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Alteration in Cngb1 Expression upon Maternal Immune Activation in a Mouse Model and Its Possible Association with Schizophrenia Susceptibility

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Objective: The cyclic nucleotide-gated channel (Cng) regulates synaptic efficacy in brain neurons by modulating Ca^{2+} levels in response to changes in cyclic nucleotide concentrations. This study investigated whether the expression of Cng channel, cyclic nucleotide-gated channel subunit beta 1 (Cngb1) exhibited any relationship with the pathophysiology of schizophrenia in an animal model and whether genetic polymorphisms of the human gene were associated with the progression of schizophrenia in a Korean population.
Methods: We investigated whether Cngb1 expression was related to psychiatric disorders in a mouse model of schizophrenia induced by maternal immune activation. A case-control study was conducted of 275 schizophrenia patients and 410 controls with single-nucleotide polymorphisms (SNPs) in the 5′-near region of *CNGB1*.
Results: Cngb1 expression was decreased in the prefrontal cortex in the mouse model. Furthermore, the genotype frequency of a SNP (rs3756314) of *CNGB1* was associated with the risk of schizophrenia.
Conclusion: Our results suggest that *CNGB1* might be associated with schizophrenia susceptibility and maternal immune activation. Consequently, it is hypothesized that *CNGB1* may be involved in the pathophysiology of schizophrenia.

KEY WORDS: Cyclic nucleotide-gated channel subunit beta 1; Single nucleotide polymorphism; Maternal immune activation; Animal model; Schizophrenia.

INTRODUCTION

Cyclic nucleotide-gated channels (CNGs) play an important role in signaling systems by mediating sensory transduction in retinal rods and olfactory neurons [1]. The roles were characterized based on the conduction of cation currents in response to changes in the intracellular levels of cGMP, mediation of the electrical response to light in retinal rods, [2] the response to changes in internal cAMP levels, and the electrical response to odorants in olfactory receptor neurons [3]. Cyclic nucleotides regulate several important aspects of neuronal function including gene transcription [4,5], neural development, and synaptogenesis [6,7], and were shown to be involved in synaptic strength and transmitter release in a variety of models of neural plasticity [8-10]. The importance of CNGs in the transduction of sensory stimuli through the activation of G-protein-coupled cascades into electrical membrane signals has previously been investigated [11,12]. CNG channel proteins are distributed in various tissues, including the kidney [13] and brain [1]. Native retinal rod CNGs are known to be heterotetramers formed exclusively by the co-assembly of two different subunits, the CNG subunit alpha (CNGA) and the CNG subunit beta (CNGB). Several studies on retinal bipolar and ganglion cells, hippocampal neurons, and a sympathetic neuron cell line reported a widespread distribution of CNG channels in the nervous system [1,14,15]. It has also been demonstrated that CNG channels are important targets for the action of diffusible messengers such as nitric oxide (NO) and car-

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bon monoxide (CO), thus providing a pathway for these messengers to alter Ca^{2+} levels within the neurons [16,17]. Because of the known properties of CNG channels and their role as effectors of cyclic nucleotide-mediated signaling cascades, it has been postulated that they may play an important role as modulators of neuronal activity and synaptic plasticity in the nervous system [18].

Despite the potentially important roles of CNG channels, the association between CNG proteins and the pathophysiology of schizophrenia as a neurodevelopmental disorder has not been reported. Therefore, the objective of this study was to investigate whether the expression of CNGB1, a CNG channel protein, exhibited any relationship with the pathophysiology of schizophrenia in an animal model and whether genetic polymorphisms in *CNGB1* were associated with the progression of schizophrenia in a Korean population. In the present study, we investigated a mouse model of schizophrenia induced by maternal immune activation (MIA) to examine the expression of Cngb1 in specific brain regions such as the prefrontal cortex. Furthermore, we screened for genetic variations in the 5′-near region of human *CNGB1* gene by direct sequencing and selected two single-nucleotide polymorphisms (SNPs; rs73545191, rs16959613). Although these two SNPs have been reported in *CNGB1* (http://www.ensembl.org), they have not yet been investigated in the context of a genetic association with schizophrenia. To investigate the association between the 5′-near region of the SNPs of *CNGB1* and schizophrenia in a Korean population, we conducted genotype, allele, and haplotype analyses. Subsequently, a luciferase activity assay was performed to determine the functional effects of the identified SNPs.

