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Biol Psychiatry. Author manuscript; available in PMC 2009 March 1.

Published in final edited form as:
Biol Psychiatry. 2008 March 1; 63(5): 441–448.

MEGF10 Association with Schizophrenia

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Abstract

Background—The 5q21-31 region has been implicated in many linkage studies to harbor risk genes for schizophrenia. In our previous report of stepwise fine-mapping of this region, the *MEGF10* gene was one of the genes showing consistent associations in our screening subsample. In this report, we carried out independent replication and expression studies of the *MEGF10* gene.

Methods—Association studies with 8 SNPs in the *MEGF10* gene were performed in the Irish case-control study of schizophrenia (ICCSS) sample (652 cases and 617 controls). The expression of *MEGF10* was also compared between healthy controls and schizophrenic patients using postmortem brain cDNA libraries.

Results—In the ICCSS sample, associations with the disease were found in the same risk alleles and haplotypes as that observed in our fine-mapping studies. The major allele (A) of rs27388 was overrepresented in affected individuals ($p = 0.0169$), which remained significant after correction for multiple testing. In expression studies, *MEGF10* had higher expression levels in the affected than the unaffected ($p = 0.015$). Schizophrenic patients with a 1/1 genotype at rs27388 had higher expressions than those patients with 1/2 and 2/2 genotypes ($p = 0.0008$).

Conclusions—Evidence from both association and expression studies suggests that *MEGF10* is likely associated with schizophrenia. The major allele and 1/1 genotype at rs27388 impose higher risks for the disease.

Keywords

Linkage disequilibrium test; association; MEGF10; schizophrenia; Irish families; case control study; gene expression

INTRODUCTION

Schizophrenia is a complex mental disorder in which both genetic and environmental factors play an important role. In the last several years, many genes have been studied as candidate genes and shown to have associations with schizophrenia (1-3). Chromosome 5q21-33 is a region implicated in many linkage studies. In this broad region, only a few candidate genes are

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reported (4;5) including the SPEC2/PDZ-GEF2/ACSL6 (6) and IL3 (7) genes reported in our Irish family and case control samples. In our previous linkage disequilibrium (LD) fine-mapping of the Irish study of high density of schizophrenia families (ISHDSF), we found several other regions showing consistent associations with the disease in addition to the SPEC2/PDZ-GEF2/ACSL6 region (6). The multiple epidermal growth factor like domains 10 (*MEGF10*) is one of these genes. In our fine-mapping of the 5q21-31 region, we used a stepwise approach and divided our ISHDSF into two parts: the screening subsample containing 61 families selected by family NPL scores (≥ 1.41) and the replication subsample containing the rest of ISHDSF (205 families) showing minimal linkage signals to the 5q region. In our fine-mapping of the linkage region, we found that *MEGF10* was consistently associated with schizophrenia in the screening subsample, but did not show association in the replication subsample. The negative results in the replication subsample could be a consequence of our selection strategy, i.e. the replication subsample was depleted of genetic signal at this locus. Since the fine-mapping was mainly focused on the *SPEC2, PDZ-GEF2* and *ACSL6* locus, in this study, we focused on the *MEGF10* gene. Given the results from our fine-mapping study, a reasonable approach to follow up with *MEGF10* would be to seek independent evidence from other samples. Therefore, we examined this gene in the Irish case-control study of schizophrenia (ICCSS) sample and in the postmortem brain cDNAs of schizophrenia, bipolar disorder and unaffected subjects from the Stanley Medical Research Institute (http://www.stanleyresearch.org/).

METHODS AND MATERIALS

The ISHDSF sample

The ISHDSF was collected in Northern Ireland, United Kingdom and the Republic of 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 (8) 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 1350 subjects had DNA sample for genotyping. Of them, 515 were diagnosed with the narrow definition, 634 were diagnosed with the intermediate definition and 686 were diagnosed with the broad definition. Detailed descriptions of the sample were published previously (9).

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 652 (433 males and 219 females) affected subjects and 617 (348 males, 269 females) controls. 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 poor-outcome schizoaffective disorder by DSM-III-R criteria, and the diagnosis 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

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schizophrenia. However, since the controls were not screened by clinicians, this is a potential weakness in our design. 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 association 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. The *MEGF10* gene is about 170 kb, containing 25 exons. In our fine-mapping studies, we typed a total of 35 SNPs in *MEGF10*. The associations were observed in a small segment around rs27388-rs27652. Based on the results of these SNPs, we selected 8 markers around this segment to replicate in the ICCSS sample. Based on HaploView program (10), these SNPs tag major haplotypes (with frequency > 1%) in this segment.

We used two techniques for SNP genotyping in our fine-mapping. For the ISHDSF sample (including both screening and replication subsamples), the SNPs were typed with the FP-TDI method (11;12). 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 (13) and PCR and FP-TDI extension primers were designed by the PRIMER 3 program (14). 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. The ICCSS sample was typed with the TaqMan assay (15). The assays used were either validated assays or custom designed assays developed by Applied BioSystems Corporation (Foster city, CA). We also performed cross genotyping to check if the two different methods produce the same results. We typed 5 96-well plates for 3 SNPs using both methods and the genotypes obtained had a discrepancy of 0-1% amongst the tested subjects and SNPs. These results assured us that the two methods produced consistent results. All markers typed were checked for deviation from the Hardy-Weinberg Equilibrium (HWE) and Mendelian errors by the PEDSTATS program (16).

