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Investigation of single-nucleotide variants in *MBD5* associated with autism spectrum disorders and schizophrenia phenotypes

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ABSTRACT

MBD5 (Methyl-CpG-binding domain 5) is a critical gene for normal development. While deletion or duplication of *MBD5* may contribute to a genetic predisposition to autism spectrum disorders (ASD), intellectual disability, or epilepsy, the impact of rare *MBD5* single nucleotide variants (SNVs) on neurodevelopmental features, particularly features with late onset, has not been fully explored. In this study, we conducted exon-targeted resequencing of *MBD5* with next-generation sequencing technology in 562 Japanese patients (192 with idiopathic ASD and 370 with schizophrenia (SCZ)) and detected 16 *MBD5* SNVs with allele frequencies of ≤1%. We then performed phenotype analyses with 12 novel variants of these 16 SNVs. SCZ patients with these variants exhibited mainly within normal development ranges until the first psychosis and ASD patients with SNVs did not precisely overlap with the core characteristics described in previous literature as being associated with *MBD5* SNVs. Our results suggested that *MBD5* variants might contribute to a broad spectrum of neurodevelopmental pathophysiology. Further research and assessment of clinical diagnostic screening are necessary for understanding the burden of rare *MBD5* SNVs for these neurodevelopmental disorders.

Key Words: neurodevelopmental disorders, rare variants, next-generation sequencing technology, genotype-phenotype correlations

INTRODUCTION

Recent epidemiologic and genetic studies support substantial overlap of risk genes across neurodevelopmental component in autism spectrum disorders (ASDs) and schizophrenia (SCZ) pathogenesis.[1-6] Common genetic variants may explain less than half of the total variation responsible for increased risk of developing either condition,[7] and understanding shared genetic architectures has been challenging. Recent cross-disorder approaches to identify rare copy-number variants (CNVs) and single nucleotide variants (SNVs) have provided additional information about shared genomic risks and trait variability.[8-11]

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In this study, we focused on *MBD5* (methyl-CpG-binding domain 5). *MBD5* encodes a member of the MBD family, which plays critical roles in transcriptional regulation and development. *MBD5* contains two mRNA isoforms; the longer one (isoform 1) is highly expressed in brain; the shorter one (isoform 2) is highly expressed in oocytes. Both isoforms might have a role in cerebral functions and in epigenetic reprogramming after fertilization.[12] Gene expression changes suggest that *MBD5* is a dosage-sensitive gene critical for normal development.[13] Recently, *MBD5* is regarded as the causal gene in the pathogenesis of 2q23.1 microdeletion and duplication syndrome (OMIM 156200), which is a neurodevelopmental disorder characterized by ASD, intellectual disability, severe speech impairment, seizures, behavioral problems, microcephaly, hypotonia, and short stature.[14-16] To date, significant excess of rare SNVs in *MBD5* coding exon have been also detected in ASD patients.[14, 17] The core features observed in *MBD5* SNVs are similar with the 2q23.1 microdeletion and duplication syndrome having a milder phenotype.[14] Therefore, deep sequencing of *MBD5* in one cohort of patients with either ASD or SCZ might be a good way for elucidating the common pathogenesis of these disorders.[18]

To understand the impact of rare *MBD5* SNVs on neurodevelopmental etiologies, we conducted exon-targeted resequencing of *MBD5* in a cohort of Japanese patients with ASD or SCZ followed by phenotyping and mRNA expression analysis for the *MBD5* SNVs. Our results suggested that *MBD5* variants contributed to broad phenotypes involving neurodevelopmental features.

MATERIALS AND METHODS

Study samples

The targeted-resequencing discovery cohort comprised 192 individuals with ASD (mean age ± SD = 16.3 ± 8.4 years; 77.6% male) and 370 with SCZ (mean age ± SD, 49.7 ± 14.8 years; 53.0% male). For expression analysis, 30 control subjects (mean age ± SD, 42.9 ± 10.8 years; 50.0% male) were added. All subjects were unrelated, living on the mainland of Japan, and self-identified as Japanese. All patients fulfilled the criteria listed in *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition (DSM-5) for ASD or SCZ. Healthy control subjects were selected from the general population and had no history of mental disorders based on questionnaire responses from the subjects themselves during the sample inclusion step. The study was explained to each participant and/or the parents both verbally and in writing. Written informed consent was obtained from the participants and from the parents for patients under 20 years old. All procedures performed in this study involving human participants were approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and were conducted in accordance with the Helsinki Declaration of 1975 and its later amendments or comparable ethical standards.

