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ORIGINAL ARTICLE

Further evidence for the association between G72/G30 genes and schizophrenia in two ethnically distinct populations

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Recently, the nested genes *G72* and *G30* on chromosome 13q32–q33 have been implicated in the etiology of schizophrenia. We genotyped six single-nucleotide polymorphisms (SNPs: rs3916965, rs3916967, rs2391191, rs778294, rs779293 and rs3918342), which span approximately 82.5 kb in the region encompassing the *G72/G30* genes in 1176 Han Chinese subjects (588 cases and 588 controls) and 365 Scottish subjects (183 cases and 182 controls). Significant association between an allele of marker rs778293 and schizophrenia was found in our Chinese samples ($P = 0.0013$), and was replicated in the Scottish samples ($P = 0.022$). LD analysis revealed that four SNPs between rs3916965 and rs778294 were in LD, called block I, and the two distal SNPs (rs778293 and rs3918342) constituted a block II in both the Chinese and Scottish samples. We selected one SNP from each block (rs778294 from block I and rs778293 from block II), and then analyzed the haplotypes. A significant difference was observed for the common haplotype *GC* in the Chinese sample ($P = 0.0145$), and was replicated in the Scottish sample ($P = 0.003$). On meta-analysis, we separately analyzed the studies in Asian and European populations because of significant heterogeneity in the homogeneity test. We found a statistically significant association between rs778293 and schizophrenia in Asian populations, but no difference was found between cases and controls in the European populations. Overall, our data give further support to the existing evidence that *G72/G30* genes are involved in conferring susceptibility to schizophrenia.
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Introduction

Schizophrenia (MIM 181500) is a common mental disease that affects approximately 1% of the world's population and has a devastating effect on patients' lives.[1] Understanding the etiology and pathogenesis of schizophrenia is one of the most important challenges facing psychiatry.

The currently prevailing view is that schizophrenia is a neurodevelopmental disorder leading to abnormalities of synaptic connectivity; individual differences in liability are largely genetic, with heritability estimates of around 80%.[2] However, identification of specific susceptibility genes for schizophrenia has been difficult, probably owing in large part to genetic heterogeneity and multigenic inheritance of genes of small effect.[3]

Evidence of linkage of schizophrenia to chromosome 13q22–q34 has been demonstrated in multiple studies.[4–8] Recently, two overlapping genes, *G72* and *G30*, which are transcribed in the brain, spanning a 65 kb segment from chromosome 13q34 were shown to be significantly associated with schizophrenia by using several individual single-nucleotide polymorphisms (SNPs) and haplotypes at the *G72/G30* locus in a case–control study involving a French–Canadian samples, and a Russian cohort of samples.[9] Interestingly, *G72* protein interacts with the gene for D-amino acid oxidase (DAAO) on 12q24 to regulate glutaminergic signaling through the *N*-methyl-D-aspartate (NMDA) receptor pathway.[10] Consistent

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associations have been reported between *G72/G30* and schizophrenia by subsequent studies.[11–15] However, Mulle *et al*.[16] recently reported a negative association for this finding in a family-based study. Furthermore, association of the *G72/G30* gene locus with bipolar disorder has also been reported.[11,17,18]

To further investigate the role of the *G72/G30* gene locus in schizophrenia susceptibility, we studied several SNPs and haplotypes in the region of the *G72/G30* gene using Chinese and Scottish case–control samples. In addition, we performed a meta-analysis of all published previous studies to deal with the ambiguities raised by inconsistent results among molecular genetic studies.

Materials and methods

Subjects

The study was approved by the local psychiatry research ethics committees, and informed consent was obtained from all subjects.

Chinese samples. A total of 1176 subjects participated, including 588 schizophrenics (318 males, mean age = 32.7 ± 19.7 years, age at onset = 25.6 ± 13.6 years; 270 females, mean age = 31.0 ± 15.4 years, age at onset = 27.2 ± 16.7 years) and 588 normal controls (189 males, mean age = 37.1 ± 21.7 years; 399 females, mean age = 39.9 ± 19.4 years). All patients were diagnosed by the Anhui Mental Health Center according to DSM-III-R criteria for schizophrenia using a combination of examination of psychiatric case records and clinical interview, using the Chinese version of the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L). The diagnosis was checked and verified by two independent senior psychiatrists who reviewed the psychiatric case records. The controls were drawn from a combination of local volunteers and blood transfusion donors. None had a history of neurological or major mental illness. All subjects were *Han* Chinese in origin.

