χ^2 distributions. We used the HAPLOVIEW program (Barrett et al. 2005) to estimate pairwise LD and to illustrate haplotype blocks. The haplotype blocks were partitioned by the confidence interval algorithm (Gabriel et al. 2002).

For the samples from the Stanley Institute, potentially confounding variables (Table 1) were tested for association with *HINT1* expression in all individuals using generalized linear regression analysis with the SPSS software (version 10 for Windows). For the schizophrenia and healthy control samples, we found that brain pH was significantly correlated with *HINT1* expression level ($B=0.306$, $p=0.018$) (Table 1), therefore, brain pH was used as a covariate in subsequent analyses. ANCOVA analyses were used to compare the expression levels between the controls and schizophrenia patients. Based on the results of our association analyses of both ISHDSF and ICCSS samples, sex was used as a covariate in the ANCOVA analyses.

4 RESULTS

4.1 LD and haplotype structure

We examined the LD and haplotype structure for both ISHDSF and ICCSS with the HAPLOVIEW program. In both samples, most markers shared high LD with each other, but only markers 1–5 were grouped in a single LD block. The overall pattern of the two samples was similar (Figure 2).

4.2 Association analyses of the ISHDSF sample

Marker information and allele frequencies were presented in Table 2. The genotype frequencies of all 8 markers studied were in accordance with the Hardy–Weinberg equilibrium (HWE). Using the PDT to examine the associations, we found that three markers (rs7728773, rs4696 and rs7735116) reached 5% nominal significance.

Based on our previous studies of the *SPEC2/PDZ-GEF2/ACSL6* and *IL3* loci, where the associations were sex-specific, we performed sex-stratified analyses. In these analyses, we found that several markers (rs3891636, rs7728773, rs2189663, rs3864283 and rs2526303) were nominally significant in the female subjects, but not significant in the male subjects (Table 2). These were consistent with the signals observed in the *SPEC2/PDZ-GEF2/ACSL6* and *IL3* loci.

We also conducted sex-stratified analyses for multi-marker associations to identify significant intervals according to LD structure. In multi-marker haplotype analyses, many multi-marker combinations were significant in the females (Table 3), but not in the males (data not shown). The most significant combination was 5-7-8, with a global P -value of 0.0021. In this combination, the most significant haplotype ($p=0.0014$), C-T-G, was undertransmitted, another haplotype, C-A-C, was overtransmitted to the affected subjects. In all combinations,

undertransmitted haplotypes were more significant than the overtransmitted haplotypes (Table 3).

4.3 Association analyses of the case control (ICCSS) sample

For the ICCSS sample, we typed the same 8 SNPs. For all markers typed, no HWE deviations were observed. The allele frequencies of these 8 SNPs were similar as those of the family sample (Table 4). No significant associations were observed when the entire sample was analyzed. In sex stratified analysis, we found that rs3864283 showed association in allelic tests in the male subjects (Table 4). To be consistent with our findings in the ISHDSF, we conducted sex-stratified haplotype analyses, however, no significant associations were observed (data not shown).

4.4 False positive rate evaluation

To evaluate whether our findings are likely false, we used the Q-value program (Storey and Tibshirani 2003) to estimate the proportion of true null in our association analyses. Of the 365 tests performed for the family and case control samples, including single marker tests, haplotype tests for 2-, 3-, 4-, and 5-marker combinations, the true null proportion (π_0) estimated was between 0.531–0.607 depending on the parameters used. While no test had a q value less than 0.05, 54 tests had a q values ≤ 0.15 , and these tests all had a p value ≤ 0.04 . In other words, about 85% of these 54 tests are likely to be true findings. These included female specific tests for rs3891636, rs7728773, rs2189663, rs3864283 and rs2526303 and all haplotype tests listed in Table 3.

