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Original article:

N-METHYL-D-ASPARTATE RECEPTOR NR1 SUBUNIT GENE (*GRIN1*) G1001C POLYMORPHISM AND SUSCEPTIBILITY TO SCHIZOPHRENIA : A META-ANALYSIS

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ABSTRACT

A comprehensive literature search was conducted to identify all case-control studies investigating the association between *GRIN1* G1001C polymorphism and schizophrenia susceptibility (MIM: 138249; dbSNP: rs 11146020). A total of 6 eligible studies (including 1639 schizophrenia cases and 1489 controls) were identified for the meta-analysis. Including all studies, there was significant heterogeneity between studies. In overall the GC (OR=1.00, 95 % CI: 0.0.85-1.19) and CC (OR=1.09, 95 % CI: 0.67-1.79) genotypes were not associated with schizophrenia risk compared with the GG genotype. In one study patients were diagnosed using DSM-IIIR criteria and in another study the genotypic frequencies of control subjects showed significant deviation from the expected frequencies according to the Hardy-Weinberg equilibrium. After excluding these studies from the meta-analysis, the heterogeneity between studies dramatically decreased. Statistical analysis showed that the GC genotype compared with the GG genotype significantly increased the risk of schizophrenia (OR=1.85, 95 % CI: 1.43-2.42, P<0.0001). The CC versus GG genotype significantly increased the schizophrenia risk (OR=2.46, 95% CI: 1.17-6.84, P=0.017). There was significant linear trend for presence of 0, 1, and 2 of the C allele and risk of schizophrenia (χ^2=25.45, P<0.0001). In conclusion, the C variant allele may be associated with an increased risk for developing schizophrenia.

Keywords: case-control study; genetic polymorphism; *GRIN1*, meta-analysis

INTRODUCTION

Among many theories proposed for the pathogenesis of schizophrenia, dysfunction of the glutamate neurotransmitter system attracts much attention (Belsham, 2001). Several genes that encode N-methyl-D-aspartate (NMDA) receptors have been cloned, including *GRIN1* (MIM: 138249) (Brett et al., 1994). Decreased expression of *GRIN1* has been observed in various brain regions in schizophrenic patients (Sokolov, 1998; Gao et al., 2000; Ibrahim et al., 2000). Also, reduced expression of *Grin1* leads to behavioral abnormalities in mice that are similar to those observed in pharmacologically induced models of schizophrenia (Mohn et al., 1999).

In literature, several single nucleotide polymorphisms were described in *GRIN1*, including G1001C in the promoter region (dbSNP: rs 11146020). Previous studies investigating the association between this polymorphism and risk of schizophrenia have provided inconsistent results (Begni et al., 2003; Martucci et al., 2003; Qin et al., 2005; Zhao et al., 2006; Georgi et al., 2007; Li et al., 2008; Galehdari et al., 2009; Betcheva et al., 2009). The lack of concordance across the studies might have been due to individual studies, that have no suf-

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ficient statistical power to detect the effect of polymorphism on the schizophrenia susceptibility, differences between the study populations, or differences in the study design. Meta-analysis can summarize results from different studies by producing a single estimate of the major effect with enhanced precision. One of the major advantages of a meta-analysis is that it decreases the probability that random error will produce false-negative or false-positive association. Therefore, a meta-analysis could assist in estimating population-wide effects of polymorphisms in human disease and provide more reliable results than a single study. No meta-analysis investigating the association between *GRIN1* G1001C polymorphism and susceptibility to schizophrenia has been conducted to date. Therefore the present study was done.

MATERIALS AND METHODS

Literature search strategy

Eligible studies were identified by searching the MEDLINE (National Library of Medicine, Washington, DC, USA), Scopus, EBSCOhost Research Databases, ProQuest, EMBASE, Directory of Open Access Journals (DOAJ) and CAB Abstract for relevant reports before September 2009. Search terms were different combinations of "*GRIN1*", "schizophrenia", and "polymerphism". The following criteria were used for the literature selection for the meta-analysis: (1) The article should be published in English; (2) Only case-control studies were considered; (3) The association study of G1001C polymorphism and schizophrenia risk were considered; (4) Only published articles in scientific journals were considered, therefore meeting abstracts and unpublished reports were not considered.

Data extraction

I extracted the following information from each article: author(s), year of publication, country of origin, criteria used for schizophrenia diagnosis, ethnicity, genotyping information, and number of cases and controls. For studies including subjects of different populations, data were extracted separately.

Reports of Martucci et al. (2003) and Betcheva et al. (2009) were not included in the study because raw data were not mention in these articles. One study was not included in the analysis because the article was published in Chinese language (Li et al., 2008). The report of Galehdari et al. (2009) that included two different case-control groups was considered as two studies, in the meta-analysis. The application of these criteria yielded 6 case-control studies eligible for meta-analysis (Begni et al., 2003; Qin et al., 2005; Zhao et al., 2006; Georgi et al., 2007; Galehdari et al., 2009).

Statistical analysis

Pearson's χ^2 test was used to determine whether the observed frequencies of genotypes for control group in each study conformed to the Hardy-Weinberg equilibrium. The heterogeneity between studies was determined using Cochran's Q test, and the heterogeneity was considered significant for $P<0.05$. The strength of the associations between schizophrenia and the G1001C polymorphism was estimated by odds ratios (ORs) and 95 % confidence interval (CI). I estimated the risk of the genotypes GC and CC in comparison with the putative low risk GG genotype. A random effects model (DerSimonian and Laird, 1986) was selected to pool data if there is significant heterogeneity, otherwise, the Mantel-Haenszel fixed effects model was used (Mantel and Haenszel, 1959). Also the linear trend for presence of 0, 1, and 2 high risk variant allele (C allele) and risk of schizophrenia was performed using χ^2 test. The P-value less than 0.05 considered statistically significant.

