Genome-Wide Variants Shared Between Smoking Quantity and Schizophrenia on 15q25 Are Associated With *CHRNA5* Expression in the Brain

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Cigarette smokers with schizophrenia consume more cigarettes than smokers in the general population. Schizophrenia and smoking quantity may have shared genetic liability. Genome-wide association studies (GWASs) of schizophrenia and smoking quantity have highlighted a biological pleiotropy in which a robust 15q25 locus affects both traits. To identify the genetic variants shared between these traits on 15q25, we used summary statistics from large-scale GWAS meta-analyses of schizophrenia in the Psychiatric Genomics Consortium 2 and smoking quantity assessed by cigarettes smoked per day in the Tobacco and Genetics Consortium. To evaluate the regulatory potential of the shared genetic variants, expression quantitative trait loci analysis in 10 postmortem brain regions was performed using the BRAINEAC dataset in 134 neuropathologically normal individuals. Twenty-two genetic variants on 15q25 were associated with both smoking quantity and schizophrenia at the genome-wide significance level ($P < 5.00 \times 10^{-8}$). Major alleles of all variants were associated with higher smoking quantity and risk of schizophrenia. These genetic variants were associated with *PSMA4*, *CHRNA3*, and *CHRNA4* expression in specific brain regions (lowest $P = 4.81 \times 10^{-4}$) and with *CHRNA5* expression in multiple brain regions (lowest $P = 8.70 \times 10^{-6}$). Risk-associated major alleles of these variants were commonly associated with higher expression in several brain regions, excluding the medulla, at the transcript level. In addition, the risk-associated major allele at rs637137 was associated with higher *CHRNA5* expression at the specific exon level in multiple brain regions (lowest $P = 2.37 \times 10^{-5}$). Our findings suggest that genome-wide variants shared between smoking quantity and schizophrenia contribute to a common pathophysiology underlying these traits involving altered *CHRNA5* expression in the brain.

Key words: schizophrenia/number of cigarettes smoked per day/gene expression/GWAS/*CHRNA5*/postmortem brain

Introduction

The prevalence of cigarette smoking in patients with schizophrenia is estimated to be 2- to 5-fold higher than in the general population worldwide.^{1,2} In addition, smokers with schizophrenia consume larger quantities of cigarettes than smokers in the general population.³ Although schizophrenia is highly comorbid with cigarette smoking and nicotine dependence,⁴ the underlying neurobiological basis of this comorbidity is unknown. The self-medication hypothesis suggests that patients with schizophrenia smoke to alleviate their psychiatric symptoms and cognitive impairments and/or antipsychotic-induced side effects.^{5,6} However, in addition to being a consequence of self-medication, smoking may also be a causal factor in the etiology of schizophrenia.⁷

Schizophrenia, nicotine dependence, and smoking-related behaviors have a high estimated heritability of approximately 50%–80%.^{8–10} Large-scale genome-wide association study (GWAS) meta-analyses have identified risk genes for many complex human diseases and traits, including schizophrenia, nicotine dependence, and smoking-related behaviors such as smoking quantity.^{11–13} Smoking quantity as estimated by the number of cigarettes smoked per day (CPD) has been widely used in genetic association studies because heavy smoking is commonly considered a proxy for nicotine dependence.^{11,14,15} Among smoking-related phenotypes (CPD, ever/never smoked, age at initiation, and cessation), associations between 3 genomic loci—15q25, 10q25, and 9q13—and CPD have been revealed in the largest GWAS meta-analysis to

date from the Tobacco and Genetics Consortium (TAG) ($n > 140\,000$).¹¹ In contrast, the largest GWAS meta-analysis from the Schizophrenia Working Group of the Psychiatric Genomics Consortium 2 (PGC2), using 36 989 patients with schizophrenia and 113 075 controls, has identified 108 distinct genomic loci related to the risk of schizophrenia.¹² Polygenic risk score and linkage disequilibrium (LD) score regression analyses from these large GWASs have indicated a genetic correlation between greater smoking quantity and risk of schizophrenia,^{16–18} consistent with the well-known comorbidity between schizophrenia and nicotine dependence. Therefore, schizophrenia and smoking quantity may have shared genetic liability that predisposes individuals to both traits. Remarkably, a robust 15q25 locus that includes the *CHRNA5-CHRNA3-CHRNA4* nicotinic acetylcholine receptor (nAChR) gene cluster is commonly associated with both smoking quantity and schizophrenia. Associations between smoking quantity and other major psychiatric disorders such as bipolar disorder, major depression disorder, autism spectrum disorder, and attention deficit hyperactivity disorder have never been detected at a genome-wide significance level.¹⁹ Therefore, schizophrenia is the only one psychiatric disorder that has strong association with smoking risk variants.