4.5 Expression studies of the *HINT1* gene in the brain

We used Q-PCR to determine the expression levels of the *HINT1* gene. The C_T values for all subjects were obtained from the BioRad iCycler software. First, variables potentially affecting the expression of postmortem mRNAs were evaluated separately on the expression of the *HINT1* gene by linear regression analysis. No variables had significant relation with the expression of *HINT1* gene for the entire samples, which include the schizophrenia patients, bipolar disorder patients and unaffected controls (data not shown). However, when schizophrenia patients and controls were compared, brain pH had significant effect on the expression of the *HINT1* gene ($B=0.306$, $p=0.018$). Based on this finding, we used brain pH and sex as covariates in subsequent analyses. No significant difference was observed between patients and controls (Table 5) However, significant interaction between sex and diagnosis was observed with or without the inclusion of the genotypes of rs3864283. As expected, sex has significant influence on the expression of the gene. It was interesting that the results became more significant when patients of bipolar disorder were included in this analysis (Data not shown).

5 Discussion

Multiple linkage studies have implicated chromosome 5q21-33, where *HINT1* is located, as a region likely to harbor risk genes (Schwab et al. 1997), including the study of ISHDSF (Straub et al. 1997; Straub et al. 2002). Gene expression of *HINT1* in prefrontal cortex was found to decrease in schizophrenia and male patients has lower level expression of *HINT1* (Vawter et al. 2002; Vawter et al. 2004). Recently *HINT1* was also suggested to be associated with dysregulation of postsynaptic dopamine transmission (Barbier et al. 2007), thus suggesting a potential role in several neuropsychiatric diseases.

In this work we conducted association studies with family and case control samples. In the family sample, ISHDSF, we found three markers (rs7728773, rs4696 and rs7735116) were nominally significantly associated with schizophrenia. We also found that five markers

(rs3891636, rs7728773, rs2189663, rs3864283 and rs2526303) were significant in the female subjects. The sex-stratified analyses showed stronger association signals, suggesting that almost all association signals were derived from female offspring, which was consistent with the result of our previous study of genes in 5q22-31 region (Chen et al. 2007b; Chen et al. 2006). In the case control sample, there was no significant association when the entire sample was analyzed. In sex stratified analyses, rs3864283 showed nominally significant association in the male subjects, several other markers showed a trend. This is different than the family sample. However, it is clear that both samples showed clear sex-specific associations. It is worth noting that markers rs3864283 showed opposite effects in the two sexes. In our previous study of genes in the 5q22-31 region or *CSF2RB* gene (Chen et al. 2006), similar sexual heterogeneity was observed. In sex stratified haplotype analyses, we found that many multi-marker combinations were significant in family samples. The most significant combination was 5-7-8, with a global P-value of 0.0021. In this combination, the most significant haplotype ($p=0.0014$), T-G-C, was undertransmitted to the affected subjects. Another haplotype C-A-C, with frequency of 0.188, was overtransmitted to the affected subjects ($p = 0.0304$). Similar results were observed in other marker combinations that undertransmitted haplotypes were more significant than the overtransmitted haplotypes (Table 3). But no significant haplotype association was found in the case control sample. The discrepancies were puzzling but not surprising. Some promising candidate genes identified for schizophrenia in recent years had similar inconsistencies (Levitt et al. 2006; Riley and Kendler 2006; Williams et al. 2005; Tosato et al. 2005; Camargo et al. 2007; Norton et al. 2006).

To compare with other genes in this linkage region directly, we analyzed markers used in this study with those reported in the *SPEC2/PDZ-GEF2/ACSL6* locus (Chen et al. 2006) in the family and case-control samples. In the family sample, these 8 markers in *HINT1* region have high LD with those positive markers reported in the previous study (Supplementary data, Figure 1S and Table 1S). For example, markers rs2549012, rs3756295, rs1291602, rs31251, rs152815 and rs2240525 are all in high LD with markers used in this study. Similar analyses were also done for the case control sample (Supplementary data, Figure 2S and Table 2S), and the LD pattern was similar to that of the family sample. These results suggest that those significant markers in *HINT1* may have the same origin as those positive markers in *SPEC2/PDZ-GEF2/ACSL6* locus. In other words, the association signals observed at the *HINT1* gene are the same as that at the *SPEC2/PDZ-GEF2/ACSL6* locus. Therefore, we cannot differentiate these genes with confidence although the strength of signals is weaker at the *HINT1* gene.