RESULTS AND DISCUSSION

For the present meta-analysis 5 published articles dealing with 6 case-control studies were selected (Begni et al., 2003; Qin et al., 2005; Zhao et al., 2006; Georgi

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et al., 2007; Galehdari et al., 2009). From these studies, 4, and 2 studies were carried out in Asian and European countries, respectively. Data were available from 6 studies, including 1639 patients and 1489 control subjects (Table 1). The numbers in the case-control studies varied considerably (range 178 to 1373 individuals). In all of the studies, patients were diagnosed using DSM-IV criteria except one study that used DSM-IIIR criteria (Zhao et al. 2006). In all studies the distribution of genotypes in the control group was consisted with Hardy-Weinberg equilibrium (Begni et al., 2003; Qin et al., 2005; Zhao et al., 2006; Georgi et al., 2007), except for one study (Galehdari et al., 2009). There was significant difference in frequency of the variant C allele between Asian and European populations. Overall, the prevalence of the C allele among Asian controls was significantly higher than that in Caucasians (Table 1).

Including all studies, there was significant heterogeneity between the studies (Table 2). In overall, the GC genotype was not associated with schizophrenia risk compared with the GG genotype (OR=1.00, 95 % CI: 0.85-1.19). The same result obtained in comparison of CC versus GG genotypes (OR=1.09, 95 % CI: 0.67-1.79).

In order to find the source of heterogeneity between the studies, we excluded one case-control study from the Galehdari et al. (2009) report, because its control group was not at Hardy-Weinberg equilibrium (χ^2=39.50, df=1, P<0.0001). However, the heterogeneity did not decrease significantly. In the study of Zhao et al. (2006) patients were diagnosed using DSM-IIIR criteria, whereas, in the other studies DSM-IV criteria was used. After excluding these studies from the analysis, the heterogeneity between the studies dramatically decreased (Table 2). Statistical analysis showed that the GC genotype compared with the GG genotype significantly increased the risk of schizophrenia (OR=1.85, 95 % CI: 1.43-2.42, P<0.0001). The CC versus GG genotypes significantly increased the schizophrenia risk (OR=2.46, 95 % CI: 1.17-6.84, P=0.017). There was significant linear trend for presence of 0, 1, and 2 of the C allele and risk of schizophrenia (χ^2=25.45, P<0.0001).

Table 1: Studies included in the meta-analysis

			Control					Case		
Study (year)	**Place**	**ethnicity**	GG	GC	CC	C allele	χ^2 HWE**	GG	GC	CC
Begni et al., 2003	Italy	Caucasians	121	23	1	0.0862	0.01	99	36	4
Qin et al., 2005	China	Asians	100	35	5	0.1607	0.77	156	92	5
Zhao et al., 2006	China	Asians	412	244	25	0.2158	2.31	505	174	13
Georgi et al., 2007	Germany	Caucasians	279	42	2	0.0712	0.11	287	64	4
Galehdari et al., 2009	Iran	Mixed	28	83	0	0.3738	39.50***	22	84	5
Galehdari et al., 2009	Iran	Mixed	53	35	1	0.2078	3.32	24	56	9

Note: * Criteria used for schizophrenia diagnosis
** χ^2 for testing Hardy-Weinberg equilibrium
*** P<0.05

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Table 2: Summary of meta-analysis of case-control studies examining *GRIN1* G1001C polymorphism and schizophrenia risk

		GC vs GG			CC vs GG		
Study	**df**	**Q statistic**	**OR**	**95 % CI**	**Q statistic**	**OR**	**95 % CI**
All of studies	5	48.96*	1.00	0.85-1.19	17.73*	1.09	0.67-1.79
All after excluded Galehdari 2009 study	4	48.35*	0.99	0.83-1.17	15.85*	0.95	0.57-1.59
All of studies after excluding 2 studies	3	5.14	1.85	1.43-2.42	8.23	2.46	1.17-6.94

Note: * There is heterogeneity between studies P<0.05.

We know that schizophrenia is a multifactorial trait with multiple genetic and environmental factors that contribute to the risk of disorder. In pathogenesis of such traits the influence of genetic and environmental factors and also their interactions may be different between ethnic groups. Several meta-analyses indicated that some genetic polymorphisms might be associated with altered risk of some types of cancers in some ethnicity (Hu et al., 2005; Kiyohara et al., 2006; Saadat, 2006; Saadat and Ansari-Lari, 2009). Based on the present study the relationship between this genetic variation and schizophrenia risk among different ethnic groups is an open question.

Because the authors of the original articles used in the present meta-analysis did not mention clearly whether they had used hospital based and/or population based controls or other risk factors for schizophrenia, stratification of the studies is impossible. It is strongly recommended that in future studies, the investigators considering the "STrengthening the REporting of Genetic Association studies (STREGA)" statement (Little et al., 2009). It may be concluded that the relationship between the *GRIN1* genotypic variation and susceptibility to schizophrenia is still an open question. In this field further larger case-control studies with multi-ethnic population are required.

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