Pleiotropy is the phenomenon in which a single locus or gene affects multiple phenotypes.^{20,21} The molecular mechanisms of pleiotropy can be dichotomized into multiple molecular functions of a single gene product and multiple consequences of a single molecular function.²⁰ Pleiotropy accounts for a subset of genetic mechanisms shared between human behaviors and diseases. A specific gene on 15q25 may affect smoking behavior and risk of schizophrenia, and nAChRs may be involved in the etiology of smoking quantity and schizophrenia.

Genetic variants identified by GWASs as being associated with common diseases and phenotypes are enriched in regulatory genomic regions,²² and genome-wide significant genetic variants may contribute to disease and phenotype susceptibility by altering the expression of the proxy gene.^{23,24} Brain function depends on the precise regulation of gene expression,²⁵ and gene expression is differentially regulated across brain regions.²⁶ Therefore, a critical step in the elucidation of the shared genetic basis of smoking quantity and schizophrenia is to investigate the spatial dynamics of the human brain transcriptome for genes at the 15q25 locus. Genome-wide significant genetic variants on 15q25 may mediate the risks of smoking quantity and schizophrenia by altering the expression of the nAChR gene cluster in a specific brain region.

In this study, we identified genetic variants on 15q25 shared between smoking quantity and schizophrenia at a genome-wide significance level. To reveal the molecular effects of these GWAS-implicated genetic variants on 15q25, we investigated the anatomical expression patterns of the proxy genes for genetic variants on 15q25

and whether these genetic variants affect expression at the exon and gene levels in comprehensive 10 human brain regions studied postmortem.

Materials and Methods

Subjects in TAG and PGC2

GWAS summary statistics from TAG¹¹ and PGC2¹² were available directly in a public database (<https://www.med.unc.edu/pgc/results-and-downloads>). Genetic variants on 15q25 associated with both smoking quantity and risk of schizophrenia were examined using the TAG and the PGC2 datasets.

A detailed description of genotyping, quality control (QC), and imputation from TAG¹¹ and PGC2¹² is described in [Supplementary methods](#). TAG assessed smoking quantity by measuring CPD and performed a GWAS of smoking quantity among 38 181 subjects of European ancestry. Imputed allele dosages for each genetic variant were tested for association with smoking quantity under an additive linear regression model. The dependent variable was smoking quantity, and the independent variables were the imputed allele dosage for a genetic variant plus an indicator variable for whether a subject was classified as a case in the primary study, which adjusted for case status to reduce potential confounding. The analysis was performed separately for males and females. A fixed-effect meta-analysis for smoking quantity was performed on the TAG studies¹¹.

The primary GWAS samples from the Schizophrenia Working Group of PGC2 consisted of 32 405 patients with schizophrenia and 42 221 controls of European ancestry, 1 836 patients with schizophrenia and 3 383 controls from East Asia, and 1235 parent affected–offspring trios of European ancestry.¹² Criteria from the *Diagnostic and Statistical Manual of Mental Disorders* and the *International Classification of Diseases* were used to diagnose these patients. In each GWAS dataset, association testing under an additive logistic regression model was conducted using imputed marker dosages as independent variables, diagnostic status as a dependent variable, and principal components as covariates. The results were combined using an inverse-weighted fixed-effects model.

Postmortem Brain Tissue Samples

Associations of the smoking-quantity- and schizophrenia-associated genetic variants with the expression of 5 genes on 15q25 were examined in 10 postmortem brain regions using the BRAINEAC dataset from the UK Brain Expression Consortium (<http://www.braineac.org/>²⁷). Brain tissues from 134 neuropathologically normal individuals of European ancestry free of neurodegenerative disorders were collected by the Medical Research Council Sudden Death Brain and Tissue Bank, United Kingdom, and the Sun Health Research Institute, United States,

as previously described.^{28,29} A detailed description of the samples, tissue processing, and dissection has been previously described.³⁰ To cover a broad range of brain areas, 10 brain regions, including cerebellar cortex (CRBL), frontal cortex (FCTX), hippocampus (HIP), medulla (MEDU), occipital cortex (OCTX), putamen (PUTM), substantia nigra (SNIG), temporal cortex (TCTX), thalamus (THAL), and intralobular white matter (WHMT), were extracted per brain. A detailed description of mRNA quantification, genotyping, QC, and imputation were described in [Supplementary methods](#).

Informed consent was obtained from all participants and/or their families in each database. This study was performed according to the World Medical Association's Declaration of Helsinki and was approved by the Research Ethical Committee of Kanazawa Medical University.