Sample preparation, Target resequencing and Data analysis

Sample preparation, library preparation, resequencing, data processing, variant calling, and variant annotation performed were previously described in detail.[19]

Candidate variants were defined as exonic nonsynonymous or splice-site variants with allele frequencies of ≤1% in our cohort and the following three public exome databases: dbSNP Build 144 (http://www.ncbi.nlm.nih.gov/projects/SNP/), the 1000 Genomes Project (http://www.1000genomes.org) and the Exome Aggregation Consortium (http://exac.broadinstitute.org). We then examined two databases as a reference of Japanese controls: the Tohoku Medical Megabank Organization of Tohoku University (ToMMo) (https://ijgvd.megabank.tohoku.ac.jp)[20] and the Human Genetic Variation Database (HGVD) (http://www.genome.med.kyoto-u.ac.jp/SnpDB/). When available,

parents were sequenced to determine inheritance patterns. For variants within coding regions, prediction of significance was performed with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/)[21] and MutationTaster (http://www.mutationtaster.org).[22] Additional clinical variant annotations were obtained from NCBI ClinVar (last accessed March 2016; http://www.ncbi.nlm.nih.gov/clinvar/).[23] The MBD5 amino-acid sequence (Q9P267) was retrieved from UniProt database (http://www.uniprot.org/uniprot/). All candidate variants were confirmed by Sanger sequencing with the ABI 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA).

Phenotypic analysis

The clinical features of patients with rare *MBD5* variants were examined retrospectively from medical records. Each case with phenotypic information was assessed for overlap with and divergence from the core characteristics associated with *MBD5* SNVs previously reported.[13, 24] All comorbidities were diagnosed by experienced psychiatrists according to DSM-5 criteria.

Gene expression analysis

Lymphoblastoid cell lines (LCLs, human lymphocytes transformed with Epstein-Barr virus) from each subject with an *MBD5* SNV detected in this study, 30 SCZ patients without an *MBD5* variant (overlapping the targeted-resequencing discovery cohort, mean age ± SD, 43.6 ± 11.2 years; 50.0% male) and each control subject were prepared and cultured according to standard methods. Total RNA was extracted from LCLs using RNAqueous Kit (Ambion, Austin, Texas) and treated with DNase to remove contaminate genomic DNA using TURBO DNA-free Kit (Ambion, Austin, Texas); RNA was then reverse transcribed to cDNA with High capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, California). β-2-microglobulin (B2M) and glucuronidase-β (GUSB), two house keeping genes, were selected as internal control genes for normalization of the polymerase chain reaction (PCR). Quantitative real-time PCR (qPCR) was performed on an ABI prism 7900HT Real-Time PCR System (Applied Biosystems, Foster City, California) using predesigned TaqMan Gene Expression Assay probes (Hs00289233_m1 for MBD5, Hs99999907_m1 for B2M and Hs99999908_ml for GUSB; Applied Biosystems). Measurement of the cycle threshold was performed in duplicate. The data, including amplifying efficiency and relative expression on quantification, were analyzed using the comparative cycle threshold (Ct) method. Expression levels in subjects with SNVs were compared with those in the SCZ group without SNVs or the control group using the two-tailed z test. A P value of < 0.05 was considered statistically significant.

RESULTS

Variation screening of all MBD5 exons

We sequenced *MBD5* exons and exon-intron boundaries in genomic DNA isolated from Japanese patient sample (n = 562). Nucleotide sequence data reported here have been deposited in the DNA Data Bank of Japan (DDBJ) databases (http://www.ddbj.nig.ac.jp) under the accession number DRA004490. Overall, 3.4% (19/562) of cases harbor 16 *MBD5* SNVs. There was neither statistically significant difference (p = 0.16, P values were calculated by one-tailed Fisher's exact test) in the frequency of rare SNVs in the cohort of ASD (2.1%, 4/192) and SCZ (4.1%, 15/370) individuals as carriers. A total of 16 heterozygous SNVs, three in the 5'-UTR and 13 in coding exons, were detected. Each of 13 SNVs in a coding exon was a missense variant. No splice-site, nonsense, or frameshift variants were found. No variants were located in conserved motifs such as the MBD or PWWP (pro-trp-trp-pro) domains (Fig. 1). We were able to determine

