Investigation of single-nucleotide variants in \textit{MBD5} associated with autism spectrum disorders and schizophrenia phenotypes

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\textbf{ABSTRACT}

\textit{MBD5} (Methyl-CpG-binding domain 5) is a critical gene for normal development. While deletion or duplication of \textit{MBD5} may contribute to a genetic predisposition to autism spectrum disorders (ASD), intellectual disability, or epilepsy, the impact of rare \textit{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 \textit{MBD5} with next-generation sequencing technology in 562 Japanese patients (192 with idiopathic ASD and 370 with schizophrenia (SCZ)) and detected 16 \textit{MBD5} SNVs with allele frequencies of \textless{}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 \textit{MBD5} SNVs. Our results suggested that \textit{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 \textit{MBD5} SNVs for these neurodevelopmental disorders.

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

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\textbf{INTRODUCTION}

Recent epidemiologic and genetic studies support substantial overlap of risk genes across neurodevelopmental component in autism spectrum disorders (ASDs) and schizophrenia (SCZ) pathogenesis.\textsuperscript{1-6} Common genetic variants may explain less than half of the total variation responsible for increased risk of developing either condition,\textsuperscript{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.\textsuperscript{8,11}

Received: July 13, 2016; accepted: September 1, 2016
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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. Gene expression changes suggest that MBD5 is a dosage-sensitive gene critical for normal development. 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. To date, significant excess of rare SNVs in MBD5 coding exon have been also detected in ASD patients. The core features observed in MBD5 SNVs are similar with the 2q23.1 microdeletion and duplication syndrome having a milder phenotype. 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.

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.

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) 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/)\textsuperscript{21} and MutationTaster (http://www.mutationtaster.org).\textsuperscript{22} Additional clinical variant annotations were obtained from NCBI ClinVar (last accessed March 2016; http://www.ncbi.nlm.nih.gov/clinvar/).\textsuperscript{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.\textsuperscript{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 housekeeping 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 Hs99999808_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 PWPP (pro-trp-trp-pro) domains (Fig. 1). We were able to determine
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 | TAmmO* (HC) | HGVD* (HC) | PolyPhen-2 | ClinVar |
|---------------|-------------------|--------------------|-------------------|-------------------|------|--------|-------------------|------------|-------------|-------------|------------|---------|
| 2 exon 4      | 149099019         | c.-825A>G          | 5'-UTR            |                   | 1 SCZ | M      | ND                | 14/124     | 0.00089     |             |            |         |
| 2 exon 6      | 149217385         | c.-448G>A          | 5'-UTR            | p.E149V           | 1 ASD | M      | Maternal          | 1/124      | 0.00089     |             |            |         |
| 2 exon 9      | 149222072         | c.-606A>T          | 5'-UTR            | rs574394341       | 2 SCZ | IF 1M  | ND                | 2/124      | 0.0012     |             |            |         |
| 2 exon 9      | 149222072         | c.709A>G           | p.I237V           | rs751251720        | 1 ASD | M      | ND                | 0.00089    | 0.0012     | 0.57       | 1/858      | 0.0012  |
| 2 exon 2      | 149222774         | c.845G>A           | p.G282D           | rs759201974        | 1 ASD | M      | Maternal          | 0.00089    | 0.0012     | 0.57       | 1.746      | 0.0013  |
| 2 exon 9      | 149222774         | c.906G>A           | p.T302I           | rs755788566        | 1 ASD | F      | Paternal          | 0.00089    | 0.0012     | 0.57       | 1/746      | 0.0013  |
| 2 exon 2      | 149223430         | c.1327C>T          | 5'-UTR            | rs758838720        | 1 SCZ | F      | ND                | 0.00089    | 0.0012     | 0.57       | 2/1124     | 0.0012  |
| 2 exon 10     | 149240770         | c.2750A>G          | p.I850M           | rs756787235        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 4/124      | 0.0012  |
| 2 exon 10     | 149240875         | c.2750A>G          | p.I850M           | rs756787235        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 2/124      | 0.0012  |
| 2 exon 10     | 149240875         | c.2750A>G          | p.I850M           | rs756787235        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 2/124      | 0.0012  |
| 2 exon 11     | 149243391         | c.2750A>G          | p.I850M           | rs756787235        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 2/124      | 0.0012  |
| 2 exon 12     | 149247342         | c.3442A>G          | 5'-UTR            | rs758476304        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 1/858      | 0.0012  |
| 2 exon 12     | 149247545         | c.4045G>A          | 5'-UTR            | rs758476304        | 1 SCZ | M      | ND                | 0.00089    | 0.0012     | 0.57       | 1/858      | 0.0012  |

