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Gender-Specific Associations between *CHGB* Genetic Variants and Schizophrenia in a Korean Population

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Purpose: Schizophrenia is a devastating mental disorder and is known to be affected by genetic factors. The *chromogranin B (CHGB)*, a member of the chromogranin gene family, has been proposed as a candidate gene associated with the risk of schizophrenia. The secretory pathway for peptide hormones and neuropeptides in the brain is regulated by chromogranin proteins. The aim of this study was to investigate the potential associations between genetic variants of *CHGB* and schizophrenia susceptibility.
Materials and Methods: In the current study, 15 single nucleotide polymorphisms of *CHGB* were genotyped in 310 schizophrenia patients and 604 healthy controls.
Results: Statistical analysis revealed that two genetic variants (non-synonymous *rs910122*; *rs2821* in 3'-untranslated region) were associated with schizophrenia [minimum p=0.002; odds ratio (OR)=0.72], even after correction for multiple testing (p_{corr}=0.02). Since schizophrenia is known to be differentially expressed between sexes, additional analysis for sex was performed. As a result, these two genetic variants (*rs910122* and *rs2821*) and a haplotype (*ht3*) showed significant associations with schizophrenia in male subjects (p_{corr}=0.02; OR=0.64), whereas the significance disappeared in female subjects (p>0.05).
Conclusion: Although this study has limitations including a small number of samples and lack of functional study, our results suggest that genetic variants of *CHGB* may have sex-specific effects on the risk of schizophrenia and provide useful preliminary information for further study.

Key Words: Single nucleotide polymorphisms, *chromogranin B*, schizophrenia, gender-specific marker

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INTRODUCTION

Schizophrenia is a devastating mental disorder and is characterized by several symptoms related to abnormalities in perception and expression, such as delusion, hallucination, and disorganized thinking.[1] Although evidence on gender differences in schizophrenia development is inconclusive,[2] the prevalence of the disease is different between the sexes, with about a 1.4-fold higher risk in men than in women.[3] The heritability of schizophrenia is reported to be around 80%, suggesting that genetic factors are strongly involved in the risk for the disorder.[4] The putative susceptible genes for schizophre-

nia are *dystrobrevin-binding protein 1*, *neuregulin 1 (NRG1)*, *D-amino-acid oxidase (DAOA)*, and *regulator of G-protein signaling 4 (RGS4)*.[5] Recently, *chromogranin B (CHGB)* has also been identified as a candidate gene influencing the risk of schizophrenia,[6-10] although robust evidence has not been reported. In a genome-wide association study (GWAS) performed in a Japanese population, a genetic variant (microsatellite: D20S95) near *CHGB* was discovered as a potential genetic marker for schizophrenia development.[11] In addition, our GWAS of schizophrenia in a Korean population (in submission) has identified three markers of *CHGB* that were significantly associated with schizophrenia (minimum p=3.2×10^{-6} at rs2821). Inspired by these results, this study performed a replication study of the association between schizophrenia development and *CHGB* using additional single nucleotide polymorphisms (SNPs).

Accumulating evidence indicates that synaptic disturbance or damage may be important in the etiology of schizophrenia. Granins (secretogranins/chromogranins) are a family of soluble proteins that are stored in and released from the secretory large dense-core vesicles of the synapse. The CHGB, also known as secretogranin I, is an acidic glycoprotein that is present in secretory granules of neuroendocrine cells and neurons.[12] In genetic association studies performed in Asian populations (Japanese and Chinese) genetic makers in *CHGB* (including several non-synonymous variants of rs6133278, rs910122, rs236152, rs236153, and rs74621755) have been reported to be associated with the risk of schizophrenia.[9,10,13]

In order to investigate potential genetic markers of *CHGB* for schizophrenia, we analyzed the associations of polymorphisms in *CHGB* with the risk of the disease in a Korean population.