Statistical Analyses

Pairwise LD analyses, expressed in terms of r^2 and D' , were applied to investigate intermarker relationships for the 22 variants in the 1000 Genomes Project Phase 1 Utah residents with Northern and Western European ancestry (CEU) population using SNP Annotation and Proxy Search (SNAP; <http://archive.broadinstitute.org/mpg/snap/ldsearch.php>). Pairwise LD correlations among the 22 genetic variants in the BRAINEAC samples were analyzed using Haploview 4.2 (<https://www.broadinstitute.org/haploview/haploview>). The number of effectively independent genetic variants among the 22 genetic variants in the BRAINEAC samples was estimated with correction for multiple testing according to the spectral decomposition method of Nyholt using SNPSpD³¹ and the TAGGER algorithm (<http://www.broad.mit.edu/mpg/tagger>) with a criterion of r^2 greater than 0.8 in “pair-wise

tagging only” mode (implemented in Haploview 4.2). The effective number of independent marker loci was 3 in both methods. Therefore, the P -values for the following analyses were adjusted using the Bonferroni correction. P -values less than .017 ($\alpha = .05/3$ independent genetic variants) were considered to be nominally significant. Furthermore, P -values less than 5.00×10^{-3} ($\alpha = .05/10$ brain regions) were considered to be significant to more stringently control type I error.

The following statistical analyses were performed using R 3.1.1 (<http://www.r-project.org/>) and IBM SPSS 24.0 software (IBM Japan, Tokyo, Japan). Differences in the expression of each gene among the 10 brain regions were analyzed using a repeated-measures ANOVA with the expression levels of each gene as the repeated-measures factor and brain region as the within-subjects factor. The expression quantitative trait loci (eQTL) analysis in the 10 brain regions was run for each expression profile (either gene level or exon level) against every genetic variant under a linear regression model, with each profile's expression as the dependent variable and allele dosage for each genetic variant as the independent variable.

Results

Genetic Variants Associated With Both Smoking Quantity and Schizophrenia

We first searched for genetic variants associated with both smoking quantity and risk of schizophrenia in TAG and PGC2 at the 15q25 locus (± 200 kb) peripheral to rs12914385, which has been reported to have the strongest association with smoking quantity¹¹ ([figure 1](#)). The regions (± 200 kb peripheral to rs12914385) encompassed chr15:78803032-78926732 (hg19) in PGC2. In

Fig. 1. Regional association plot of 15q25 locus for smoking quantity (red circles) and risk of schizophrenia (blue circles). P -values ($-\log_{10}$) are shown in regions peripheral to rs12914385 (± 200 kb). The genetic variants most strongly associated with smoking quantity and schizophrenia are labeled. The red line shows the genome-wide significance level (P -value = 5.00×10^{-8}).

the genomic region, 121 genetic variants were associated with smoking quantity at a genome-wide significance level ($4.03 \times 10^{-8} \leq P \leq 4.23 \times 10^{-35}$), while 82 genetic variants were associated with risk of schizophrenia at this level ($3.98 \times 10^{-8} \leq P \leq 1.87 \times 10^{-12}$). Due to differences in genotyping and imputation methods between consortia, several genetic variants obtained from PGC2 were not obtained from TAG [60/82 single nucleotide polymorphisms (SNPs)]. In contrast, all genetic variants, except for rs4887082, used in TAG were present among the genetic variants used in PGC2 (120/121 SNPs). Of the 120 genetic variants, 22 were associated with both smoking quantity and risk of schizophrenia at the genome-wide significance level ($P < 5.00 \times 10^{-8}$; [table 1](#)). Major alleles of all 22 genetic variants were associated with higher smoking quantity and risk of schizophrenia. The most significant smoking quantity-associated genetic variant on 15q25, rs12914385, was not associated with risk of schizophrenia at a genome-wide significance level (odds ratio [OR] = 1.052, $P = 2.15 \times 10^{-6}$), whereas the most significant schizophrenia-associated genetic variant, rs8042374, was associated with smoking quantity at a genome-wide significance level ($\beta = .981$, $P = 2.38 \times 10^{-24}$). The LD relationships among the 22 genetic variants in the CEU population are shown in [figure 2](#). These genetic variants, except for 2 genetic variants (rs518425 and rs12443170), were in high LD. These genetic variants were located on or around 5 genes: *HYKK*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4*.

Spatial Expression Patterns of the 5 Genes in 10 Brain Regions

We next investigated the spatial expression patterns of *HYKK*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4* in 10 brain regions postmortem: CRBL, FCTX, HIP, MEDU, OCTX, PUTM, SNIG, TCTX, THAL, and WHMT ([figure 3](#)). All genes were highly differentially expressed among brain regions (*HYKK*, $F_9 = 83.3$, $P < 2.0 \times 10^{-16}$; *PSMA4*, $F_9 = 15.8$, $P < 2.0 \times 10^{-16}$; *CHRNA5*, $F_9 = 31.4$, $P < 2.0 \times 10^{-16}$; *CHRNA3*, $F_9 = 317.9$, $P < 2.0 \times 10^{-16}$; and *CHRNA4*, $F_9 = 33.3$, $P < 2.0 \times 10^{-16}$).