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, minor 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 | p.N621I | p.G640C | p.M675I | p.H909Y | p.H912Q | p.V1349M | p.V1349M | p.V1349M | p.S1357N | SCZ (n = 357) | ASD (n = 190) |
|----------|-----------|-----------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Patient  | SCZ | ASD | SCZ | SCZ | ASD | SCZ | SCZ | SCZ | SCZ | SCZ | SCZ | SCZ | SCZ | SCZ |
| Gender   | M | M | F | M | F | F | M | M | M | F | F | M | M | 53% male |
| Age of evaluation (years) | 53 | 23 | 18 | 47 | 6 | 31 | 66 | 30 | 57 | 36 | 46 | 36 | 36 | 50.0 (14.7) |
| Developmental delay | N | - | - | - | - | - | - | - | - | - | - | - | - | 9% |
| Motor delay | N | - | - | - | - | - | - | - | - | - | - | - | - | 27% |
| Language impairment | N | - | - | - | - | - | - | - | - | - | - | - | - | 9% |
| Behavioral problems | N | + | - | - | - | - | - | - | - | - | - | - | - | 27% |
| Autistic-like symptoms | - | + | - | - | + | - | - | - | - | - | - | - | - | 9% |
| Withdrawal behaviors | - | - | - | - | - | - | - | - | - | - | - | - | - | 27% |
| Repetitive behaviors | - | + | - | - | + | - | - | - | - | - | - | - | - | 9% |
| Seizures | - | - | - | - | - | - | - | - | - | - | - | - | - | 27% |
| Educational years | 9 | 16 | 10 | 12 | N | 16 | 12 | 13 | 9 | 10 | N | 18 | N | 14 | 12.3 (2.8) |
| Age of psychosis onset (years) | 34 | N | 15 | 20 | N | 28 | 42 | 18 | 17 | 16 | 28 | N | 37 | 27 | 25 | 25.0 (8.8) |
| Other neurodevelopmental features | 1 | | | | | | | | | | | | | | (3%) |
| Other physiological features | 2 | 3 | 4 | | | | | | | | | | | 34% | 6% |
| Psychiatric family history | 5 | | | | | | | | | | | | | | |

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
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. MBD5 contains a PWWP domain; this domain is thought to be important in cell division, growth, and differentiation. 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.\textsuperscript{26, 27)} While MBD5 is suggested to be a dosage-sensitive gene,\textsuperscript{23} 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.\textsuperscript{28)} To minimize possible bias due to population-specific rare variant patterns,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{3, 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,\textsuperscript{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,\textsuperscript{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 \textit{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.

\textbf{ACKNOWLEDGEMENTS}

We are grateful to all of the patients and their families who contributed to this study.
COMPETING INTERESTS

The authors have no conflicts of interest to declare.

FUNDING

This work was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the Strategic Research Program for Brain Sciences from the Japan Agency for Medical Research and Development, AMED; the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) program at AMED; the Innovative Areas “Glial Assembly: a new regulatory machinery of brain function and disorders” program; and the Innovative Areas “Comprehensive Brain Science Network program.

REFERENCES

1) Rapoport J, Chavez A, Greenstein D, Addington A, Gogtay N. Autism spectrum disorders and childhood-onset schizophrenia: clinical and biological contributions to a relation revisited. *J Am Acad Child Adolesc Psychiatry*, 2009; 48: 10–18.
2) Sullivan PF, Daly MJ, O’Donovan M. Genetic architectures of psychiatric disorders: the emerging picture and its implications. *Nat Rev Genet*, 2012; 13: 537–551.
3) Sullivan PF, Magnusson C, Reichenberg A, Boman M, Dalman C, Davidson M, et al. Family history of schizophrenia and bipolar disorder as risk factors for autism. *Arch Gen Psychiatry*, 2012; 69: 1099–1103.
4) Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *The Lancet*, 2013; 381: 1371–1379.
5) Moreno-De-Luca A, Myers SM, Challman TD, Moreno-De-Luca DE, David WL, David H. Developmental brain dysfunction: revival and expansion of old concepts based on new genetic evidence. *Lancet Neurol*, 2013; 12: 406–414.
6) Hommer RE, Swedo SE. Schizophrenia and autism-related disorders. *Schizophr Bull*, 2015; 41: 313–314.
7) Gibson G. Rare and common variants: twenty arguments. *Nat Rev Genet*, 2011; 13: 135–145.
8) Mowry BJ, Gratton J. The emerging spectrum of allelic variation in schizophrenia: current evidence and strategies for the identification and functional characterization of common and rare variants. *Mol Psychiatry*, 2013; 18: 38–52.
9) Stein JL, Parikshak NN, Geschwind DH. Rare inherited variation in autism: beginning to see the forest and a few trees. *Neuron*, 2013; 77: 209–211.
10) Zuk O, Schaffner SF, Samocha K, Do R, Hechter E, Kathiresan S, et al. Searching for missing heritability: designing rare variant association studies. *Proc Natl Acad Sci U S A*, 2014; 111: E455–464.
11) Gonzalez-Mantilla AJ, Moreno-De-Luca A, Ledbetter DH, Martin CL. Cross-disorder method to identify novel candidate genes for developmental brain disorders. *JAMA Psychiatry*, 2016; 73: 275–283.
12) Laget S, Joulie M, Le Masson F, Sasai N, Christians E, Pradhan S, et al. The human proteins MBD5 and MBD6 associate with heterochromatin but they do not bind methylated DNA. *PLoS ONE*, 2010; 5: e11982.
13) Mullegama SV, Rosenfeld JA, Orellana C, van Bon BW, Halbach S, Repnikova EA, et al. Reciprocal deletion and duplication at 2q23.1 indicates a role for MBD5 in autism spectrum disorder. *Eur J Hum Genet*, 2014; 22: 57–63.
14) van Bon BW, Koolen DA, Brueton L, McMullan D, Lichtenbelt KD, Ades LC. The 2q23.1 microdeletion syndrome: clinical and behavioural phenotype. *Eur J Hum Genet*, 2010; 18: 163–170.
15) Taikowski ME, Mullegama SV, Rosenfeld JA, van Bon BW, Shen Y, Repnikova EA. Assessment of 2q23.1 microdeletion syndrome implicates MBD5 as a single causal locus of intellectual disability, epilepsy, and autism spectrum disorder. *Am J Hum Genet*, 2011; 89: 551–563.
16) Mullegama SV, Alaime JT, Chen L,elsea SH. Phenotypic and molecular convergence of 2q23.1 deletion syndrome with other neurodevelopmental syndromes associated with autism spectrum disorder. *Int J Mol Sci*, 2015; 16: 7627–7643.
17) Bonnet C, Ali Khan A, Bresso E, Vigouroux C, Beri M, Lejczak S. Extended spectrum of MBD5 mutations
in neurodevelopmental disorders. *Eur J Hum Genet*, 2013; 21: 1457–1461.