MATERIALS AND METHODS

Study subjects

A total of 310 patients with schizophrenia (mean age=44.7 years; range=23–73 years; 185 males and 125 females) were recruited from Seoul National University, Jinju Mental, Soonyoung University, Hadong Wooridle, and Keyo Hospitals. A total of 604 healthy controls (mean age=48.79 years; range=8–84 years; 254 males and 350 females) were simultaneously recruited from an unselected population who had come in for routine health checkups in the same regional areas. Healthy controls were recruited from Seoul National University and Hallym University Hospitals. Trained psychiatrists diagnosed schizophrenia patients based on the criteria set forth by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV).[14] Patients with complicating diagnoses of mental retardation, organic brain damage, neurological disorders, drug or alcohol abuse, autoimmune disorders, and low comprehension skills were excluded from the study. To ensure no history and present evidence of psychiatric illness, each control subject underwent additional evaluation by trained psychiatrists using the Structured Clinical Interview from DSM-IV, non-patient edition. The ethnicity of all patients and healthy controls was Korean. Informed written consent was obtained from all subjects before blood was drawn. The study protocol was approved by the Institutional Review Board of each hospital.

SNP selection and genotyping

Candidate SNPs of *CHGB* were selected from genotype data from Japanese and Han Chinese populations in the 1000 Genomes database (http://browser.1000genomes.org/index.html) based on the following conditions: 1) minor allele frequency (MAF) >5%, 2) linkage disequilibrium (LD) status [LD coefficient (r^2) >0.98], 3) position within the gene, and 4) amino acid changes. In addition, previously reported SNPs were included. A total of 15 SNPs of *CHGB* (rs226137, rs236139, rs236141, rs16991480, rs76791154, rs236145, rs446659, rs6085323, rs6085324, rs6133278, rs910122, rs881118, rs742711, rs74621755, rs2821) were genotyped in 310 schizophrenia patients and 604 healthy controls using the TaqMan assay on the ABI Prism 7900HT (Applied Biosystems, Foster City, CA, USA). Genotyping quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates >99.5%). Selected SNPs and their probe information are available in Supplementary Table 1 (only online).

Statistical analysis

LD was obtained using the Haploview v4.2 software from the Broad Institute (http://www.broadinstitute.org/mpg/haploview), with examination of Lewontin's D' (|D'|) and the r^2 between all pairs of bi-allelic loci.[15] Haplotypes (*ht*s) were estimated using the PHASE software.[16] Comparison of genotype distributions between schizophrenia patients and healthy controls was carried out with a logistic regression model adjusted for age (continuous value) and sex (male=0, female=1) as covariates using SAS, version 9.4 (SAS Inc., Cary, NC, USA). The effective number of independent marker loci was calculated for multiple testing corrections using SNPSpD (https://gump.qimr.edu.au/general/daleN/SNPSpD/), a program that is based on the spectral decomposition of matrices of pair-wise LD between SNPs.[17] The total sum of the tested independent marker loci in the gene was calculated as 10.9139, which was applied to correct for multiple testing (p value×10.9139).

RESULTS

Genotyping and haplotypes of *CHGB* polymorphisms

A total of 15 *CHGB* genetic variants, including six non-synonymous SNPs (rs6085324, rs6133278, rs910122, rs881118, rs742711, and rs74621755), were selected from Asian populations (Chinese and Japanese) in the 1000 Genomes database, based on the selection criteria, and were genotyped (Fig. 1A). Detailed information about 15 investigated polymorphisms in

