Association study between the NMDA receptor 2B subunit gene (*GRIN2B*) and schizophrenia: A HuGE review and meta-analysis

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Schizophrenia is a severe mental illness to which hypofunction of the N-methyl-D-aspartate receptors has been linked. Association studies have implicated the N-methyl-D-aspartate receptor 2B subunit gene (*GRIN2B*) as a candidate for schizophrenia. Subsequent studies have attempted to replicate the association, but the results have been mixed and thus inconclusive. It is necessary to explain the inconsistency of these results and to clarify the contribution of the *GRIN2B* gene to schizophrenia. The current meta-analysis covers all published association studies up to January 2006 using systematic allelic and genotypic analyses involving five polymorphisms. The results show evidence of a statistically significant association for *GRIN2B*. The association seems weaker, but nonetheless interesting. The meta-analysis supports the involvement of the glutamate system of the brain in the pathogenesis of schizophrenia. This may be the first systematic meta-analysis study focusing on *GRIN2B*. ***Genet Med* 2007:9(1):4–8.**

Schizophrenia is a severe mental illness characterized by hallucinations, delusions, and disorganized thought and behavior.[1,2] A glutamate and N-methyl-D-aspartate (NMDA) receptors hypofunction model based on pharmacologic and genetic approaches has been suggested as a possible approach in the study of the disease.[3–5] Glutamate is one of the major neurotransmitters in the vertebrate central nervous system and is bound by the NMDA receptors. Functional NMDA receptors are composed of a common glutamate receptor, an ionotropic NMDA receptor 1 subunit (GRIN1), and one of four NMDA receptor 2 subunits (GRIN2 [A–D]) in an undetermined ratio to make up the receptor complex.[6] The NMDA receptor antagonists phencyclidine and ketamine can induce schizophreniform psychosis in healthy volunteers and exacerbate psychotic symptoms in patients with schizophrenia.

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GENE VARIANTS

The *GRIN2B* gene, at 12p12 and 419 kb in size, consists of 13 exons. It is expressed in the hippocampus, basal ganglia, and cerebral cortex.[7] The *GRIN1* gene has been found to be underexpressed in the cortex of schizophrenics[8] and has been suggested as a likely candidate in the pharmacogenetics of typical and atypical antipsychotics.[9] Evidence of studies on functional expression[10,11] and a mouse model[12] support the *GRIN2B* gene as a candidate for schizophrenia. Five polymorphisms including T-200G, 366C/G, 2664C/T, 4197T/C, and 5988T/C of *GRIN2B* have been the subject of schizophrenia association studies. The overall description of each polymorphism is shown in Table 1.

Objectives

Independent studies have attempted to replicate these initial positive findings focusing on each polymorphism. However, a number of these studies have produced contrary findings, resulting in an uncertain overall picture. We have attempted to reconcile the inconsistency in these findings, to measure the magnitude of the effect of the risk alleles, and to elucidate the genetic relationship between the *GRIN2B* gene and schizophrenia. The meta-analysis, has, therefore, combined results from all published association studies up to January 2006 using both fixed and random effects methods.

METHODS

Selection criteria

Eligible studies had to meet all of the following criteria: (1) were published in a peer-reviewed journal and were independent studies using original data, (2) provided sufficient data to

Table 1
Overall description of each polymorphism

Markers	dbSNP rs id	Contig position	Region	Function	Codon	Protein residue
2664C/T	rs1806201	6476482	Exon 13	Synonymous	ACC/ACT	Thr888/Thr888
366C/G	rs7301328	6777751	Exon 2	Synonymous	CCC/CCG	Pro122/Pro122
T-200G	rs1019385	6893817	5′ upstream	Locus	—	—
4197T/C	rs1805247	6474949	Exon 13	Synonymous	CAT/CAC	His1399/His1399
5988T/C	rs1805502	6473155	Exon 13	Untranslated	—	—

The three coding SNPs (2664C/T, 366C/G, and 4197T/C) do not determine an amino acid substitution, and they are silent polymorphisms. Contig accession: NT_009714.

calculate the odds ratio (OR) with confidence interval (CI) and *P* value, (3) investigated one or more of the five polymorphisms, (4) described the genotyping primers, machines, and protocols or provided reference to them, (5) diagnosed schizophrenic patients according to the International Classification of Diseases, Diagnostic and Statistical Manual [of Mental Disorders], or Chinese classification of mental disorders systems, and (6) used healthy individuals as controls. Authors were contacted in cases in which there were queries regarding their studies.

