

Genetic Evidence for the Association between the Early Growth Response 3 (*EGR3*) Gene and Schizophrenia

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Abstract

Recently, two genome scan meta-analysis studies have found strong evidence for the association of loci on chromosome 8p with schizophrenia. The early growth response 3 (*EGR3*) gene located in chromosome 8p21.3 was also found to be involved in the etiology of schizophrenia. However, subsequent studies failed to replicate this finding. To investigate the genetic role of *EGR3* in Chinese patients, we genotyped four SNPs (average interval ~2.3 kb) in the chromosome region of *EGR3* in 470 Chinese schizophrenia patients and 480 healthy control subjects. The SNP rs35201266 (located in intron 1 of *EGR3*) showed significant differences between cases and controls in both genotype frequency distribution ($P=0.016$) and allele frequency distribution ($P=0.009$). Analysis of the haplotype rs35201266-rs3750192 provided significant evidence for association with schizophrenia ($P=0.0012$); a significant difference was found for the common haplotype AG ($P=0.0005$). Furthermore, significant associations were also found in several other two-, and three-SNP tests of haplotype analyses. The meta-analysis revealed a statistically significant association between rs35201266 and schizophrenia ($P=0.0001$). In summary, our study supports the association of *EGR3* with schizophrenia in our Han Chinese sample, and further functional exploration of the *EGR3* gene will contribute to the molecular basis for the complex network underlying schizophrenia pathogenesis.

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Introduction

Schizophrenia (OMIM 181500) is a severe mental disorder with a lifetime risk of about 1%, characterized by hallucinations, delusions and cognitive deficits. Family, twin and adoption studies have consistently demonstrated an important genetic component to schizophrenia (~80%), in addition to developmental and environmental influences [1,2].

Identification of susceptibility genes associated with schizophrenia has been difficult. This is presumably due to the etiological complexity and abnormalities in development of this psychiatric disease. In spite of these difficulties, chromosome region 8p has been shown to be the most well-established association with schizophrenia. Recently, two genome-scan meta-analysis studies for psychiatric diseases have found strong evidence for the association of 8p with schizophrenia [3,4]. In addition, a genome-scan study with a large sample of pedigrees has added further evidence for the hypothesis that at least one schizophrenia susceptibility gene may be located on chromosome 8p [5]. More recently, a number of liability genes located in chromosome 8p were found to be involved in the etiology of schizophrenia. *EGR3* is one of these compelling susceptibility genes that have been associated with schizophrenia in various ethnic populations [6,7,8,9,10,11].

Early growth response (*EGR*) genes (*EGR1*, *EGR2*, *EGR3*, and *EGR4*) are a family of immediate early gene transcription factors that

are important for neuronal responses [12]. All the family members share a highly conserved DNA-binding domain composed of three zinc-finger motifs. They play an important role in the mediation of gene transcription in neuronal development and are involved in the regulation of synaptic plasticity, learning and memory process [13,14]. *EGR1* and *EGR3* are the most abundant *EGR* proteins in the brain, and their expression is up-regulated by synaptic activity in the brain [15]. *EGR3* has an essential role in learning and memory processing of both short- and long-term hippocampus-dependent memory; it also mediates adaptation to stress and novelty [15,16]. Therefore, *EGR3* is a compelling candidate gene for schizophrenia from functional and positional perspective.

Expression of *EGR3* has been reported to be significantly lower in postmortem hippocampus' of schizophrenic patients compared to the control subjects [17]. Expression of *EGR3* was also reported to be down-regulated in the dorsolateral prefrontal cortex of patients with schizophrenia. Moreover, pedigree and case-control analyses in a study utilizing a Japanese sample found that *EGR3* was significantly associated with schizophrenia; this result was also replicated in a Korean population study [8,11]. However, subsequent studies utilizing a Japanese sample and a Chinese sample failed to replicate this finding [18,19].

As a result of the "winner's curse" phenomena, a seemingly high proportion of false positive reports caused by the overestimation of genetic effects were published. A stringent criterion for

interpreting association studies is that an association based on one study should be viewed as tentative until it has been independently replicated in at least one other study [20]. Hence, we performed a study to further explore the relationship between *EGR3* and schizophrenia in a case-control sample of Chinese.