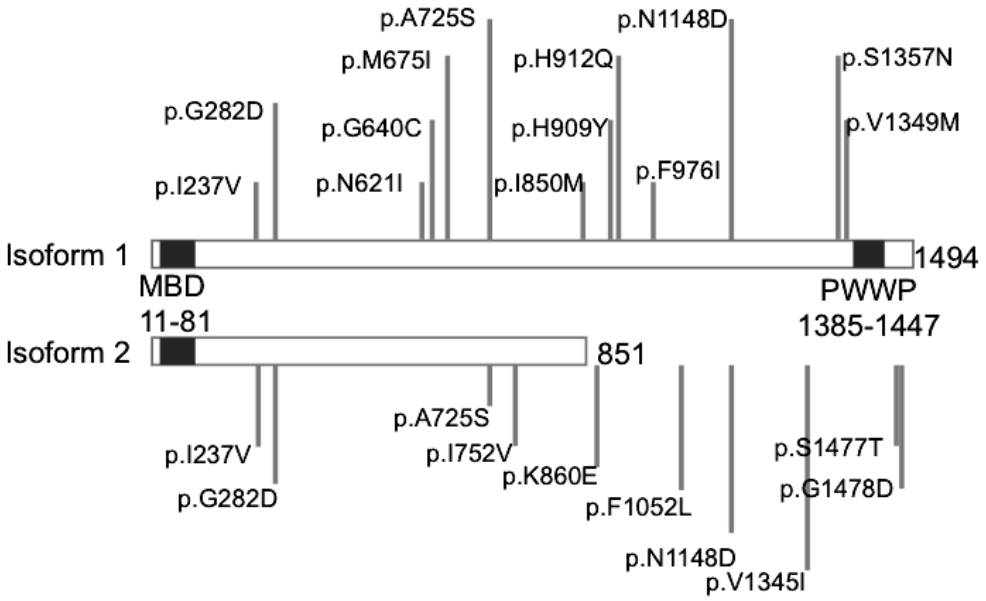

Fig. 1 Location for each variant of interest
The *MBD5* protein structure depicted here is based on the NCBI reference sequence NP_018328 and UniProt KB Q9P267 (http://www.uniprot.org/uniprot/). Variants at the upper side are identified in cases; those at the lower side, controls.
MBD: methyl-CpG-binding domain, PWWP: PWWP domain

inheritance status for three ASD cases. We did not identify any *de novo* variants. Among these three cases, two involved transmission from a depressed mother to her son, and one involved transmission from a healthy father to his daughter. The frequencies of four missense variants (p.I237V, p.G282D, p.A725S, and p.N1148D) did not differ statistically from their frequencies in both ToMMo and HGVD. Therefore, we regarded three SNVs in the 5'-UTR and nine of 13 missense variants as novel ones (Table 1).

Clinical features in patients with MBD5 SNVs

Comparison of the clinical characteristics of the novel 12 *MBD5* SNV cases in previously reported[13, 24] to our cohort, and a summary of SNV-negative cases in this study are presented in Table 2.

Gene dosage of MBD5

We could assess the level of *MBD5* expression in cells from two patients with *MBD5* SNVs (p.I850M and p.H909Y). The data showed that *MBD5* mRNA expression level for these variants was neither decreased nor increased relative to normal controls or to SCZ patients without *MBD5* SNVs (Fig. 2).