Expression studies

The expression studies were carried out with postmortem brain cDNAs from the Stanley Medical Research Institute (http://www.stanleyresearch.org/). The messenger RNA and reverse transcription to cDNA were carried out by the Stanley researchers. The Stanley panel consisted of 104 subjects, of them 35 were diagnosed (DSM-IV criteria) as schizophrenia, 34 as bipolar disorder and 35 were unaffected controls. There were 69 males and 35 females in the panel. Real-time quantitative PCRs were conducted with TaqMan expression probe (Hs01002798_m1) for the *MEGF10* 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 μL of PCR mixture containing the FAM-labeled *MEGF10* 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; 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). For each sample the average of the triplicate C_T values was used. The relative expression levels between the *MEGF10* and *TBP* were calculated by the $2^{\Delta C_T}$ method, where $\Delta C_T = C_T^{MEGF10} - C_T^{TBP}$. Genomic DNAs for the same subjects were also obtained from the Stanley Institute and genotyped for rs27388, rs26946, rs26947 and rs27652. Univariate ANCOVA implemented with general linear model in the SPSS software (version 15 for Windows) was used to compare

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the expressions between schizophrenia subjects and unaffected controls. The effects of the brain pH of the samples, lifetime usage of alcohol, drugs, antipsychotics and smoking status at death on the expression of *MEGF10* gene were evaluated separately to determine whether to include them as covariates in the ANCOVA analyses.

Statistical analyses

We used the pedigree disequilibrium test (PDT) (17) as implemented in the UNPHASED program (version 2.4, PDTPHASE module) (18) to analyze the ISHDSF sample. For each typed SNP, the PDT program was run for the narrow, intermediate and broad disease definitions. 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 multi-locus haplotype analyses, we used 10 restarts for the expectation-maximization (EM) algorithm (19) and used 1% as the cutoff for minor haplotypes. For the case control sample, the UNPHASED program (version 3.06) (18) was used to analyze both single marker and multi-marker haplotype associations with sex as covariate. As in the family sample, haplotypes with frequencies less than 1% were aggregated, and only haplotypes observed at least once in the samples were used (the observed option in the program). 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 SNP spectral decomposition (SNPSpD) method (20) to estimate the number of independent tests, and used Bonferroni correction for multiple testing. We used the HAPLOVIEW program (version 3.32) (10) to estimate pairwise LD and to illustrate haplotype blocks. The haplotype blocks were partitioned by the confidence interval algorithm (21). The 8-marker haplotype was reconstructed by MERLIN (22) and FUGUE (http://www.sph.umich.edu/csg/abecasis/FUGUE/) programs.

To test the hypothesis that association signals differed between the screening subset and the remaining sample, we performed an analysis extending the principles of ordered subset analysis to a family-based framework (23-25). The test was performed by sequentially adding pedigrees based on the selection criterion for inclusion in the screening set, evidence for linkage (NPL score), and recalculating association statistics at each sequential addition. Significance was assessed by randomly sorting the sample and performing the analysis on unordered subsets of the same size to generate a null distribution of test statistics. In this context, this analysis helped to validate the selection of the screening sample and explained the difference in association evidence between the screening and validation samples.

RESULTS

LD and haplotype structure of MEGF10

We compared the LD and haplotype structures amongst the screening, replication and case control samples for the typed SNPs. All markers showed high levels of LD in all 3 samples when LDs were measured by D' (Figure 1). However, there were some differences in LD blocks amongst the 3 samples. For both the screening and ICCSS samples, all 8 markers were in a single LD block. For the replication subsample, markers 1-2 were partitioned in one block and markers 4-8 in another block. For r^2 measurements, marker 3, or rs246897, showed consistently lower LD with other markers in the replication subsample as compared with both the screening and ICCSS samples. When the haplotypes were examined, the two most abundant haplotypes were identical in all 3 samples. It was interesting that the screening subsample had substantially more minor haplotypes than the other 2 samples despite it being the smallest of the samples.

Association analyses of the ISHDSF sample

In our previous fine-mapping of the 5q21-31 linkage peak, the ISHDSF was divided into two mutually exclusive subgroups, the screening and replication subsamples. As reported