Results

We genotyped four SNPs (average interval ~ 2.3 kb) in the chromosome region of *EGR3* in 470 Chinese schizophrenia patients and 480 control subjects (Figure 1). Hardy-Weinberg equilibrium (HWE) was tested separately in case and control samples. The genotype distribution of SNPs rs35201266, rs3750192, and rs1877670 were found to be in HWE ($P > 0.05$) both in controls and cases. The genotype distribution of rs1008949 was not in HWE in patients ($P < 0.05$). All SNPs were highly polymorphic in both groups.

Single-marker analysis

The genotype and allele frequency distribution of all the SNPs in schizophrenia patients and controls are shown in Table 1. SNP rs35201266 showed significant differences between cases and controls both in the genotype frequency distribution ($\chi^2 = 8.25$, $P = 0.016$) and the allele frequency distribution (the A-allele: $\chi^2 = 6.829$, $P = 0.009$, odds ratio (OR) = 1.37, 95% confidence intervals (CI) = 1.07–1.75). Moreover, in the genetic model analysis (Table 2), a significantly positive result was observed for the A-allele of rs35201266 in the additive model ($\chi^2 = 7.09$, $P = 0.0078$, OR = 1.46), and weakly positive results were found in the dominant model ($\chi^2 = 7.09$, $P = 0.0258$, OR = 1.36, 95% CI = 1.04–1.79) and the recessive model ($\chi^2 = 5.21$, $P = 0.0224$, OR = 2.69, 95% CI = 1.11–6.50). After Bonferroni correction, the difference observed for rs35201266 ($P = 0.036$) in the allele frequency distribution and the additive model ($P = 0.031$) remained significant.

Haplotype analysis

Figure 2 presents the results of Linkage disequilibrium (LD) tests between pairs of SNP markers for the respective control groups. Owing to the medium LD ($D' > 0.8$) between rs1008949 and rs35201266, we selected the rs35201266 as the relevant tagging SNP for haplotype analyses. Analysis of haplotype rs35201266-rs3750192 showed significant association with schizophrenia (global $P = 0.0012$). A significant difference was found for the common haplotype AG ($P = 0.0005$, OR = 1.77, 95% CI = 1.27–2.47) which was more prevalent in cases compared to controls (11.4% vs 6.8%). After correction for multiple testing (4 haplotypes and 4 allelic comparisons), the difference observed for the AG haplotype ($P = 0.004$) remained significant. In addition, several other two- and three-SNP tests of haplotype association were also significant (Table 3). Haplotypes containing the A allele of rs35201266, were higher in frequency in cases compared to controls.

Age at onset (AAO) analysis

Since the marker rs35201266 showed significant association with schizophrenia, and schizophrenia onset is quite concentrated for people between the ages of 15 and 45 [21], we stratified the patients into 4 subgroups by AAO to examine if there were any clinical differences between the patients carrying allelic variants of rs35201266 (Table 4). However, no significant differences were found between the risk allele (A) carriers and the G homozygotes of rs35201266 (Global $P = 0.2764$). When the allele frequencies were compared in different subgroups between the A carriers and the G homozygous carriers, no significant differences were observed ($P > 0.1$).

Gender analysis

Samples were stratified by gender according to the study of Zhang *et al.* [22] and marker rs35201266 was analyzed in patients. For the A carriers and the G homozygotes of rs35201266, there was no significant difference ($P = 0.0794$, OR = 0.71, 95% CI = 0.48–1.06) between males and females (Table 4).

Comparing our results with previous association studies

A comparison of our results with the previous genetic studies have been performed, and the A-allele of rs35201266 showed the positive results in Chinese ($P = 0.009$), Korean ($P = 0.0008$), and a part of Japanese population ($P = 0.0009$) (Table 5). We also found that the MAF of rs35201266 was lower in the Chinese population compared to other populations (Table 5).

When we compared our results with previous GWASs on schizophrenia (Table 6), the positive association between chromosome 8p and disease was identified at least in four different ethnic populations from three GWASs [23,24,25].

Meta-analysis

For the meta-analysis, we combined and analyzed data from six populations (including this study). We utilized the schizophrenia research forum (<http://www.szgene.org>) and identified five independent case-control studies that tested for associations between various SNPs of *EGR3* and schizophrenia [8,11,18,19,26]. The study of Mansour *et al.* [26] did not provide enough information to calculate an effect of size, and therefore, was excluded from further analysis. We combined SNP data from the remaining studies and separately examined three SNPs (rs1008949, rs35201266 and rs3750192) in the *EGR3* gene locus. Summary statistics of the meta-analysis for these three SNPs are shown in Figure 3.