METHODS

Animals

C57BL6/J mice (eight weeks old) were purchased from DBL Animal, Inc. (Seoul, Korea). The mice were mated in groups of one male and two females. When a vaginal plug was observed during daily checks, the female mice were considered pregnant and separated. All mice were housed under standard conditions of a 12/12-h light/dark cycle (lights on at 06:30) with free access to food and water. All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals provided by the US National Institutes of Health [19].

The experimental procedures were approved by the Institutional Animal Care and Use Committee of Soonchunhyang University (approval no. SCH20-0008).

Maternal Immune Activation and Drug Administration to Mice

Pregnant dams on gestation day 9 received either a single injection of polyinosinic-polycytidylic acid (Poly I:C) or control (saline) solution intravenously into the tail vein under mild physical constraint [20,21]. Poly I:C (potassium salt) was obtained from Sigma-Aldrich (Buchs, St Gallen, Switzerland) and dissolved in isotonic 0.9% NaCl solution to obtain the desired dosage (5 mg/kg based on calculations for the pure form of Poly I:C). All animals were returned to their home cages immediately after the injection procedure and left undisturbed until weaning of the offspring. The offspring were weaned 23 days after birth and housed in groups. Male offspring were selected and used for further experiments. A total of four experimental groups of adolescents were tested: 1) control (CON) group comprised of the offspring of normal mothers; 2) CON-clozapine (CLZ) group comprised of offspring treated with clozapine during the adolescence period; 3) MIA group comprised of the offspring of mothers treated with Poly I:C; and 4) the MIA-CLZ group comprised of MIA offspring treated with clozapine during the adolescence period. Each group was comprised of 10 male mice. The clozapine-treated offspring were treated during the adolescence period. Clozapine (Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in 0.1N HCl and administered at 5 mg/kg/day. The animals were orally treated with clozapine solution on postnatal day 35 for three weeks until postnatal day 65 [20,21]. Extremely large or small litters were eliminated from the study.

Pre-pulse Inhibition Test

The Pre-pulse Inhibition (PPI) test was performed as described previously [21,22]. First, the mice were placed in a small Plexiglas cylinder within a larger sound-attenuating chamber (SR-LAB; San Diego Instruments, San Diego, CA, USA). The cylinder was seated on a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. The test session consisted of the

following components: a 5-minute acclimation period to 68-dB background noise that began when the animals were placed in the chambers and continued throughout the entire session; 14 pulse-alone trials in which a 40 ms, 120 dB broadband noise burst was presented; 30 pre-pulse + pulse trials in which the onset of a 20 ms pre-pulse broadband noise preceded the onset of the 120 dB pulse by 100 ms, 10 for each pre-pulse intensity of 3, 6, and 12 dB above the background noise, and eight non-stimulus trials consisting of only background noise. The pre-pulse intensities used in our protocol did not induce a startle reaction. All the trials were presented in a pseudo-random order at an average of 22 seconds (15−30 seconds range) inter-trial intervals. Four 120 dB pulse trials were presented at the beginning and end of the test session (for a total of 60 trials) but were not used in the calculation of the PPI values. The PPI levels were calculated as a percentage score for each pre-pulse [21,22]. The equation for this calculation was: %PPI = 100 − {[(startle response for pre-pulse + pulse trial) / (startle response for pulse alone trial)] × 100}. The global PPI was considered an overall measure of the observed treatment for which the percentage PPI was averaged across three pre-pulse measurements for each mouse [21,22].

Western Blot Analysis

All mice used for experiments in this study were euthanized by decapitation. Brain slices, 8−12 mm from the frontal pole of the prefrontal cortex were collected using a brain matrix device (ASI Instruments, Warren, MI, USA). From these, we isolated the prefrontal region of the cerebral cortex. The prefrontal cortex region was isolated by removal of the olfactory tracts and cutting 1 mm posterior to the bregma. Four whole hemispheres of the prefrontal cortexes of each mouse were used for Western blotting. The prefrontal cortex tissues were lysed in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitors and then centrifuged at 14,000 rpm for 10 min at 4°C. To identify CNGB1 protein, 100 μg of the lysed protein was placed on 10 and 8% sodium dodecyl sulfate (SDS) gels and transferred to polyvinylidene difluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). After blocking with 5% skim milk, the membranes were probed with anti-Cngb1 (1:100; sc-13705; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-β-tubulin (Tubb; 1:3,000; Thermo Fisher Scientific, Inc., Rockford, IL, USA) antibodies overnight at 4°C followed by incubation with anti-goat (1:3,000; #7074; Cell Signaling Technology, Inc., Seoul, Korea) or anti-mouse secondary antibodies (1:10,000; Sigma) for 1 hour at room temperature. The immunoreactive bands were detected using an Enhanced Chemiluminescence Kit (Elpis Biotech Inc., Daejeon, Korea).