Table 1 Rare exonic variants in *MBD5* identified in this study

Chr.	Position	Location (GRCh37)	Transcript Variant	Amino Acid Variant	dbSNP (release 144)	Case	Gender	Inheritance Status	Our cohort[a)] (ASD+SCZ)	MAF	ToMMo[a)] (HC)	MAF	*P* value[2]	HGVD[a)] (HC)	MAF	*P* value[b)]	PolyPhen-2	Mutation Taster	ClinVar
2	exon 4	148990819	c.-825A>G	5'-UTR	-	1 SCZ	M	ND	1/1124	0.00089	-	-	-	-	-	-	-	-	-
2	exon 6	149215885	c.-443G>A	5'-UTR	-	1 ASD	M	Maternal	1/1124	0.00089	-	-	-	-	-	-	-	-	-
2	exon 6	149216042	c.-286G>A	5'-UTR	rs574249341	2 SCZ	1F 1M	ND	2/1124	0.0018	-	-	-	-	-	-	-	-	-
2	exon 9	149226221	c.709A>G	p.I237V	rs751251720	1 ASD	M	ND	1/1124	0.00089	1/2140	0.00047	0.57	1/858	0.0012	0.68	Benign	Disease causing	-
2	exon 9	149226357	c.845G>A	p.G282D	rs759201974	1 ASD	M	Maternal	1/1124	0.00089	3/2140	0.0014	0.57	1/746	0.0013	0.64	Probably damaging	Disease causing	-
2	exon 9	149227374	c.1862A>T	p.N621I	rs755768566	1 ASD	F	Paternal	1/1124	0.00089	-	-	-	-	-	-	Possibly Damaging	Disease causing	-
2	exon 9	149227430	c.1918G>T	p.G640C	_	1 SCZ	F	Not Paternal	1/1124	0.00089	-	-	-	-	-	-	Probably Damaging	Disease causing	-
2	exon9	149227537	c.2025G>C	p.M675I	rs758838720	1 SCZ	F	ND	1/1124	0.00089	-	-	-	-	-	-	Benign	Disease causing	-
2	exon 9	149227685	c.2173G>T	p.A725S	rs747127657	1 SCZ	M	ND	1/1124	0.00089	1/2136	0.00047	0.57	4/600	0.0067	0.053	Benign	Polymorphism	Uncertain significance
2	exon 10	149240710	c.2550A>G	p.I850M	rs756787235	1 SCZ	M	ND	1/1124	0.00089	-	-	-	-	-	-	Possibly Damaging	Disease causing	-
2	exon 10	149240885	c.2725C>T	p.H909Y	rs747447962	1 SCZ	M	ND	1/1124	0.00089	-	-	-	2/858	0.0023	0.40	Benign	Disease causing	-
2	exon 10	149240896	c.2736C>A	p.H912Q	rs756653034	1 SCZ	M	ND	1/1124	0.00089	-	-	-	-	-	-	Benign	Disease causing	-
2	exon 11	149243391	c.2926T>A	p.F976I	-	1 SCZ	M	ND	1/1124	0.00089	-	-	-	-	-	-	Probably damaging	Disease causing	-
2	exon 12	149247342	c.3442A>G	p.N1148D	rs764746304	1 SCZ	M	ND	1/1124	0.00089	4/2140	0.0019	0.44	1/858	0.0012	0.68	Benign	Disease causing	-
2	exon 12	149247945	c.4045G>A	p.V1349M	-	3 SCZ	2F 1M	ND	3/1124	0.0027	1/2138	0.00047	0.12	-	-	-	Probably damaging	Disease causing	-
2	exon 12	149247970	c.4070G>A	p.S1357N	-	1 SCZ	M	ND	1/1124	0.00089	-	-	-	-	-	-	Probably Damaging	Disease causing	-

Amino acid position was determined based on the NCBI reference sequence NP_018328.

Chr, chromosome; SCZ, schizophrenia; ASD, autism spectrum disorders; M, male; F, female; MAF, mainor allele frequency; ToMMo, the Tohoku Medical Megabank Organization of Tohoku University; HC, healthy controls; HGVD, the Human Genetic Variation Database; PolyPhen-2, Polymorphism Phenotyping v2; ClinVar, NCBI ClinVar (last accessed April 2016); ND, not determined.