Effects of Smoking-Quantity- and Schizophrenia-Associated Genetic Variants on Gene Expression in 10 Brain Regions

We investigated the effects of 22 genetic variants associated with both smoking quantity and schizophrenia on the expression of *HYKK*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4* on 15q25 in 10 brain regions. These genetic variants were associated with *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4* expression in specific brain regions ($P < 0.017$ and $P < 5.00 \times 10^{-3}$;

[figure 4](#)). These genetic variants were associated with *PSMA4* expression only in FCTX and WHMT (lowest $P = 3.80 \times 10^{-3}$); *CHRNA5* expression in 8 brain regions (lowest $P = 8.70 \times 10^{-6}$), excluding MEDU and THAL; *CHRNA3* expression only in MEDU and PUTM (lowest $P = 4.81 \times 10^{-4}$); and *CHRNA4* expression only in MEDU (lowest $P = 6.83 \times 10^{-4}$). Risk-associated major alleles of these genetic variants were commonly associated with higher expression than nonrisk minor alleles in several brain regions ([figure 4](#), red colors), whereas the opposite association was detected only in MEDU ([figure 4](#), blue colors). The genetic variant rs12443170, which did not have a high LD with the other 21 genetic variants, was not associated with the expression of any gene ($P > .017$). None of the genetic variants were associated with *HYKK* expression ($P > .017$).

Effects of Smoking-Quantity- and Schizophrenia-Associated Genetic Variants on CHRNA5 Exon Expression in the Brain

As these genetic variants were broadly associated with *CHRNA5* expression in 8 brain regions, excluding MEDU and THAL, we further focused on *CHRNA5* exon-specific expression. The *CHRNA5* gene spans 29.8 kb and has 6 exons and 4 transcripts: *CHRNA5-001* (reference sequence ENST00000299565.5), *CHRNA5-002* (ENST00000394802.4), *CHRNA5-003* (ENST00000559554.1), and *CHRNA5-004* (ENST00000559576.1) ([figure 5A](#)). *CHRNA5-001* and *CHRNA5-003* have 6 exons, *CHRNA5-002* has 5 exons, and *CHRNA5-004* has 2 exons. In the genomic region located on *CHRNA5*, antisense RP11-650L12.2-001 (ENST00000567141.1) is located around exon 5 of *CHRNA5*. On the basis of genotyping data for the 22 variants in the BRAINEAC samples, 3 LD-tagging genetic variants were selected. The 3 tagging SNPs were rs637137, rs518425, and rs12443170. Remarkably, rs637137 captured the other 19 genetic variants. Although rs518425 and rs12443170 were not associated with *CHRNA5* expression in brain regions ([figure 4](#)), these 2 genetic variants were used as negative control. Of these 3 genetic variants, rs637137 was specifically associated with *CHRNA5* exon expression ($P < .017$, lowest $P = 2.37 \times 10^{-5}$, exon-specific probesets 3–8 and 11, [figures 5B and 5C](#)) in 8 brain regions, excluding MEDU and THAL. The risk-associated major allele was associated with higher expression than the nonrisk minor allele. This genetic variant was not associated with the 5' untranslated region (5' UTR) (probeset 1), exon 1 (probeset 2), or the 3' UTR (probeset 12) of *CHRNA5* or RP11-650L12.2-001-specific probesets (9 and 10) in any brain regions ($P > .017$). These findings suggest that genome-wide variants shared between smoking quantity and schizophrenia may be preferentially associated with expression of the *CHRNA5-002* transcript.

Table 1. Genetic Variants Associated With Both Smoking Quantity and Risk of Schizophrenia at the Genome-Wide Significance Level