Subjects

A case-control study was conducted to determine the genetic association between CNGB1 SNPs and schizophrenia. All psychiatric diagnoses were established according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria following a review of case notes and a direct examination of the cases. Well-trained psychiatrists diagnosed 275 Korean patients with schizophrenia (mean age ± standard deviation, 45.9 ± 10.3 years; male/female = 171/104) based on the DSM-IV criteria. A total of 410 control subjects (49.1 ± 11.2 years; male/female = 189/221) were recruited from individuals who participated in a general health checkup program. Blood samples and demographic data were provided by the biobank of the College of Medicine, Soonchunhyang University. The study was approved by the Ethics Review Committee of the SCH biobank (SCHIRB-BIO-150004).

SNP Selection and Genotyping

To establish whether any functional SNPs affected gene expression, the promoter region of *CNGB1* was screened and searched for all known promoter SNPs (2,000 bp upstream) in the human *CNGB1* region from human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP) and the HapMap database (https://ftp.ncbi.nlm.nih.gov/hapmap/). Among these, we included SNPs with > 5% minor allele frequency, > 10% heterozygosity, and genotype frequencies in an Asian population, and excluded SNPs with unknown heterozygosity. We ultimately selected two *CNGB1* SNPs (rs73545191, rs16959613) that were hypothesized to affect CNGB1 expression for further analysis. DNA was isolated from the peripheral blood using a DNA purification kit (Nanohelix Co., Ltd., Seoul, Korea) [23]. The primer sequences of the *CNGB1* SNPs were as follows: rs73545191 (sense: 5′-GATCTAGGAGGAGAGGGTGCAA-3′ and anti-sense 5′-GCATCCAGGAGTTTGAGAGG-3′, resulting in a 248-bp polymerase chain reaction (PCR) product); and rs16959613 (sense: 5′-ATGATGGGGCTT

GGACTATG-3′ and anti-sense 5′-CCCAGCCTCTCTCAG ACATC-3′, resulting in a 244-bp PCR product), that comprised different polymorphisms. The PCR products were sequenced using an ABI PRISM 3730xl DNA analyzer (PE Applied Biosystems, Foster City, CA, USA). The sequence data were analyzed using SeqManII software (DNASTAR, Inc., Madison, WI, USA).

Transfection and Luciferase Assay

SH-SY5Y and HeLa cells were employed for assessing promoter activity. The cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS and 100 U/ml penicillin/streptomycin. The cultures were maintained in a humidified incubator at 37°C in an atmosphere of 5% CO_2 and 95% air. The cell culture medium was changed every two days.

Genomic DNA samples with two identified SNPs were amplified using the primer sequences as indicated in the "SNP selection and genotyping" section. The promoter inserts were excised by restriction digestion using KpnI and XhoI and the resulting fragments were cloned into the pGL3-Basic vector (Promega, Mannheim, Germany) containing a luciferase reporter gene. To analyze the effect of SNPs on the expression, dual-luciferase reporter assays (Promega) were performed according to the manufacturer's instructions. Briefly, cell lysates were obtained 24 hours after Lipofectamine (Invitrogen) transfection of the pGL3 vector constructs into the SH-SY5Y and HeLa cells, using 1x phosphate-buffered saline washing buffer and RIPA lysis buffer. Typically, 100 µl of luciferase assay reagent was added to the lysate (20 µl) and after incubation with the Stop & Glo reagent, luciferase activity was determined using a luminometer (Berthold LB952, Bad Wildbad, Germany). An empty pGL3 vector was used as a negative control. The activity of co-transfected renilla luciferase was used to normalize the values (the firefly luciferase values were divided by the renilla luciferase values).

