Abstract

Schizophrenia is a devastating neuropsychiatric disorder with genetically complex traits. Genetic variants should explain a considerable portion of the risk for schizophrenia, and genome-wide association study (GWAS) is a potentially powerful tool for identifying the risk variants that underlie the disease. Here, we report the results of a three-stage analysis of three independent cohorts consisting of a total of 2,535 samples from Japanese and Chinese populations for searching schizophrenia susceptibility genes using a GWAS approach. Firstly, we examined 115,770 single nucleotide polymorphisms (SNPs) in 120 patient-parents trio samples from Japanese schizophrenia pedigrees. In stage II, we evaluated 1,632 SNPs (1,159 SNPs of \( p < 0.01 \) and 473 SNPs of \( p < 0.05 \) that located in previously reported linkage regions). The second sample consisted of 1,012 case-control samples of Japanese origin. The most significant \( p \) value was obtained for the SNP in the ELAVL2 [(embryonic lethal, abnormal vision, Drosophila)-like 2] gene located on 9p21.3 (\( p = 0.00087 \)). In stage III, we scrutinized the ELAVL2 gene by genotyping gene-centric tagSNPs in the third sample set of 293 family samples (1,163 individuals) of Chinese descent and the SNP in the gene showed a nominal association with schizophrenia in Chinese population (\( p = 0.026 \)). The current data in Asian population would be helpful for deciphering ethnic diversity of schizophrenia etiology.

Introduction

Schizophrenia is a debilitating mental disorder characterized by psychotic manifestations including hallucinations, delusions, and cognitive deficits. Despite the high heritability of the disease estimated at up to 80%, key molecules and/or molecular pathways underlying the disease are still elusive. Candidate gene-based analyses have an inherent limitation, that is, we do not know the precise pathophysiological basis for the disease. However, through the rapid development of genotyping technology, it has become feasible to genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) covering the whole human genome. A shift to genomewide association study (GWAS) from a gene-based approach is accelerated. To date, a number of GWASs of psychiatric disorders, including schizophrenia, have been reported [1,2,3,4,5,6,7]. These have produced substantial evidence for the association of the disease with specific risk loci. For instance, O’Donovan et al. reported the evidence for association around \( ZNF804A \) (\( p = 1.61 \times 10^{-7} \)) [2]. However, the protein encoded by \( ZNF804A \) is uncharacterized and its function is unknown. No functional candidate genes that stemmed from current understanding of schizophrenia pathophysiology surpassed the genome-wide significance level in that study. It is also noteworthy that many GWASs so far have potentially missed the true association of the genes with small effect, because of a stringent threshold. Conversely, a liberal threshold requires follow-up studies to eliminate false positives from genuine associations. Therefore, a simple procedure for overcoming this problem is the use of a multistage screening approach, using a modest threshold in each stage. In addition, case-control design is liable to population stratification, which can cause spurious associations. To eliminate false positives due to population stratification and other confounding factors, the transmission disequilibrium test (TDT) design that uses patients and their parents (trios) is preferable as an alternative approach.

In this study, starting from a whole genome association survey of trio families, we carried out a staged association study for schizophrenia by analyzing three sets of samples, two from Japanese cohort and one from Chinese population, which is ethnically close to Japanese. All three sets of our samples showed a nominally significant association with a SNP on the ELAVL2 gene.

This Asian GWAS of schizophrenia is hoped to provide a broader view of the genetic basis of schizophrenia, because schizophrenia GWASs to date are much accumulated in European descent.

Results

Stage I: GWAS of Japanese trio samples

Because of concerns regarding population stratification and other unknown confounding factors, we performed the first-stage screening restricted to pedigree trio samples comprising 120...
families, each consisting of a patient with schizophrenia and their parents. All the subjects were Japanese and diagnosis of schizophrenia was carried out by at least two experienced psychiatrists according to DSM-IV criteria, on the basis of interview and medical records.