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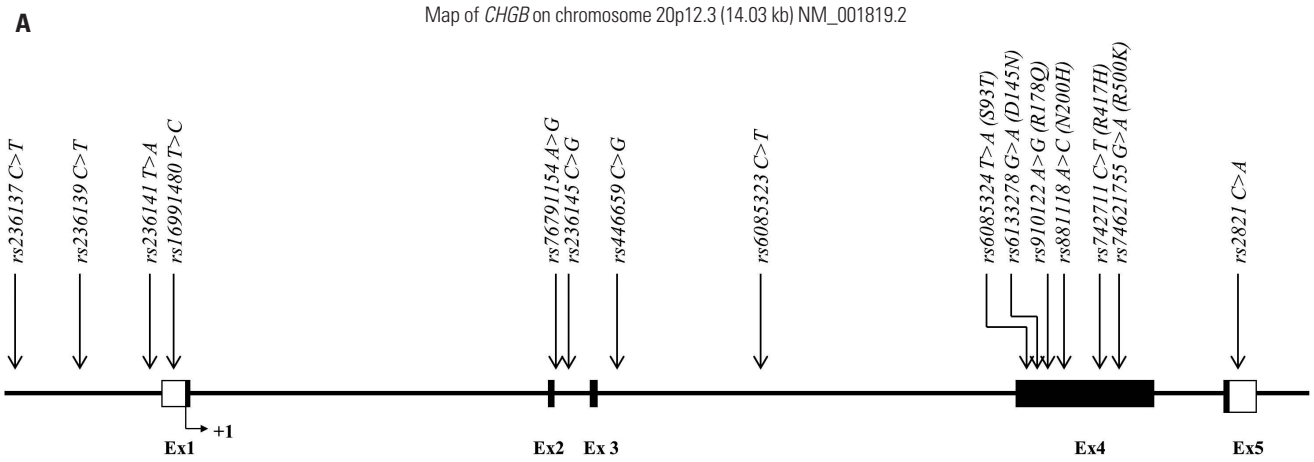
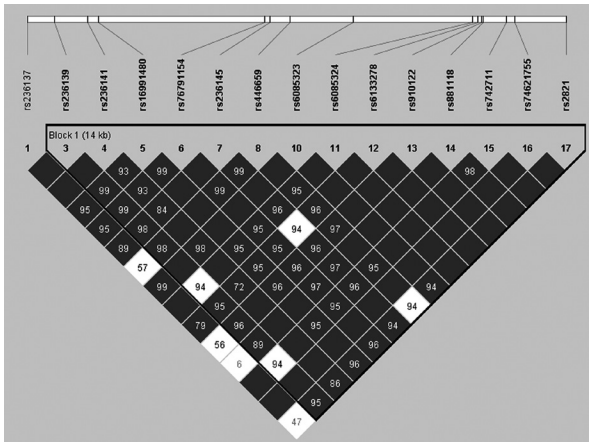


B LDs among *CHGB* polymorphisms



C Haplotype of *CHGB*

ht	*rs236139*	*rs236141*	*rs16991480*	*rs76791154*	*rs236145*	*rs446659*	*rs6085323*	*rs6085324*	*rs6133278*	*rs910122*	*rs881118*	*rs742711*	*rs74621755*	*rs2821*	Freq.
ht1	T	T	T	A	C	C	C	A	G	A	A	A	G	C	0.398
ht2	C	T	C	G	G	G	C	T	A	G	A	G	G	A	0.237
ht3	C	T	T	A	C	C	C	T	G	A	C	G	G	C	0.138
ht4	C	A	T	A	C	G	T	T	G	G	A	G	G	A	0.083
ht5	C	T	T	A	G	G	C	T	A	G	A	G	A	A	0.079
others															0.065

Fig. 1. Schematic physical map of *CHGB*. (A) Polymorphisms of *CHGB* investigated in this study. Black blocks indicate coding exons; white blocks, 5′- and 3′-untranslated regions. First base of translation site is denoted as nucleotide +1. (B) LDs among *CHGB* polymorphisms. (C) Haplotypes (*hts*) of *CHGB* in a Korean population. *CHGB, chromogranin B;* LD, linkage disequilibrium.

this study (such as allele, position, MAF, heterozygosity, and Hardy-Weinberg equilibrium) is presented in Table 1. LDs among SNPs were measured by calculating |D'| and r^2 values. Among the investigated genetic variants, one SNP (rs236137) was excluded from the LD block construction due to its low frequency (MAF <5%), whereas the other SNPs with MAF over 5% were used for LD block construction (Fig. 1B). The LD block was composed of five major *ht*s with a frequency over 5% (Fig. 1C). Since the haplotype *ht5* was equivalent to rs74621755, *ht5* was excluded from further statistical analysis.