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) was assessed using SNPStats (https://www.snpstats.net/). SNPStats was also used to evaluate the odds ratios (ORs), 5% confidence intervals (CIs), and p values. Multiple logistic regression analysis adjusted for age and gender as covariables was also performed. In the logistic regression analysis for each SNP, the following models were used that assumed either co-dominant inheritance (where the relative hazard differed between the subjects with one minor allele and those with two minor alleles), dominant inheritance (subjects with one or two minor alleles had the same relative hazard for the disease), or recessive inheritance (subjects with two minor alleles were at increased risk of the disease). Bonferroni correction was applied by multiplying the p values by the number of SNPs (n = 2). The χ^2 test was used to compare allele frequencies between the groups. To avoid the chance findings due to multiple testing, Bonferroni correction was applied by decreasing the significance levels to $p = 0.025$ ($p = 0.05/2$) for each of the two SNPs. To examine whether the genetic variants influenced the transcription factor binding sites, we compared the transcription factor binding sites using the online program AliBaba 2.1 (http://www.gene-regulation.com/pub/programs/alibaba2). The quantitative measurements of CNGB1 and Tubb proteins were obtained using ImageJ software, as previously described [24].

RESULTS

Pre-pulse Inhibition

We tested whether an atypical antipsychotic treatment (clozapine) was effective in preventing the emergence of sensorimotor gating deficiency following MIA during adulthood. The PPI level was measured using the acoustic startle response (ASR) test in the offspring in the control, clozapine-treated, MIA, and clozapine-treated MIA groups. The results revealed a significant group effect on PPI between the controls and the MIA model. Significant pre-pulse facilitation was detected in the MIA model at lower pre-pulse stimulus intensities and in the startle response (Fig. 1). MIA significantly ($p < 0.05$) increased the acoustic startle amplitude at 120 dB compared to the control group (Fig. 1A). Clozapine administration decreased the ASR levels in MIA offspring ($p < 0.05$, Fig. 1A). Peripubertal clozapine treatment significantly ($p < 0.05$) elevated the PPI trial scores in MIA offspring to levels found in the control offspring (Fig. 1C). The MIA induced PPI disruption in 120 dB pulse stimulus trials (Fig. 1A) was consistently seen across all the pre-pulse levels, leading to significant ($p < 0.05$) decreases in the mean percentage PPI in MIA offspring compared to the control group (Fig. 1B). This result indicated that MIA induced sensorimotor

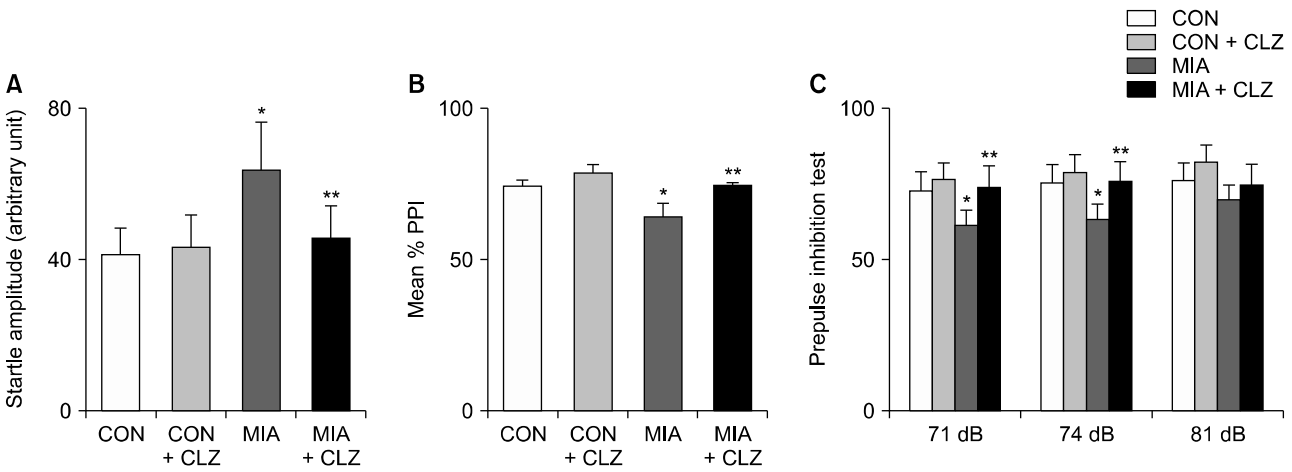

Fig. 1. Modulation of PPI and acoustic startle response by clozapine treatment in MIA mice. (A) Startle amplitude was calculated to 120 dB of alone stimuli in all the performed trials. (B) PPI is presented as a mean PPI percentage value in startle amplitude as a function of the magnitude of the prepulse stimulus. (C) Inhibition of startle reflex to a 120 dB stimulus. A stimulus was achieved through prepulse of 71, 74, and 80 dB, corresponding to 3, 6, and 12 dB above the background noise, respectively.

