Association of *G72/G30* polymorphisms with early-onset and male schizophrenia

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To explore the effect of *G72/G30* polymorphisms on the clinical manifestations of schizophrenia, especially on the age at onset and sex of patients, we examined three single nucleotide polymorphisms in 216 schizophrenic patients and 321 healthy controls. Significant associations of schizophrenia with the A allele of rs947267 ($P = 0.012$) and haplotype A-A-G (rs2391191-rs947267-rs778294) ($P = 0.008$) were found in early-onset schizophrenic patients. So did the same allele ($P = 0.034$) and haplotype ($P = 0.009$) as mentioned above in male patients. These findings suggest that the *G72/G30* gene may modulate the age at onset and there might be a potential interaction between this locus and sex in the pathogenesis of schizophrenia. *NeuroReport* 17:1899–1902

Keywords: age at onset, association study, *G72/G30*, polymorphism, schizophrenia

Introduction

Schizophrenia is a major psychiatric disorder that affects almost 1% of the world's population and accounts for about 2.5% of healthcare costs [1]. Symptoms usually begin in late adolescence or early adulthood. Involvement of neurodevelopmental mechanism, especially glutamatergic transmission via *N*-methyl-D-aspartate receptors and synaptic plasticity in schizophrenia, has been recently suggested, on the basis of several lines of evidence [2]. Linkage analyses on diverse samples have provided accumulating evidence that chromosome 13q32-q33 may be involved in susceptibility to schizophrenia. A meta-analysis of genomewide linkage studies further identified 13q32-q33 as significantly linked to schizophrenia [3].

Chumakov *et al.* [4] first reported that genetic variations near *G72* (MIN 607408) and *G30* (MIN 607415) locus on 13q34 were associated with schizophrenia in French-Canadian and Russian cohorts. *G72* and *G30* overlap on complementary chromosomal strands and are therefore transcribed in opposite directions. By yeast two-hybrid experiments, Chumakov *et al.* [4] also identified that the G72 protein interacts with the enzyme D-amino acid oxidase (MIN 124050), which regulates glutamatergic signaling through an *N*-methyl-D-aspartate receptor pathway. Gene expression analysis of G72 and G30 exhibited correlations between expression levels of the *G72* and *G30* genes, as well as a tendency toward overexpression of G72 mRNA in schizophrenic post-mortem dorsolateral prefrontal cortex of 44 schizophrenic patients compared with 44 control participants [5].

Hattori *et al.* [6] suggested that a susceptibility variant for both bipolar illness and schizophrenia exists in the vicinity of the *G72/G30* gene in two series of pedigrees. Findings from several subsequent studies tend to advance a role for G72/G30 in the overall risk for schizophrenia and bipolar disorder from various populations, including a meta-analysis study and a research based on a sample of child-onset schizophrenia [7–14]. The majority of replication studies have, however, been inconsistent with respect to the associated alleles or haplotypes. Moreover, negative associations of *G72/G30* polymorphisms with schizophrenia were also reported [15,16].

Nevertheless, *G72/G30* locus may be candidate genes for the pathogenesis of schizophrenia or disease modification. The present investigation is an association study to test the hypothesis that *G72/G30* polymorphisms are related to schizophrenia susceptibility and/or age at schizophrenic onset or sex.

Materials and methods

Our study sample consisted of 216 patients with schizophrenia (120 men and 96 women; mean age: 30 ± 10 years) and 321 healthy controls (184 men and 137 women; mean age: 31 ± 11 years) who were group-matched for age, sex and ethnicity. All of the participants were Chinese Han descendants. A part of this sample has been used in our previous study [17]. The age of clinical symptoms onset (13–53 years, mean 25.67 years) was retrospectively estimated as the time of emergence of any schizophrenic symptoms according to the International Classification of Diseases-10. The objectives and procedures of the study were explained to all participants and written informed consent was obtained. Research ethics committee approval was obtained from the Ethical Committee of Peking University Health Science Center.

Three single nucleotide polymorphisms (SNPs) (rs2391191, rs947267, r778294) were genotyped either with the polymerase chain reaction (PCR)-based restriction fragment length polymorphism genotyping or with direct DNA sequencing. The construction of 25 μl PCR reaction mixture and the condition of PCR amplification were performed to be the same as that reported in our previous study [17], with annealing temperatures of 55–62°C. Fifteen microliter PCR products were digested completely with 2U of restriction enzyme (*Hae*III for rs947267, *Bsr*I for rs778294) and electrophoresed subsequently on 2–4% agarose gels stained with ethidium bromide. For rs2391191, the PCR products were sequenced on an ABI PRISM 377-96 DNA Sequencer (Applied Biosystem, Foster City, California, USA).

Deviation of the genotype counts from the Hardy–Weinberg equilibrium was tested using a χ^2 goodness-of-fit test. The pairwise linkage disequilibrium (LD) analysis was applied to detect the inter-marker relationship, using D'-value. The case–control association analysis was performed by SHEsis (Bio-X Life Science Research Center, Shanghai, China) a powerful software platform for analyses of LD, haplotype construction and genetic association at polymorphism loci [18]. Results were considered significant at two-tailed $P < 0.05$.