Scottish samples. In all, 183 cases (131 males, mean age = 30.9 ± 19.0 years, age at onset = 26.9 ± 13.7 years; 52 females, mean age = 28.8 ± 19.4 years, age at onset = 23.5 ± 14.5 years) and 182 controls (106 males, mean age = 39.4 ± 19.6 years; 76 female, mean age = 36.8 ± 21.5 years) were recruited for the study in Scotland.[19] All patients fulfilled a DSM-III-R diagnosis of chronic schizophrenia on the basis of examination of psychiatric case notes and clinical interview using the SADS-L. The diagnosis was checked and verified by two independent senior psychiatrists who reviewed the psychiatric case records. Controls were recruited from a large general practice in Scotland, the blood transfusion service and from local volunteers with no history of schizophrenia or other major psychiatric disorders. All subjects were *Scottish* in origin.

Genotyping

We selected five SNPs (rs3916965, rs3916967, rs2391191, rs778293 and rs3918342) around the *G72/G30* gene locus from a list showing a significant allelic association with schizophrenia in the French–Canadian population,[9] and one SNP (rs778294) situated in the *G30* exon1 between rs2391191 and rs778293.

Genotyping was accomplished by allele-specific PCR, in which primers were designed to specifically amplify the reference allele or its variant in separate PCR reactions.[20] The assay used in this study combines kinetic (real-time quantitative) PCR with allele-specific amplification, which is described elsewhere.[21] PCR primers used in this study were designed by a tetra-primer ARMS-PCR primer design program.[22] The primers sequences are listed in Table 1. In real-time PCR, two PCR reactions were performed for each sample, containing 10 ng genomic

Table 1 Markers and primers used for allele-specific PCR

Marker[a]	*Physical location*[b]	*Primer sequence*		*Annealing temperature (°C)*
		Forward[c]	*Reverse*	
rs3916965 (M12)	104 901 361	5′-GCTTGTAGGATTACTCATTTACA/G	5′-AATACAGGGAAAAAAGTGATGACA	58
rs3916967 (M14)	104 915 349	5′-GGGTCCCTGGCTAATCTTTCAACTA/G	5′-GTTATTCTTCTCTCCTCATATTCAA	58
rs2391191 (M15)	104 917 447	5′-CTACTTCATAGGTTTTCCAAA/G	5′-AGATAAAGAGTAACATACCAATAGA	56
rs778294 (M19)	104 940 236	5′-TTCTTCAAGCCTGTAAGGAGATTCA/G	5′-ACACTTGACTCCGGTGATGAGGTTA	56
rs778293 (M22)	104 967 200	5′-AAAATTCAGCTTTAAAATCACTCT/C	5′-TAGGATGTCAGACTTTATTCTAA	58
rs3918342 (M23)	104 983 750	5′-CATTCACTATCTTAGCATGACCCA/G	5′-GGATATAGGATACTAAAATCTGAG	58

[a]Markers rs3916965, rs3916967, rs2391191, rs778294, rs778293 and rs3918342 (rs3916965: located in 15.2 kb upstream of *G72* exon 1; rs3916967: located in 1.2 kb upstream of *G72* exon 1; rs2391191: located in *G72* exon2; rs778294: located 15 bp upstream of the last *G72* exon; rs778293: located 25.8 kb downstream of the last *G72* exon; rs3918342: located 42.4 kb downstream of the last *G72* exon) correspond to M-12, M-14, M-15, M-19, M-22 and M23, respectively, of Chumakov *et al*.[9]
[b]UCSC Browser, July 2005; http://genome,ucsc.edu/cgi-bin/hgGateway.
[c]An additional mismatch was deliberately put at position −3 from the 3′ terminus of the allele-specific primer to confer the specificity of PCR amplification.

DNA, 2.5 μl TaqMan® universal PCR master mix (Applied Biosystems), 0.2 μM allele-specific primer, 0.2 μM common primer and 0.2 × SYBR® Green I (Molecular Probe, Inc.) in a total volume of 5 μl. To reduce well-to-well variability in PCR reaction conditions, an automated dispenser (Hydra® microdispenser, Robbins Scientific) and digital multichannel pipettes (Thermo Labsystems) were used. Kinetic PCR reactions were performed on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems). After an initial 2-min incubation step at 50°C, and an enzyme heat activation step of 12 min at 95°C, 55 cycles consisting of 15 s at 95°C and 40 s at annealing temperature were performed, followed by a final stage of dissociation to check the PCR product. Allele calling was manually performed.

To ensure that the obtained genotypes were valid, regenotyping was performed on 48 random DNA samples for each of the six SNPs. All genotypes were in agreement with the first round of genotyping. In addition, the same DNA samples were subjected to direct sequencing for the two SNPs rs3916967 and rs778294. No genotyping errors were disclosed.

