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Interaction between Interleukin 3 and Dystrobrevin-Binding Protein 1 in Schizophrenia

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Abstract

Schizophrenia is a common psychotic mental disorder that is believed to result from the effects of multiple genetic and environmental factors. In this study, we explored gene-gene interactions and main effects in both case-control (657 cases and 411 controls) and family-based (273 families, 1350 subjects) datasets of English or Irish ancestry. Fifty three markers in 8 genes were genotyped in the family sample and 44 markers in 7 genes were genotyped in the case-control sample. The Multifactor Dimensionality Reduction Pedigree Disequilibrium Test (MDR-PDT) was used to examine epistasis in the family dataset and a 3-locus model was identified (permuted $p = 0.003$). The 3-locus model involved the *IL3* (rs2069803), *RGS4* (rs2661319), and *DTNBP1* (rs2619539) genes. We used MDR to analyze the case-control dataset containing the same markers typed in the *RGS4*, *IL3* and *DTNBP1* genes and found evidence of a joint effect between *IL3* (rs31400) and *DTNBP1* (rs760761) (Cross-validation consistency 4/5, balanced prediction accuracy = 56.84%, $p = 0.019$). While this is not a direct replication, the results obtained from both the family and case-control samples collectively suggest that *IL3* and *DTNBP1* are likely to interact and jointly contribute to increase risk for schizophrenia. We also observed a significant main effect in *DTNBP1*, which survived correction for multiple comparisons, and numerous nominally significant effects in several genes.

Keywords

Epistasis; Association; Multifactor Dimensionality Reduction algorithm; Pedigree Disequilibrium Test

2 Introduction

Schizophrenia is a complex disorder in which both genetic and environmental factors are likely involved. Heritability estimates are as high as 80% (Sullivan et al. 2003), and several

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chromosomal regions have been linked to the trait (Owen et al. 2004b). Due to this observed heterogeneity, some of the etiology of schizophrenia is likely the result of both biological and statistical epistasis (O'Donovan et al. 2003). Biological epistasis (Bateson 1909) is the result of physical interactions of biomolecules in individuals (Moore and Williams 2002), while statistical epistasis, or gene-gene interaction, initially described by R.A. Fisher, is a deviation from additivity in a mathematical model (Fisher 1918). Statistical epistasis is thereby a population-level event measured on average outcomes among samples, whereas biological epistasis is only observable in individuals. It has been suggested that variation among individuals in biologically epistatic processes is likely to yield statistically epistatic effects (Moore and Williams 2005).

Interest in statistical epistasis among Schizophrenia investigators has recently increased. Schizophrenia samples have been examined for statistical epistasis using several approaches (Macgregor and Khan 2006;Zammit et al. 2004;Qin et al. 2005b;Ott et al. 2005;Sullivan et al. 2001). Some studies of epistasis rely exclusively on regression and relatively restricted hypotheses (Norton et al. 2006;Norton et al. 2002;De, V et al. 2006;Corvin et al. 2007;Nicodemus et al. 2007), and sometimes couple statistical results with functional studies (Talkowski et al. 2007). Other investigations of statistical epistasis in schizophrenia use non-parametric methods that do not rely on the strict modeling assumptions of regression to detect and test for interaction. One such method which has been shown to have good power in studies with binary categorical outcomes is the multifactor dimensionality reduction method (MDR) (Ritchie et al. 2001;Hahn et al. 2003;Hahn and Moore 2004;Moore 2004;Moore 2007;Ritchie et al. 2003). MDR is a nonparametric method that performs an exhaustive search of all possible interactions and maps the data into a single dimension relevant to association. Critical values for significance are obtained via permutation testing, which inherently adjusts for the multiple comparisons from the search performed. Several recently published studies have applied MDR to the statistical epistasis problem in schizophrenia (Qin et al. 2005a;Yasuno et al. 2007;Vilella et al. 2007;Zhao et al. 2007).

In this study, we employ the recently developed multifactor dimensionality reduction pedigree disequilibrium test (MDR-PDT) (Martin et al. 2006) method to screen for potential interactions in our Irish Study of High Density Schizophrenia Families (ISHDSF) sample and use an independent case-control sample, the Irish Case Control Study of Schizophrenia (ICCS) to verify the finding using MDR. MDR-PDT functions very similarly to MDR, searching through all possible interactions for association with the genotype pedigree disequilibrium test (Martin et al. 2003a). This method is especially useful for family-based samples that have been collected for the purposes of linkage analysis. Some statistical epistasis investigation is possible with linkage analysis, as in (Schulze et al. 2004). However, association analysis is required to map susceptibility to combinations of specific genes rather than chromosomal regions (Risch and Merikangas 1996). Our results suggest that this approach may be effective to identify real interactions for biological testing.