Associations of *CHGB* SNPs and haplotypes with schizophrenia

In order to investigate associations between *CHGB* genetic variants and schizophrenia in a Korean population, logistic regression analysis was performed. As a result, four SNPs, rs446659, rs6133278 (Asp145Asn), rs910122 (Arg178Gln), and rs2821, showed statistically significant associations with the risk of schizophrenia under the co-dominant model [minimum p=0.002 and odds ratio (OR)=0.72 at rs2821] (Table 2). Although significance at rs446659 and rs6133278 disappeared, significant signals at rs910122 and rs2821 were retained even after correction for multiple testing (p_{corr}<0.05). Due to different prevalences between men and women in schizophrenia, additional statistical analyses in both sexes were performed to determine the effect of sex on disease susceptibility. Interestingly, different significances in genetic associations were observed according to sex: five polymorphisms (rs236141, rs446659, rs6085323, rs910122, and rs2821) and one haplotype (*ht4*) showed significant associations with schizophrenia in male subjects (minimum p=0.002 and OR=0.64 at rs2821) (Table 2), even after the correction for multiple testing (minimum p_{corr}= 0.02 at rs2821). However, no significant association was observed in female subjects (p>0.05).

In silico analysis

In order to predict the functional impact of the significantly associated polymorphisms (rs446659, rs6085323, rs6133278, rs910122, and rs2821; p<0.05) (Table 2), *in silico* analyses were performed. Among the investigated polymorphisms, several

Table 1. Information of *CHGB* Polymorphisms among Subjects Investigated in This Study (n=914)

SNP	Allele	Position	AA change	Genotype			MAF	Heterozygosity	HWE		
				C/C	C/R	R/R			Patients	Controls	Total
rs236137	C>T	Promoter		689	212	10	0.127	0.222	0.668	0.048	0.155
rs236139	C>T	Promoter		304	458	147	0.414	0.485	0.438	0.362	0.243
rs236141	T>A	Promoter		746	160	8	0.096	0.174	0.273	0.352	0.857
rs16991480	T>C	5′ UTR (exon 1)		513	344	57	0.251	0.376	0.294	0.574	0.947
rs76791154	A>G	Intron 2		514	342	58	0.251	0.376	0.294	0.440	0.912
rs236145	C>G	Intron 2		408	382	111	0.335	0.446	0.521	0.035	0.144
rs446659	C>G	Intron 3		306	435	166	0.423	0.488	0.849	0.701	0.601
rs6085323	C>T	Intron 3		766	140	8	0.085	0.156	0.107	0.710	0.569
rs6085324	T>A	Exon 4	Ser93Thr	302	463	148	0.416	0.486	0.414	0.269	0.184
rs6133278	G>A	Exon 4	Asp145Asn	421	376	115	0.332	0.444	0.890	0.012	0.032
rs910122	A>G	Exon 4	Arg178Gln	288	442	178	0.439	0.493	0.742	0.970	0.719
rs881118	A>C	Exon 4	Asn200His	669	219	26	0.148	0.253	0.158	0.431	0.121
rs742711	G>A	Exon 4	Arg417His	297	459	148	0.418	0.486	0.335	0.323	0.187
rs74621755	G>A	Exon 4	Arg500Lys	781	122	11	0.079	0.145	0.011	0.192	0.015
rs2821	C>A	3′ UTR (exon 5)		303	432	177	0.431	0.490	0.340	0.691	0.302

AA, amino acid; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; UTR, untranslated region; *CHGB, chromogranin B.*
C/C, C/R, and R/R refer to the common homozygote, heterozygote, and minor homozygote, respectively.

functional motifs were predicted on/nearby two non-synonymous SNPs (rs6133278 and rs910122) (Table 3) using the ELM resource for functional site prediction program (http://elm.eu.org/index.html). In particular, a motif (MOD_PIKK_1), including rs6133278 (D145N) is known as a phosphorylation site for phosphatidylinositol 3-kinase-related kinase (PIKK) family members that are recently implicated in brain development and functioning.