The trios were initially genotyped using Affymetrix GeneChip Mapping 100 K Arrays. Out of a total of 115,770 SNPs, 97,963 SNPs were successfully genotyped. The rest, 17,807 SNPs, were nonpolymorphic in the Japanese population or failed at the genotyping stage. They were excluded from further analyses. We ranked genotyped SNPs on the basis of strength of association using the allelic association test. Nominally significant results were detected for 1,159 SNPs (p<0.01).

Genotyping data yielded an average call rate of 96.6%, and apparent inheritance errors in trio samples were detected in <0.2% of all SNPs. A quantile-quantile (QQ) plot for association results is provided in Figure 1. The group of SNPs that slightly deviated from a diagonal straight line in the QQ plot are considered to reflect SNPs with weak genetic effects, and from the plot, it seems that there is not gross inflation of false-positive results derived from genotyping errors. The most significant p value was obtained for marker rs2174623 at 4q28.1 (p = 6.11×10^{-6}), followed by markers rs83955 at Xq24 (p = 7.10×10^{-6}) and rs10499985 at 7p15.1 (p = 3.14×10^{-5}). However, no human reference gene was located at these regions. P values for the TDT analyses of schizophrenia trios for all 97,963 SNPs are shown in a Manhattan plot (Figure 2).

Stage II: Replication in Japanese case-control samples

We selected 1,632 SNPs from the first-stage screening [1,159 SNPs of p<0.01, and 473 SNPs of p<0.05 located in previously reported linkage regions [8,9]]. In the second-stage analysis, we have taken advantage of an affordable multiplex genotyping platform (Illumina Bead Array). The second sample consisted of 506 patients with schizophrenia and 506 age- and sex-matched controls of Japanese origin. Control subjects were recruited from hospital staff and volunteers who had no family history of psychoses. They showed no current or past evidence of psychoses, during brief interviews by expert psychiatrists. In this experiment, 120 SNPs have dropped owing to the low designability of target SNPs and 40 SNPs could not be genotyped. Accordingly, 1,472 SNPs were successfully genotyped in an independent Japanese case-control sample. Sixty-nine SNPs located on autosomes and 17 SNPs located on chromosome X showed a nominal significance of p<0.05 (Tables S1, S2). It may be reasonable not to declare a compelling association regarding these SNPs because of multiple testing; therefore, the interpretation of data must be made with caution. The top 20 SNPs are listed in Table 1. The most significant p value in the second-stage analysis was obtained for the SNP in the ELAVL2 [embryonic lethal, abnormal vision, Drosophila]-like 2] gene located on 9p21.3 (p = 0.000097).

Stage III: Replication in Chinese family samples collected by NIMH

Japanese and Chinese are genetically close, but apparently different populations. In addition, the gene-based approach provides more information than single-SNP analysis, because a high-density mapping could capture the potential risk-conferring variations, which is difficult by examining sparse-density SNPs on the GeneChip.

Accordingly, to confirm association signals in a gene-based manner, we performed a follow-up study of the ELAVL2 gene whose SNP showed the most compelling association in the case-control study using Japanese population, by densely genotyping 293 pedigree samples (284 quad and 9 trio samples, consisting of 1,163 family members) from Chinese population. We analyzed 56 tagSNPs located in and around the ELAVL2 gene. This gene has not been reported to be genetically associated with schizophrenia to date.

As shown in Figure 3, single marker analysis in the third set showed a nominally significant association with four SNPs on the gene (lowest p = 0.026). Three SNPs are clustered in the intron 1 of the gene. They lose significance when conservative Bonferroni’s correction was applied. The transmitted/non-transmitted and overrepresentation/underrepresentation relationship of the allele revealed consistent risk of the minor C allele of the initial marker SNP (rs10491817) in each stage sample. The significant SNPs on the ELAVL2 gene showed no deviation from Hardy-Weinberg disequilibrium (based on the data from independent parents in the Chinese sample set).