In the last several years, promising candidate genes (Stefansson et al. 2002;Brzustowicz et al. 2004;Pimm et al. 2005;Chowdari et al. 2002;Hodgkinson et al. 2004;Duan et al. 2004) have been identified, including dystrobrevin-binding protein 1 (*DTNBPI*) (Straub et al. 2002a), the *SPEC2/PDZ-GEF2/ACSL6* locus (Chen et al. 2006) and interleukin 3 (*IL3*) (Chen et al. 2007b) which were reported from observations made in the ISHDSF.

Our group has been working with the ISHDSF for many years now and found suggestive linkages at several chromosome regions (Straub et al. 2002b). Following up these linkages, several candidate genes, i.e. *DTNBPI* (Straub et al. 2002a), the *SPEC2/PDZ-GEF2/ACSL6* locus (Chen et al. 2006) and *IL3* (Chen et al. 2007b) have been identified. We have also studied the regulator of G-protein signaling 4 (*RGS4*) (Chen et al. 2004a) and catechol-o-

methyltransferase (*COMT*)(Chen et al. 2004b) genes in this sample, and modest associations were also observed. These studies, in detecting multiple associations from the same sample, provided an opportunity to examine whether these genes work in concert and collectively contribute to increase the risk of schizophrenia. Combined with the ICCSS samples, this study has the capability to replicate multilocus associations in two independent study designs and significantly advance the understanding of the genetic etiology for these functional and positional candidates in schizophrenia.

3 Methods and Materials

3.1 Subjects

3.1.1 The ISHDSF sample—The ISHDSF was collected in Northern Ireland, United Kingdom and the Republic of Ireland. Phenotypes were assessed using DSM-III-R. Individuals in the study were determined to be cases if they were diagnosed as having schizophrenia, poor-outcome schizoaffective disorder, simple schizophrenia, schizotypal personality disorder, schizophreniform disorder, delusional disorder, atypical psychosis and good-outcome schizoaffective disorder. The final inclusion criteria for pedigrees in the ISHDSF sample required two or more first, second or third degree relatives with a diagnosis above, one or more of whom had a schizophrenia, poor-outcome schizoaffective disorder or simple schizophrenia diagnosis. The sample contained 273 pedigrees and about 1350 subjects had a DNA sample for genotyping. Of these, 654 were diagnosed as cases (427 males and 227 females). Detailed descriptions of the sample were published previously (Kendler et al. 2000).

3.1.2 The ICCSS sample—The ICCSS sample was collected in the same geographic regions as that of the ISHDSF sample. In this study, we used 657 affected subjects (436 males and 221 females) and 411 controls (233 males and 178 females). The affected subjects were selected from in-patient and outpatient psychiatric facilities in the Republic of Ireland and Northern Ireland. Subjects were eligible for inclusion if they had a diagnosis of schizophrenia or poor-outcome 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. The broader trait definition for the ISHDSF was used to provide more cases and offset the lesser sensitivity per sample for family-based tests of association.

3.2 Marker selection and genotyping

In this study, we selected to use a total of 53 markers from several genes that had shown significant associations in the ISHDSF sample. These markers covered the *CSF2RB*, *DTNBP1*, *RGS4*, *SPEC2*, *PDZ-GEF2*, *FNIP1*, *ACSL6* and *IL3* genes. For the ICCSS sample, we typed the same markers in the *DTNBP1*, *RGS4*, *SPEC2*, *PDZ-GEF2*, *FNIP1*, *ACSL6* and *IL3* genes. The details for the markers typed in the ISHDSF and ICCSS are listed in Supplemental Table I.

We used two techniques for SNP genotyping. A majority of SNPs were typed by the TaqMan method (Livak 1999). SNPs typed by this method were either validated assays or custom designed assays developed by Applied BioSystems Corporation (Foster city, CA). The remaining SNPs were typed using the FP-TDI protocol (Chen et al. 1999; van den Oord et al. 2003a). For the FP-TDI procedures, DNA sequences of SNPs obtained from dbSNP [<http://www.ncbi.nlm.nih.gov/SNP/index.html>] were masked by the RepeatMasker program (Bedell et al. 2000) and PCR and FP-TDI extension primers were designed by the PRIMER 3 program (Rozen and Skaletsky 2000). We used the FP-TDI genotyping products from the Perkin Elmer Corporation (Boston, MA) and followed the recommended procedures for PCR

and single base extension. All markers typed were checked for deviation from the Hardy-Weinberg Equilibrium (HWE) and Mendelian errors by the PEDSTATS program (Wigginton et al. 2005).

3.3 Statistical analyses

3.3.1 ISHDSF sample—We used the allele and genotype pedigree disequilibrium sum tests (PDT, Geno-PDT) (Martin et al. 2000; Martin et al. 2003a) to analyze single marker association in the entire ISHDSF sample. Analyses of these markers using the PDT averaged statistics were reported elsewhere (Chen et al. 2004a; Chen et al. 2006; Straub et al. 2002a; Chen et al. 2007b). These analyses were performed in this manner due to the use of the PDT-sum statistics in the current implementation of MDR-PDT, and the desire for consistency among signals detected by multilocus and single-locus methods. Additionally, the PDT averaged statistics can be biased to the alternate hypothesis in some pedigrees and the sum statistics are generally more powerful (Martin et al. 2001). PDT methods and MDR-PDT do not support covariate analysis of non-SNP variables, and although sex may be associated with schizophrenia, these data contain extended pedigrees that cannot be partitioned by sex. To address this issue the whole data was analyzed to assess for the presence of signals that could affect PDT and MDR-PDT results and inferences.