GWAS Rank			TAG			PGC2			Gene		
TAG	PGC2	SNP ID	Bp	A1/A2	FRQ_A1	$\beta \pm SE$	P	OR $\pm SE$	P	Closest	
45	76	rs9788682	78802586	A/G	0.22	-1.005 ± 0.101	2.44×10^{-23}	0.908 ± 0.013	1.88×10^{-8}	HYKK Intron	
34	71	rs7164594	78803057	T/C	0.22	-1.009 ± 0.099	3.10×10^{-24}	0.907 ± 0.013	1.26×10^{-8}	HYKK Intron	
29	47	rs7163730	78814681	A/G	0.78	1.010 ± 0.099	2.21×10^{-24}	1.078 ± 0.013	2.84×10^{-9}	HYKK Intron	
32	51	rs4461039	78817447	A/T	0.78	1.007 ± 0.099	2.59×10^{-24}	1.077 ± 0.013	3.23×10^{-9}	HYKK Intron	
44	60	rs2036534	78826948	T/C	0.78	0.979 ± 0.098	1.49×10^{-23}	1.077 ± 0.013	4.06×10^{-9}	HYKK 3' UTR	
43	42	rs3813570	78832832	T/C	0.77	0.994 ± 0.099	1.24×10^{-23}	1.079 ± 0.013	1.86×10^{-9}	PSMA4 5' UTR	
25	37	rs684513	78858400	C/G	0.77	1.040 ± 0.101	5.87×10^{-25}	1.085 ± 0.013	7.88×10^{-10}	CHRNA5 Intron	
36	34	rs667282	78863472	T/C	0.76	0.995 ± 0.098	3.65×10^{-24}	1.081 ± 0.012	4.11×10^{-10}	CHRNA5 Intron	
38	24	rs637137	78873976	A/T	0.24	-0.987 ± 0.098	5.32×10^{-24}	0.925 ± 0.012	2.33×10^{-10}	CHRNA5 Intron	
75	29	rs518425	78883813	A/G	0.72	0.802 ± 0.092	2.11×10^{-18}	1.077 ± 0.012	3.24×10^{-10}	CHRNA5 Intron	
28	20	rs12910984	78891627	A/G	0.77	0.983 ± 0.096	1.84×10^{-24}	1.083 ± 0.012	1.46×10^{-10}	CHRNA3 Intron	
21	2	rs3743078	78894759	C/G	0.23	-0.997 ± 0.096	4.22×10^{-25}	0.916 ± 0.012	2.03×10^{-12}	CHRNA3 Intron	
27	28	rs938682	78896547	A/G	0.77	0.984 ± 0.096	1.76×10^{-24}	1.081 ± 0.012	3.10×10^{-10}	CHRNA3 Intron	
19	22	rs11637630	78899719	A/G	0.77	0.998 ± 0.096	3.95×10^{-25}	1.082 ± 0.012	1.90×10^{-10}	CHRNA3 Intron	
20	8	rs7177514	78907406	C/G	0.77	0.998 ± 0.096	3.95×10^{-25}	1.090 ± 0.012	3.65×10^{-12}	CHRNA3 Intron	
23	6	rs6495308	78907656	T/C	0.77	0.997 ± 0.096	4.69×10^{-25}	1.091 ± 0.012	2.95×10^{-12}	CHRNA3 Intron	
87	68	rs12443170	78907736	A/G	0.14	-1.157 ± 0.134	7.34×10^{-18}	0.906 ± 0.017	1.04×10^{-8}	CHRNA3 Intron	
22	7	rs8042059	78907859	A/C	0.77	0.997 ± 0.096	4.57×10^{-25}	1.090 ± 0.012	3.35×10^{-12}	CHRNA3 Intron	
30	1	rs8042374	78908032	A/G	0.77	0.981 ± 0.096	2.38×10^{-24}	1.091 ± 0.012	1.87×10^{-12}	CHRNA3 Intron	
49	10	rs4887069	78909070	A/G	0.77	0.982 ± 0.100	6.52×10^{-23}	1.087 ± 0.013	1.83×10^{-11}	CHRNA3 Intron	
48	27	rs6495309	78915245	T/C	0.22	-0.972 ± 0.098	4.51×10^{-23}	0.923 ± 0.013	2.95×10^{-10}	CHRNA3 Intergenic	
39	63	rs12440014	78926726	C/G	0.74	0.996 ± 0.099	6.02×10^{-24}	1.077 ± 0.013	5.53×10^{-9}	CHRNA4 Intron	

Note: GWAS, genome-wide association study; TAG, Tobacco and Genetics Consortium; PGC, Psychiatric Genomics Consortium; SNP, single nucleotide polymorphism; Bp, nucleotide location; A, allele; FRQ, frequency; SE, standard error of β or odds ratio (OR); *HYKK*, hydroxylase kinase; *PSMA4*, proteasome subunit alpha 4; *CHRNA5*, cholinergic receptor nicotinic alpha 5 subunit; *CHRNA3*, cholinergic receptor nicotinic alpha 3 subunit; *CHRNA4*, cholinergic receptor nicotinic beta 4 subunit. *P*-values $< 5.00 \times 10^{-8}$ are shown in boldface.

Fig. 2. Linkage disequilibrium (LD) structure for all combinations of the 22 genetic variants associated with both smoking quantity and schizophrenia. For each pair of genetic variants, the D' in the Utah residents with Northern and Western European ancestry (CEU) samples in the 1000 Genomes Project is shown below the diagonal and the r^2 is shown above the diagonal. High levels of LD are represented by red (r^2) and blue (D') colors, with increasing color intensity shown by the color bars. Bp, nucleotide location. The 3 genetic variants in boldface are tagged among the 22 genetic variants.

Fig. 3. Spatial expression patterns of the *HYKK*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4* genes in 10 brain regions studied postmortem. Box plots indicate degrees of each gene expression at each brain region. CRBL, cerebellar cortex; FCTX, frontal cortex; HIPP, hippocampus; MEDU, medulla; OCTX, occipital cortex; PUTM, putamen; SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus; WHMT, intralobular white matter.