Statistical analysis

Markers were tested for deviation from Hardy–Weinberg equilibrium in all samples using the Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). For the case–control analysis, χ^2 tests were performed to compare overall allele frequency of the polymorphic marker distributions between patients and controls using the CLUMP program,[23] which is useful for any 2*N contingency table, especially where N is large and the table is sparse. The significance was established using Monte Carlo simulations. In this study, the normal χ^2 (T1) was used to generate an empirical P-value using 10 000 simulations. Values for D', the normalized linkage disequilibrium statistic, were calculated using 2LD software.[24] Haplotype frequencies were estimated using the program PHASE vision 2.2,[25] a software that implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. The distribution of global haplotype frequencies in patients and controls was compared using the 2002 version of Epi_Info (http://www.cdc.gov/epiinfo/). Bonferroni corrections were applied for all multiple statistical tests.

Meta-analysis

The studies included in the meta-analysis were identified using Medline with the search terms 'G72' and 'Schizophrenia'. All the data analyzed had been previously published. Association studies were analyzed by random-effects meta-analysis. The significance of the pooled odds ratio (OR) was determined by Z test, and the heterogeneity of the group of ORs was assessed using a χ^2 test of goodness of fit. All statistical analyses were performed using the program STATA vision 8.2 (http://www.stata.com).

For family-based association studies, we calculated a transmission ratio of the risk allele from heterozygous parents in the trio family samples from each transmission/disequilibrium test (TDT), which was equivalent to an OR calculated from the McNemar test. The χ^2 statistic was used to calculate P-values for the TDT tests.[26]

Results

All the SNPs were highly polymorphic in both samples. The comparison analyses between the two populations of 588 Chinese controls and 182 Scottish controls demonstrate strong significant differences in allele frequency for SNPs rs3916965 ($\chi^2 = 77.78$, $P < 0.0001$), rs3916967 ($\chi^2 = 69.64$, $P < 0.0001$), rs2391191 ($\chi^2 = 63.12$, $P < 0.0001$) and rs778294 ($\chi^2 = 67.13$, $P < 0.0001$), except for SNPs rs778293 and rs3918342. A departure from Hardy–Weinberg equilibrium (HWE) was tested separately in case and control samples and the genotype distributions of all six SNPs in both cases and controls were in HWE in the two populations.

Allele frequency

Allele frequency of the SNPs located in the *G72/G30* region in the two populations is shown in Table 2.

(1) Chinese samples: Between the 588 case and 588 control individuals, we observed statistically significant differences in allele distributions on SNP marker rs778293 ($\chi^2 = 10.43$, $P = 0.0013$; OR = 0.76, 95% CI = 0.64–0.90). Furthermore, rs778293 still showed significant difference after Bonferroni correction ($P = 0.0078$).

(2) Scottish samples: Significant differences in allele frequency between the 183 case and 182 control groups were observed in three of the SNPs: rs778294 ($\chi^2 = 5.04$, $P = 0.025$; OR = 0.69, 95% CI = 0.50–0.97), rs778293 ($\chi^2 = 5.21$, $P = 0.022$; OR = 0.71, 95% CI = 0.52–0.96) and rs3918342 ($\chi^2 = 12.11$, $P = 0.0005$; OR = 0.60, 95% CI = 0.44–0.81). After Bonferroni correction, rs3918342 still showed significant difference ($P = 0.003$).

LD analysis

Table 3 presents the results of LD tests between pairs of SNP markers for the respective control groups. The LD analysis revealed that four SNPs between rs3916965 and rs778294 were in LD block I ($D' > 0.3$), and that the two distal SNPs (rs778293 and rs3918342) constituted block II ($D' > 0.3$) in both Chinese and Scottish samples. Furthermore, the three SNPs (rs3916965, rs3916967 and rs2391191) could also constitute block I-1 for a strict standard ($D' > 0.9$) in the Chinese sample.