Search strategy

The literature included in the current analysis was selected using PubMed and focusing on the keywords "schizophrenia," "NMDAR2B," and "NMDA receptor 2B subunit," and the abbreviation of the gene "*GRIN2B*." All references cited in these studies and published reviews were reviewed to identify additional work not indexed by MEDLINE. Some raw data, unavailable in articles, were obtained from the authors. The analyzed data cover those from all English-language publications up to January 2006.

Assessment of study quality: Extended Quality Score

For association studies with inconsistent results on the same polymorphisms, the methodologic quality should be assessed by appropriate criteria to limit the risk of introducing bias into meta-analyses or systematic reviews. The technique known as "Extended Quality Score"[13] was developed to assess the quality of association studies. Each article is scored on an extended-quality scale that designates them as "high," "median," or "poor" quality.

Data collection and statistical analyses

Data from each case-control study were used to construct two-by-two tables in which subjects were classified by diagnostic category and type of allele. The Cochran's χ^2-based Q statistic test was performed to assess heterogeneity and thus to ensure that each group of studies was suitable for meta-analysis. On condition that heterogeneity was found, the random effects model, which yields wider CIs, was adopted; otherwise, both the fixed and random effects models were considered appropriate. We assessed publication bias by using an ancillary procedure attributed to Egger et al.,[14] which uses a linear regression approach to measure funnel plot asymmetry on the natural logarithm of the OR. The larger the deviation from the funnel curve of each study, the more pronounced the asymmetry. The results from small studies tend to scatter widely at the bottom of the graph, with the spread narrowing among larger studies. The significance of the intercept is evaluated using the *t* test.

ORs and relative risk were pooled according to both the fixed and random effects methods of DerSimonian and Laird, and 95% CIs were constructed using Woolf's method. The significance of the overall OR was determined by the *z* test. For the sensitivity analysis, each study was removed in turn from the combined total, and the remaining studies were reanalyzed. This procedure was used to ensure that no individual group was biasing the combined result. The analysis was conducted by Comprehensive Meta Analysis (Version 1.0.23, BIOSTAT, Englewood, NJ). The type I error rate was set at 0.05.

We calculated both OR and ln(OR) with CIs. Compared with OR, ln(OR) has a more symmetric CI, which is advantageous in meta-analysis.[15] An R-project program was used to depict the degree of differences and trend of association of risk allele frequency from controls to patients. If the vector arrow had the same direction, this indicated the same kind of association (and vice versa).

Haplotype construction, counting, and linkage disequilibrium (LD) structure defining were performed using 30 CEPH trios (Utah residents) on Haploview software (www.hapmap.org). The multiallelic D' was computed by performing a series of pairwise D' calculations using each haplotype in turn as an allele, with all other haplotypes at the locus serving as the other allele. This was then repeated for each haplotype at each locus and averaged by haplotype frequency. Maximum likelihood haplotype structures were calculated using an expectation-maximization algorithm.

RESULTS

Study inclusion and characteristics

The combined search yielded 62 references. After overlapping references and those that clearly did not meet the criteria were discarded, 9 references were retained. These references were then filtered to ensure conformity with the inclusion cri-

teria. One reference[16] was excluded for insufficient and equivocal data, one reference[17] was excluded for association with clozapine, and one reference[18] was excluded on the grounds of being a linkage study. Finally, six references[5,19–23] met our criteria for inclusion. The references included 1166 cases and 1080 controls. The studies finally included were of median-to-high quality ("Extended Quality Score"[13]) and included no "poor quality" study.

Associations

For T-200G of the *GRIN2B* gene, the results showed a significant *P* value of .005 without evidence of heterogeneity ($P = .38$), and the overall OR was 0.71 (0.56–0.9) (supplementary Table 1, available online only). Furthermore, the genotypic analysis also showed positive results without heterogeneity no matter whether the T allele or G allele was combined (supplementary Table 1, available online only). For 4197T/C, a weak association was found in genotypic analysis with a *P* value of .017 (overall OR = 0.47; 0.26–0.87) with no evidence of heterogeneity ($P = .57$) (supplementary Table 1, available online only). However, no statistically significant association was found for three other single nucleotide polymorphisms (SNPs) in allelic or genotypic analyses (Table 2). The forest plots of allelic analysis are shown in Figure 1. No publication bias was found [no *P* (T) < .05].

Retrospective analysis

The asymptote lines of the analysis in retrospect based on the publication year showed that cumulative synthesis of the *GRIN2B* 2664C/T tended to be stable after 2003 (Fig. 2), similar to those of our meta-analysis. However, more replications were suggested for other SNPs because of the instability of asymptotic slopes.