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previously, in the screening subsample, we typed a total of 289 SNPs, and two promising loci, i.e. the *MEGF10* and the *SPEC2, PDZ-GEF2* and *ACSL6* locus, were identified (6). In the *MEGF10* gene, we typed a total of 35 SNPs in our screening subsample, with an average of ~5 kb/SNP. We observed associations in a 37 kb fragment from intron 2 to intron 5 in the *MEGF10* gene (6). In this 37 kb interval, 6 SNPs produced nominal p values < 0.05 by the PDT program (Table 2). Associations with the intermediate and broad disease definitions showed same trend, but the signals were weaker (data not shown). As reported in the fine-mapping study, TDT (26) and FBAT (27) programs produced similar results for these SNPs (see supplementary table in reference 6). These results suggested consistent associations in the *MEGF10* gene in the screening subsample and met the criteria (several tests with nominal $p \leq 0.05$ with multiple diagnoses in the same gene) set for replication in our stepwise study design for fine-mapping this linkage region. In all 6 markers, the major allele was overtransmitted to the affected individuals. In an effort to verify the association, we typed 8 markers selected from this interval for the replication subsample according to our stepwise fine-mapping design. However, we did not observe association in the replication subsample. While pondering the results, we realized that there was a possibility that the replication subsample was depleted of association signals because our screening subsample was designed to enrich linkage signals to increase the power. The lack of power in the replication subsample could therefore be a predicted outcome. A close examination of the replication subsample showed that 7 out of 8 of the SNP markers, the results were in the same direction for the same alleles as those seen in the screening sample although individual results were far weaker and not close to significant. In post hoc genotype association analysis, the 1/1 genotypes were showed a trend of association (compare supplementary Tables 1 and 2). To seek further evidence, we performed two simulation tests. First, we selected N families with the highest NPL scores and conducted PDT tests for these families for markers showing associations at this locus. This test would tell us how many families were required to produce the maximal associations at this locus. From our tests, we found that 46-48 families with the highest NPL scores produced the maximal associations. All of these 48 families were included in our screening subsample. Second, we performed 1000 permutations by randomly selecting 61 families (the number of families in the screening subsample) from the entire sample without regard to their linkage signal on 5q and conducting PDT tests at markers rs27388 and rs26946. If we rarely found replicates producing p values less than or equal to the p values observed in our screening subsample, we could conclude that our selection of families on the basis of their 5q linkage scores indeed significantly enriched for association signals. In these permutations, there were only 3 and 7 tests respectively with a p value reaching that found in the screening subsample for rs27388 ($p = 0.0017$) and rs26946 ($p = 0.0056$). These results indicated that our selection of the screening subsample on the basis of family linkage indeed significantly enriched for association signals which could explain the lack of association found in the replication subsample.

The association at the *MEGF10* gene showed a characteristic of the linkage signal observed in the ISHDSF. In the linkage study of ISHDSF, the linkage was most significant with the narrow disease definition. The associations at the *MEGF10* gene were the same, the associations with the intermediate and broad definitions were substantially weaker (see Supplementary Table 1 in reference 6).

We did not perform independent multiple testing correction in the screening subsample, the reason was that this sample was used as hypothesis-generating sample in our fine-mapping project. The signals we sought were consistent associations as judged by multiple nominal significances in a single gene with multiple testing statistics and diagnoses (see reference 6 for details). The *MEGF10* gene clearly met these criteria for replication.

Based on these results from single marker analyses, we limited our haplotype analyses to the screening subsample with the narrow disease definition. As summarized in Table 3, we

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observed significant multi-marker associations. Significant association (global $p = 0.00328$) was observed for markers 4-5 or rs246896 and rs27388. Consistent with the single marker analyses, we found that the major haplotype, 1-1 or C-A, was overtransmitted ($p = 0.00522$) to the affected individuals, and haplotype 2-2 or T-G were undertransmitted ($p = 0.00165$) to the affected individuals.

Replication in the ICCSS sample

To verify the findings observed in the screening subsample, we genotyped the same 8 SNPs in an independent case control sample collected from the same geographic region. In single marker analyses, we found that three of the 8 markers reached 5% significance. The other 6 markers demonstrate trend results (i.e. $p < 0.10$) (Table 4). In all markers, the associated alleles were the same as that observed in the screening subsample. Given that all overrepresented alleles in the affected subjects were the same as that observed in the screening subsample, criterion for one-tailed test could be applied. To correct for multiple testing, we used the SNPSpD method to estimate the number of equivalent independent tests for these 8 SNPs, and the program produced a number of 2. Based on these rationales, the corrected significance threshold should be 0.1/4 (2 independent tests for the 8 SNPs, and 2 for haplotype tests), i.e. 0.025. Judging by this criterion, rs27388 remained significant after multiple testing correction.

We also carried out multi-marker analyses in the ICCSS sample. We found that the same marker combinations and the same haplotypes were associated with schizophrenia (Table 5). It was interesting that the protective haplotypes tend to be more significant. Both protective haplotypes from combinations 4-5 and 1-4-5 remained significant after multiple testing correction.

Expression studies of the MEGF10 gene

We used quantitative PCR experiments to determine the expression levels of the *MEGF10* gene. The C_T values for all subjects were obtained from BioRad iCycler software. First, factors potentially affecting the expression of postmortem mRNAs were evaluated separately on the expression of the *MEGF10* gene. Of the factors evaluated, the amount of lifetime antipsychotics use was the only factor that had significant effect on the expression of *MEGF10* gene ($p = 0.025$), therefore, it was included in subsequent analyses. We then focused our analyses on the expressions between the controls and schizophrenia subjects. We found that the expression levels between these two groups were significantly different ($p = 0.015$), (Figure 2A). Furthermore, we found that the effect of genotypes on the expression levels was significant in schizophrenia subjects (Figure 2B). In particular, schizophrenia subjects with 1/1 genotypes for rs27388 had significantly higher expression ($p = 0.0008$) than those affected subjects with 1/2 and 2/2 genotypes. Similar effects were observed from other markers (data not shown). Based on these findings, we used the geno-PDT method (28) to conduct genotype association analyses for the ISHDSF retrospectively. We found, indeed, that the 1/1 genotypes were overtransmitted to the affected subjects (see Supplementary Table 1). For example, for rs246896 and rs27388, the nominal p values for the 1/1 genotype were 0.043 and 0.086 respectively. For the ICCSS sample, for the same two markers, the nominal p values for the 1/1 genotype were 0.0370 and 0.0243 respectively (Supplementary Table 3).