PPI, prepulse inhibition; MIA, offspring of mice injected with poly I:C group; MIA + CLZ, clozapine-treated MIA group; CON, control group; CON + CLZ, Clozapine-treated CON group.

Data are presented as the mean ± standard error of the mean; *$p < 0.05$ vs. CON; **$p < 0.05$ vs. MIA.

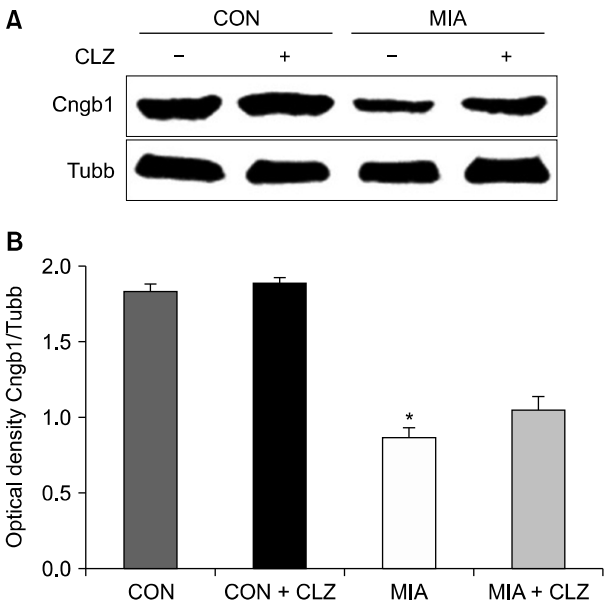

Fig. 2. Protein expression of CNGB1 in MIA model. (A) CNGB1 expression was detected by Western blot analysis with an anti-β-tubulin antibody (Tubb) as an internal control. Animal models induced by poly I:C exhibited decreased CNGB1 expression in the prefrontal cortex. (B) Quantitative analysis of Western blot analysis data for CNGB1 expression showed a significant difference in CNGB1 levels between CON and MIA groups (*$p < 0.05$).

CON, control group; CON + CLZ, Clozapine-treated CON group; MIA, offspring of mice injected with poly I:C group; MIA + CLZ, clozapine-treated MIA group.

gating deficits. Moreover, the MIA induced PPI disruption was significantly ($p < 0.05$) improved by treatment with clozapine.

Western Blots

To investigate the Poly I:C-induced regulation of Cngb1 proteins, Western blotting of the prefrontal cortex areas from the brains of control, clozapine-treated, MIA, and clozapine-treated MIA offspring was performed (Fig. 2). The quantities of Cngb1 proteins in the prefrontal cortex were significantly lower in the MIA model compared to the controls ($p < 0.05$; Fig. 2). However, the change in Cngb1 expression was not affected by antipsychotic treatment ($p > 0.05$; Fig. 2).

SNP Genotype Distribution

After identifying Cngb1 as an MIA-related protein in mice, we extended our study to human subjects. The genotype distributions of two SNPs (rs73545191 and rs16959613) were identified by HWE ($p > 0.05$, control group). As shown in Table 1, the genotype frequency of rs73545191 was statistically associated with the development of schizophrenia in the codominant model (OR = 1.51, 95% CI: 1.07−2.15, $p = 0.020$, corrected p value, $p^c = 0.040$) after Bonferroni correction. In the codominant model, the GG, GA, and AA genotype frequencies

Table 1. Genotype and allele frequencies of CNGB1 SNPs in control and schizophrenia subjects

SNPs	Genotype/ Allele	Control		Schizophrenia		Model	OR (95% CI)	p value	p value[c]
		Freq.	%	Freq.	%				
rs73545191	GG	309	75.37	188	68.36	Co-dominant	1.51 (1.07−2.15)	0.020	0.040
	GA	98	23.90	87	31.64		0.00 (0.00−NA)		
	AA	3	0.73	0	0.00	Dominant	1.48 (1.04−2.09)	0.029	0.058
						Recessive	0.00 (0.00−NA)	0.120	0.240
	G	716	87.32	463	84.18		0.77 (0.57−1.05)	0.101	0.202
	A	104	12.68	87	15.82				
rs16959613	GG	308	75.12	187	68.00	Co-dominant	1.43 (0.99−2.05)	0.130	0.260
	GC	92	22.44	78	28.36		1.43 (0.57−3.61)		
	CC	10	2.44	10	3.64	Dominant	1.43 (1.01−2.02)	0.045	0.090
						Recessive	1.31 (0.52−3.28)	0.570	1.000
	G	708	86.34	452	82.18		0.85 (0.79−1.32)	0.193	0.386
	C	112	13.66	98	17.82				

SNPs, single-nucleotide polymorphisms; Freq., frequency; OR, odds ratio; CI, confidence intervals; NA, not applicable; p value[c], p value corrected using Bonferroni's method.