Table 2
Overall results of allelic and genotypic analyses for 2664C/T and 366C/G polymorphisms

Markers/types	OR (95% CI)	*P* (Z)	*P* (Q)
2664C/T[a] (5)[b]			
1/2	0.92 (0.81–1.04)	0.1881	0.3861
(11+12)/22	0.95 (0.76–1.2)	0.6732	0.1277
11/(12+22)	0.85 (0.7–1.03)	0.1000	0.9565
366C/G (4)			
1/2	0.96 (0.83–1.1)	0.5119	0.0800
(11+12)/22	1 (0.66–1.51)	0.9877	0.0250
11/(12+22)	0.89 (0.72–1.11)	0.3177	0.5966

OR, odds ratio; CI, confidence interval.
[a]1 = the first allele; 2 = the second allele. If OR >1, the first allele is the risk allele; if OR <1, the second allele is the risk allele.
[b]The number of studies included are indicated in parentheses.
P (Z): Z test used to determine the significance of the overall OR.
P (Q): Cochran's χ^2-based Q statistic test used to assess the heterogeneity.
P (T): T test used to evaluate the significance of publication bias. No *P* (T) < 0.05 (not shown).
The results of the heterozygotes with one or the other group of homozygotes are shown. The results of each genotype compared with each of the others are shown in supplementary Table 1 (available online only).

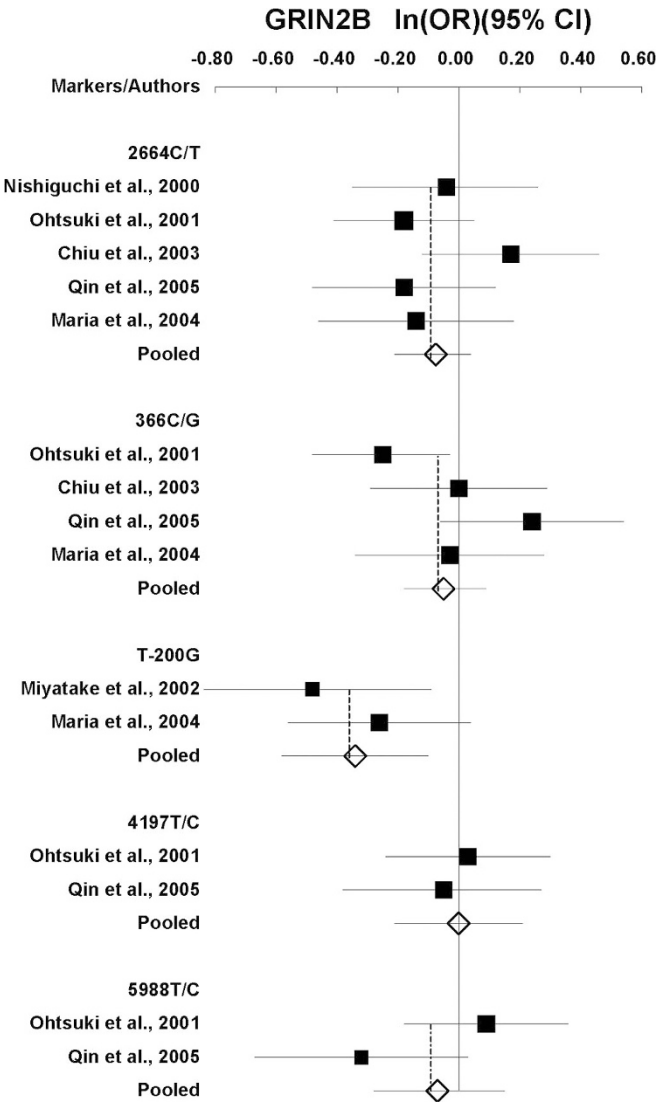

Fig. 1. Forest plots of ln(OR) with 95% CIs for each of the five polymorphisms of the NMDA receptor 2B subunit gene (*GRIN2B*). The ln(OR) (*black squares*), with the size of the square inversely proportional to its variance, and 95% CIs (*horizontal lines*). Pooled results (*unshaded black diamond*).

The trend of allele frequency by R project and funnel plots are shown as supplementary figures (supplementary Fig. 1 and Fig. 2, available online only). The results of genotypic analysis of each genotype compared with each of the others are shown in supplementary Table 2 (available online only). The results of demography of the included studies and individual studies based on allele and genotype data are presented in supplementary Tables 3, 4, and 5, respectively (available online only). Other data are available on request.