CONCLUSION AND DISCUSSION

In our search for schizophrenia susceptibility genes in the 5q21-31 linkage region, we selected families showing linkage to this region as a screening sample, reasoning that this strategy could increase our power since this selection should enrich the association signals specific to this region. Using this procedure, we found consistent associations in two loci (*MEGF10* and *SPEC2, PDZ-GEF2* and *ACSL6*). The previous report was focused on the *SPEC2, PDZ-*

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GEF2 and *ACSL6* locus (6). In this study, we focused on the *MEGF10* locus. In *MEGF10*, we observed consistent signals in the screening subsample, but we found no association in the replication subsample. There could be two different explanations to these results. One is that the original *MEGF10* results represented a false positive. The other possibility is that the result could be a consequence of our selection strategy in that association signals specific to this region were depleted in the remaining families. To distinguish the possibilities, we decided to perform independent association study with ICCSS sample. We also performed simulation studies with the IHDSF. In this case control sample, ICCSS, we found that all tested markers showed nominal associations at 10% level, and identical risk and protective haplotypes to that of the screening subsample were observed. In simulation studies, we found that our selection of the screening subsample indeed enriched association signals. For the replication study of ICCSS sample, rs27388 remained significant after multiple testing correction. The two protective haplotypes in combinations 4-5 and 1-4-5 also survived multiple testing correction. Based on a simulation study by Sullivan, replications with the same marker and same allele, or with same marker combination and same haplotype would have the lowest rate of false positive (29). The ICCSS replication met this criterion. Furthermore, we also carried out expression studies with postmortem brain cDNAs of schizophrenia and unaffected controls. This study confirmed that the expression levels of the *MEGF10* gene differed between the affected and unaffected subjects and that affected subjects with genotypes overtransmitted to and overrepresented in the Irish samples had significantly higher expressions. These three lines of independent evidence were consistent and mutually supportive of the hypothesis that the *MEGF10* gene is associated with schizophrenia.

Our study of the *MEGF10* gene raises an interesting question. How many candidate genes are under a single linkage peak? Should we pursue a second candidate after we identified a candidate in the same linkage peak? From a classic, Mendelian genetics perspective, a linkage seems to suggest a single susceptibility gene. However, it is not all clear that this can be applied for complex diseases. In the studies of model organisms, it seems that a linkage peak to a complex trait could have multiple susceptibility genes (30;31). There is also a study of human disease that several genes under a single linkage peak may be associated with the same disease (32). These studies indicate that it is possible that multiple susceptibility genes may be located under a single linkage peak. The two loci identified in the 5q linkage region in the ISHDSF may not be unprecedented. Given the 5 million basepair genomic distance between the *MEGF10* gene and the *SPEC2, PDZ-GEF2* and *ACSL6* locus, it seems unlikely that the associations observed at these genes are the same signals.

In a recent study, *MEGF10* was identified as the human ortholog of ced-1 (33), a gene in *C. elegans* that participates in the engulfment and clearance of apoptotic cells. In that study, the authors demonstrated that all 3 components of this engulfment pathway, i.e. *ced-1, ced-6* and *ced-7*, have their counterparts (*MEGF10, GULP1* and *ABCA1*) in humans and these human orthologs work in the similar pathway as *ced-1, ced-6* and *ced-7* do in *C. elegans*. In a separate study, *ced-1* mutations cause abnormal axon branching and navigation patterns (34). The *ced-1* orthologs in *Drosophila* and mouse play an essential role in axon pruning during brain development (35;36). Given the fact that this pathway is highly conserved in evolution, it is likely that *MEGF10* may be involved in similar functions in humans, i.e., engulfment and clearance of apoptotic cells, axon navigation and pruning process. Since neuron apoptosis, axon navigation and pruning are critical in shaping the developing brain, we can speculate that dysfunction of these processes will cause inappropriate neuron connection and circuitry, leading to the pathophysiology of schizophrenia. In fact, there is a neurodevelopmental hypothesis of schizophrenia (37), and some structural abnormalities are observed (38).

In the last several years, we have seen several candidate genes for schizophrenia identified. In subsequent replication studies, every one of these promising candidate genes has some

inconsistencies or conflicting reports (39-44). In this study, we find nearly identical results in the screening subsample and the case control sample, meeting the criteria set for the replication of association studies proposed in recent literature (29;45;46). In addition, in expression study, we found that affected subjects had different expression than that of healthy controls. These results suggest that *MEGF10* is likely involved in schizophrenia and further study of this gene is necessary.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This study is supported by a research grant (RO1MH41953) to KSK from National Institute of Mental Health and by a young investigator award to XC from the National Alliance for Research on Schizophrenia and Depression. We thank the patients and their families for participating in this study. The Northern Ireland Blood Transfusion Service assisted with collection of control sample. The postmortem brain cDNA specimens were donated by The Stanley Medical Research Institute Brain Collection courtesy of Drs. Michael B. Knable, E. Fuller Torrey, Maree J. Webster, and Robert H. Yolken.