Haplotype analysis was performed using the Association in the Presence of Linkage (APL) (Martin et al. 2003b; Chung et al. 2006) statistic and HBAT (Horvath et al. 2004) with the $-e$ option for regions of known linkage for the ISHDSF samples. These analyses were conducted with a cutoff frequency of 1% for rare haplotypes.

The MDR-PDT (Martin et al. 2006) was used to explore multi-locus associations in the ISHDSF sample. The MDR-PDT is a within-family measure of indirect or direct association between genotype and disease. As described previously (Martin et al. 2006), the PDT statistic (Martin et al. 2003a) functions within the framework of the MDR algorithm. Genotypes are classified as high and low-risk by comparing the genoPDT statistic to a threshold of 0, where positive statistics indicate evidence for association at that genotype. The MDR-PDT statistic is then calculated for the pooled high-risk genotypes for each set of loci. The models are ordered and evaluated by MDR-PDT statistics. A permutation test is applied to estimate the significance of the result, which inherently adjusts for the size of the search performed. The MDR-PDT procedure is illustrated in Figure 1 and includes the following steps: 1. All possible discordant sib pairs (DSPs) are generated within each sibship and pooled; 2. Each genotype is determined to be high or low risk by comparing the geno-PDT statistic for each multilocus genotype to a threshold τ ($\tau = 0$); where whether above or below the threshold indicates positive or negative association with affected status; 3. Statistics for the pooled high-risk genotypes are calculated using the PDT. This is the MDR-PDT statistic for this model. A classification error (CE), calculated as the number of low-risk affecteds plus high-risk unaffecteds divided by the total sample, is also calculated for the model; 4. Steps 2–3 are repeated for all possible combinations of loci, calculating an MDR-PDT statistic and CE for each, choosing the largest MDR-PDT statistic as the final result; And 5. A permutation test is performed to determine the distribution of the statistic under the null hypothesis, to which the result from step 4 is compared for significance assessment.

To examine whether the best model of a given order that is observed by MDR-PDT is a real signal or is likely to be the result of sampling error, a permutation test is conducted. The permutation test consists of randomizing status for offspring, holding the proportion of affected individuals constant across permutations, calculating the statistic, and repeating many times to estimate the distribution of the null hypothesis. The test based on the permutation procedure should have the correct type I error, even for sparse data. This validity is due to all contingency table cells from each permutation containing the same number of observations as those from

the unpermuted data. In this study, we limit our search to include only 2- and 3-locus interactions.

3.3.2 ICCSS sample—Hardy-Weinberg equilibrium (HWE) and single marker allele and genotype frequency association analyses were performed using chi-squared statistics from Powermarker v3.25 statistical software (Liu and Muse 2005). A Fisher's exact test was used when cell counts in the table relating genotypes to status for a marker were less than 5. Where we observed Hardy-Weinberg disequilibrium, we calculated inbreeding coefficients separately in cases and controls to determine whether the disequilibrium was evidence of association (Wittke-Thompson et al. 2005). We used the MDR method with 5-fold cross-validation to screen for potential interactive effects. As in the family sample, we limited our search to 2- and 3-locus interactions. Permutation testing is used to estimate the statistical significance of an MDR result. Haplotype trend regression from Powermarker with permutation testing (Liu and Muse 2005) and the Haplo.Stats (Schaid 2004) module from the R project for the ICCSS. Again these analyses were conducted with a cutoff frequency of 1% for rare haplotypes.

3.3.3 Multiple Testing Corrections—Multiple testing was accounted for depending on the type of analysis. MDR and MDR-PDT both inherently correct for the search conducted with permutation testing. Haplotype analysis significance in the ICCSS and ISHDSF was also adjusted with permutation testing with either the HBAT -p option or in haplotype trend regression. Multiple tests of main effects were corrected for using Nyholt's SNPSpD method (Nyholt 2004) with the modification of (Li and Ji 2005). The effective number of tests for the 44 markers which were in both datasets was 17.133 for the family data and 17.277 for the controls from the case-control data, showing the similarity of correlation among SNPs for these independent samples. For the full 53 markers from the family data, there were 24.148 effectively independent tests. For significance assessment for tests of main effects where any null may be rejected from either sample to observe association, there are $17.277 + 24.148 = 41.425$ independent hypothesis tests, for an alpha threshold of 0.0012. This threshold is determined by the Sidak correction for multiple tests (Sidak 1967) which is slightly more liberal than the Bonferroni correction but provides the exact correction necessary to return the experiment-wise error rate to the desired level.