Discussion

Of multiple loci identified by large-scale GWAS meta-analyses of smoking quantity and schizophrenia, 15q25 was a common genetic locus associated with these traits.

Twenty-two genetic variants, including 3 independent tagging variants, on 15q25 were associated with both traits at a genome-wide significance level. These genetic variants were located on or around 5 genes: *HYKK*,

Fig. 4. Influences of risk-associated major alleles for genetic variants associated with smoking quantity and schizophrenia on the expression of 5 genes (*HYKK*, *PSMA4*, *CHRNA5*, *CHRNA3*, and *CHRNA4*) on 15q25 in 10 brain regions. The color bar shows the β -values corresponding to the color in the figures. Red indicates that individuals with the risk-associated major allele showed higher expression than those with the nonrisk minor allele. β -values with nominal significant P -values ($\alpha = .05/3$) are shown with a thick border. * $P < 5.00 \times 10^{-3}$. CRBL, cerebellar cortex; FCTX, frontal cortex; HIPP, hippocampus; MEDU, medulla; OCTX, occipital cortex; PUTM, putamen; SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus; WHMT, intralobular white matter.

PSMA4, *CHRNA5*, *CHRNA3*, and *CHRNA4*. These genes were differentially expressed across brain regions. These genome-wide shared variants were associated with *PSMA4*, *CHRNA3*, and *CHRNA4* expression in specific brain regions and *CHRNA5* expression in many brain regions at the gene level. Risk-associated major alleles of these shared variants were commonly associated with higher expression in several brain regions, excluding the MEDU. Furthermore, the risk-associated major allele at rs637137 that was a tagging variant for these genetic variants was associated with higher *CHRNA5* expression at the specific exon level in several brain regions (CRBL, FCTX, HIPP, OCTX, PUTM, SNIG, TCTX, and WHMT). Our findings provide support for shared genetic variation between smoking quantity and schizophrenia and suggest that genome-wide shared variants contribute to the common etiology of these traits by altering *CHRNA5* expression in the brain.

Nicotine binds and activates nAChRs located on neurons in the brain's mesolimbic reward system.³² The reward system comprises dopaminergic neurons originated in the ventral tegmental area (VTA) and releases the neurotransmitter dopamine in regions such as the nucleus accumbens (NAc), HIPP, amygdala, and prefrontal cortex (PFC). Chronic nicotine exposure induces decreased dopamine and glutamate neurotransmission in the brain's reward system (VTA and NAc), which results

in the development of nicotine dependence. Varenicline tartrate, a partial $\alpha 4 \beta 2$ nAChR agonist, is approved as one of the smoking-cessation medications. The drug can promote smoking cessation by attenuating the reinforcing effects of nicotine in the mesolimbic reward system. However, the *CHRNA4* (20q13) and *CHRNA2* (1q21) genes that encode the $\alpha 4$ and $\beta 2$ nAChR subunits, respectively, are not included in the 15q25 locus. Considering that the *CHRNA5* is expressed in whole brain regions (Supplementary figure S1), brain regions other than mesolimbic reward system might contribute to the pathogenesis of schizophrenia and nicotine dependence. Cognitive functions that are impaired in patients with schizophrenia and nicotine dependence are modulated by nAChR activation by cholinergic inputs.³³ Acetylcholine (ACh) release plays a major role in the cognitive functions in PFC.³⁴ The *CHRNA5* gene encodes the $\alpha 5$ nAChR subunit. The $\alpha 5$ nAChR subunit is found in the PFC.³⁵ The dysfunction of ACh signaling via the $\alpha 5$ subunit-containing nAChRs in the PFC could contribute to diminished cognitive functions observed in schizophrenia and nicotine dependence.³⁶

The nonsynonymous variant (Asp398Asn; rs16969968) in *CHRNA5* has been reported as a functional genetic variant associated with smoking-related phenotypes in several genetic association studies.^{11,37,38} The aspartic acid (G allele; Asp) to asparagine (A; Asn) substitution is

Fig. 5. Influences of risk-associated major alleles of 3 genetic variants associated with smoking quantity and schizophrenia on *CHRNA5* expression at the exon level in 10 brain regions. **(A)** A schema of *CHRNA5* transcripts based on Ensembl GRCh37 release 91. The locations of exon-specific probesets are indicated by arrows (1–12). **(B)** P -values ($-\log_{10}$) for associations between exon expression in 10 brain regions and the 3 single nucleotide polymorphisms (SNPs) are shown. The blue line shows the nominal significance level ($\alpha = .05/3$, $P = .017$). The red line shows the significance level ($\alpha = .05/10$, $P = 5.00 \times 10^{-3}$). **(C)** The color bar shows the β -values corresponding to the color in the figures. Red indicates that individuals with the risk-associated major allele showed higher expression than those with the non-risk-associated minor allele. β -values with significant P -values ($\alpha = .05/3$) are marked with a thick border. $*P < 5.00 \times 10^{-3}$. CRBL, cerebellar cortex; FCTX, frontal cortex; HIPP, hippocampus; MEDU, medulla; OCTX, occipital cortex; PUTM, putamen; SNIG, substantia nigra; TCTX, temporal cortex; THAL, thalamus; WHMT, intralobular white matter.