FINANCIAL DISCLOSURES

All authors declared no conflict of interests in this study, both biomedical and financial.

REFERENCE LIST

1. Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. Am J Hum Genet 2002;71:337–348. [PubMed: 12098102]
2. Chowdari KV, Mirnics K, Semwal P, Wood J, Lawrence E, Bhatia T, et al. Association and linkage analyses of RGS4 polymorphisms in schizophrenia. Hum Mol Genet 2002;11:1373–1380. [PubMed: 12023979]
3. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 2002;71:877–892. [PubMed: 12145742]
4. Pimm J, McQuillin A, Thirumalai S, Lawrence J, Quested D, Bass N, et al. The epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. Am J Hum Genet 2005;76:902–907. [PubMed: 15793701]
5. Petryshen TL, Middleton FA, Tahl AR, Rockwell GN, Purcell S, Aldinger KA, et al. Genetic investigation of chromosome 5q GABAA receptor subunit genes in schizophrenia. Mol Psychiatry 2005;10:1074–88. 1057. [PubMed: 16172613]
6. Chen X, Wang X, Hossain S, O'Neill FA, Walsh D, Pless L, et al. Haplotypes spanning SPEC2, PDZ-G EF2 and ACSL6 genes are associated with schizophrenia. Hum Mol Genet 2006;15:3329–3342. [PubMed: 17030554]
7. Chen X, Wang X, Hossain S, O'Neill AF, Walsh D, van den Oord EJ, et al. Interleukin 3 and schizophrenia: The impact of sex and family history. Mol Psychiatry 2007;12:273–282. [PubMed: 17179997]
8. Kendler KS, McGuire M, Gruenberg AM, O'Hare A, Spellman M, Walsh D. The Roscommon Family Study. III. Schizophrenia-related personality disorders in relatives. Arch Gen Psychiatry 1993;50:781–788. [PubMed: 8215802]
9. Kendler KS, Myers JM, O'Neill FA, Martin R, Murphy B, MacLean CJ, et al. Clinical features of schizophrenia and linkage to chromosomes 5q, 6p, 8p, and 10p in the Irish Study of High-Density Schizophrenia Families. Am J Psychiatry 2000;157:402–408. [PubMed: 10698816]
10. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265. [PubMed: 15297300]

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11. Chen X, Levine L, Kwok PY. Fluorescence polarization in homogeneous nucleic acid analysis. Genome Res 1999;9:492–498. [PubMed: 10330129]
12. van den Oord EJ, Jiang Y, Riley BP, Kendler KS, Chen X. FP-TDI SNP scoring by manual and statistical procedures: a study of error rates and types. Biotechniques 2003;34:610–20. 622. [PubMed: 12661166]
13. Bedell JA, Korf I, Gish W. MaskerAid: a performance enhancement to RepeatMasker. Bioinformatics 2000;16:1040–1041. [PubMed: 11159316]
14. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 2000;132:365–386. [PubMed: 10547847]
15. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–149. [PubMed: 10084106]
16. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet 2005;76:887–893. [PubMed: 15789306]
17. Martin ER, Monks SA, Warren LL, Kaplan NL. A test for linkage and association in general pedigrees: the pedigree disequilibrium test. Am J Hum Genet 2000;67:146–154. [PubMed: 10825280]
18. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003;25:115–121. [PubMed: 12916020]
19. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12:921–927. [PubMed: 7476138]
20. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–769. [PubMed: 14997420]
21. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–2229. [PubMed: 12029063]
22. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002;30:97–101. [PubMed: 11731797]
23. Hauser ER, Watanabe RM, Duren WL, Bass MP, Langefeld CD, Boehnke M. Ordered subset analysis in genetic linkage mapping of complex traits. Genet Epidemiol 2004;27:53–63. [PubMed: 15185403]
24. Macgregor S, Craddock N, Holmans PA. Use of phenotypic covariates in association analysis by sequential addition of cases. Eur J Hum Genet 2006;14:529–534. [PubMed: 16538225]
25. Perdry H, Maher BS, Babron M-C, McHenry T, Clerget-Darpoux F, Marazita ML. An ordered subset approach to including covariates in TDT. BMC Genet. 2007In press
26. Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. Am J Hum Genet 1999;65:1161–1169. [PubMed: 10486335]
27. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. Genet Epidemiol 2000;19(Suppl 1):S36–S42. [PubMed: 11055368]
28. Martin ER, Bass MP, Gilbert JR, Pericak-Vance MA, Hauser ER. Genotype-based association test for general pedigrees: the genotype-PDT. Genet Epidemiol 2003;25:203–213. [PubMed: 14557988]
29. Sullivan PF. Spurious genetic associations. Biol Psychiatry 2007;61:1121–1126. [PubMed: 17346679]
30. Pasyukova EG, Vieira C, Mackay TF. Deficiency mapping of quantitative trait loci affecting longevity in Drosophila melanogaster. Genetics 2000;156:1129–1146. [PubMed: 11063689]
31. Yalcin B, Willis-Owen SA, Fullerton J, Meesaq A, Deacon RM, Rawlins JN, et al. Genetic dissection of a behavioral quantitative trait locus shows that Rgs2 modulates anxiety in mice. Nat Genet 2004;36:1197–1202. [PubMed: 15489855]
32. Paschou P, Feng Y, Pakstis AJ, Speed WC, DeMille MM, Kidd JR, et al. Indications of linkage and association of Gilles de la Tourette syndrome in two independent family samples: 17q25 is a putative susceptibility region. Am J Hum Genet 2004;75:545–560. [PubMed: 15303240]
33. Hamon Y, Trompier D, Ma Z, Venegas V, Pophillat M, Mignotte V, et al. Cooperation between Engulfment Receptors: The Case of ABCA1 and MEGF10. PLoS ONE 2006;1:e120. [PubMed: 17205124]
34. Schmitz C, Kinge P, Hutter H. Axon guidance genes identified in a large-scale RNAi screen using the RNAi-hypersensitive Caenorhabditis elegans strain nre-1(hd20) lin-15b(hd126). Proc Natl Acad Sci U S A 2007;104:834–839. [PubMed: 17213328]