However, we also would like to assess significance where two tests have been performed for the same null hypothesis in the independent samples. For this analysis, requiring that both tests reject at the level established for any individual test is conservative. To investigate the combined evidence for association across all the data, we used Fisher's method (Fisher 1950) to merge p-values from each SNP in different samples. We then compared the merged p-value to the threshold for significance for the effective number of independent tests established by SNPSpD for the ICCSS controls, $\alpha = 0.0021$, since this was the higher of the two estimated effective independent tests for the two samples. We note here that these tests are supplemental to the single hypothesis tests described above, using the same data, and thus are not independent tests requiring further corrections.

We also report all nominally significant findings here for the use of other investigators looking at these genes.

4 Results

4.1 Single locus results

4.1.1 ISHDSF sample—Based on our previous studies, we selected a total of 53 SNPs to study in the ISHDSF. These SNPs cover the *DTNBPI*, *RGS4*, *SPEC2*, *PDZ-GEF2*, *FNIP1*, *ACSL6*, *IL3* and *CSF2RB* genes. These genes were reported to be associated with schizophrenia in our ISHDSF sample (Straub et al. 2002a;Chen et al. 2004a;Chen et al. 2006;Chen et al.

2007b;Chen et al. 2007a). Of these markers, many are nominally associated with schizophrenia at the allele and/or genotype level in the ISHDSF (Table 1). The most significant results were observed in the *SPEC2* and *DTNBP1* genes. In *SPEC2*, both allele and genotype tests exceeded the threshold for multiple tests at rs3756295 and in *DTNBP1* rs760761 exceeded the threshold for genotype test.

4.1.2 ICCSS sample—In the ICCSS, 44 markers in *RGS4*, *DTNBP1*, and the chromosome 5 linkage region including, *SPEC2*, *PDZ-GEF2*, *FNIP1*, *ACSL6*, and *IL-3* were genotyped. Four markers were nominally significantly associated with disease at either alleles or genotypes, and 3 additional markers showed a trend (Table 2). Some of these markers deviated slightly from Hardy-Weinberg equilibrium; however, this can be considered evidence of association if the disequilibrium of heterozygotes is in opposite directions in cases and controls (rs2549012, genotype $p = 0.02$, control $F = 0.05$, case $F = -0.13$; rs3756295, genotype $p = 0.08$, control $F = 0.02$, case $F = -0.11$; rs31251, genotype $p = 0.027$, control $F = 0.06$, case $F = -0.10$; and rs2240525 in genotype $p = 0.06$, control $F = 0.05$, case $F = -0.09$). However, only one marker, rs760761, in *DTNBP1* exceeded the threshold for multiple testing.

The chromosome 5 markers rs2549012, rs3756295, rs31251 and rs2240525 and the chromosome 6 markers rs2005976 and rs760761 all showed evidence of association from both the ISHDSF and the ICCSS. The association at *DTNBP1* marker rs760761 exceeded the threshold for multiple tests for all tests from either sample.

4.1.3 Merged results—Fisher's method was employed to obtain combined estimates of significance for all tests which examined a single null hypothesis in both independent samples (Supplementary Table 2). The threshold for significance for these tests is the more stringent of the thresholds established by SNPSpD for either sample as described in section 2.5. In these results there were many nominally significant findings, several exceeded the threshold for significance taking into account multiple testing. Two tests of genotypes in *SPEC2* at rs2549012 ($p = 0.0007$) and rs3756295 ($p = 0.0004$) were strongly associated with the disease. Also the genotype test for *ACSL6* SNP rs2240525 ($p = 0.0015$) was significantly associated. Two markers in *DTNBP1* were significantly associated, rs2005976 at alleles ($p = 0.0021$) and rs760761 at alleles and genotypes (allele $p = 0.00005$, genotype $p = 0.00003$).

4.2 Haplotype results

Association signals were strong and distributed throughout the chromosome 5 region among these samples in the ISHDSF (Table 3). Previously unreported associated haplotypes were observed using a 3-locus scan with APL and testing the full haplotype with HBAT using the $-e$ option which adjusts the null hypothesis for association in regions of known linkage, and the HBAT $-p$ option which permutation tests the results for multiple testing adjustment. A 4-locus haplotype was found overlapping the intergenic space and 5' ends of *ACSL6* and *IL3* including rs3914025, rs3846726, rs3916441, and rs31400. APL and HBAT observed nominal signals for the minor allele haplotype. An adjacent region contained a 5-marker haplotype overlapping the intergenic haplotype at rs31400 and also included rs31480, rs40401, rs31481, and rs2069803. The overlap of associated haplotypes at rs31400 is relevant for the subsequent epistasis analyses, and in previous analyses this marker was found to associate with disease in females in the ISHDSF and ICCSS (Chen et al. 2007b). If the haplotypes underlying the interaction is dependent on family history and sex, then the ICCSS would be less powerful for detecting the haplotype associations because less than one third of the affected subjects had positive family history of schizophrenia. The tests for these chromosome 5 haplotypes in the ICCSS in the full sample and in females were not significant.