DISCUSSION

Studies of schizophrenia tend to be characterized by genetic heterogeneity. Different alleles or genotypes have been reported to

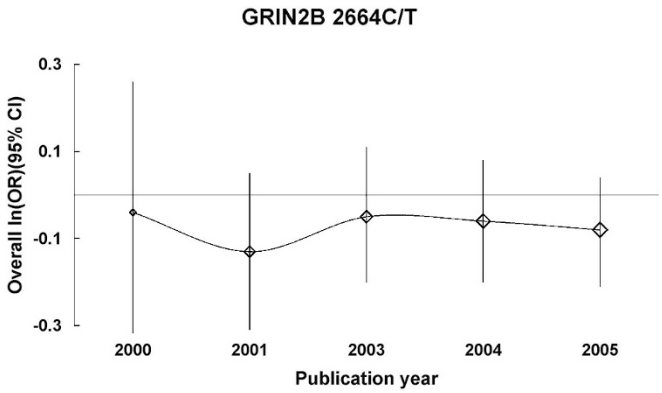

Fig. 2. Retrospective analysis, based on publication since 2000, is shown for 2664C/T; it is not shown for other SNPs, but is available on request.

be associated in different populations. The current meta-analysis adopted a variety of methods with the random effects model accounting for additional sources of interstudy variation when heterogeneity existed. It is more conservative than the fixed effects model, because the latter assumes the same true genetic effects between studies, whereas the former assumes the normally distributed effects and parameterizes the interstudies variation. We investigated OR, relative risk, and ln(OR) by both models using Mantel-Haenszel, inverse variance, and Peto one-step approaches. In fact, we found no evidence of significant heterogeneity other than weak heterogeneity in the genotypic analysis for 5988T/C and 366C/G ($P = .03$).

The functional NMDA receptors are multiple heterodimer complexes composed of *GRIN1* and *GRIN2* subtypes. The genes are expressed in various tissues and brain regions and are likely to be under a complex expression control.[11] The three coding SNPs (2664C/T, 366C/G, and 4197T/C) do not determine an amino acid substitution, and they are silent polymorphisms (synonymous mutations). The 5988T/C is in the 3′-untranslated region, whereas T-200G is in the 5′-upstream region. In regard to the LD and haplotype structure of the *GRIN2B* gene (supplementary Fig. 3, available online only), we did not find any strong haplotype structure for SNPs 2664C/T, 4197T/C, and 5988T/C, although they were previously reported to be in almost complete LD with SNPs 1665C/T and 5806A/C.[21] None of the three individual markers (366C/G, 2664C/T, and 5988T/C) provided significant evidence. Both allele and haplotype frequencies may vary around the world, and the markers could be in LD with different haplotypes in different populations. Otherwise, other at-risk polymorphisms, which were in the small strong haplotype structure with T-200G (supplementary Fig. 3, available online only), such as 15G/T,[21] should be further examined. Because the structure regions were performed using 30 CEPH trios (Utah residents), the LD results may be different in different populations. Mutation analyses of other subtypes might help to confirm the NMDA-deficiency hypothesis of schizophrenia. It is not impossible that the genes conferring susceptibility to schizophrenia interact with one another. Examination of genetic risk factors that have less of an effect or are possibly in epistasis with the genes could be interesting.

Limitations and implications

This may be the first meta-analysis based on *GRIN2B*. Although the current study detected statistically positive evidence (with small effects) in international populations, several problems, including possible effects of variables such as age, ethnicity, and gender, need to be investigated in future meta-analyses. Sensitivity analysis has not been shown because of the relatively limited data set included for the two associated polymorphisms. In addition, the two polymorphisms cannot account for much of schizophrenia because no linkage was found in the region 12p.[24] For future association studies, more accurate phenotype definition, strict selection of the patients, and much larger samples will be required. The sample size needed is difficult to predict, because it depends on the degree of association, LD, accuracy of phenotypic data, and heterogeneity of allelic frequencies. However, the low heterogeneity in the current study suggests that a relatively small sample size is sufficient. Moreover, the establishment and use of standardized criteria for the sample collection methods, DNA marker sets, assessment protocol, and application of demographic statistical methods would also be beneficial. This would enhance comparability between study outcomes, simplify collaboration among investigators, and allow the pooling of data in future multisite projects or meta-analyses.

With the results of other candidates in the glutamate-related genes, such as the *NRG1* [13] and *DTNBP1* (unpublished) genes, confirmed by meta-analyses and with the results of haplotype study of all these SNPs and additional SNPs, the current results may support the involvement of the *GRIN2B* gene related-glutamine system in the pathogenesis of schizophrenia, considering their particular features and essential role in the function of NMDA receptors.[25] The biological significance may be difficult to interpret; however, this developing understanding of glutamate and the NMDA receptor system gene will have potential research implication.

Electronic-database information

Accession numbers and uniform resource locators for data in this article are as follows:

Online Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/Omim for *GRIN2B* and *GRIN1*.

Genotype data: http://www.hapmap.org/ for *GRIN2B* and *GRIN1*.

Genome data: http://genome.ucsc.edu/ for *GRIN2B* and *GRIN1*.

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