Quantitative RT-PCR in postmortem brains from schizophrenia

The identification of ELAVL2 as a susceptibility gene for schizophrenia in both Japanese and Chinese cohorts led us to examine whether the expression levels of the gene are altered in the postmortem brains of patients with schizophrenia. In addition, the accumulating lines of evidence show that schizophrenia and bipolar disorder partly share common susceptibility genes or genetic pathways. We performed real-time quantitative RT-PCR assays for mRNA levels of the gene in the dorsolateral prefrontal cortex (DLPFC; Brodmann’s area 46) of schizophrenia, bipolar disorder and control brains.

However, the experiments showed that the expression levels of ELAVL2 were not different among brains from schizophrenics, bipolar disorder patients and control subjects (Figure 4). We did not examine the allele-specific expression levels of the transcript because the minor allele (C) frequency is very low (0.056) in Caucasian (from which the postmortem brains are derived) according to the HapMap database (http://hapmap.ncbi.nlm.nih.gov/).
Discussion

We performed a GWAS, a follow-up replication study and a gene-centric dense mapping to identify susceptibility genes and risk variants for schizophrenia in Japanese and Chinese populations. A novel candidate gene has emerged from our staged association analyses.

ELAVL2 [(embryonic lethal, abnormal vision, Drosophila)-like 2], also known as Hu antigen B (HuB) or Hel-N1, belongs to the RNA-binding Hu (Elavl) protein family. In mammals, the family consists of four highly conserved members that include the ubiquitously expressed ELAVL1 (HuA, HuR) and the neuronal-specific ELAVL2 (HuB, Hel-N1), ELAVL3 (HuC, ple21) and ELAVL4 (HuD) [10,11,12,13,14]. Neuronal Elavl proteins (nELAVL; ELAVL2, ELAVL3 and ELAVL4) have been identified as splicing regulators in neuron-like cells, and are likely to exert critical posttranscriptional control as key inducers of programmed neuronal differentiation and function in the mammalian nervous system [15,16]. Given their role in neuronal differentiation and plasticity, nELAVL proteins including ELAVL2 could be potential candidates for neurodegenerative and psychiatric diseases. Indeed, recent studies implicate ELAVL4 as a Parkinson’s disease susceptibility gene [17,18]. Noteworthy, the altered expression of GAP43, one of known targets of ELAVL4, is reported in the frontal cortices and the hippocampus of patients with schizophrenia [19,20,21,22,23]. Although their relevance to schizophrenia pathogenesis is still awaiting clarification, they are worthy of further investigation.

In this work, we have attempted to minimize several limitations often plaguing association studies in psychiatry. Firstly, to minimize population stratification and genetic heterogeneity, we have focused on trio samples of Japanese descent at the first stage. Secondly, we conducted a replication study for significant SNPs using case-control samples from the same Japanese population but an independent sample set at the second stage. Finally, to confirm the association in an ethnically close but different population, we analyzed patient-parents trios/quads of Chinese descent at the third stage. In this stage, to achieve greater coverage of genetic variations for the survived gene, we performed gene-centric analysis by selecting 56 tag SNPs throughout the entire region of the gene (150 kb).

However, several limitations must be considered. We have not achieved sufficient SNP coverage in the first stage of this study, and the number of samples is modest to detect small to medium effect of a disease-associated gene at genome-wide significance level. When Bonferroni’s correction was applied, the significant p value was 5.10 × 10^-7 for multiple tests of 97,963 SNPs (p = 0.05/97,963). The most significant p value in the first stage analysis (p = 6.11 × 10^-7) loses significance after conservative correction. In addition, a small number of genuine causal variants will be buried within a larger number of SNPs with nominal associations. Therefore, the current study will require a follow-up analysis to distinguish the small number of genuine causal variants from the high proportion of SNPs with false-positive associations. The ELAVL2 gene showed a nominal significance at each stage. However, the initial marker SNP in Japanese population (rs10491817) was not significant in Chinese population (p = 0.082). This may reflect the allelic heterogeneity of the gene, because the significant markers in both populations were not in tight linkage disequilibrium (Figures S1, S2). In this context, the gene warrants further investigation.
Recently, Ikeda and his colleagues reported the first GWAS for schizophrenia in Japanese population [1]. In the study, the strongest associations were observed at rs12218361 mapped near the 3' end of the OAT (a gene for ornithine aminotransferase) on 10q26.13 and rs11895771 mapped within the SULTB1 (a gene for sulfotransferase family, cytosolic, 6B, member 1) on 2p22.2 ($p = 7.2 \times 10^{-6}$ and $p = 6.2 \times 10^{-8}$, respectively). No significant associations for those genes were observed in this study. However, it is noteworthy that the two Japanese studies gave the same gene of nominal significance, C6orf105 (Table 1). This putative gene is reported as a candidate for non-syndromic oral clefts [24], but its exact function is unknown.