DISCUSSION

Schizophrenia is a severe mental disorder that affects one's behaviors, emotions, and thinking. In addition, schizophrenia is considered to be a complex disorder affected by genetic, neurobiological, and environmental factors. Concentration of neurotransmitters (in particular, high dopamine, and low glutamate levels) has been considered as an important link between schizophrenia and altered brain function in terms of neurobiological process.[18,19] In addition, several studies have provided evidence that CHGB, a presynaptic protein that influences neurotransmitter release in neuroendocrine system, is differentially regulated in several brain neurological disorders, including schizophrenia.[20-23] Recently, genetic associations on several neurotransmission related genes (*Dysbindin, NRG1, DAOA, COMT, DISC1*, and *CHGB*) with schizophrenia have been highlighted.[24,25] However, the exact causes of schizophrenia are not yet fully understood.

Among the proteins that participate in the neurotransmitter release process, CHGB has been reported to be associated with schizophrenia. The CHGB protein, which is also known as secretogranin I, is a widespread constituent of large dense-core vesicles and induces dopamine release by affecting the exocytosis of secretory pathway of hormones and/or neuropeptides.[26] The correlation between chromogranin and dopamine in schizophrenia has been emphasized in recent findings. A recent *in vivo* neuroimaging study has suggested that schizophrenia patients have increased synaptic dopamine concentrations in the brain when compared with a control group.[27] Although an *in vitro* study reported that dopamine release might be increased by CHGB in a dose-dependent manner,[28] another study also revealed that levels of CHGB were lower in the cerebrospinal fluid from schizophrenia patients than from healthy controls.[20] Considering these previous reports and our results, the genetic effects induced by *CHGB* variants on schizophrenia in this study (minimum p_{corr}=0.02, OR=0.72 at rs2821) might have the possibility of being related to dopamine levels by influencing neurotransmission pathway.

Post-translational processes (such as phosphorylation, methylation, and glycosylation) that regulate protein function can be influenced by genetic variants, inducing an amino acid change.[29,30] According to a previous report that examined changes in phosphorylation status induced by SNPs at a genome-wide level, the non-synonymous *CHGB* variant of rs910122 (Arg178Gln) was reported to be a phospho-variant affecting adjacent CHGB phosphorylation sites by altering kinase activity.[31] According to the study, the amino acid change from arginine to glutamine at *CHGB* rs910122 was shown to inhibit v-akt murine thymoma viral oncogene homolog kinase binding on nearby a serine residue at amino acid position 183 of CHGB. This result suggests that the non-synonymous variation may have a cis-acting effect on subsequent amino acid phosphorylation of CHGB, resulting in a considerable susceptibility to schizophrenia (OR=0.65 at rs910122). In addition, other pro-

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Table 2. Associations of *CHGB* Polymorphisms with Schizophrenia