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35. MacDonald JM, Beach MG, Porpiglia E, Sheehan AE, Watts RJ, Freeman MR. The Drosophila cell corpse engulfment receptor Draper mediates glial clearance of severed axons. Neuron 2006;50:869–881. [PubMed: 16772169]

36. Awasaki T, Tatsumi R, Takahashi K, Arai K, Nakanishi Y, Ueda R, et al. Essential role of the apoptotic cell engulfment genes draper and ced-6 in programmed axon pruning during Drosophila metamorphosis. Neuron 2006;50:855–867. [PubMed: 16772168]

37. Caruncho HJ, Dopeso-Reyes IG, Loza MI, Rodriguez MA. A GABA, reelin, and the neurodevelopmental hypothesis of schizophrenia. Crit Rev Neurobiol 2004;16:25–32. [PubMed: 15581396]

38. Roberts RC, Roche JK, Conley RR. Synaptic differences in the postmortem striatum of subjects with schizophrenia: a stereological ultrastructural analysis. Synapse 2005;56:185–197. [PubMed: 15803499]

39. Levitt P, Ebert P, Mirnics K, Nimgaonkar VL, Lewis DA. Making the case for a candidate vulnerability gene in schizophrenia: Convergent evidence for regulator of G-protein signaling 4 (RGS4). Biol Psychiatry 2006;60:534–537. [PubMed: 16860780]

40. Riley B, Kendler KS. Molecular genetic studies of schizophrenia. Eur J Hum Genet 2006;14:669–680. [PubMed: 16721403]

41. Williams NM, O'Donovan MC, Owen MJ. Is the dysbindin gene (DTNBP1) a susceptibility gene for schizophrenia? Schizophr Bull 2005;31:800–805. [PubMed: 16166606]

42. Tosato S, Dazzan P, Collier D. Association between the neuregulin 1 gene and schizophrenia: a systematic review. Schizophr Bull 2005;31:613–617. [PubMed: 16081509]

43. Camargo LM, Collura V, Rain JC, Mizuguchi K, Hermjakob H, Kerrien S, et al. Disrupted in Schizophrenia 1 Interactome: evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. Mol Psychiatry 2007;12:74–86. [PubMed: 17043677]

44. Norton N, Williams HJ, Owen MJ. An update on the genetics of schizophrenia. Curr Opin Psychiatry 2006;19:158–164. [PubMed: 16612196]

45. Neale BM, Sham PC. The future of association studies: gene-based analysis and replication. Am J Hum Genet 2004;75:353–362. [PubMed: 15272419]

46. Petryshen TL, Middleton FA, Kirby A, Aldinger KA, Purcell S, Tahl AR, et al. Support for involvement of neuregulin 1 in schizophrenia pathophysiology. Mol Psychiatry 2005;10:366–374. [PubMed: 15545978]