Recent studies show suggestive evidence of association of multiple GABA-related genes with schizophrenia [25,26]. One of the benefits obtained from GWAS is that we can examine whether a subset of genes categorized into some signaling pathway are involved in the pathogenesis of disease, beyond single genes [27]. We pursued this issue using the first-stage GWAS dataset and unveiled the accumulation of association signals from genes of GABAergic pathways in schizophrenia. Association signature on GABA-related loci was identified across several human chromosomes, which is particularly highlighted on chromosome 5q34 (Figure S3). However, most of the SNPs in and around GABA-related genes associated with schizophrenia in our first-stage dataset were not confirmed in the second-stage samples. Only rs10515831, which lies 90 kb downstream of GABRB2, 47 kb upstream of GABRA6 and 209 kb upstream of GABRA1 on 5q34, showed a nominal significant association with the disease in the second-stage analysis ($p = 0.033$). This may be due to weak genetic contributions of these genes in Japanese, suggesting the necessity of a much larger number of second-stage samples.

In summary, we provided a suggestive evidence for the contribution of ELAVL2 to the pathogenesis of schizophrenia, in both Japanese and Chinese populations. This prioritized gene deserves further evaluation to improve the understanding of schizophrenia genetics.

Materials and Methods

Samples

A three-stage analysis was performed by using two independent Japanese cohorts and an ethnically close Chinese population. In the first stage, 120 patient-parents trio samples from Japanese schizophrenia pedigrees (360 members) were analyzed. In the second stage, case–control samples consisted of 1,012 unrelated individuals (506 schizophrenia patients, mean age 49.2 ± 13.0 years; 506 age- and sex-matched controls, mean age 49.2 ± 13.0 years). In the third stage, Chinese sample consisted of 293 pedigrees (1,163 subjects: nine trios and 284 quads) collected by the NIMH initiative (http://nimhgenetics.org/). For the Japanese samples, all the subjects resided in central Japan. Consensual diagnoses were made by at least two experienced psychiatrists according to DSM-IV criteria. Written informed consent was obtained from all the participants, after the provision and an

Table 1. The top 20 signals in the two-stage association analyses.