Haplotype analysis

As the members of block I are in significant LD with each other, this may have the effect of artificially magnifying the significance level. We only performed haplotype analysis for block II. Furthermore, we

Table 2 SNP allele frequencies (%) in the case and control samples

Marker position[a]		*Allele*	*Anhui sample*		P-*value*[b]	*Odds ratio (95% CI)*	*Scotland sample*		P-*value*[b]	*Odds ratio (95% CI)*
SNP	*kb*		*Cases*[c]	*Controls*[c]			*Cases*[c]	*Controls*[c]		
rs3916965	0.0	A	58.8 (684)	59.1 (695)	0.869	0.99 (0.83–1.17)	36.9 (135)	32.7 (119)	0.224	1.20 (0.88–1.65)
		G	41.2 (480)	40.9 (481)			63.1 (231)	67.3 (245)		
rs3916967	14.0	A	38.5 (453)	39.6 (463)	0.578	0.95 (0.81–1.13)	60.4 (221)	64.6 (234)	0.235	0.83 (0.61–1.14)
		G	61.5 (723)	60.4 (705)			39.6 (145)	35.4 (128)		
rs2391191	16.1	A	58.5 (679)	58.1 (674)	0.833	1.02 (0.86–1.20)	36.3 (133)	34.2 (123)	0.540	1.10 (0.80–1.51)
		G	41.5 (481)	41.9 (486)			63.7 (233)	65.8 (237)		
rs778294	38.9	T	14.2 (167)	13.9 (163)	0.812	1.03 (0.81–1.31)	25.4 (93)	33.0 (120)	**0.025**	0.69 (0.50–0.97)
		C	85.8 (1005)	86.1 (1009)			74.6 (273)	67.0 (244)		
rs778293	65.8	A	58.9 (686)	65.4 (759)	**0.0013**	0.76 (0.64–0.90)	52.5 (191)	60.9 (218)	**0.022**	0.71 (0.52–0.96)
		G	41.1 (478)	34.6 (401)			47.5 (173)	39.1 (140)		
rs3918342	82.4	T	45.6 (534)	48.4 (567)	0.172	0.89 (0.76–1.05)	40.7 (149)	53.6 (195)	**0.0005**	0.60 (0.44–0.81)
		C	54.4 (638)	51.6 (605)			59.3 (217)	46.4 (169)		

[a]Position of SNPs are shown as distances from rs3916965, where *G72* and *G30* are located in the range of 14.9–40.0 kb and 8.0–-54.7 kb.
[b]Significant *P*-value (<0.05) are in bold face.
[c]Number of alleles for each SNP is given in parentheses.

Table 3 Pairwise LD results

	Anhui sample and Scotland sample[a,b]					
	rs3916965	*rs3916967*	*rs2391191*	*rs778294*	*rs778293*	*rs3918342*
rs3916965	—	**0.99**	**0.93**	**0.39**	0.23	0.25
rs3916967	**0.95**	—	**0.97**	**0.37**	0.23	0.27
rs2391191	**0.87**	**0.93**	—	**0.36**	0.24	0.23
rs778294	**0.92**	**0.93**	**0.96**	—	0.09	0.08
rs778293	0.13	0.11	0.07	0.16	—	**0.33**
rs3918342	0.12	0.05	0.06	0.05	**0.70**	—

[a]For each pair of SNPs, the standardized D' is shown. D'-value >0.3 are in bold face.
[b]D'-value above the subtraction sign corresponds to the Anhui sample, and below the subtraction sign to the Scotland sample.

selected one SNP from each block (rs778294 from block I and rs778293 from block II) to construct haplotypes. The results of haplotype frequency estimation and the comparison of those frequencies between case and control subjects are summarized in Table 4 for Chinese samples and in Table 5 for Scottish samples.

Chinese samples. We analyzed the frequency of the haplotypes consisting of the two SNPs rs778293 and rs3918342 and found that they were associated with schizophrenia (global $P=0.006$). When haplotypes were compared individually between cases and controls, a strongly significant difference was found for haplotype *AT* ($\chi^2=8.46$, $P=0.0036$; OR = 0.78, 95% CI = 0.65–0.92), which showed lower occurrence in cases than in controls (31.5 vs 37.1%), and a weaker significant difference was found for haplotype *GT* ($\chi^2=4.44$, $P=0.035$; OR = 1.30, 95% CI = 1.01–1.67), which showed higher occurrence in cases than in controls (14.1 vs 11.2%). After rigorous correction for multiple testing, the differences observed for haplotype *AT* ($P=0.036$) remain significant.

The results of haplotype frequency estimation for the SNPs rs778294–rs778293 showed a significant association with the disease (global $P=0.011$). In our study, significant differences were found for the haplotype *GC* ($\chi^2=5.98$, $P=0.0145$; OR = 1.24, 95% CI = 1.04–1.49), which was more prevalent in cases than in controls (33.7 vs 29.0%), and for the haplotype *GT* ($\chi^2=6.17$, $P=0.013$; OR = 0.81, 95% CI = 0.69−0.96), which was less prevalent in cases than in controls (52.0 vs 57.1%).