Additionally, a haplotype was observed in *DTNBP1* in the ISHDSF and the ICCSS spanning 4 markers including rs2005976, rs760761, rs2619522, and rs1018381 (Tables 3 and 4). This haplotype was highly significantly associated in both the ICCSS and ISHDSF, reflecting both the strength and diffuse nature of the association in this gene.

4.3 Epistasis results

Epistasis was explored in ISHDSF samples using the MDR-PDT (Figure 1). These analyses revealed a significant 3-locus model ($p = 0.003$). This model includes *IL3* SNP rs2069803, *DTNBP1* SNP rs2619539, and *RGS4* SNP rs2661319 (Figure 2).

We also used MDR with 5-fold cross-validation to evaluate 2- and 3-locus interactions in the ICCSS sample. A significant 2-locus interaction was observed by MDR between *IL3* SNP rs31400 and *DTNBP1* SNP rs760761, with a cross-validation consistency of 4/5 and average balanced prediction accuracy = 56.84%, $p = 0.019$ (Figure 3).

To test whether the signals from the interacting markers found in the ISHDSF and ICCSS came from the same genetic background, we carried out 2-locus haplotype analyses in both *IL3* and *DTNBP1* using the two loci implicated in the models. If significant, these results would indicate that both SNPs from each model were in linkage disequilibrium with the causal variant functioning in the interaction. For the ISHDSF, we used the APL statistic and observed nominally significant associations in both *DTNBP1* (global $p = 0.04$) and *IL-3* (global $p = 0.01$). For the ICCSS sample, haplotype trend regression with permutation testing was used. There was no significant risk haplotype for the *IL3* gene while a nominally significant haplotype was identified for the *DTNBP1* gene (global $p = 0.01$). These negative results in *IL3* were not unexpected, because we knew that the associations in the ICCSS for *IL3* are sex-specific and family-history dependent (Chen et al. 2007b).

5 Discussion

Schizophrenia is believed to have complex etiology and multiple risk factors may be required for the manifestation of the disease. In recent years, several candidate genes have been reported in the ISHDSF samples. Taking advantage of these data, we use the MDR-PDT method to explore potential epistatic interactions in this sample. We tested 2- and 3-locus interactions for 53 SNPs covering the *DTNBP1*, *RGS4*, *CSF2RB*, *IL3*, *SPEC2*, *PDZ-GEF2*, *FNIP1* and *ACSL6* genes. We found a 3-locus model involving *RGS4*, *IL3* and *DTNBP1*. We attempted but failed to replicate these results with an independent case-control sample (ICCSS). However, a 2-locus interaction involving both *IL3* and *DTNBP1* genes with different markers was identified. To understand whether the markers implicated in the ISHDSF and ICCSS came from the same genetic background, we conducted 2-locus haplotype analyses for rs31400-2069803 and rs2619539-rs760761 for the *IL3* and *DTNBP1* genes respectively. We found that the haplotypes in the ISHDSF sample were indeed derived from previously identified risk haplotypes in the *IL3* (Chen et al. 2007b) and *DTNBP1* genes (van den Oord et al. 2003b). For the ICCSS sample, a major haplotype (1-1) in the *DTNBP1* gene was marginally significant. This haplotype was the risk haplotype reported in a German sample (Schwab et al. 2003) and it differed from the one identified in ISHDSF. No significant haplotype was observed in the *IL3* gene when the entire ICCSS sample was used. Two-locus haplotype analyses using only those subjects with positive family history identify the same risk haplotype ($p = 0.0762$) as that observed in the ISHDSF sample (data not shown).

This study found 2 multilocus models featuring different markers in *IL3* and *DTNBP1*. In the ISHDSF sample, MDR-PDT reported a 3-locus interaction while the ICCSS reported a 2-locus interaction with MDR. The difference between the two models was the presence of an *RGS4* SNP in the MDR-PDT model. This may be due to the generally weaker associations observed

in the ICCSS. *RGS4* is a strong functional candidate for schizophrenia susceptibility, since *RGS4* transcripts are significantly less abundant in postmortem samples of dorsolateral prefrontal cortex in schizophrenics than in normal controls (Mirnics et al. 2001). Significant correlation has been observed between *RGS4* genetic variation and dorsolateral prefrontal cortex morphometry in schizophrenic patients relative to controls (Prasad et al. 2005). It was also observed that when sex was used as the outcome instead of disease status, the geno-PDT found a trend toward transmission disequilibrium among genotypes of the MDR-PDT model *RGS4* SNP rs2661319 to either sex (global $p = 0.09$). This sampling-error effect might also explain the presence of the *RGS4* SNP in the MDR-PDT model, as it may be functioning as a surrogate for the sex variable, allowing the true interaction between *IL3* and *DTNBP1* to be detected.