| Rank | SNP ID   | Position | 1st stage | 2nd stage | Gene       | HWE Control | HWE Case |
|------|----------|----------|-----------|-----------|------------|-------------|----------|
| 1    | rs10491817 | 9p21.3   | 0.00649   | 0.00087   | ELAVL2     | 0.576       | 0.879    |
| 2    | rs10507559 | 13q14.2  | 0.00933   | 0.00094   |            | 0.498       | 0.382    |
| 3    | rs9296021  | 6p21.32  | 0.01030   | 0.00158   | C6orf10    | 0.240       | 0.035    |
| 4    | rs10497106 | 2q23.3   | 0.00091   | 0.00181   | FMNL2      | 0.159       | 0.280    |
| 5    | rs1899264  | 2p12     | 0.02010   | 0.00240   |            | 0.679       | 0.972    |
| 6    | rs950651   | 5p15.32  | 0.00014   | 0.00299   |            | 0.713       | 0.256    |
| 7    | rs1449531  | 3p24.3   | 0.00024   | 0.00299   |            | 0.439       | 0.531    |
| 8    | rs970954   | 12p12.1  | 0.00511   | 0.00329   | IFLTD1     | 0.900       | 0.021    |
| 9    | rs488212   | 11q22.3  | 0.01590   | 0.00386   |            | 0.310       | 0.658    |
| 10   | rs7695870  | 4q32.1   | 0.00953   | 0.00419   | GRIA2      | 0.814       | 0.975    |
| 11   | rs600647   | 11q22.3  | 0.01510   | 0.00530   |            | 0.345       | 0.801    |
| 12   | rs10517668 | 4q32.1   | 0.00376   | 0.00549   |            | 0.875       | 0.682    |
| 13   | rs2289965  | 11p15.1  | 0.00286   | 0.00678   | IGFB2      | 0.273       | 0.711    |
| 14   | rs2292101  | 3p25.2   | 0.01140   | 0.00679   | PPARG      | 0.817       | 0.550    |
| 15   | rs2235394  | 6p24.1   | 0.01110   | 0.00697   | C6orf105   | 0.306       | 0.556    |
| 16   | rs10496761 | 2q22.1   | 0.03250   | 0.00912   |            | 0.099       | 0.678    |
| 17   | rs1048076  | 6p21.1   | 0.00225   | 0.00945   | ENPP4      | 0.974       | 0.876    |
| 18   | rs348116   | 5p15.2   | 0.00270   | 0.00952   |            | 0.207       | 0.918    |
| 19   | rs10505845 | 12p12.2  | 0.00838   | 0.01010   |            | 0.392       | 0.868    |
| 20   | rs3106653  | 2q24.1   | 0.00465   | 0.01194   | KCNJ3      | 0.710       | 0.199    |

Rank is ordered according to the results of the second-stage analysis.

SNP information is listed based on UCSC Feb. 2009 (http://genome.ucsc.edu/).

P values of the 1st stage are calculated by Transmission Disequilibrium Test (TDT).

P values of the 2nd stage are calculated by Fisher's exact test.

HWE: Hardy Weinberg Equilibrium. Departures from the assumption of HWE are evaluated based on the data from the 2nd stage case-control samples.

doi:10.1371/journal.pone.0020468.t001
explanation of study protocols and purposes. Our case samples in the current study consist of all such patients with schizophrenia who are in a remission/stable chronic state and possess the ability to agree to join the research. This study was approved by the Ethics Committee of RIKEN, and conducted according to the principles expressed in the Declaration of Helsinki.

First-stage analysis

The first-stage GWAS was performed using Affymetrix GeneChip Mapping 100 K microarrays (Affymetrix, Santa Clara, CA) following the manufacturer’s protocol. Genotype data were analyzed with the GeneSpring GT (Varia) 2.0 software package developed by Agilent Technologies (Santa Clara, CA). Transmission disequilibrium test was performed using the R program (http://www.r-project.org). We set a liberal first-stage significance level to increase the potential to detect associated genes with small effects in the subsequent stage analyses: (i) p value < 0.01, and (ii) p value < 0.05 when SNPs are located in candidate chromosomal regions detected in the meta-analysis of schizophrenia linkage studies [9] or in the reported linkage regions of Japanese population [8]. We used the Haploview 4.2 to create a Manhattan plot of p values from GWAS study (http://www.broadinstitute.org/haploview). A QQ plot of p values from GWAS was created using R scripts provided by Diabetes Genetics Initiative (http://www.broadinstitute.org/science/projects/diabetes-genetics-initiative/plotting-genome-wide-association-results). The data obtained in this study have been deposited into the NCBI’s Gene Expression Omnibus [28] and are accessible through GEO Series accession number GSE27923 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE27923).