associated with diminished nAChR function.³⁹ In addition, mice with altered gene function of the $\alpha 5$ nAChR (ie, an Asp codon converted to an Asn codon) exhibited PFC-dependent behavioral deficits in social interaction and sensorimotor gating.⁴⁰ In contradiction to the risk-associated major allele at rs637137, the risk Asn minor allele was associated with both high smoking quantity and risk of schizophrenia.³⁸ However, the Asp398Asn variant was not associated with schizophrenia at the genome-wide significance level in PGC2 (OR = 1.059, $P = 4.63 \times 10^{-7}$), although the Asp398Asn variant was strongly associated with smoking quantity in TAG ($\beta = 1.015$, $P = 4.50 \times 10^{-33}$). Although the nonsynonymous variant rs16969968 and 3 tagging variants including rs637137 are at the same genetic locus on 15q25, the LD between the rs16969968 and 3 tagging variants were weak ($r^2 = 0.11$ – 0.24 , $D' = 0.78$ – 1.00). This condition suggests that the associations at these variants are

statistically independent. Thus, we did not focus on the Asp398Asn variant for eQTL analyses due to its lack of association with schizophrenia at a genome-wide significance level (Supplementary figure S2). Further study is needed to dissociate the influences of the independent tagging variant rs637137 and the nonsynonymous variant on nAChR function.

The shared genetic variants were associated with *CHRNA5* expression in many brain regions including FCTX, TCTX, PUTM, and HIPP. The volumes and functions of these regions were impaired in patients with schizophrenia compared with healthy subjects.^{41–43} According to the Genotype-Tissue Expression (GTEx) database [GTEx Analysis Release V7 (dbGaP Accession phs000424.v7.p2), <https://www.gtexportal.org/home/gene/CHRNA5>], the *CHRNA5* is expressed in whole tissues without specificity (Supplementary figure S1). There was no difference in mean *CHRNA5* expression between

brain tissues and nonbrain tissues ($P > .05$). Although this study focused on effects of the genome-wide shared genetic variants on cerebral *CHRNA5* expression, the genetic variants may affect gene regulations in nonbrain tissues as well as brain tissues. Indeed, rs637137 was associated with *CHRNA5* expression in other nonbrain tissues, such as skeletal muscle and transformed fibroblasts, whereas associations between rs637137 and *CHRNA5* expression in several brain regions have been replicated with the same direction using the GTEx (<https://www.gtexportal.org/home/gene/CHRNA5>). Compared with nonbrain tissues, *CHRNA5* expression in brain tissues were more strongly affected by the genetic variant (Multi-tissue eQTL Comparison). It is suggested that chronic nicotine administration may reverse the hypofrontality observed in patients with schizophrenia.³⁵ However, roles of the *CHRNA5* on disease mechanism in the brain regions are not still elucidated. Further investigation of the mechanism is required.

To perform eQTL analyses in 10 brain regions, we focused on 22 shared genetic variants associated with both smoking quantity and schizophrenia. The tagging rs637137 associated with *CHRNA5* expression was intronic variant within *CHRNA5*. Generally, gene level *cis*-eQTL signals are much located within UTR, coding region, or promoter region compared to intronic regions.²⁷ Therefore, there may be genetic variants strongly related to *CHRNA5* expression without intronic regions. We further explored the effects of genetic variants that do not overlap on *CHRNA5* expression in 10 brain regions (Supplementary figure S3). In addition to genetic variants within *CHRNA5*, genetic variants at 5' promoter region were strongly associated with *CHRNA5* expression in brain regions. However, these associations should be carefully interpreted because these variants were not associated with smoking quantity and schizophrenia at a genome-wide significance level.