We tested for heterogeneity for each SNP of the combined sample, and the results were not significant [rs1008949 ($\chi^2 = 5.26$, $df = 3$, $P = 0.15$), rs35201266 ($\chi^2 = 4.40$, $df = 2$, $P = 0.11$) and rs3750192 ($\chi^2 = 4.24$, $df = 3$, $P = 0.24$)], enabling us to test for association of each of the SNPs by using a fixed-effect meta-analysis. A significant difference was found between patients and controls for the A-allele of rs35201266 (Subtotal OR = 1.27, 95% CI = 1.12–1.43, $Z = 3.87$, $P = 0.0001$), but not for rs1008949

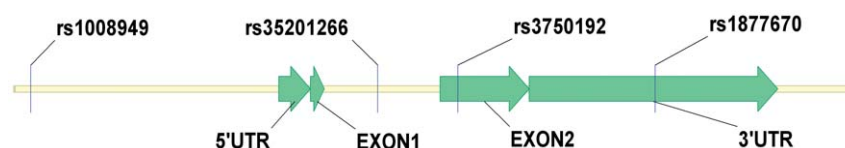


Figure 1. Organization and position of selected SNPs of *EGR3*.
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Table 1. Genotype and allele frequencies of the SNPs analyzed in cases and controls.

Marker	Genotype Distribution (%)					Allele Distribution (%)					
	Genotype	Case ^a	Control ^a	χ^2	<i>P</i> value ^b	Allele	Case ^a	Control ^a	χ^2	<i>P</i> value ^b	OR (95% CI)
rs1008949	CC	30.8(145)	32.3(155)	3.29	0.193	C	58.2(547)	57.1(548)	0.239	0.625	1.05 (0.87–1.26)
	CT	54.7(257)	49.6(238)			T	41.8(393)	42.9(412)			
	TT	14.5(68)	18.1(87)								
rs35201266	AA	3.8(18)	1.5(7)	8.25	0.016	A	20.1(189)	15.5(149)	6.829	0.009	1.37 (1.07–1.75)
	AG	32.6(153)	28.1(135)			G	79.9(751)	84.5(811)			
	GG	63.6(299)	74.4(338)								
rs3750192	GG	71.9(338)	66.3(318)	3.78	0.151	G	84.1(791)	81.3(780)	2.788	0.095	1.23 (0.96–1.57)
	GT	24.5(115)	30.0(144)			T	15.9(149)	18.7(180)			
	TT	3.6(17)	3.7(18)								
rs1877670	TT	30.0(141)	27.9(134)	0.55	0.758	T	55.3(520)	54.3(521)	0.211	0.646	1.04 (0.87–1.26)
	TC	50.6(238)	52.7(253)			C	44.7(420)	45.7(439)			
	CC	19.4(91)	19.4(93)								

^aNumber of alleles for each SNP is given in parentheses.

^bSignificant *P* value (<0.05) are in boldface.

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(Subtotal OR = 1.03, 95%CI = 0.95–1.12, *Z* = 0.75, *P* = 0.45) and rs3750192 (Subtotal OR = 1.0, 95%CI = 0.89–1.12, *Z* = 0.02, *P* = 0.98).

Since only one family-based association study yielded a significant association (*P* = 0.0009) [8], we didn't perform a family-based meta-analysis.

Discussion

In the current study, we found that SNP rs35201266 of *EGR3* and several haplotypes comprised of rs35201266 were significantly associated with schizophrenia patients of *Han* Chinese origin. Our SNP result is consistent with the findings of previous studies that

were performed utilizing a Japanese sample of family-trios and a Korean case-control sample [8,11]. A comparison of our results with the previous studies indicated that the A-allele of rs35201266 may be a risk allele for schizophrenia in Chinese, Korean, and a part of Japanese population. We also found that the MAF of rs35201266 was lower in the population we studied compared to other populations. This difference may be due to differences in ethnic backgrounds [27].

In the genetic model analysis, SNP rs35201266 showed strongly significant differences between cases and controls both in the additive model analysis (*P* = 0.0078) and the allelic comparisons (*P* = 0.009). Together with the weakly significant *P* value in the dominant model (*P* = 0.0258) and the recessive model (*P* = 0.0224),

Table 2. Analysis of the genetic models.