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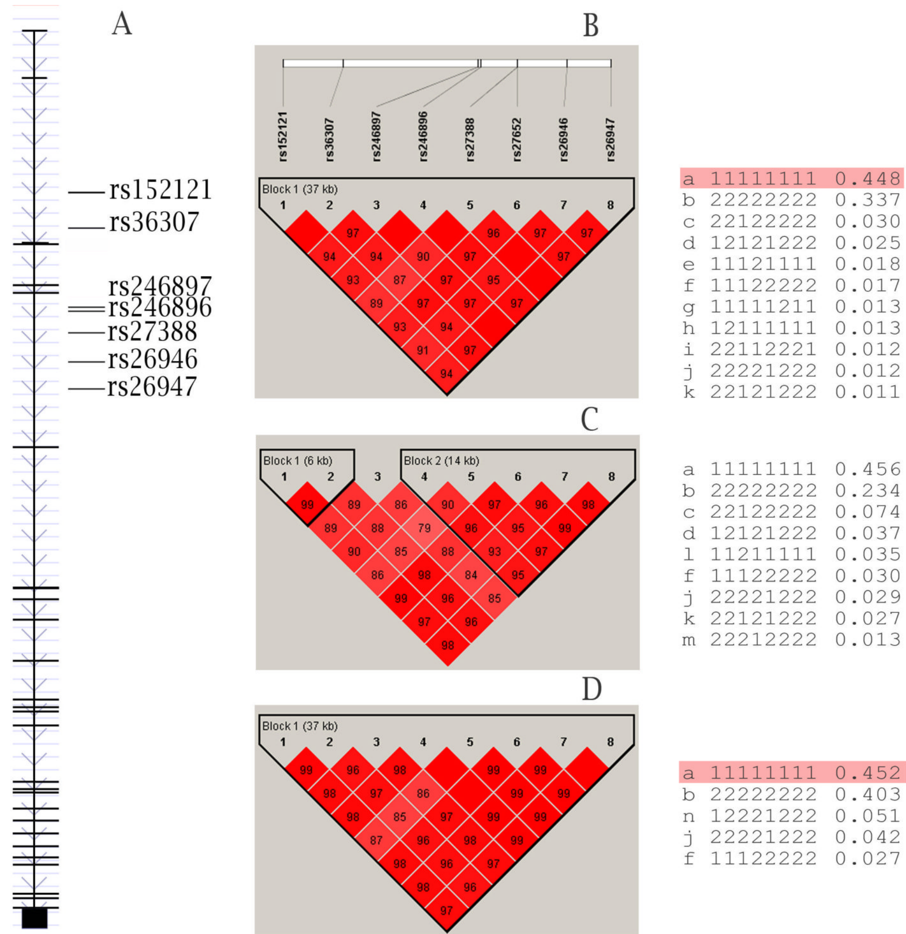

Figure1.

The *MEGF10* gene and the LD and haplotype structure of the samples used in this study. A. Gene structure and the relative position of the typed SNPs. Rs27652 was too close to rs27388, it was not shown in the picture. In the figure, exons were illustrated by the long horizontal bars. The 3′ and 5′ UTRs were illustrated by the short bars. B. LD and haplotype structures of the screening subsample. LDs shown were D'. Haplotypes were labeled by letters on the left and their frequencies were listed on the right. Risk haplotype was highlighted. C. LD and haplotype structures of the replication subsample. D. LD and haplotype structures of the ICCSS sample.

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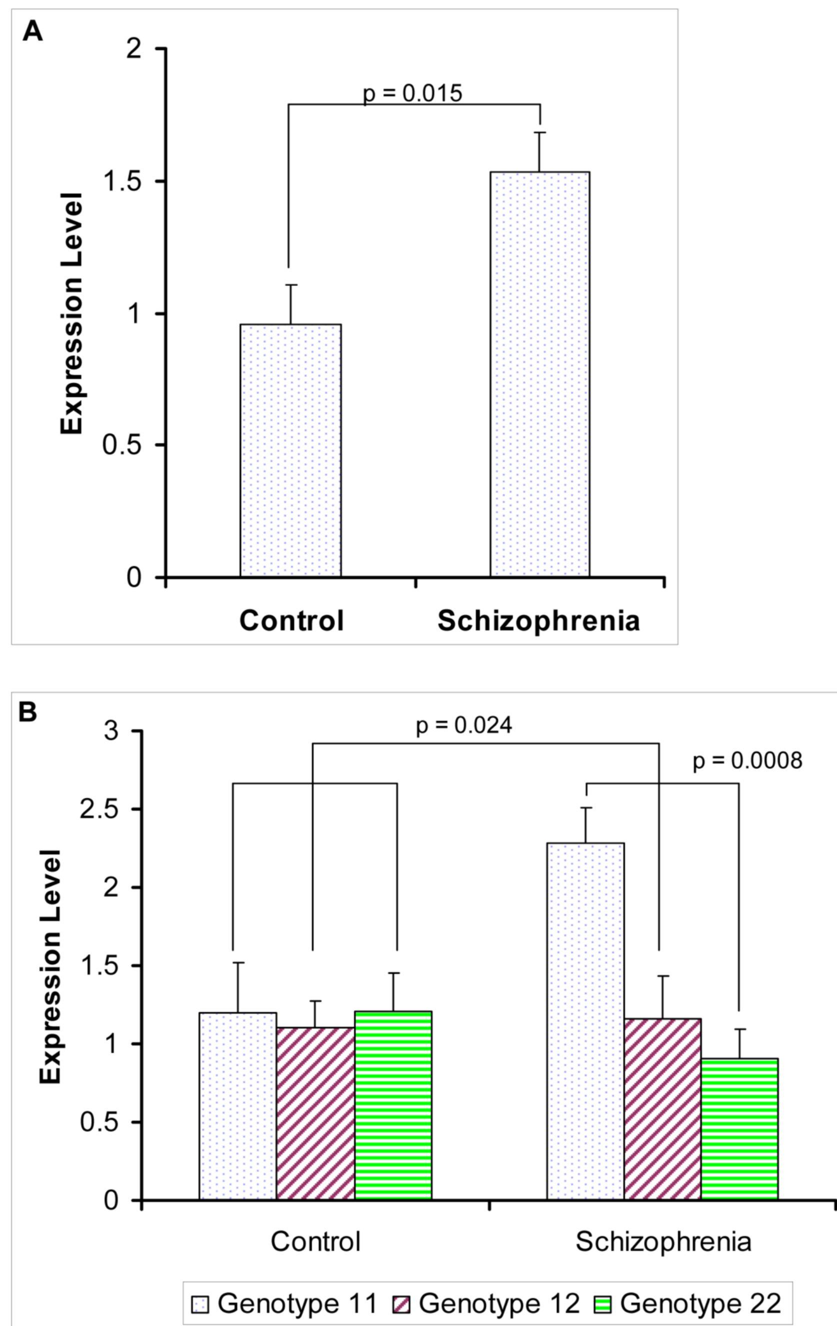