Results

Genotype frequencies of any of SNPs rs2391191, rs947267, rs778294 in case and control groups did not show significant deviations from Hardy–Weinberg equilibrium (data not shown). Neither rs2391191 nor rs778294 revealed significant allelic association in case–control samples. A significant difference in frequency of allele was, however, found in SNP rs947267 [A > C, $P = 0.006$; odds ratio (OR) = 1.43, 95% confidence interval (CI), 1.11–1.85] between schizophrenic patients and healthy controls (Table 1). With the intermarker LD calculation, the three SNPs were found to be in strong LD ($D' > 0.7$) with each other. Multilocus association analysis showed that haplotype A-A-G constructed by rs2391191, rs947267 and rs778294 was also associated with schizophrenia in the total group ($P = 0.003$, OR = 1.46, 95% CI, 1.14–1.87) (Table 2).

The results of comparison between early-onset schizophrenic (age at onset < 18 years) patients and group-matched controls (age < 18 years) also revealed a significant association of the A allele of SNP rs947267 ($P = 0.012$, OR = 1.67, 95% CI, 1.13–2.51) and haplotype A-A-G ($P = 0.008$, OR = 1.67, 95% CI, 1.13–2.29) with schizophrenia. Further analysis stratified by sex showed that the same allele ($P = 0.034$, OR = 1.45, 95% CI, 1.03–2.04) and haplotype ($P = 0.009$, OR = 1.54, 95% CI, 1.11–2.14) (Tables 1 and 2) as mentioned above were significantly associated with schizophrenia in male patients compared with male controls, but not in female patients (data not shown). Furthermore, these results, except two haplotypes, still remain significantly associated with schizophrenia, after using the permutation method to obtain empirical P values.

Discussion

In this study, we reported the association of an SNP (rs947267) and a haplotype located in the *G72/G30* gene with schizophrenia, age at onset and sex in a Chinese population. The results revealed that rs947267 and the haplotype constructed by three SNPs might increase the susceptibility of schizophrenia or might be linked with

Table 1 Allele frequencies of each SNP of *G72/G30* genes and the results of comparison between case and control groups

Group	SNP	Allele	No. patients (freq)[a]	No. controls (freq)[a]	χ^2	*P* value[b]	OR[c] (95% CI)
Total (no. patients: 216; no. controls: 321)	MI5	A	276 (0.64)	394 (0.61)	0.698	0.404	1.11 (0.87–1.42)
		G	156 (0.36)	248 (0.39)			
	MI8	A	299 (0.69)	392 (0.61)	7.483	**0.006**	1.43 (1.11–1.85)
		C	133 (0.31)	250 (0.39)			
	MI9	A	43 (0.10)	74 (0.12)	0.776	0.378	1.20 (0.79–1.82)
		G	389 (0.90)	560 (0.88)			
EOS (no. patients: 88; no. controls: 131)	MI5	A	113 (0.64)	160 (0.61)	0.441	0.507	1.14 (0.77–1.68)
		G	63 (0.36)	102 (0.39)			
	MI8	A	121 (0.69)	149 (0.57)	6.284	**0.012**	1.67 (1.13–2.51)
		C	55 (0.31)	113 (0.43)			
	MI9	A	18 (0.10)	33 (0.13)	0.574	0.449	1.26 (0.69–2.29)
		G	158 (0.90)	229 (0.88)			
Men (no. patients: 120; no. controls: 184)	MI5	A	154 (0.64)	224 (0.61)	0.671	0.413	1.15 (0.82–1.61)
		G	86 (0.36)	144 (0.39)			
	MI8	A	167 (0.70)	225 (0.61)	4.520	**0.034**	1.45 (1.03–2.04)
		C	73 (0.30)	143 (0.39)			
	MI9	A	22 (0.09)	40 (0.11)	0.460	0.498	1.21 (0.70–2.10)
		G	218 (0.91)	328 (0.89)			

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; EOS, early-onset schizophrenia; freq, frequency.
[a]The numbers of alleles (allele frequencies are shown in parentheses).
[b]The values in boldface type are nominally significant *P* value ($P < 0.05$).
[c]ORs of alleles were calculated for each reference vs. variant allele. MI5-rs2391191; MI8-rs947267; MI9-rs778294.