Marker	Genotype ^a			Dominant Model (Risk allele 1)			Recessive Model (Risk allele 1)			Additive Model (Risk allele 1)		
	11	12	22	χ^2	<i>P</i> value ^b	OR (95% CI)	χ^2	<i>P</i> value ^b	OR (95% CI)	χ^2	<i>P</i> value ^b	OR ^c
rs1008949				0.23	0.6329	1.07 (0.81–1.41)	2.33	0.1272	0.76 (0.54–1.08)	0.26	0.6129	0.94
Cases	68	257	145									
Controls	87	238	155									
rs35201266				4.97	0.0258	1.36 (1.04–1.79)	5.21	0.0224	2.69 (1.11–6.50)	7.09	0.0078	1.46
Cases	18	153	299									
Controls	7	135	338									
rs3750192				3.57	0.0590	0.77 (0.58–1.01)	0.01	0.9134	0.96 (0.49–1.89)	2.26	0.1029	0.85
Cases	17	115	338									
Controls	18	144	318									
rs1877670				0.50	0.4790	0.90 (0.68–1.20)	0.00	0.9959	1.00 (0.72–1.38)	0.22	0.6389	0.96
Cases	91	238	141									
Controls	93	253	134									

^a1: Minor allele; 2: Major allele.

^bSignificant *P* value (<0.05) are in boldface.

^cThe software program Finetti (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>) did not provide 95%CI.

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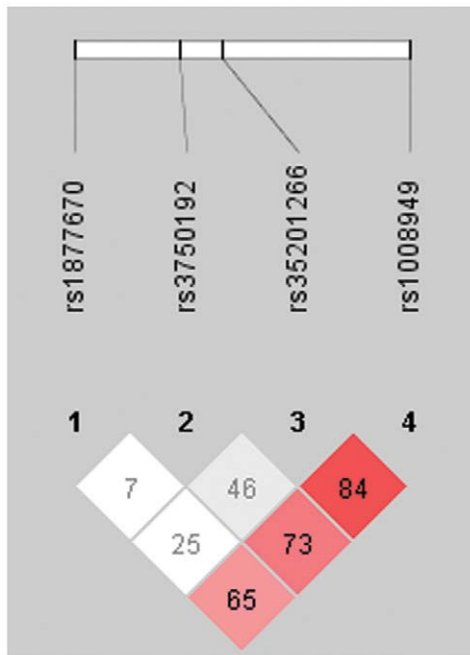


Figure 2. LD between markers genotyped in the *EGR3* gene locus in Han Chinese. LD structure (D') between marker pairs is indicated by the shaded matrices. The figure was generated using HaploView 4.1.

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there is a reasonable interpretation for these findings: the additive model or the multiplicative model (the allelic comparisons) may be more accurate to describe the effect of *EGR3* in schizophrenia etiology [28]. Actually, the ORs for different models for the A-allele of rs35201266 are in the same direction, suggesting the increased risk of schizophrenia with the possession of one or more A alleles.

Furthermore, we stratified the patients by AAO, to examine if there were any clinical differences between the patients carrying the different allele of rs35201266. No significant differences were found between the risk allele (A) carriers and the G homozygotes of rs35201266 in either the total or the subgroup allele frequency distribution. Hence, our current study did not indicate an AAO specific genetic effect.

We also stratified samples by gender, however, there was no significant difference between males and females in cases ($P=0.0794$). Thus, this result did not support a gender specific association for rs35201266 of *EGR3* with schizophrenia [29].

Our independent replication of the association of SNP rs35201266 with schizophrenia, in consideration with similar studies, reduces the likelihood that the association we observed was a false-positive and suggests that *EGR3* gene may indeed be a risk factor for schizophrenia. Recently, the genome-wide association studies (GWASs) are being widely used to study schizophrenia because they incorporate a powerful, systematic, and unbiased genetic approach to the analysis of complex diseases [2]. We summarized the findings of several GWASs that have detected associations with genetic variants in the vicinity of chromosome 8p, the same region that contains *EGR3*, and schizophrenia. Interestingly, the positive association between chromosome 8p and schizophrenia was identified at least in four different ethnic populations from three GWASs [23,24,25], however, the most significant candidate gene was not *EGR3*. Since the variants of *EGR3* locus may not surpass the level of genome-wide significance in GWA studies [30], the susceptibility of *EGR3* to schizophrenia cannot be excluded by GWASs. Hence, much work is still to be done to identify the contribution of *EGR3* to the involvement in genetic risk for schizophrenia.