Second-stage analysis

In the second stage, genotyping was performed using Illumina (San Diego, CA), through the use of their Integrated BeadArray System. We supplied Illumina with 96-well barcoded DNA microtiter plates containing 1,012 samples of DNA (4 mg each) quantified with Pico Green to be 100 ng/ml. Assay quality was as follows: sample success rate of 100%, locus success rate of 97.40%,
was conducted using an ABI 7900HT Fast Real-Time PCR System administered with anti-psychotics. Quantitative RT-PCR analysis was performed in triplicate, based on a standard curve method. The Mann-Whitney U test (two-tailed) was used to evaluate significant changes in target gene expression levels.

**Supporting Information**

**Table S1** Replication in Japanese case-control samples (autosomes). SNP information is based on the UCSC database (http://genome.ucsc.edu/). P values of the 1st stage analysis are calculated by Transmission Disequilibrium Test (TDT). P values of the 2nd stage analysis are calculated by Fisher’s exact test. HWE: Hardy Weinberg Equilibrium. Departures from the assumption of HWE are evaluated based on the data from the 2nd stage case-control samples.

**Table S2** Replication in Japanese case-control samples (chromosome X). SNP information is based on the UCSC database (http://genome.ucsc.edu/). P values of the 1st stage analysis are calculated by Transmission Disequilibrium Test (TDT). P values of the 2nd stage analysis are calculated by Fisher’s exact test.

**Figure S1** Linkage disequilibrium between markers in Chinese population. Linkage disequilibrium (LD) between markers constructed by the Haploview program is shown (based on the data from independent parents in the Chinese sample set). The number in each cell represents the LD parameter $r^2$ ($\times 100$). Each cell is painted with graduated color relative to the strength of linkage disequilibrium between markers. The rs numbers are SNP I.D. in the NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp). The significant SNPs and the genomic region surrounding these SNPs were shown in red and a red pentagon, respectively.

**Figure S2** Linkage disequilibrium between markers in Japanese population. Linkage disequilibrium (LD) between markers constructed by the Haploview program using the data from HapMap database is shown (http://hapmap.ncbi.nlm.nih.gov/). The number in each cell represents the LD parameter $r^2$ ($\times 100$). Each cell is painted with graduated color relative to the strength of linkage disequilibrium between markers. The rs numbers are SNP I.D. in the NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp). The significant SNPs and the genomic region surrounding these SNPs were shown in red and a red pentagon, respectively.

**Figure S3** Association signals on chromosome 5q GABA A receptor subunit gene cluster. The chromosome 5q risk locus contains a cluster of GABA A receptor subunit genes, GABRB2, GABRA6, GABRA1, GABRG2 and GABRP. Significant SNPs ($p<0.05$) and the corresponding genes are shown in red.