Table 2. Analysis of haplotypes consisting of CNGB1 polymorphisms in schizophrenia patients and control subjects

Haplotype		Frequency		OR (95% CI)	p value
rs73545191	rs16959613	Control	Schizophrenia		
G	G	0.856	0.822	1	-
A	C	0.119	0.158	1.48 (1.05−2.09)	0.024
G	C	0.017	0.02	0.90 (0.47−1.70)	0.74
A	G	0.008	NA	0.00 (0.00−NA)	1

OR, odds ratio; CI, confidence intervals; NA, not applicable.

were 75.37, 23.90, and 0.73% in the control group and 68.4, 31.64, and 0.00% in the schizophrenia group, respectively (Table 1). The GA genotype was associated with an increased risk of developing schizophrenia. In the dominant model, the GA genotype was weakly associated with an increased risk of developing schizophrenia (OR = 1.48, 95% CI: 1.04−2.09, p = 0.029, p^c = 0.058). In the dominant model, the genotype frequencies of GG and genotypes that contained only major and minor allele (GA-AA) frequencies were 75.37 and 23.90% in the control group and 68.36 and 31.64% in the schizophrenia group, respectively. It is hypothesized that the GA-AA genotype group might be at an increased risk of developing schizophrenia. The genotype and allele frequency of rs7939644 were not associated with the development of schizophrenia.

We evaluated the haplotype of polymorphisms within the *CNGB1* gene using SNPstats. In linkage disequilibrium analysis, the D value from rs73545191 to rs16959613 was 0.9622 and the r statistic was 0.9102. In haplotype analysis, the AC haplotype was significantly associated with the development of schizophrenia (OR = 1.48, 95% CI: 1.05−2.09, p = 0.024) (Table 2). We further investigated whether disease susceptibility-related SNPs (rs73545191 and rs16959613) altered the transcriptional activity of the *CNGB1* promoter sequence. To assess the promoter activity of each genotype of SNPs, we transfected plasmids that contained one of the genotypes and a luciferase reporter gene into human SH-SY5Y neuroblastomas. The reporter activity was compared between the two constructs that contained the two genotypes (GG and AA for rs73545191; GG and CC for rs16959613). No significant luciferase activity in cells transfected with constructs that contained rs73545191 and rs16959613 was detected (relative promoter activity in percentage): rs73545191; pGL3-allele/pRL-SV40, pGL3-basic, 100.00 ± 26%; GG, 57.92 ± 29%; AA, 86.94 ± 31%, respectively; and rs16959613; pGL3-basic, 100.00 ± 24%; GG, 107.73 ± 29%; CC, 91.36 ± 22%, respectively. The power of the sample size was calculated using a ge-

netic power calculator (http://zzz.bwh.harvard.edu/gpc/). The estimation of the sample size revealed that this case-control study was sufficiently powered for the determination of positive associations. Consequently, the genetic power for rs73545191 and rs16959613 was determined to be 0.9733 (high-risk allele frequency: 0.12; number of cases: 275; control-to-case ratio: 1.491; and the number of cases at 80% power: 142) and 0.9763 (high-risk allele frequency: 0.13; number of cases: 275; control-to-case ratio: 1.4729; and the number of cases at 80% power: 138), respectively.

DISCUSSION

The present study investigated the relationship between a gene regulated in an animal model of schizophrenia and human schizophrenia susceptibility. To the best of our knowledge, this study was the first to investigate the potential effect of Cngb1 protein and human gene polymorphisms on schizophrenia. Our results showed that the MIA schizophrenia animal model had decreased sensorimotor gating function with downregulated Cngb1 protein expression. The results of the genetic association study suggested that *CNGB1* polymorphisms in the Korean population might have increased susceptibility to developing schizophrenia. The rs73545191 GA genotype, in particular, was associated with schizophrenia development and was implicated as a risk factor for schizophrenia. However, the luciferase assay results showed that there was no significant difference in promoter activity for gene expression using the constructs that contained two SNPs (rs73545191 and rs16959613). We found no direct relationship between the SNPs and CNGB1 expression in human cell line. As such, the changes in genotype frequencies in the SNPs may be related only to the genetic susceptibility to schizophrenia. This was a limitation of the study.