A similar 2-locus interaction between *IL3* and *DTNBP1* was identified by MDR; however, the markers involved were different. As reported previously, we observed associations in multiple markers in the chromosome 5 region covering a large genomic distance and many markers in this region share high LD. Variations in signal strength were observed for individual markers between different samples. This variation may contribute to the difference of 2-locus interaction between ISHDSF and ICCSS. Another possible reason to explain the difference between the ISHDSF and ICCSS is that the ISHDSF consists of extended families with multiple affected individuals in each family, while the ICCSS consists of largely sporadic cases. If family history is important for this interaction, then the marginal interaction observed in the ICCSS might be due to the lack of power since only about 30% of the cases in the ICCSS have positive family history. This rationale is based on our previous report that the association observed in *IL3* in ICCSS is family history dependent. It may be possible that the markers associated in both the MDR and MDR-PDT models are in tight LD with the same functional variant in both *IL3* and *DTNBP1* that is contributing to increased susceptibility for Schizophrenia. Although we cannot exclude the possibility that this finding is false, since it does not meet the stringent criteria of replication (Sullivan 2007). But the interactions in the ISHDSF and ICCSS involve the same 2 genes, this does meet part of the criteria (replication at the level of gene) proposed by Neale and Sham (Neale and Sham 2004). Taken together, while the markers in the interaction observed in the ICCSS are not the same as that in the ISHDSF sample, we believe that it does support the interaction observed in the ISHDSF sample.

The biological connection between *IL3* and *DTNBP1*, however, is not obvious. This may be the case for many genetic interactions that are observed statistically. Many such interactions are observed at the population level in *S. cerevisiae* which include genes with no known physical interaction (Schuldiner et al. 2005; Tong et al. 2001). These investigations have uncovered novel biology in pathways which were previously extensively studied (Tong et al. 2004). These studies demonstrated that variation in genes which function together in and across biochemical pathways can have unforeseen effects on phenotypes, and that exhaustive searches through these spaces can reveal models which predict gene functions and phenotype outcomes (Segre et al. 2005).

IL3 is a cytokine secreted by Th0 immune cells in the immune system. Th2 activity has been shown to be increased in schizophrenic patients (Avgustin et al. 2005). Other Th2 cytokines have also been associated with schizophrenia, such as *IL2* and *IL4* (Schwarz et al. 2006). As a pleiotropic growth factor, *IL3* is essential for the development and differentiation of all hematopoietic cell types. Withdrawal of *IL3* triggers apoptosis, a process which has been implicated in schizophrenia pathophysiology (Jarskog et al. 2005; Margolis et al. 1994). While both *IL3* and *IL3* receptors are expressed in the brain (Konishi et al. 1995), little is known about their functions. Transgenic mice with over- or suppressed-expression of *IL3* all have neurological dysfunctions (Chavany et al. 1998; Chiang et al. 1996; Cockayne et al. 1994). In

animal models, *IL3* is capable of activating microglia and astrocytes in the brain (Chiang et al. 1996; Powell et al. 1999). When activated, both microglia and astrocytes participate in and modulate synaptogenesis via production of cytokines, chemokines and other permeable molecules (Bessis et al. 2007; Natarajan et al. 2004).

On the other hand, *DTNBP1* is a structural protein, and it has been shown to be expressed in the brain and associated with the synaptic complex (Weickert et al. 2004; Talbot et al. 2006). *DTNBP1* appears to play a role in cognitive function and memory (Owen et al. 2004a). Dysbindin has been observed to be present in significantly reduced concentration in schizophrenic brain tissue relative to normal controls (Weickert et al. 2004). Because of these relevant functions and impact on multiple traits, *DTNBP1* has been postulated to mediate the course of schizophrenia (McClellan et al. 2007). But its role in synapsis is not clear. Based on our current knowledge of both *IL3* and *DTNBP1*, we can only speculate that *DTNBP1* is a downstream regulatory target of *IL3*. To understand the nature and mechanism of this regulation, further studies are necessary. These results may assist investigators who study molecular mechanisms and pathophysiology to design experiments which elucidate the process by which these genes interact to produce schizophrenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Diagram illustrating the multifactor dimensionality reduction pedigree disequilibrium test (MDR-PDT) algorithm. Details of the procedure are in the text.

Figure 2.

MDR-PDT three-locus model summary of interaction between *RGS4* SNP rs2661319, *IL3* SNP rs2069803, and *DTBPN1* SNP rs2619539. Each multifactorial cell is labeled as “high risk” ($T = 3$) or “low risk” ($T < 3$). For each multilocus stratification, empirical distributions of affected (left bar in cell) and unaffected (right bar in cell) are shown. The classification accuracy for the 3-locus model is 63.31% (permuted p -value = 0.008) with a MDR-PDT statistic of 5.41 (permuted p -value = 0.003).

Figure 3.