To further confirm or exclude the implication of *EGR3* in association with schizophrenia, we performed a meta-analysis. We did not detect significant heterogeneity between the available studies, and this enabled us to test three SNPs (rs1008949, rs35201266 and rs3750192) for association with schizophrenia using a fixed-effects meta-analysis. We found a statistically significant association between rs35201266 and schizophrenia ($P=0.0001$) in various East Asian populations. The estimates of the combined OR of rs35201266 ranged from 1.12 to 1.43, suggesting that the data from Kim's study [11] and our study affected the combined estimate, whereas, the study of Yamada *et al.* also showed an increase of risk allele in cases compared to controls (34.3% vs 31.9%) [8]. The results of our meta-analysis suggest that *EGR3* may have a small, but significant effect in the susceptibility to the development of schizophrenia, at least in the tested East Asian populations [31].

Specifically, we observed that the A-allele of rs35201266, and haplotypes comprised of the A-allele, were over-represented in patients with schizophrenia. As mentioned earlier, rs35201266 (A/G) is located in intron 1 of *EGR3*. In an *in vitro* study, the A-allele was associated with reduced expression of *EGR3*, suggesting that its function may be regulatory [8]. Reduced expression of *EGR3* has also been observed in patients with schizophrenia [8,17]. Hence, the findings of our study are consistent with previous studies. Therefore, our study provides additional support for the possibility of SNP rs35201266 possessing a biological function in schizophrenia susceptibility.

EGR3 may play a very important role in the pathological mechanism underlying schizophrenia. *EGR3*^{-/-} mice were

Table 3. Estimated frequency of haplotype and association significance.

No. of Markers	Haplotype	Global P^a	Alleles Increasing in Cases	Estimated Haplotype Frequency(%)		P value ^a	OR (95% CI)
				Cases	Controls		
2	rs1008949-rs35201266	0.0330	C-A	17.8	14.5	0.0445	1.29 (1.00–1.65)
	rs35201266-rs3750192	0.0012	A-G	11.4	6.8	0.0005	1.77 (1.27–2.47)
3	rs1008949-rs35201266-rs3750192	0.0246	C-A-G	9.3	6.1	0.0083	1.59 (1.11–2.27)
	rs35201266-rs3750192-rs1877670	0.0180	A-G-C	7.1	4.5	0.0135	1.64 (1.08–2.47)

^aSignificant P values (<0.05) are in boldface.

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Table 4. Age at onset and gender analysis of rs35201266 (A/G) in cases.

Age at onset	Main effect		P value	OR (95% CI)	Gender	Main effect		P value	OR (95% CI)
	A carriers ^a	G homozygote ^a				A carriers ^a	G homozygote ^a		
<15	1.75 (3)	3.34 (10)	0.3119	0.52 (0.11–2.06)	Male	46.78 (80)	55.18 (165)	0.0794	0.71 (0.48–1.06)
15 ≤ and <30	78.36 (134)	78.34 (217)	0.1651	1.37 (0.86–2.19)	Female	53.22 (91)	44.82 (134)		
30 ≤ and <45	16.95 (29)	22.41 (67)	0.1586	0.71 (0.42–1.18)					
45 ≤	2.92 (5)	1.67 (5)	0.3656	1.77 (0.44–7.16)					
Global P			0.2764						

^aNumber of alleles for each SNP is given in parentheses.

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reported to display heightened reactivity to stress and novelty, abnormalities in social interactions, and deficits in synaptic plasticity, which model the cognitive deficits of schizophrenia [16]. The impact of the antipsychotic medication clozapine inhibiting the increased aggression and impulsivity behavior of *EGR3*^{-/-} mice is very similar with the effects of antipsychotic medications in schizophrenia patients [32].

In addition to *EGR3*'s essential function for neuron activity, *EGR3* also interacts with a number of factors implicated in the risk and pathogenesis of schizophrenia. Such factors include proteins and microRNAs (miRNAs) that have been reported to-affect/be-affected by the expression of *EGR3*. Proteins that have been reported to affect the expression of *EGR3* by induction include N-methyl-D-aspartate receptors (NMDARs), calcineurin (CN), brain-derived neurotrophic factor (BDNF), and neuregulin (NRG1). *EGR3* can be induced by NMDARs [33], which are highly permeable to Ca²⁺ (Ca²⁺ influx through NMDARs is essential for synaptic plasticity). Hypofunction of the NMDAR pathway has been reported to contribute to the etiology of schizophrenia in a number of studies [34,35,36].