In expression study, we observed no difference between schizophrenia patients and healthy controls. But we did find significant interactions between diagnosis and sex with or without the inclusion of the genotypes of rs3864283 (Table 5), which showed sex-specific associations in both family and case-control samples. These results indicated that sex has different influences on the expression for affected and unaffected subjects and supported the notion that sex is involved in the associations observed at this gene.

Our association and expression studies suggest that *HINT1* may be associated with schizophrenia and the association is sex specific. In recent years there is increasing evidence that sex may play a significant role in the etiology of schizophrenia. Sex specific associations with schizophrenia have been reported for the catechol o-methyltransferase gene (Shifman et al. 2002) and several other genes like Nogo and GPR50 (Tan et al. 2005; Thomson et al. 2005). It has been argued recently that sex may play a more important role in brain structure and psychiatric disorders than commonly acknowledged (Salem and Kring 1998; Czlonkowska et al. 2005; Cahill 2006). Our finding is consistent with this converging evidence.

With this study, we have examined multiple genes spanning over 1 million basepair genomic distance in the linkage region of the ISHDSF sample. In these studies, consistent sex-specific

associations were observed across the region although the strength of the signals varied. Since PDT does not exclude linkage from association, we analyzed the association with FBAT –e option (Laird et al. 2000) that is designed to exclude linkage in family based association test. Of the 8 markers tested in this study, 3 of them (rs7728773, rs4696 and rs7735116) have nominal significance (p values = 0.0450, 0.0197 and 0.0250 respectively) (data not shown). These results are comparable with what we observed with PDT program (Table 2). We have also assessed the likelihood that findings in this study are false by a false positive discovery rate method (Storey and Tibshirani 2003). In this assessment, a majority of the tests with a p value ≤ 0.04 are likely to be true, which include those significant single marker tests for the female subjects and all haplotype tests listed in Table 3. Based on this assessment, we incline to conclude that the associations found at *HINT1* are more likely to be true than to be false.

The associations we observed in multiple genes in this region raise an interesting question. How many risk genes are there under a single linkage peak? What is the relationship amongst these genes? In our study of the 5q21-23 linkage region, we have observed sex-specific associations in several genes covering over 1 million basepairs. While there are many genes in this interval the association signals are originated from markers sharing extensive high LD (see supplementary Figure 1S and 2S). In this sense, the multiple association signals in these genes are really the same signal. Of note, the sharing of LD is higher in the family sample than the case-control sample (comparing Figure 1S and 2S). This may be one of the reasons that the associations found in the family sample are more consistent across genes in this region. Although there are differences in strength of association signals, we cannot distinguish these genes with confidence because association test is based on underlying LD at the testing site. From a functional perspective, it is also possible that multiple functional elements (either regulatory elements or genes) in this region contribute collectively to the risk to schizophrenia. In recent literature, there is evidence that multiple elements in a large genomic interval coordinate the expression of multiple genes (Maeda and Karch 2007; Cleard et al. 2006; Gierman et al. 2007; Noordermeer et al. 2008). We can speculate that if schizophrenia is caused by the disorganization and coordination of multiple genes spanning a relatively large genomic distance, then multiple signals over many genes in a chromosomal region would be reasonable, or even likely, since chromatin is organized in domains and chromosome territories and multiple genes in the domains and chromosome territories are regulated coordinately. Functional studies of this region, including gene function and genomic and chromatin structures, may be necessary to understand the underlying mechanisms.