a) minor allele count / total allele count

b) *P* values were calculated by one-tailed Fisher's exact test

Table 2 Clinical characteristics in individuals with *MBD5* SNVs

Variant	c.-825A>G	c.-443G>A	c.-286A>G	c.-286A>G	p.N621I	p.G640C	p.M675I	p.I850M	p.H909Y	p.H912Q	p.F976I	p.V1349M	p.V1349M	p.V1349M	p.S1357N	without SNV	without SNV
Patient	SCZ	ASD	SCZ	SCZ	ASD	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ	SCZ (n = 357)	ASD (n = 190)
Gender	M	M	F	M	F	F	F	M	M	M	M	F	F	M	M	53% male	77% male
Age of evaluation (years)	53	23	18	47	6	31	66	47	30	50	57	36	46	38	36	50.0 (14.7)[a]	16.4 (8.3)[a]
Developmental delay	N	-	-	-	-	-	-	-	-	-	-	N	-	+	-	5%	27%
Moter delay	N	-	-	-	-	-	-	-	-	-	-	N	-	+	-		
Language impairment	N	-	-	-	-	-	-	-	-	-	-	-	-	+	+		
Behavioral problems	N	+	-	-	-	-	-	-	-	-	-	-	-	+	-		
Autistic-like symptoms	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-		
Withdrawal behaviors	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-		
Repetitive behaviors	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-		
Seizures	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-		
Educational years	9	16	10	12	N	16	12	13	9	10	N	10	18	N	14	12.3 (2.8)[a]	N
Age of psychosis onset (years)	34	N	15	20	N	28	42	18	17	16	28	N	37	27	25	25.0 (8.8)[a]	N
Other neurodevelopmental features		1															13%[b]
Other physiological features			2			3				4						34%[c]	6%
Psychiatiric family history			5										6				

SCZ, schizophrenia; ASD, autism spectrum disorders; M, male; F, female; +, feature present; –, feature absent; N, not reported or patient too young to determine

1, Attention deficit hyperactivity disorder; 2, Dystonia; 3, Hyperthyroidism; 4, Water intoxication; 5, Intellectual disability: Maternal sister; 6, SCZ: Mother

[a] Mean (SD); [b] mainly ADHD and seizures; [c] mainly diabetes and hypertension

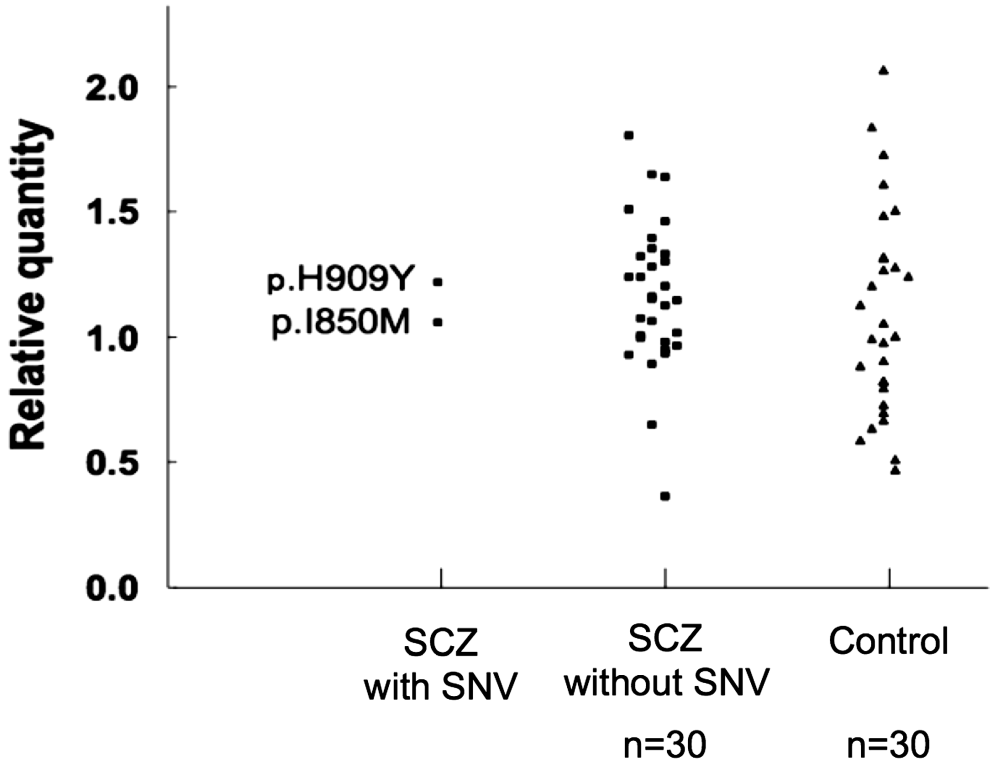

Fig. 2 *MBD5* mRNA expression
Each dot represents the relative expression value of each sample as calculated via the 2−ΔΔCT method. Square: SCZ, Triangle: controls, SCZ: schizophrenia, SNV: single nucleotide variant