EGR3 has been reported to be induced by CN [37,38,39]. CN is a Ca²⁺ and calmodulin-dependent phosphatase participating in many cellular processes and Ca²⁺-dependent signal transduction pathways [40]. Human genetic association studies and behavioral analysis of mouse models have provided evidence for the involvement of CN signaling in schizophrenia susceptibility [7,41]. CN as an upstream gene for *EGR3* might be triggered by calcium influx through NMDARs [42]. *EGR3* is also a target gene for *Brain-derived neurotrophic factor* (BDNF) and *Neuregulin 1* (NRG1) [43,44,45], both of which are schizophrenia susceptibility genes

[6,46,47,48,49]. Induction of *EGR3* by *BDNF* enables *EGR3* to control the expression of *Gamma-aminobutyric acid receptor* (GABAR) [50], which is also a candidate risk gene for the schizophrenia [51].

In addition to being downstream and upstream of susceptibility genes, *EGR3* has been linked to a number of miRNAs that have been associated with schizophrenia. miRNAs are a class of non-coding small RNAs that negatively regulate gene expression in numerous biological processes by promoting mRNA degradation and/or repressing translation through sequence-specific interactions with the 3' UTRs of target mRNAs [52,53]. The miR-15 family was reported to be significantly up-regulated in the cerebral cortex of schizophrenia patients and was therefore hypothesized to have a biological influence in the cortex of schizophrenia patients by influencing genes involved in cortical structure and neural plasticity [54]. *EGR3* is one of the targets genes for miR-15 family as predicated by the Miranda (<http://www.microna.org/microna/home.do>) and TargetScan (<http://www.targetscan.org/>) websites, consistent with the findings of elevated expression of miRNAs and decreased expression of *EGR3* in schizophrenia patients. Guo *et al.* proposed a model highlighting *EGR3* and miRNAs involved in signaling pathways and regulatory networks in the nervous system [55]. Further exploration regarding functional relationship between miR-15 family and *EGR3* gene involved in the pathogenesis of schizophrenia will be worthwhile.

We found that rs3750192 was not significantly associated with schizophrenia, in agreement with two other studies [11,19] and in contrast to another study [8]. Yamada *et al.* utilizing a Japanese sample demonstrated that rs3750192 was associated with schizophrenia ($P=0.0171$) [8]. The discrepancy of the results

Table 5. Comparison of rs35201266 between current study and the previous studies.

Polymorphism	Studies	Ethnicity	Sample	MAF ^b in Control (Allele)	Risk Allele	P value ^c
rs35201266 (A/G)	Current Study	Chinese	Case-Control	0.155 (A)	A	0.009
	Kim et al., 2010 [11]	Korea	Case-Control	0.364 (A)	A	0.0008
	Yamada et al., 2007 [8]	Japanese	Family-trois	Unknown	A	0.0009
		Japanese	Case-Control	0.319 (A)	A	0.234
	Hapmap ^a	European	Control	0.392 (A)	Unknown	Unknown
		Nigeria (African)	Control	0.203 (A)	Unknown	Unknown

^aHapmap:<http://hapmap.ncbi.nlm.nih.gov/>.

^bMAF: Minor Allele Frequency.

^cSignificant P value (<0.05) are in boldface.

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Table 6. Summary of GWA studies on chromosome 8p.

GWA Studies	Ethnicity	Sample	Region	Number of Positive Markers	Position (dbSNP 132)	P value
Shi et al., 2009 [27]	European Ancestry	Case-Control	8p23.3-8p21.2	22	549,908–25,655,470	$<9.27 \times 10^{-4}$
	African American	Case-Control	8p23.2-8p21.1	22	2,724,897–29,297,518	$<9.26 \times 10^{-4}$
Yamada et al., 2011 [28]	Japanese	Family-trios	8p23.3-8p21	37	1,817,045–27,577,392	<0.05
Ma et al., 2011 [29]	Chinese	Case-Control	8p23.1-8p22	7	10,022,938–10,062,543	$<5.0 \times 10^{-5}$

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between studies may be due to different study designs and/or different ethnic groups. We also found that SNP rs1008949 was not associated with schizophrenia, consistent with the findings of another study (Liu et al.) utilizing a Chinese case-control sample [18].