Scottish samples. We tested the two-locus haplotype rs778293–rs3918342 and found a significant difference in the distribution of the global haplotypes between case and control individuals (global $P=0.0003$). In particular, a strongly significant difference was found for haplotype *AT*

Table 4 Estimated haplotype frequencies (%) and association significance of the Anhui sample

Haplotype[a]	*Anhui sample*		χ^2	P-*value*[b]	*OR (95% CI)*
	Cases	*Controls*			
rs778293–rs3918342			12.41	**0.006**	
AT	31.472 (370)	37.139 (437)	8.46	**0.0036**	0.78 (0.65–0.92)
GC	26.906 (316)	23.353 (275)	3.80	0.051	1.20 (0.99–1.46)
AC	27.520 (324)	28.299 (333)	0.17	0.680	0.96 (0.80–1.16)
GT	14.102 (166)	11.209 (132)	4.44	**0.035**	1.30 (1.01–1.67)
rs778294–rs778293			11.15	**0.011**	
GC	33.712 (396)	28.970 (341)	5.98	**0.0145**	1.24 (1.04–1.49)
GT	52.032 (612)	57.119 (672)	6.17	**0.013**	0.81 (0.69–0.96)
AC	7.345 (86)	5.596 (66)	2.81	0.094	1.33 (0.94–1.87)
AT	6.911 (81)	8.315 (98)	1.75	0.186	0.81 (0.59–1.12)

[a]Number for each haplotype is given in parentheses.
[b]Significant *P*-values (<0.05) are in boldface.

Table 5 Estimated haplotype frequencies (%) and association significance of the Scotland sample

Haplotype[a]	*Scotland sample*		χ^2	P-*value*[b]	*OR (95% CI)*
	Cases	*Controls*			
rs778293–rs3918342			18.72	**0.0003**	
AT	31.910 (117)	47.310 (172)	17.80	**0.00002**	0.52 (0.38–0.72)
GC	38.824 (142)	32.394 (118)	3.24	0.072	1.32 (0.96–1.81)
AC	20.465 (75)	14.035 (51)	5.36	**0.021**	1.58 (1.05–2.38)
GT	8.801 (32)	6.261 (23)	1.54	0.215	1.42 (0.79–2.57)
rs778294–rs778293			11.88	**0.0078**	
GC	32.665 (120)	23.085 (84)	8.53	**0.003**	1.63 (1.16–2.29)
GT	41.925 (153)	43.948 (160)	0.34	0.557	0.92 (0.68–1.24)
AC	14.842 (54)	16.061 (58)	0.20	0.658	0.91 (0.60–1.39)
AT	10.568 (39)	16.906 (62)	6.22	**0.013**	0.58 (0.37–0.91)

[a]Number for each haplotype is given in parentheses.
[b]Significant *P*-values (<0.05) are in boldface.

($\chi^2=17.80$, $P=0.00002$; OR = 0.52, 95% CI = 0.38–0.72), which was less frequent in cases than in controls (31.9 vs 47.3%), and a weaker significant difference was found for haplotype *AC* ($\chi^2=5.36$, $P=0.021$; OR = 1.58, 95% CI = 1.05–2.38), which was more frequent in cases than in controls (20.5 vs 14.0%). After correction for multiple testing, the differences observed for haplotype *AT* ($P=0.0002$) remain significant.

Analysis of the frequency of haplotypes consisting of the SNPs rs778294–rs778293 in cases and controls showed that the frequencies were significantly different (global $P=0.0078$). When the haplotypes were compared individually between cases and controls, a strongly significant difference was found for the haplotype *GC* ($\chi^2=8.53$, $P=0.003$; OR = 1.63, 95% CI = 1.16–2.29), which was more prevalent in cases than in controls (32.7 vs 23.1%), and a weak significant difference was found for the haplotype *AT* ($\chi^2=6.22$, $P=0.013$; OR = 0.58, 95% CI = 0.37–0.91), which was less prevalent in cases than in controls (10.6 vs 16.9%). After rigorous correction for multiple testing, the differences observed for the haplotype *GC* ($P=0.03$) remain significant.

Meta-analysis

Case–control studies. We found five independent samples, from four different studies that looked for an association between *G72/G30* genes and schizophrenia.[9,11–13] When our study was included, the number of independent samples was seven. This allowed us to separately examine 2063 schizophrenic patients and 2162 controls for SNPs rs3916965 and rs3918342. We also calculated the pooled OR for the SNP marker rs778293 (1764 cases and 1862 controls), as it showed significant differences in both of our sets

of samples. The ORs and 95% CIs for the case–control studies are shown in Table 6.