Loci	All subjects					Gender group								
						Male					Female			
	MAF		OR (95% CI)	*p* value	p_{corr}*	MAF		OR (95% CI)	*p* value	p_{corr}*	MAF		OR (95% CI)	*p* value
	Case (n=310)	Control (n=604)				Case (n=185)	Control (n=254)				Case (n=125)	Control (n=350)		
rs236137	0.117	0.132	0.87 (0.63–1.18)	0.36	-	0.104	0.144	0.67 (0.43–1.05)	0.08	-	0.136	0.124	1.13 (0.73–1.75)	0.59
rs236139	0.434	0.403	1.11 (0.90–1.36)	0.34	-	0.449	0.404	1.22 (0.92–1.63)	0.17	-	0.412	0.402	0.99 (0.73–1.35)	0.96
rs236141	0.089	0.100	0.82 (0.58–1.16)	0.26	-	0.081	0.118	0.63 (0.39–1.01)	0.05	-	0.100	0.087	1.11 (0.68–1.82)	0.67
rs16991480	0.229	0.262	0.86 (0.68–1.09)	0.21	-	0.227	0.258	0.85 (0.62–1.18)	0.34	-	0.232	0.264	0.87 (0.62–1.23)	0.43
rs76791154	0.229	0.262	0.86 (0.68–1.09)	0.22	-	0.227	0.258	0.85 (0.62–1.18)	0.34	-	0.232	0.264	0.87 (0.62–1.23)	0.43
rs236145	0.304	0.352	0.83 (0.67–1.03)	0.09	-	0.306	0.338	0.88 (0.66–1.18)	0.39	-	0.300	0.362	0.78 (0.57–1.07)	0.12
rs446659	0.383	0.443	0.78 (0.63–0.96)	0.02†	NS	0.380	0.451	0.74 (0.55–0.98)	0.04†	NS	0.388	0.438	0.82 (0.61–1.11)	0.19
rs6085323	0.079	0.089	0.80 (0.56–1.16)	0.24	-	0.073	0.110	0.60 (0.36–0.98)	0.04†	NS	0.088	0.073	1.14 (0.68–1.91)	0.61
rs6085324	0.442	0.402	1.17 (0.95–1.44)	0.14	-	0.446	0.396	1.26 (0.94–1.68)	0.12	-	0.436	0.407	1.08 (0.80–1.46)	0.61
rs6133278	0.293	0.352	0.78 (0.64–0.97)	0.02†	NS	0.296	0.343	0.83 (0.62–1.10)	0.20	-	0.288	0.360	0.74 (0.54–1.01)	0.06
rs910122	0.392	0.464	0.74 (0.60–0.91)	0.004†	0.04†	0.381	0.482	0.65 (0.49–0.87)	0.003†	0.04†	0.408	0.451	0.85 (0.63–1.14)	0.28
rs881118	0.166	0.139	1.28 (0.97–1.68)	0.08	-	0.173	0.126	1.42 (0.98–2.08)	0.07	-	0.156	0.149	1.14 (0.77–1.69)	0.52
rs742711	0.445	0.403	1.18 (0.96–1.46)	0.12	-	0.446	0.396	1.25 (0.94–1.68)	0.13	-	0.444	0.408	1.10 (0.81–1.50)	0.52
rs74621755	0.065	0.086	0.74 (0.51–1.09)	0.13	-	0.070	0.079	0.90 (0.55–1.48)	0.69	-	0.056	0.091	0.57 (0.31–1.05)	0.07
rs2821	0.378	0.458	0.72 (0.59–0.88)	0.002†	0.02†	0.370	0.476	0.64 (0.48–0.85)	0.002†	0.02†	0.391	0.444	0.81 (0.61–1.09)	0.17
Frequency														
ht1	0.422	0.387	1.13 (0.92–1.40)	0.24	-	0.430	0.386	1.21 (0.91–1.61)	0.20	-	0.411	0.388	1.06 (0.78–1.43)	0.73
ht2	0.223	0.245	0.91 (0.72–1.15)	0.43	-	0.219	0.244	0.88 (0.63–1.21)	0.42	-	0.230	0.246	0.95 (0.67–1.34)	0.75
ht3	0.155	0.129	1.28 (0.97–1.69)	0.08	-	0.159	0.114	1.42 (0.96–2.09)	0.08	-	0.149	0.140	1.15 (0.77–1.72)	0.50
ht4	0.073	0.088	0.76 (0.52–1.10)	0.14	-	0.062	0.108	0.52 (0.31–0.88)	0.01†	NS	0.089	0.073	1.15 (0.69–1.93)	0.60

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
p value and OR are analyzed under the co-dominant model. Logistic analysis is adjusted by age and sex as covariates in case-control analysis and by age in gender group analysis. *Haplotype 5 (ht5)* is equivalent to rs74621755.
*p_{corr} value after multiple testing corrections with the effective number (10.9139) of independent marker loci, †Statistical significance of $p<0.05$.