MDR two-locus model of *IL3* SNP rs31400 and *DTNBP1* SNP rs760761. Distribution of cases (*left bar in cell*) and controls (*right bar in cell*) stratified by multilocus genotype. Each multifactorial cell is labeled as “high risk” or “low risk”. Average balanced prediction accuracy for the model was 56.84%, permuted $p = 0.019$.

Table 1
Single-locus association results from the ISHDSF grouped by gene.

Marker	Position	Alleles (minor)	Gene	Role	Risk allele	P-Value	
						Allele- PDT	Geno- PDT
rs2619539 ^l	chr6:15728834	C/G (G)	DTNBP1	Intron	-	0.415	0.282
rs16876738 ^l	chr6:15735532	A/G (A)	DTNBP1	Intron	-	0.317	0.053
rs1011313 ^l	chr6:15741411	A/G (G)	DTNBP1	Intron	G	0.861	0.049
rs2005976 ^l	chr6:15758781	A/G (G)	DTNBP1	Intron	G	0.006	0.008
rs760761 ^l	chr6:15759111	C/T (T)	DTNBP1	Intron	T	0.006	0.001 ^d
rs2619522 ^l	chr6:15761628	C/A (A)	DTNBP1	Intron	T	0.021	0.026
rs1018381 ^l	chr6:15765049	C/T (T)	DTNBP1	Intron	-	0.116	0.155
rs2072707	chr22:35643581	G/T (T)	CSF2RB	Intron	-	0.217	0.496
rs2284031	chr22:35645580	C/T (T)	CSF2RB	Intron	-	0.063	0.200
rs17811365	chr22:35647320	G/T (G)	CSF2RB	Intron	-	0.389	0.555
Rs909486	chr22:35648488	C/T (T)	CSF2RB	Intron	-	0.059	0.096
rs2075936	chr22:35653251	A/G (G)	CSF2RB	Intron	-	0.730	0.787
rs11705394	chr22:35654176	C/T (C)	CSF2RB	Intron	-	0.387	0.133
rs7285064	chr22:35654499	C/T (T)	CSF2RB	S/S	-	0.277	0.031
Rs131840	chr22:35658294	C/T (T)	CSF2RB	P/P	-	0.310	0.583
Rs131842	chr22:35660273	C/T (C)	CSF2RB	3' UTR	-	0.285	0.507
rs1030271 ^l	chr5:130660554	C/G (C)	SPEC2	Intron	C	0.024	0.095
rs2549012 ^l	chr5:130687657	A/C (C)	SPEC2	Intron	C	0.005	0.004
rs4706020 ^l	chr5:130701975	A/G (A)	SPEC2	Intron	A	0.022	0.087
rs3756295 ^l	chr5:130720739	C/G (G)	SPEC2	Intron	G	0.001 ^d	0.001 ^d
Rs40396 ^l	chr5:130735943	C/G (G)	SPEC2	Intron	G	0.009	0.037
Rs27708 ^l	chr5:130771440	A/G (G)			G	0.019	0.065
rs4996522 ^l	chr5:130789369	A/G (A)	PDZ-GEF2	3' UTR	G	0.004	0.023
rs1291602 ^l	chr5:130794561	C/T (T)	PDZ-GEF2	Q/R	-	0.148	0.321
rs739952 ^l	chr5:130824467	A/T (T)	PDZ-GEF2	Intron	T	0.009	0.036
rs244739 ^l	chr5:130834773	A/C (C)	PDZ-GEF2	Intron	C	0.005	0.021
Rs31251 ^l	chr5:130861845	A/G (G)	PDZ-GEF2	Intron	G	0.045	0.009