When all of the studies were taken into account, we found significant heterogeneity in the homogeneity analysis for these SNPs: rs3916965 ($\chi^2 = 16.39$, df = 6, $P = 0.01$), rs778293 ($\chi^2 = 26.07$, df = 5, $P < 0.01$) and rs3918342 ($\chi^2 = 45.80$, df = 6, $P < 0.01$). Thus, we separately analyzed the studies in Asian and European populations to limit ethnic heterogeneity.

In the Asian population, significant difference was found between patients and controls for SNP rs778293 (pooled $OR_{Asian} = 0.81$, 95% CI = 0.72–0.92, $Z = -3.33$, $P = 0.001$), but not for rs3916965 (pooled $OR_{Asian} = 1.10$, 95% CI = 0.97–1.24, $Z = 1.52$, $P = 0.128$) and rs3918342 (pooled $OR_{Asian} = 1.0$, 95% CI = 0.80−1.27, $Z = 0.05$, $P = 0.963$). There was no heterogeneity for the two SNPs (rs3916965: $\chi^2 = 3.36$, df = 1, $P = 0.07$; rs778293: $\chi^2 = 1.42$, df = 1, $P = 0.23$) within the Asian pooled studies, but a trend for heterogeneity was detected for marker rs3918342 ($\chi^2 = 4.01$, df = 1, $P = 0.05$).

In the European populations, the meta-analysis found no difference between cases and controls (rs3916965: pooled $OR_{European} = 1.02$, 95% CI = 0.81–1.29, $Z = 0.15$, $P = 0.885$; rs778293: pooled $OR_{European} = 1.13$, 95% CI = 0.79−1.62, $Z = 0.68$, $P = 0.494$; rs3918342: pooled $OR_{European} = 1.12$, 95% CI = 0.74–1.71, $Z = 0.54$, $P = 0.587$), however, we still found significant heterogeneity for every marker (rs3916965: $\chi^2 = 12.43$, df = 4, $P = 0.01$; rs778293: $\chi^2 = 15.12$, df = 3, $P < 0.01$; rs3918342: $\chi^2 = 41.41$, df = 4, $P < 0.01$).

Family-based studies. In three family-based association studies with a total of 480 family trios, we performed meta-analysis for SNPs rs3916967 and rs2391191, separately.[14–16] The study of Korostishevsky *et al.* found significant evidence for the association of schizophrenia with *G72/G30* genes

Table 6 Meta-analysis of case–control association studies between *G72/G30* and schizophrenia

Case–control studies	*Number of*		*Ethnicity*	*rs3916965*		*rs778293*		*rs3918342*	
	Cases	*Controls*		*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*	*Odds ratio*	*95% CI*
Chumakov *et al.*[9]	213	241	European	0.68	0.52–0.89	1.49	1.13–1.96	1.38	1.07–1.80
	183	183	European	1.08	0.81–1.45	1.08	0.80–1.46	1.44	1.08–1.93
Schumacher *et al.*[11]	299	300	European	1.23	0.98–1.56			0.76	0.60–0.95
Wang *et al.*[12]	537	538	Asian	1.23	1.04–1.46	0.88	0.74–1.05	1.14	0.96–1.34
Korostishevsky *et al.*[13]	60	130	European	0.98	0.63–1.53	1.51	0.97–2.35	2.17	1.39–3.38
Current study	588	588	Asian	0.99	0.84–1.16	0.76	0.64–0.90	0.89	0.76–1.05
	183	182	European	1.20	0.89–1.63	0.71	0.53–0.95	0.60	0.44–0.80
Pooled	2063	2162	All	1.05	0.90–1.22	1.00	0.79–1.26	1.07	0.84–1.38
Significance				$Z = 0.60$, $P = 0.547$		$Z = -0.04$, $P = 0.967$		$Z = 0.56$, $P = 0.574$	
Heterogeneity				$\chi^2 = 16.39$, $P = 0.01$		$\chi^2 = 26.07$, $P < 0.01$		$\chi^2 = 45.80$, $P < 0.01$	
$Pooled_{Asian}$	1125	1126	Asian	1.10	0.97–1.24	0.81	0.72–0.92	1.0	0.80–1.27
Significance				$Z = 1.52$, $P = 0.128$		$Z = -3.33$, $P = 0.001$*		$Z = 0.05$, $P = 0.963$	
Heterogeneity				$\chi^2 = 3.36$, $P = 0.07$		$\chi^2 = 1.42$, $P = 0.23$		$\chi^2 = 4.01$, $P = 0.05$	
$Pooled_{European}$	938	1036	European	1.02	0.81–1.29	1.13	0.79–1.62	1.12	0.74–1.71
Significance				$Z = 0.15$, $P = 0.885$		$Z = 0.68$, $P = 0.494$		$Z = 0.54$, $P = 0.587$	
Heterogeneity				$\chi^2 = 12.43$, $P = 0.01$		$\chi^2 = 15.12$, $P < 0.01$		$\chi^2 = 41.41$, $P < 0.01$	

*$P < 0.05$.