The limitation of this study was that our sample was moderate in size and that there was not sufficient coverage of SNPs to span the whole sequence of *EGR3*. However, our results clearly demonstrate that SNP rs35201266 is significantly associated with schizophrenia, largely consistent with previous reports. We also showed that several haplotypes comprised of rs35201266 were significantly associated with schizophrenia.

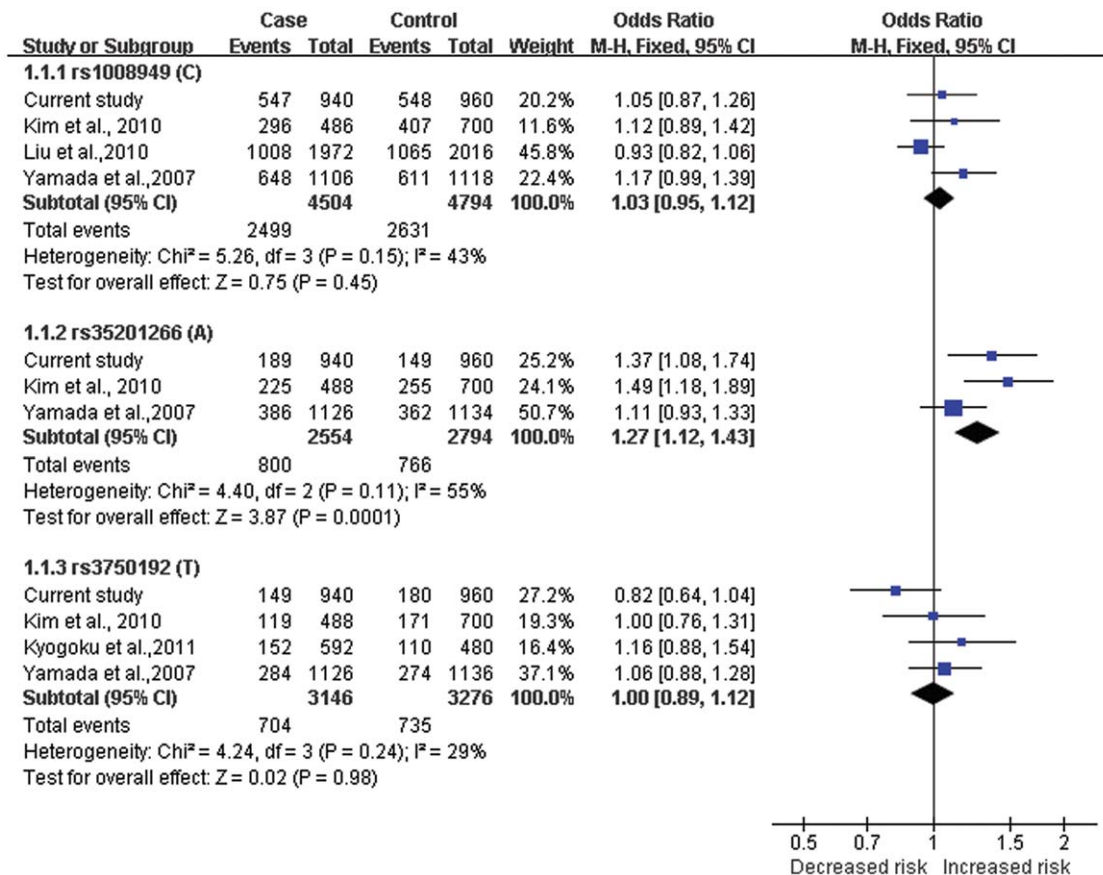
In summary, our study provides evidence for the association of the *EGR3* locus and schizophrenia in the Han Chinese population. Considering the core position of *EGR3* in the CN signaling pathway, and its interaction with a number of candidate risk

factors implicated with schizophrenia, it strongly warrants investigation of the molecular basis of *EGR3* in relationship to the pathogenesis of schizophrenia.