DISCUSSION

Previous studies on *MBD5* variants have focused on neurodevelopmental disorders with early-onset phenotypes. Our study is the first to illustrate possible relationships between *MBD5* and neurodevelopmental features with both early- and late-onset. We analyzed the sequence of *MBD5* in a cohort comprising 192 individuals with ASD and 370 with SCZ. Overall, 3.4% of these individuals harbored rare *MBD5* SNVs. No statistically significant difference in SNV frequency between ASD and SCZ was observed. Each candidate variant was inherited. Phenotypic analysis with 12 novel variants of these 16 SNVs show that almost all SCZ cases with SNVs exhibited normal development at least up to the age of 15 years old; onset occurred at age of 42 years in one case. Additionally, each of the two individuals with ASD was within normal intelligence and lacked each of the core phenotypes associated with ASD reported previously including intellectual disability, seizures, and behavioral problems. Comorbid attention deficit hyperactivity disorder (ADHD), which is also regarded as a neurodevelopmental disorder, is observed in one out of two ASD cases. Expression analysis revealed that these SNVs did not change mRNA expression levels.

MBD5 play critical roles in transcriptional regulation and development.[15] *MBD5* contains a PWWP domain; this domain is thought to be important in cell division, growth, and differentiation.[25] *MBD5* is expressed not just in early developmental periods, but also in the adult periods;

this finding confirmed the notion that *MBD5* may have certain roles in adult neuropsychiatry.[26, 27] While *MBD5* is suggested to be a dosage-sensitive gene,[13] normal mRNA expression levels are also observed in patients carrying an inherited intronic deletion in the 5'-UTR of *MBD5* and exhibiting phenotypes typical of the 2q23.1 deletion syndrome.[28] To minimize possible bias due to population-specific rare variant patterns,[29, 30] it may be a good way to examine the public data of more than 1000 Japanese healthy controls as a reference. Based on the Residual Variation Intolerance Score, which is based upon allele frequency data as represented in whole exome sequence, *MBD5* is among the top 2% of the most intolerant genes.[31] Taken together, the SNVs in our case cohort, particularly patient-specific ones, could indicate broad contributions of these *MBD5* variants to neurodevelopmental features. Especially, the SNVs located *MBD5* isoform 1, which is highly expressed in the brain, might be critical for late onset neurodevelopmental phenotypes.[24]

Each variant was definitively inherited or of unknown origin; this finding is similar to previous findings. Inherited *MBD5* variants have been implicated in contributing substantially to ASD.[5, 11, 24, 32] Although prenatal problems were not reported in our cohort, interactions of genetic variants with maternal infection during pregnancy have been implicated in autistic symptomatology,[33, 34] and such findings may explain, to some extent, the incomplete penetrance and the global nature of the phenotypes. We find it interesting that in two cases a candidate variant was transmitted from a depressed mother to an affected son. Considering high penetrance, heterogeneous, and broad pathogenic effects of *MBD5* disruption,[32, 35, 36] our result indicates an association of this locus with risk for depression. These inherited variants could increase susceptibility to development of neurodevelopmental disorders.

The current study has several limitations. First, the lack of DNA from a majority of patient family members of these patients did not allow us to monitor variant segregation. Secondly, we reported only neurological and behavioral characteristics because we could not obtain detailed clinical information such as growth, craniofacial, or skeletal features, which may have enhanced the evaluation of the impact of *MBD5* variants on carriers. Finally, although the SNVs identified in this study were predicted to be protein-disrupting based on *in silico* analysis, the exact molecular mechanisms and networks affected by *MBD5* variants in ASD and/or SCZ remain unclear. Gene expression levels observed in LCLs may not necessarily reflect the impact of *MBD5* on neurodevelopmental disorders. Molecular and functional studies will be needed to provide insight into the underlying biological pathways.

In summary, we investigated the impact of *MBD5* SNVs in Japanese patients with ASD or SCZ. Our findings indicated that rare *MBD5* heterozygous variants were associated with the clinical heterogeneity evident in a broad range of neurodevelopmental disorders including ASD, SCZ, ADHD, and depression. Careful phenotyping across the lifespan of individuals affected by these conditions will be needed to establish fine genotype-phenotype correlations and determine the impact of rare *MBD5* variants on psychopathology. Further studies both in patients and carriers are required to reveal the contribution of *MBD5* to the broad risk for neurodevelopmental disorders.

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COMPETING INTERESTS

The authors have no conflicts of interest to declare.

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