Table 7 Meta-analysis of 3 family-based association studies between *G72/G30* and schizophrenia

Family-based studies[a]	*Number of families*	*Ethnicity*	*rs3916967 (A)*		*Transmission Ratio*	P-*value*	*rs2391191 (G)*		*Transmission ratio*	P-*value*
			T	*NT*			*T*	*NT*		
Addington *et al.*[14]	88	Mixed[b]	37	19	1.95	0.016	36	20	1.80	0.033
Zou *et al.*[15]	233	Asian	96	113	0.85	0.24	92	130	0.71	0.011
Mulle *et al.*[16]	159	Mixed[c]	62	66	0.94	0.724	63	69	0.91	0.60
Pooled	480		195	198	0.98[d]	0.88	191	219	0.87[e]	0.17

[a]T: transmitted; NT: not transmitted.
[b]Consisted of ∼50% Caucasians, ∼25% African Americans, and a mix of Hispanics, Asians, and Indians.
[c]Consisted of 84% Caucasians, 9% African Americans, and a mix of different races.
[d]Pooled $OR_{TDT} = 0.98$, TDT $\chi^2 = 0.02$, $P = 0.88$.
[e]Pooled $OR_{TDT} = 0.87$, TDT $\chi^2 = 1.91$, $P = 0.17$.

in Palestinian Arabs.[27] As they failed to provide enough information to calculate an effect of size, this study has not been included. The results of meta-analysis are shown in Table 7.

We found no statistically significant evidence for overtransmission from parents to their schizophrenic offspring (rs3916967: pooled $OR_{TDT} = 0.98$, $P = 0.88$; rs2391191: pooled $OR_{TDT} = 0.87$, $P = 0.17$).

Discussion

The purpose of the present study was to investigate the relationship between the *G72/G30* locus and schizophrenia. Six SNPs spanning approximately 82.5 kb of the chromosome 13q32–q33 encompassing the *G72/G30* genes were genotyped in two ethnically distinct case–control samples.

Significant association between alleles of marker rs778293 and schizophrenia was found in our Chinese samples ($P = 0.0013$), and replicated in the ethnically distinct Scottish samples ($P = 0.022$). The SNP rs778293 situated 25.8 kb downstream of the last *G72* exon and 11.1 kb upstream of *G30* exon 1 has been reported as being the most closely associated marker ($P = 0.003$) by Chumakov *et al*.[9] A comparison of our results with the study of Chumakov *et al*. shows that all six SNPs were analyzed in both the studies. Of these, rs3916965, rs3916967 and rs2391191 showed significant associations in the single-marker analysis in the Chumakov study ($P = 0.007$, $P = 0.038$ and $P = 0.032$, respectively), but not in our own study. On the other hand, rs778294 was significant in our Scottish samples ($P = 0.025$), but not in their study. Furthermore, we found significant difference for SNP rs3918342 in our Scottish population ($P = 0.0005$), but not in our Chinese samples.

There is a reasonable explanation for these findings: Schizophrenia is heterogeneous, with the markers in different ethnic groups having different informational content. The distribution of alleles in the Chinese controls for rs3916965, rs3916967, rs2391191 and rs778294 was significantly different from that observed in the Scottish controls. In addition, one should note that our Scottish sample, which was modest in size, would more easily have unstable allele frequencies than the Chinese sample in this study, and after Bonferroni correction, only one marker showed significant difference in each sample. On the other hand, the ORs of the SNPs, that is, rs778294, rs778293 and rs3918342 in the Scottish subjects were similar to that of rs778293 in the Chinese subjects. Therefore, the Scottish samples did not confer a bigger risk from *G72/G30* to schizophrenic etiology than the Chinese samples.