In summary, we have found nominally significant associations in both the family and case control samples in the *HINT1* gene and the association seems sex specific. We have also observed interactions on the expression level of *HINT1* amongst sex, diagnosis and rs3864283 genotypes. All this suggests that sex plays a significant role in the associations found in *HINT1* and this is consistent with the results at the *SPEC2/PDZ-GEF2/ACSL6* locus reported previously. Given the high LD between the *HINT1* gene and the *SPEC2/PDZ-GEF2/ACSL6* locus and the same sex specific associations, we believe that the signals observed at *HINT1* should be considered the same as that observed in the *SPEC2/PDZ-GEF2/ACSL6* locus. Therefore, we may not be able to exclude *HINT1* as a susceptibility gene for schizophrenia despite the weak associations cannot sustain multiple testing correction at the *HINT1* gene in both samples. Independent replications and functional studies may be necessary to differentiate these genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Genomic structure and location of genotyped SNPs in the HINT1 gene. HINT1 spans over 6 kb and is composed of 3 exons (vertical bars). The gene is transcribed from right to left. Eight markers were genotyped in this study.

Figure 2.
Comparison of LDs between the ISHDSF (A) and ICCSS (B) samples.

Table 1
Regression analysis of confounding variables on HINT1 expression in schizophrenia and healthy control.

Factor	B	P-value
Age	0.053	0.689
Sex	0.243	0.061
Brain pH	0.306	0.018
PMI	0.135	0.303
Brain weight	0.185	0.157
Smoking status at time of death	0.030	0.845
Lifetime alcohol use	0.000	1.000
Lifetime drug use	-0.144	0.280
Lifetime antipsychotics	-0.244	0.060

Table 2
Marker characteristics and single marker associations (*p* values) in the ISHDSF

Marker ID	Marker	Polymorphism	Distance to next marker (bp)	MAF	HWE <i>P</i> *	Total	Male	Female
1	rs3891636	C/T	0	0.213	0.316	0.103	0.871	0.012
2	rs7728773	T/C	7328	0.225	0.360	0.040	0.756	0.005
3	rs2189663	A/T	44217	0.257	0.677	0.185	0.970	0.016
4	rs2551038	C/G	57048	0.120	0.623	0.188	0.731	0.054
5	rs3864283	C/T	58337	0.259	0.251	0.428	0.662	0.035
6	rs4696	G/A	61751	0.033	0.315	0.024	0.295	0.206
7	rs2526303	A/G	64195	0.346	0.278	0.340	0.465	0.014
8	rs7735116	A/C	66670	0.040	1.000	0.005	0.025	0.660

Polymorphism, minor allele is in the left. MAF, Minor allele frequency; HWE, Hardy-Weinberg equilibrium.

Table 3
Sex stratified haplotype association in the ISHDSF sample (females)

Marker Combination	Global p	Haplotype	T(cnt)	NT(cnt)	Haplotype p
2-3-4	0.0051	C-T-G	18+133	27+146	0.0120
		T-A-G	12+42	6+33	0.0204
5-7-8	0.0021	T-G-C	23+134	34+154	0.0014
		C-A-C	14+39	9+30	0.0304
4-5-7-8	0.0019	G-T-G-C	20+97	30+111	0.0012
		G-C-A-C	11+34	7+25	0.0210

Table 4

Allelic association (*p* values) of the ICCSS sample

SNP	MAF	HWE	Total	Male	Female
rs3891636	0.191	0.889	0.217	0.055	0.620
rs7728773	0.200	0.429	0.358	0.098	0.576
rs2189663	0.235	0.455	0.221	0.060	0.659
rs2551038	0.099	0.517	0.721	0.916	0.521
rs3864283	0.236	0.408	0.156	0.027	0.577
rs4696	0.032	0.498	0.226	0.189	0.931
rs2526303	0.329	0.058	0.157	0.138	0.805
rs7735116	0.037	0.852	0.483	0.395	0.800

Table 5
Diagnosis, sex and rs3864283 genotypes influence HINT1 expression

Source	Wald Chi- Square	df	P-Value
(Intercept)	1.055	1	0.304
Diagnosis	0.061	1	0.806
rs3864283 genotype	5.524	2	0.063
Brain pH	2.779	1	0.095
sex	7.963	1	0.005
Diagnosis * rs3864283 genotype	4.383	2	0.112
Diagnosis * rs3864283 genotype * sex	3.933	1	0.047
Diagnosis * sex	5.767	1	0.016
rs3864283 genotype * sex	0.996	2	0.608