Materials and Methods

Subjects

The study was approved by the genetic research ethics committees of Xi'an Jiaotong University School of Medicine. The informed consent was written and obtained from all participants. Subjects of the case-control samples consisted of 470 patients with schizophrenia (245 males, mean age = 34.5 ± 12.1 , AAO = 24.2 ± 7.2 ; 225 females, mean age = 32.0 ± 13.9 , AAO = 24.1 ± 8.3) and 480 healthy control subjects (275 males, mean age = 29.4 ± 14.3 ; 205 females, mean age = 29.6 ± 14.1). All participants in this study were biologically

**Figure 3.** Meta-analysis of case-control studies between *EGR3* gene and schizophrenia.

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unrelated individuals. The patients were diagnosed by the Psychiatry Department of the First Affiliated Hospital of Xi'an Jiaotong University School of Medicine according to the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)* criteria for schizophrenia. The diagnosis was checked and confirmed by two independent senior psychiatrists who reviewed the psychiatric case records and excluded those with organic brain disease, or short-term drug-induced psychoses, or other symptomatic psychoses. The normal controls were drawn from a combination of local volunteers and blood transfusion donors. Subjects with a personal or family history of mental illness and with current or past evidence of psychoses were ruled out by psychiatric colleagues. All subjects were Han Chinese in origin.

Genotyping

We selected four SNPs (rs1008949, rs35201266, rs3750192, and rs1877670) around the *EGR3* gene locus from the dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and the previous studies [8,11]. These four SNPs span approximately 7.0 kb, with average interval of approximately 2.3 kb, and the density of SNPs is better than those GWASs using the 500 K chips (average interval ~6.0 kb) [56]. In addition, two SNPs (rs35201266 and rs3750192) have been associated with schizophrenia in previous studies [8,11]. The marker rs1008949 was studied both in Korean and Chinese populations [11,18]. SNP rs1877670 was located in the 3'UTR of the *EGR3* gene and may play an important role in expression of *EGR3* [57]. DNA was extracted from whole blood according to a standard protocol of the DNA Isolation Kit for Mammalian Blood (Tiangen Biotech CO., LTD). Genotyping was accomplished by allele-specific PCR, methods have been described elsewhere [58]. PCR primers used in this study were designed by a tetra-primer ARMS-PCR primer design program (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The primers sequences are listed in Table S1. To ensure that the obtained genotypes were valid, re-genotyping was performed on 50 random DNA samples for each of the four SNPs. All genotypes were in agreement with the first round of genotyping, and no genotyping errors were found.

Statistical Analysis

Primary analyses. Hardy-Weinberg equilibrium (HWE) and genetic models of all the SNPs were assessed using the software program Finetti (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hw1.pl>). Genotype frequencies were analyzed using the software program Epi_Info (<http://www.cdc.gov/epiinfo/>). Allele frequencies and pair-wise marker LD were analyzed using the software program Haploview 4.1 [59]. Haplotype frequencies were estimated using the software program PHASE version 2.2 [60]. Rare haplotypes (i.e., found in less than 5% of both case and

control subjects) were excluded from analysis. The distribution of global haplotype frequencies in cases and controls was compared using the software program Epi_Info. Bonferroni corrections were applied to all multiple statistical tests. The software program G*Power program [61] was used to determine statistical power of the case-control sample. Taking into account sample size, the case-control sample had >86% power to detect a significant association ($\alpha < 0.05$), when an effect size index corresponding to a "weak" effect (0.2) was used. Furthermore, we applied a more precise method (<http://www.stat.ubc.ca/~rollin/stats/ssize/caco.html>) to calculate our study power according to the ORs and MAFs of SNP rs35201266 reported by Kim *et al.*, [11] and found that our current sample had >84% power to detect a significant association ($\alpha < 0.05$) (two sided test).

Secondary analyses. According to the genotyping data of rs35201266, the patients were separated into two groups: the risk allele (A) carriers [heterozygous and homozygous for the allele (A)] and non-risk allele carriers [homozygous for the allele (G)]. To assess potential clinical differences (i.e., AAO and gender) in association, chi-squares, OR, and 95% CI for comparison between subgroups were calculated using the Epi_Info software. Furthermore, we compared our results with previous association studies.

Meta-analysis. The studies included in the meta-analysis were identified using Medline with the search terms 'EGR3' and 'Schizophrenia'. All the data analyzed had been previously published. The significance of the subtotal OR was determined by Z test, and the heterogeneity of the group of ORs was assessed using a chi-square test. All statistical analyses were performed using the software program RevMan version 5.0 (<http://www.cochrane.org/revman>).

Supporting Information

Table S1 Markers and primers used for allele-specific PCR.

(DOC)

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Author Contributions

Conceived and designed the experiments: JM. Performed the experiments: RZ LM ZM. Analyzed the data: RKV TG JM. Contributed reagents/materials/analysis tools: RZ LT WZ. Wrote the paper: SL JM. Recruited, diagnosed and gathered patients: LM JT SL. Contributed to the collection and preparation of DNA samples: ZX WZ.

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