The LD blocks were designated as block I and block II in our two ethnically distinct samples. Similar LD blocks have been observed in other populations.[9,13,17,27] The results of haplotype frequency estimation for block II showed a significant association with the disease in the two ethnic populations. When each haplotype was individually compared in cases and controls, significant differences were found to be in the most common haplotype *AT* and for the smallest haplotype *GT* in the Chinese sample. Our positive findings have been replicated in ethnically distinct case–control samples from Scotland. In our Scottish samples, the significant differences were also found in the most common haplotype *AT* and for a small haplotype *AC*. In our samples, the haplotype *AT*, which was the main reason for the association of two-marker haplotypes with schizophrenia, was significantly more frequent in controls than in cases. This may be explained by a protective effect of the haplotype.[11]

Interestingly, the SNPs constituting block II were not located in the genes themselves, in the coding region or in the untranslated regions (UTRs), but rather in the vicinity of the genes.[13] These findings indicate that more than one susceptibility locus in this region cannot be ruled out. On the other hand, it is unlikely that another nearby gene is involved, because chromosome 13 shows striking features of low gene density compared to other autosomes, with an average of 6.5 genes per Mb.[28] A reasonable explanation for this situation might be that an apparently gene-less region might indeed contain hidden coding sequences, and small genes are easily overlooked.[13]

Furthermore, we selected one SNP from each block, and then analyzed the haplotypes. The results of haplotype frequency estimation for the SNPs rs778294–rs778293 showed a significant association with the disease. In our Chinese samples, significant differences were found with the haplotype *GC*, which was more prevalent in cases than in controls, and with the haplotype *GT*, which was less prevalent in cases than in controls. The positive findings were also replicated in the Scotland samples. A strongly significant difference was found with the haplotype *GC* which was more prevalent in cases than in controls and a weak significant difference was found with the haplotype *AT*, which was less prevalent in cases than in controls. Our analysis implies that the *GC* haplotype may be a risk haplotype and can be predictive of schizophrenia. In addition, one should pay attention that the SNP rs778294 (T to C) is located 15 bp upstream of the last *G72* exon, an area for an acceptor splicing site. The nucleic acid polymorphisms of rs778294 may have a functional effect, with T to C affecting the splicing of the *G72* transcript.[9] At the same time, rs778294 situated in the *G30* exon 1, is a nonsynonymous change, leading to a serine to glycin substitution. Unfortunately, we know little about *G30* for the moment, despite there being a significant association between rs778294 and schizophrenia in the Scottish sample.

To confirm or exclude the implication of *G72/G30* in the pathogenesis of schizophrenia, we performed a meta-analysis. There is statistical evidence for heterogeneity between studies because of ethnical differences between samples. When studies on Asian

subjects were separated from those on European subjects, we found a statistically significant association between rs778293 and schizophrenia in the Asian population, and a trend for heterogeneity was detected for marker rs3918342. The estimates of the combined OR of rs778293 ranged from 0.72 to 0.92, suggesting that our study affects the combined estimate, whereas the study of Wang *et al.*[12] also showed a trend of association ($P = 0.16$). In contrast, the findings from case–control studies of European samples did not detect a significant association of schizophrenia and *G72/G30* genes, but we still found significant heterogeneity for every marker. The heterogeneity among the studies suggests that some unknown factor may lead to the association of several SNPs with the disease in some contexts but not in others.[29] The effect of SNPs on schizophrenia risk may be moderated by sample or study characteristics not addressed in the present analyses. In three family-based association studies, we did not find significant evidence for association of the SNPs rs3916967 and rs2391191 with schizophrenia. Actually, the lack of association in the family-based analyses may be due to the relatively smaller sample size compared with the analyses involving unrelated controls.

There is no simple interpretation from our meta-analysis, but a possible conclusion may be that there is a small but significant effect of *G72/G30* in susceptibility to schizophrenia, at least in Asian population. As new studies emerge, the present findings can be updated, and, eventually, more reliable estimates of this association in both ethnic groups may be obtained.[30]

It is important to note that although we have not attempted to genotype a set of SNPs in and around the *G72/G30* locus, but we have selected a subset of SNPs. The justification for this subset may be weak and the SNPs cannot be considered representative. We have not found any genotyping error in this study; however, genotyping errors cannot always be fully ruled out because they are generally unobtrusive. Inconsistencies in genotype data can occur for many reasons, including imperfect genotyping technologies, manual mistakes, sample mix-up and pedigree errors.[31] For family-based data, genotyping errors can increase both type I and II errors. For case–control studies, genotyping errors can increase type II errors and thereby decrease power. Additionally, genotyping errors can bias LD measurements.[32]

Overall, our data provide further evidence for a positive association between the *G72/G30* locus and schizophrenia, and support six previous reports implicating *G72/G30* as a susceptibility gene for the disease possibly by the attenuation of NMDA receptor activity via the oxidation of D-serine by DAAO, at least in some patients.[9,11–15]

Further research is needed to determine the functional variation underlying these findings and to relate this to the pathophysiology of schizophrenia.

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