**Acknowledgments**

Data and biomaterials were collected in three projects that participated in the National Institute of Mental Health (NIMH) Schizophrenia Genetics Initiative. From 1991–97, the Principal Investigators and Co-Investigators were: Harvard University, Boston, MA, U01 MH46318, Ming T. Tsuang, M.D., Ph.D., D.Sc., Stephen Faraone, Ph.D., and John Pepple, Ph.D.; Washington University, St. Louis, MO, U01 MH46276, C. Robert Cloninger, M.D., Theodore Reich, M.D., and Dragan Svrakic, M.D.;
Columbia University, New York, NY, U01 MH46289; Charles Kaufmann, M.D., Dolores Malaspina, M.D., and Jill Harkavy Friedman, Ph.D. Other participants in the US were Stephen V. Faraone, Ph.D. (Co-Principal Investigator), Shaio Zhu, M.D. (Project Coordinator), and Jing Hua Cui, M.D. (Project Coordinator) The project leaders in Taiwan were Hsi-Wen Hwu, M.D. (Taiwan Principal Investigator, National Taiwan University Hospital), Wei J. Chen, M.D. Sc.D. (Taiwan Co-Principal Investigator). Other participants in Taiwan were: Chen-Min Liu, M.D., Shih-Kai Liu, M.D., Ming-Hsien Shieh, M.D., Tsung-Jeng Huang, M.D., M.P.H., Ming-Ming Tsuang, M.D., Wen Chen On Yang, M.D., Ph.D., Chun-Ying Chen, M.D., Chwen-Cheng Chen, M.D., Ph.D., Ju-Jin Lin, M.D., Frank Huang-Chih Chou, M.D., Ph.D., Ching-Mo Chueh, M.D., Wei-Ming Liu, M.D., Chiao-Chyieh Chen, M.D., Hsin-Ju Lo, M.D., Jia-Fu Lee, M.D., Ph.D., Seng Shih, M.D., Yung Feng, M.D., Shin-Fin Lin, M.D., Shi-Chin Guo, M.D., Ming-Cheng Kuo, M.D., Liang-Jen Chou, M.D., Chi-Pin Lu, M.D., Deng-Yi Chen, M.D., Huan-Kwang Feng, M.D., Nan-Ying Chi, M.D., Wen-Kun Chen, M.D., Tien-Cheng Lee, M.D., Hsin-Pei Tang, M.D., Yih-Dar Lee, M.D., Wu-Shih Wang, M.D., For-Wey Long, M.D., Ph.D., Tao-Lai Huang, M.D., Jung-Kwang Wen, M.D., Cheng-Sheng Chen, M.D., Wen-Hsiang Huang, M.D., Shu-Yu Yang, M.D., M.D., Ph.D., Xiaoming He, M.D., Ph.D., Xiaoling He, M.D., Ph.D., Xiaolin He, M.D., Ph.D., Xiaoying Zhuo, M.D., Ph.D., Bing Jing Liu, M.D., De-Dong Wang, M.D., Ph.D., Meng Wang, M.D., Tiansheng Guo, M.D., Xiaqi Shen, M.D., Ph.D., Jinghua Yang, M.D. ENH/Northwestern University, Evanston, IL, MH059571, Pablo E. Olvea, M.D. (Collaboration Coordinator; PI), Alan R. Sanders, M.D.; Emory University School of Medicine, Atlanta, GA, MH05957, Farooq Amin, M.D. (PI); University of California, San Francisco, CA,MH060670, William Byerley, M.D. (PI); University of Iowa, Iowa, MA, MH059566, Raymond Crowe, M.D. (PI), Donald Black, M.D.; Washington University, St. Louis, MO, U01, MH060879, C. Robert Cloninger, M.D. (PI); University of Colorado, Denver, CO, MO059565, Robert Freedman, M.D. (PI), Norm Olney, M.D.; University of Pennsylvania, Philadelphia, PA, MH061675, Douglas Levinson M.D. (PI), Nancy Buccka APRN, B.C., M.S.N., New Orleans, Louisiana; University of Queensland, Queensland, Australia, MOH059568, Bryan Movory, M.D. (PI); Mt. Sinai School of Medicine, New York, NY, MH059586, Jeremy Silverman, Ph.D. (PI).

Author Contributions
Conceived and designed the experiments: KY TY. Performed the experiments: YI EH. Analyzed the data: KY KI TK. Contributed reagents/materials/analysis tools: TT TO HOM. Wrote the paper: KY TY.