The *CHRNA5* gene has 4 transcript isoforms [*CHRNA5-001* (ENST00000299565.5), *CHRNA5-002* (ENST00000394802.4), *CHRNA5-003* (ENST00000559554.1), and *CHRNA5-004* (ENST00000559576.1)], and antisense RP11-650L12.2 is located around exon 5 of the *CHRNA5* (figure 5A). The genome-wide shared variant rs637137 was specifically associated with exon-specific probesets 3, 4, 5, and 11 but not probesets 1, 2, 9, 10, and 12 on *CHRNA5* in 8 brain regions (CRBL, FCTX, HIPPO, OCTX, PUTM, SNIG, TCTX, and WHMT) (figures 5B and 5C). Probesets 1 and 2 are specific to the 5' UTR and exon 1 of *CHRNA5-001* and *CHRNA5-003*. Probeset 12 is specific to the 3' UTR of *CHRNA5-001*, *CHRNA5-003*, and *CHRNA5-004*. Probesets 9 and 10 are specific to antisense RP11-650L12.2. These findings suggest that the genome-wide shared variant could regulate *CHRNA5-002* isoform expression in a broad set of brain regions. According to exon expression data for *CHRNA5* in the GTEx database,

the *CHRNA5-002* isoform shows the highest expression of the 4 transcript isoforms in Epstein–Barr virus-transformed lymphocytes and testes but not sigmoid colon. Although there was no information about isoform expression in the brain, the *CHRNA5-002* isoform may also exhibit the highest expression of these 4 transcript isoforms in the brain.

The Fagerström Test for Nicotine Dependence (FTND) is commonly used to assess smoking quantity and nicotine dependence. Hancock et al¹³ performed a GWAS meta-analysis for nicotine dependence, defined by the FTND, in ever smokers of European ancestry ($n = 17\,074$); they reported associations between nicotine dependence and 20q13 (*CHRNA4*), 8p11 (*CHRNA6-CHRNA3*), and 15q25 (*CHRNA5-CHRNA3-CHRNA4*), whereas PGC2 has reported associations between schizophrenia and 15q25 but not 20q13 (*CHRNA4*) or 8p11 (*CHRNA6-CHRNA3*).¹² The FTND consists of continuous and dichotomized items. Item-level twin analyses of the FTND have shown that certain items such as CPD have high heritability, whereas other items have heritability near zero.¹¹ Genetic variants on 8p11 (*CHRNA6-CHRNA3*) are more strongly associated with FTND-based nicotine dependence than CPD.⁴⁵ In contrast, genetic variants on 15q25 (*CHRNA5-CHRNA3-CHRNA4*) are equally associated with FTND-based nicotine dependence and CPD.⁴⁶ Therefore, considering associations between schizophrenia and 15q25 in PGC2, a simple self-reported phenotype (CPD) measuring smoking quantity and nicotine dependence may be more useful than the FTND to evaluate comorbidity between smoking behaviors and schizophrenia.

This study has several limitations. We used summary statistics from GWAS meta-analyses of schizophrenia in the PGC2 and smoking quantity in the TAG to identify shared genetic variants. The GWAS meta-analyses of schizophrenia consisted of 36 989 patients with schizophrenia and 113 075 controls, and the GWAS meta-analyses of smoking quantity consisted of 73 853 subjects. These subjects were derived from primary GWAS and the independent replication analysis. We used only summary statistics from primary GWAS meta-analyses of schizophrenia and smoking quantity (<https://www.med.unc.edu/pgc/results-and-downloads>) because summary statistics from independent replication analysis were not available. Compared with sample sizes of the PGC2 and TAG, the BRAINEAC samples were relatively small. In addition, it is unclear whether these variants were associated with similar expression effects in postmortem brains from individuals with schizophrenia or smoking dependence. A larger dataset using not only healthy individual but also individuals with schizophrenia or smoking dependence would lead to more robust effects. Considering polygenic risk score and LD score regression results for smoking quantity and schizophrenia indicated polygenic effect shared,^{16–18} other overlapping regions except for

15q25 may also affect these phenotypes. As the genotyping and imputation methods were different between consortia, a unified method may yield more accurate findings. Although nicotine content varies among different types of cigarettes, TAG did not consider nicotine content, and heavy smokers were defined as smoking a high daily number of cigarettes. In addition, smoking behaviors may be influenced by socioeconomic and educational factors as well as gender.⁴⁷ TAG adjusted for gender but did not adjust for socioeconomic and educational factors. Considering that patients with schizophrenia have a higher prevalence of current smoking than healthy subjects and that PGC2 or BRAINEAC samples did not adjust for smoking status, the shared genetic finding on 15q25 may be due to bias from smoking behaviors. Because there were no information about smoking habits for postmortem brain samples, altered gene expressions (ie, *CHRNA5*) could be affected (up-/downregulations of their receptors) by their daily smoking habits.⁴⁸ Future studies are needed to dissect the mechanism of associations by stratifying 3 groups: schizophrenia patients who smoke, schizophrenia patients who do not smoke, and smokers without psychiatric disorders.

In conclusion, 22 genetic variants were shared between smoking quantity and schizophrenia at the genome-wide significance level, suggesting that a causal mechanism underlying the smoking–schizophrenia association might arise from common genetic factors. Genome-wide shared variants might contribute to the pathogenesis of these traits via regulation of *CHRNA5* expression in the brain. Further studies will help identify molecular targets for drug development.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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