Considerable evidence now exists that CNG channels represent a novel major target for cyclic nucleotide action in the central nervous system (CNS). The functional expression of CNG channels, especially of rod and olfactory types, has been demonstrated in neurons and astrocytes of the rodent brain [25-27]. In neurons, cation influx through CNG channels mediates membrane depolarizations [26,27]. Moreover, it has been established that Ca^{2+} influx through activated CNG channels enhances neurotransmitter release and influences synaptic plasticity [28,29]. Previous studies have also suggested a role of CNG channels in CNS development. In rats, both molecular and electrophysiological experiments detected CNG channel expression in immature hippocampal neurons before synapse formation [15,30], suggesting that CNG channels could function at the early stages of neural development. In the visual cortex, the rod and olfactory subtypes are developmentally regulated and present at the time of migration and rapid dendritic outgrowth [31]. In this context, several experimental findings also support a specific role of CNG channels as guidance molecules in growth cone formation and the synaptic bouton maturation of neurons [32,33]. Adult neurogenesis in the hippocampus contributes to learning and memory [34,35], and increasing evidence has revealed that its alteration was associated with neurodegenerative and neuropsychiatric diseases, including Huntington's disease [36], Parkinson's disease [37], Alzheimer's disease [38], schizophrenia, and depression [39-41]. There is evidence indicating that neurogenesis is positively affected by cGMP and cGMP-sparing agents such as sildenafil and nitroarginine-methyl ester [42,43]. Some cGMP effects have been linked to the activation of intracellular pathways by NO [44], which is a well-recognized mediator involved in the regulation of adult neurogenesis both in basal conditions and following brain injury [45,46]. Podda *et al.* [47] suggested that hippocampal neural stem cells express CNG channels and their activation by cGMP promoted neuronal differentiation. These results, besides highlighting the relevance of the cGMP signaling pathway in adult neurogenesis, revealed an additional role of CNG channels in the CNS in the regulation of an important aspect of brain physiology [47].

Taken together with functional positional evidence, the present experimental results indicate that mutations or polymorphisms in and/or nearby the *CNGB1* gene may play a role in mediating genetic susceptibility for and the development of schizophrenia. Thus, the association between the functional SNPs of *CNGB1* and schizophrenia was examined. The present findings demonstrated that a SNP in the promoter regions was associated with the susceptibility for developing schizophrenia. Based on the *in silico* promoter binding prediction of the rs73545191 SNP, the transcription factor C/EBP can bind to G allele but not to A allele. However, the functional alterations

that may have been induced by the polymorphism were not detected by the luciferase assay, which is a limitation of this study. The MIA model provided substantial evidence for experimental investigations of the predictive validity of psychosis-related dysfunction in schizophrenia. Psychosis-related behavioral abnormalities in adult life have etiological significance from the neurodevelopmental perspective of schizophrenia [48,49]. In the present study, we also identified decreased sensorimotor gating function and the downregulation of Cngb1 protein levels in an MIA-induced schizophrenia animal model. The changes in sensorimotor gating function were recovered by clozapine treatment. However, the changes in Cngb1 protein were not. Clozapine treatment may not influence the potential role of Cngb1 during neurodevelopment in the MIA mouse model.

The study findings suggest that prenatal immune activation might affect Cngb1 expression and influence the development of the fetal brain and that this influence could result in an increased risk of MIA-related psychiatric diseases such as schizophrenia. *CNGB1* might be one of several genes that play a role in the polygenic susceptibility to schizophrenia. Future research studies using cellular and animal models are needed to fully characterize the function of *CNGB1* in the pathophysiology of schizophrenia.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Conceptualization: Hwayoung Lee, Hak-Jae Kim. Data acquisition: Hwayoung Lee, Hyeonjung Jung. Formal analysis: Sung Wook Kang, Young Ock Kim. Funding: Hak-Jae Kim. Supervision: Hak-Jae Kim. Writing−original draft: Hwayoung Lee. Writing−review & editing: Young Ock Kim, Jun-Tack Kwon, Hak-Jae Kim.

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