References
1. Ikeda M, Aleksic B, Kinoshita Y, Okochi T, Kawashima K, et al. (2010) Genomic-Wide Association Study of Schizophrenia in a Japanese Population. Biol Psychiatry doi:10.1016/j.biopsych.2010.07.010.
2. O’Donovan MC, Craddock N, Norton N, Williams H, Peirce T, et al. (2008) Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40: 1053–1055.
3. Purcell SM, Wray NR, Stone JL, Visscher PM, O’Donovan MC, et al. (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460: 745–752.
4. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, et al. (2009) Common variants on chromosome 6p22.2 are associated with schizophrenia. Nature 460: 753–757.
5. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, et al. (2007) Common variants conferring risk of schizophrenia. Nature 460: 744–747.
6. Stefansson H, Rujescu D, Cichon S, Pettilainen OP, Ingason A, et al. (2008) Large recurrent microdeletions associated with schizophrenia. Nature 455: 232–236.
7. Xu B, Roos JL, Levy S, van Rensburg EJ, Gogos JA, et al. (2008) Strong association of de novo copy number mutations with sporadic schizophrenia. Nat Genet 40: 1053–1055.
8. Arinami T, Ohtsuki T, Ishiguro H, Ujike H, Tanaka Y, et al. (2005) Genetic investigation of chromosome 5q GABAA receptor subunit genes in sporadic schizophrenia. Mol Genet 26: 264–271.
9. Petryshen TL, Middleton FA, Tahl AR, Rockwell GN, Purcell S, et al. (2005) Reduced GAP-43 mRNA in dorsolateral prefrontal cortex of patients with schizophrenia. Cereb Cortex 11: 136–147.
10. Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Biol Psychiatry. doi:10.1016/j.biopsych.2010.07.010.
11. Replication of association between ELAVL4 and Parkinson disease: the GenePD study. Hum Genet 124: 95–99.
12. Southerland HM, Peden M, Kalaris K, Kurki T, et al. (2008) Association between the neuron-specific RNA-binding protein ELAVL4 and bipolar disorder. Cell Mol Life Sci 65: 128–140.
13. Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Biol Psychiatry. doi:10.1016/j.biopsych.2010.07.010.
14. Bleniewski R, Bogdanovic N, Göttschi CG, Davidson P (1999) The growth-associated protein GAP-43 is increased in the hippocampus and in the gyrus cinguli in schizophrenia. J Mol Neurosci 13: 101–109.
15. Perrone-Bizzozero NJ, Sower AC, Bird ED, Benowitz LI, Evans KJ, et al. (1996) Levels of the growth-associated protein GAP-43 are selectively increased in association cortices in schizophrenia. Proc Natl Acad Sci U S A 93: 14182–14187.
16. Tian SY, Wang JF, Bezchlibnyk YB, Young LT (2007) Immunoreactivity of 45 kDa growth-associated protein is decreased in postmortem hippocampus of bipolar disorder and schizophrenia. Neuropsi Lett 411: 123–127.
17. Webster MJ, Shannon Weickert C, Herman MM, Hyde TM, Kleinman JE (2001) Synaptophysin and GAP-43 mRNA levels in the hippocampus of subjects with schizophrenia. Schizophrenia Res 48: 89–98.
18. Weickert CS, Webster MJ, Hyde TM, Herman MM, Bachus SE, et al. (2001) Reduced GAP-43 mRNA in dorsolateral prefrontal cortex of patients with schizophrenia. Cereb Cortex 11: 136–147.
19. Park JW, Cai J, McIntosh I, Jabs EW, Fallin MD, et al. (2006) High throughput SNP and expression analyses of candidate genes for non-syndromic oral clefts. Journal of Medical Genetics 43: 598–608.
20. Levin DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6: 312–324.
21. Petryshen TL, Middleton FA, Talal AH, Rockwell GN, Purcell S, et al. (2005) Genetic investigation of chromosome 5q GABAA receptor subunits genes in schizophrenia. Mol Psychiatry 10: 1074–1088, 1057.
22. Yamada K, Gerber DJ, Iwamoto Y, Ohnishi T, Ohbha H, et al. (2007) Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. Proc Natl Acad Sci U S A 104: 2815–2820.
23. Edgar R, Domrachev M, Lash AE (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nuclear Acids Res 30: 207–210.
24. Kim S, Webster MJ (2009) Postmortem brain tissue for drug discovery in psychiatric research. Schizophr Bull 35: 1031–1033.
25. Kim S, Webster MJ (2010) The Stanley neuropsychopathology consortium integrative database: a novel, web-based tool for exploring neuropathological markers in psychiatric disorders and the biological processes associated with abnormalities of those markers. Neuropsychopharmacology 35: 473–482.

Genome-Wide Association Study of Schizophrenia