Table 2 Results of haplotype of *G72/G30* genes distribution and analysis in case and control groups

Group	Marker	Haplotype	Freq in case[a]	Freq in control[a]	*P* value[b]	Global *P* value[b] (d.f.)[c]	OR (95% CI)[d]
Total (no. patients: 216; no. controls: 321)	MI5-MI8	A-A	0.54	0.47	**0.039**	**0.028** (3)	1.25 (1.01–1.54)
	MI5-MI9	A-G	0.58	0.55	0.433	0.335 (3)	1.10 (0.87–1.40)
	MI8-MI9	A-G	0.67	0.61	**0.027**	**0.023** (3)	1.33 (1.03–1.71)
	MI5-MI8-MI9	A-A-G	0.50	0.45	**0.003**	**0.006** (7)	1.46 (1.14–1.87)
EOS (no. patients: 88; no. controls: 131)	MI5-MI8	A-A	0.56	0.45	**0.017**	**0.023** (3)	1.59 (1.09–2.33)
	MI5-MI9	A-G	0.57	0.55	0.673	0.281 (3)	1.09 (0.73–1.63)
	MI8-MI9	A-G	0.69	0.59	**0.031**	**0.040** (3)[e]	1.56 (1.04–2.34)
	MI5-MI8-MI9	A-A-G	0.53	0.40	**0.008**	**<0.001** (7)	1.67 (1.13–2.29)
Men (no. patients: 120; no. controls: 184)	MI5-MI8	A-A	0.55	0.45	**0.017**	**0.024** (3)	1.45 (1.04–2.02)
	MI5-MI9	A-G	0.59	0.56	0.543	0.281 (3)	1.11 (0.79–1.55)
	MI8-MI9	A-G	0.67	0.58	**0.039**	**0.042** (3)[e]	1.42 (1.02–1.98)
	MI5-MI8-MI9	A-A-G	0.53	0.42	**0.009**	**<0.001** (7)	1.54 (1.11–2.14)

Data of other rare haplotypes (< 1%) in both case and control groups are not presented.
OR, odds ratio; CI, confidence interval; EOS, early-onset schizophrenia; freq, frequency.
[a]Haplotype frequencies are shown in cases and controls, respectively.
[b]Global haplotype represents the haplotype using all possible variants. The value in boldface type is a nominally significant *P* value ($P < 0.05$).
[c]The d.f. values are shown in parentheses.
[d]ORs of haplotypes were calculated for each haplotype vs. all others.
[e]These permutation *P* values > 0.05.

the disease locus along with other genetic markers. The negative association results of rs2391191 and rs778294, which were in strong LD with rs947267, may be due to their minor effects compared with the whole effects of haplotypes. The three SNPs in our study, rs2391191, rs947267 and rs778294, were all tested in Chumakov's study (named M15, M18, M19, respectively), in which none of the markers was associated with schizophrenia in either French-Canadian or Russian populations [4]. Subsequently, several replication studies have been inconsistent with regard to the association of the above SNPs with schizophrenia or bipolar disorder [5–8,10,12–16]. In a meta-analysis study, Detera-Wadleigh and McMahon [11] reported that rs2391191 was associated with schizophrenia, but not with bipolar disorder, and neither rs947267 nor rs778294 was associated with schizophrenia or bipolar disorder. In a recent report, the SNP rs947267 has been found to be associated with schizophrenia in male patients [19].

There might be several explanations about the inconsistent association findings. First, the pathogenic mutations of *G72/G30* are not exactly the same across populations. For example, in both European (CEU) and Chinese (CHB) populations, rs2391191, which gives rise to a missense mutation, and the frequencies of major allele is the allele G in European populations (0.658 in CEU, HapMap), in Chinese populations it is the allele A (0.522, CHB, HapMap). Furthermore, Detera-Wadleigh and McMahon [11] reported the difference in LD among polymorphisms between different continental populations. Second, there is a positive association between the above SNPs and schizophrenia in some degree, owing to potential LD between the above polymorphisms and any other functional variants located in or adjacent to the *G72/G30* gene. Third, phenotypic heterogeneities, such as sex, age at onset and other clinical markers, may influence the genetic loading of participants' vulnerability to schizophrenia [20]. Stratification by age of onset and sex may serve to identify a more homogeneous patient group, less confounded by potential secondary effects of illness and with a more salient genetic risk [21].

Several implications exist for our findings that *G72/G30* polymorphisms are associated with schizophrenia in early-onset and male patients. The neurodevelopmental hypothesis of schizophrenia is based, at least in part, on the presence of premorbid impairments seen in children and adolescents who later develop schizophrenia. Aberrant neurodevelopment may be even more relevant for schizophrenia with early-onset schizophrenic [22]. Epidemiological surveys of schizophrenia have demonstrated that the two genders differ in age at onset, course of illness, clinical symptoms and outcome of disease. Many factors may account for this sex difference. Besides the sex steroid hormones, genetic factors might also be implicated. An apparent excess of sex chromosome aneuploidies (XXY and XXX) has been reported in populations of patients with schizophrenia, and schizophrenic sib-pairs are more often of the same sex than of the opposite sex [23]. Furthermore, there might be an interaction between the gene and sex on the pathogenesis of schizophrenia. The biological function of the above SNPs of *G72/G30* still remains unknown and probing into the potential function of polymorphisms might be helpful in exploring their effects on the development of schizophrenia.

Summary
Our result demonstrated a significant association between *G72/G30* polymorphisms and schizophrenia, so did in the early-onset and male patients with schizophrenia. Although more work is needed to explain the differences in alleles and haplotypes across studies, the overall evidence strongly supports association of the *G72/G30* locus with schizophrenia. Identification of functional variants will probably require biological as well as additional genetic assays.

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