Marker	Position	Alleles (minor)	Gene	Role	Risk allele	P-Value	
						Allele- PDT	Geno- PDT
rs6873582 [/]	chr5:130920640	A/G (A)	PDZ-GEF2	Intron	G	0.018	0.074
rs1422871 [/]	chr5:130970380	C/T (T)	PDZ-GEF2	Intron	T	0.011	0.043
rs152815 [/]	chr5:131054117	A/G (A)	KIAA1961	Intron	A	0.005	0.028
Rs13164236 [/]	chr5:131107707	C/T (C)	KIAA1961	Intron	T	0.019	0.072
rs1566427 [/]	chr5:131219571	A/G (G)	ACSL6	Intron	G	0.011	0.027
rs1355095 [/]	chr5:131276668	A/G (G)	ACSL6	Intron	-	0.478	0.650
rs667437 [/]	chr5:131303408	A/G (A)	ACSL6	Intron	-	0.065	0.242
rs477086 [/]	chr5:131312509	C/T (T)	ACSL6	Intron	-	0.074	0.264
rs615305 [/]	chr5:131319755	A/G (G)	ACSL6	Intron	G	0.013	0.040
rs2240525 [/]	chr5:131343783	A/G (G)	ACSL6	Intron	G	0.009	0.003
rs399714 [/]	chr5:131346687	C/T (T)	ACSL6	Intron	T	0.019	0.095
Rs77938 [/]	chr5:131350393	C/T (C)	ACSL6	Intron	-	0.161	0.349
rs440970 [/]	chr5:131364186	G/T (G)	ACSL6	Intron	-	0.263	0.626
rs246999 [/]	chr5:131376070	A/G (G)	ACSL6	Intron	G	0.029	0.076
rs3914025 [/]	chr5:131381184	C/T (T)	ACSL6	Intron	-	0.344	0.635
rs3846726 [/]	chr5:131386898	A/G (T)	ACSL6	Promoter	-	0.289	0.790
rs3916441 [/]	chr5:131397140	A/G (A)			-	0.412	0.564
Rs31400 [/]	chr5:131417406	C/T (T)	IL3	Promoter	-	0.454	0.779
Rs31480 [/]	chr5:131424231	C/T (T)	IL3	Promoter	T	0.029	0.109
Rs40401 [/]	chr5:131424377	C/T (T)	IL3	P/S	T	0.017	0.082
Rs31481 [/]	chr5:131425101	A/G (A)	IL3	Intron	-	0.074	0.179
rs2069803 [/]	chr5:131428332	C/T (C)	IL3	3' UTR	T	0.005	0.026
Rs10917670 [/]	chr1:159764500	C/T (T)	RGS4	Promoter	-	0.569	0.442
rs951436 [/]	chr1:159765000	A/T (T)	RGS4	Promoter	-	0.906	0.459
rs951439 [/]	chr1:159765349	C/T (T)	RGS4	Promoter	-	0.871	0.315
rs2661319 [/]	chr1:159771435	A/G (G)	RGS4	Intron	-	0.359	0.704

[/]Markers also genotyped in the ICCSS

^aExceeds the threshold for significance for any single-locus test from either sample

Table 2
Association detected with χ^2 tests on alleles and genotypes from the ICCSS

Gene (Role)	Marker	Minor Allele Freq.		HWE P-Value		χ^2 P-Value	
		Controls	Cases	Controls	Cases	Allele	Genotype
No gene	rs2549012	0.386	0.389	0.008	0.23	0.877	0.017
SPEC2 (prom)	rs3756295	0.373	0.36	0.023	0.548	0.538	0.078
PDZ-GEF2 (intron)	rs31251	0.395	0.381	0.039	0.113	0.514	0.027
ACSL6 (intron)	rs2240525	0.389	0.377	0.054	0.207	0.555	0.06
DTNBP1 (intron)	rs16876738	0.12	0.094	0.32	0.817	0.062	0.172
DTNBP1 (intron)	rs2005976	0.199	0.162	0.409	0.055	0.039	0.071
DTNBP1 (intron)	rs760761	0.247	0.181	0.495	0.987	0.001^a	0.002 ^b

^aExceeds the threshold for significance for any single-locus test for either sample

^bExceeds the threshold for significance for tests within the ICCSS

Table 3
APL and HBAT-e haplotype analysis for associated blocks in ISHDSF

Analysis	Gene	Markers	Haplotype	Frequency	P-Value
APL	-	rs3914025-rs3846726-rs3916441	T-A-A	0.392	0.001
APL	-	rs3846726-rs3916441-rs31400	A-A-T	0.356	0.025
HBAT -e	-	rs3914025-rs3846726-rs3916441-rs31400	T-A-A-T	0.361	0.046
HBAT -p	-	rs3914025-rs3846726-rs3916441-rs31400	T-A-A-T	0.361	0.038
APL	IL3	rs31400-rs31480-rs40401	T-C-C	0.392	0.043
APL	IL3	rs40401-rs31481-rs2069803	C-G-T	0.341	0.011
HBAT -e	IL3	rs31400-rs31480-rs40401-rs31481-rs2069803	T-C-C-G-T	0.344	0.037
HBAT -p	IL3	rs31400-rs31480-rs40401-rs31481-rs2069803	T-C-C-G-T	0.344	0.032
APL	DTNBP1	rs2005976-rs760761-rs2619522	G-T-A	0.158	0.045
APL	DTNBP1	rs760761-rs2619522-rs1018381	T-A-C	0.072	0.067
HBAT -e	DTNBP1	rs2005976-rs760761-rs2619522-rs1018381	G-T-A-C	0.091	0.057
HBAT -p	DTNBP1	rs2005976-rs760761-rs2619522-rs1018381	G-T-A-C	0.091	0.078

Table 4
Haplo.stats results for haplotype association from the ICCSS

Gene	Markers	Haplotype	Frequency		OR	95% CI	P-value	Global P
			Controls	Cases				
-	rs3914025-rs3846726-rs3916441-rs31400	T-A-A-T	0.401	0.384	0.935	0.78-1.12	0.430	0.533
IL3	rs31400-rs31480-rs40401-rs31481-rs2069803	T-C-C-G-T	0.342	0.336	0.942	0.75-1.13	0.280	0.702
DTNBP1	rs2005976-rs760761-rs2619522-rs1018381	G-T-A-C	0.089	0.12	1.49	1.14-1.73	0.001	0.0001