Figure 2.
ANCOVA analyses of the *MEGF10* gene expressions in the Stanley subjects. The expression level was the ΔC_T between *MEGF10* and the house keeping gene *TBP*. The error bars were standard errors. A. Comparison of expressions between the control and schizophrenia subjects. B. Comparison of expressions between the control and schizophrenia subjects with the inclusion of the genotypes of rs27388.

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Table 1

Marker characteristics

Marker	ID	Distance (bp)	Polymorphism	Minor allele	MAF*	HWE P*
RS152121	1	0	C/A	C	0.391	0.5288
RS36307	2	6822	C/T	T	0.447	0.8527
RS246897	3	15363	C/T	C	0.442	0.5323
RS246896	4	295	C/T	T	0.473	0.1752
RS27388	5	4112	G/A	G	0.379	0.8635
RS27652	6	74	C/T	T	0.484	0.4206
RS26946	7	5606	T/A	T	0.489	0.7164
RS26947	8	4948	T/A	T	0.473	0.9844

* Both minor allele frequency (MAF) and Hardy-Weinberg Equilibrium (HWE) *p* values were from ISHDSF sample.

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Table 2

Single marker association (PDT *p* values) with the narrow disease definition in the ISHDSF sample

Marker	Screening subsample				Replication subsample			
	Transmitted cnt (trio + sib)	Untransmitted cnt (trio + sib)	T/UT ratio	*P* value	Transmitted cnt (trio + sib)	Untransmitted cnt (trio + sib)	T/UT ratio	*P* value
RS152121	35 + 332	24 + 306	1.11	**0.0317**	49 + 505	49 + 486	1.04	0.9294
RS36307	29 + 297	26 + 270	1.10	0.1859	40 + 354	44 + 369	0.95	0.4786
RS246897	28 + 298	22 + 264	1.14	0.1429	49 + 425	51 + 415	1.02	0.8871
RS246896	32 + 279	24 + 225	1.25	**0.0017**	40 + 405	36 + 402	1.02	0.8911
RS27388	45 + 349	36 + 314	1.13	**0.0047**	51 + 521	51 + 501	1.04	0.8257
RS27652	30 + 253	22 + 210	1.22	**0.0056**	37 + 380	33 + 374	1.02	0.9876
RS26946	28 + 295	20 + 266	1.13	**0.0190**	49 + 434	48 + 433	1.00	0.8421
RS26947	25 + 267	19 + 230	1.17	**0.0382**	53 + 441	49 + 440	1.01	0.6478

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Table 3

Haplotype associations (*p* values) in the screening subsample*

Marker combination	Global *p* (df)	Haplotype	Transmitted cnt (trio + sib)	Untransmitted cnt (trio + sib)	T/UT ratio	Haplotype *p*
4-5	**0.00328 (3)**	1-1	32 + 274	21 + 224	1.25	**0.00522**
		2-2	21 + 119	26 + 153	0.78	**0.00165**
1-4-5	**0.00255 (5)**	1-1-1	28 + 247	18 + 188	1.33	**0.00142**
		2-2-2	20 + 99	23 + 118	0.84	**0.02611**

*Results were obtained with the narrow definition.

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Table 4

Single marker associations (*p* values) in the ICCSS sample

Marker	Case freq	Ctrl freq	OR	*P* value
RS152121	0.5625	0.5284	1.06	**0.0415**
RS36307	0.5103	0.4789	1.07	0.0792
RS246897	0.5031	0.4743	1.06	0.1080
RS246896	0.4792	0.4542	1.06	0.0577
RS27388	0.5773	0.5461	1.06	**0.0169**
RS27652	0.4837	0.4515	1.07	**0.0298**
RS26946	0.4764	0.4538	1.05	0.0805
RS26947	0.4791	0.4536	1.06	0.0693

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Table 5

Haplotype associations (*p* values) in the ICCSS sample

Marker combination	Global p (df)	Haplotype	Case freq	Ctrl freq	OR	Haplotype p
4-5	**0.04854 (2)**	1-1	0.4783	0.4535	1.05	0.05434
		2-2	0.4234	0.4561	0.93	**0.01419**
1-4-5	0.06006 (5)	1-1-1	0.4719	0.4476	1.05	0.05135
		2-2-2	0.3938	0.4323	0.91	**0.01798**