Table 3. Motif Search Analysis of rs6133278 and rs910122

SNP (amino acid change)	Amino acid position in CHGB	Matched sequence		Motif (ELM name)	Description
		Including SNP site	Close to SNP site		
rs6133278 (D145N)	137-142	-	PQWSLY	MOD_NEK2_2	NEK2 phosphorylation motif
	137-144	-	PQWSLYPS	MOD_GSK3_1	GSK3 phosphorylation recognition site
	143-149	PSDSQVS	-	MOD_PIKK_1	PIKK family members phosphorylation site
	146-152	-	SQVSEEV	MOD_CK1_1	CK1 phosphorylation site
rs910122 (R178Q)	179-185	-	GEDSSEE	MOD_CK2_1	CK2 phosphorylation site

ELM, eukaryotic linear motif; SNP, single nucleotide polymorphism; *CHGB, chromogranin B.*
Motif search analysis is performed using the ELM resource for functional site prediction program.

tein kinases of PIKKs that were predicted to phosphorylate the motif including rs6133278 (Asp145Asn) in our *in silico* analysis (Table 3) have been reported to be associated with several neural activities, such as neuronal development and synaptic plasticity.[32,33] Among the PIKKs, the mammalian target of rapamycin kinase signaling is related to several cognitive disorders, including schizophrenia.[34] Considering evidence that shows different phosphorylation patterns between schizophrenia patients and healthy controls,[35] regulation of CHGB phosphorylation sites may play a role in schizophrenia development.

Previously, several studies have reported gender-specific differences in prevalence and development of schizophrenia.[3,36] Moreover, gender-specific genetic markers in schizophrenia (such as rs2812393 and rs821616 of *DISC1*; rs175174 of *ZDHHC8*, for female patients) have been examined in recent genetic association studies.[37,38] Including the previously reported phospho-variant rs910122,[31] different genetic effects of *CHGB* variants on the risk of schizophrenia were also observed depending on sex in this study (Table 2). Furthermore, considering that *CHGB* rs236141 with a nominal association with schizophrenia (p=0.05 and OR=0.63 in male subjects) has been found to be a gender-specific marker for blood pressure disease,[39] our findings suggest that *CHGB* could be a gender-specific marker for schizophrenia development.

In other studies on Chinese and Japanese populations for the association between *CHGB* SNPs and schizophrenia susceptibility,[9,13] rs910122 was observed as a consistent association signal with schizophrenia susceptibility in a Chinese population with significance, but not in a Japanese population. The rs2821 also showed significance in Chinese and Korean schizophrenia patients, but not in a Japanese population (Supplementary Table 2, only online). Instead, other *CHGB* SNPs were found to have significant signals in the Japanese patients, suggesting variations in *CHGB* may have different genetic effects depending on ethnicity.

There are study limitations of an insufficient number of samples and different proportions in age range (23–73 vs. 8–84) and sex ratio (M/F=185/125 vs. 254/350) between patients and controls, respectively, as well as no functional evaluation. Although logistic analysis of this study was adjusted by sex and age as covariates in case-control analysis and by age in gender group analysis, we also do not rule out a possibility that the significant association signals may have resulted from the study limitations. However, considering the different incidences of schizophrenia according to sex and age (such as male: female ratio of ~1.4:1 and earlier mean age at onset for schizophrenia in males),[2,40] further replication studies in a larger cohort may be required.

In summary, this study replicated the association between *CHGB* polymorphisms and schizophrenia susceptibility. To our knowledge, this is the first study to identify the potential sex-specific association of *CHGB* genetic variants with schizophrenia. Despite study limitations, our findings may provide useful information on the genetic etiology of schizophrenia.

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