18) Lee S, Abecasis GR, Boehnke M, Lin X. Rare-variant association analysis: study designs and statistical tests. *Am J Hum Genet*, 2014; 95: 5–23.

19) Ishizuka K, Kimura H, Wang C, Xing J, Kushima I, Arioka Y, et al. Investigation of Rare Single-Nucleotide PCDH15 Variants in Schizophrenia and Autism Spectrum Disorders. *PLoS ONE*, 2016; 11: e0153224.

20) Nagasaki M, Yasuda J, Katsuoka F, Nariai N, Kojima K, Kawai Y, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. *Nat Commun*, 2015; 21: 8018.

21) Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods*, 2010; 7: 248–249.

22) Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods*, 2014; 11: 361–362.

23) Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*, 2014; 42: D980–985.

24) Cukier HN, Lee JM, Ma D, Young JI, Mayo V, Butler BL, et al. The expanding role of MBD genes in autism: identification of a MECP2 duplication and novel alterations in MBDS, MBDD, and SETDB1. *Autism Res*, 2012; 5: 385–397.

25) Qin S, Min J. Structure and function of the nucleosome-binding PWWP domain. *Trends Biochem Sci*, 2014; 39: 536–547.

26) Maussion G, Diallo AB, Gigek CO, Chen ES, Crapper L, Theroux JF, et al. Investigation of genes important in neurodevelopment disorders in adult human brain. *Hum Genet*, 2015; 134: 1037–1053.

27) Gigek CO, Chen ES, Ota VK, Maussion G, Peng H, Vaillancourt K, et al. A molecular model for neurodevelopmental disorders. *Transl Psychiatry*, 2015; 5: e565.

28) Mullegama SV, Elsea SH. Intragenic MBD5 familial deletion variant does not negatively impact MBD5 mRNA expression. *Mol Cytogenet*, 2014; 7: 80.

29) Mowry BJ, Gratten J. The emerging spectrum of allelic variation in schizophrenia: Current evidence and strategies for the identification and functional characterization of common and rare variants. *Mol Psychiatry*, 2012; 18: 38–52.

30) Nelson MR, Wegmann D, Ehms MG, Kessner D, St Jean P, Verzilli C, et al. An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people. *Science*, 2012; 337: 100–104.

31) Petrovski S, Wang Q, Heinzlen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet*, 2013; 9: e1003709.

32) Du X, An Y, Yu L, Liu R, Qin Y, Guo X, et al. A genomic copy number variant analysis implicates the MBD5 and HNRNP genes in Chinese children with infantile spasms and expands the clinical spectrum of 2q23.1 deletion. *BMC Med Genet*, 2014; 15: 62.

33) Schwartzer JJ, Careaga M, Onore CE, Rushakoff JA, Berman RF, Ashwood P. Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. *Transl Psychiatry*, 2013; 3: e240.

34) Mazina V, Gerdts J, Trinh S, Ankenman K, Ward T, Dennis MY, et al. Epigenetics of autism-related impairment: copy number variation and maternal infection. *J Dev Behav Pediatr*, 2015; 36: 61–67.

35) Wagenstaller J, Spranger S, Lorenz-Depiereux B, Kazmierczak B, Nathrath M, Wahl D, et al. Copy-number variations measured by single-nucleotide-polymorphism oligonucleotide arrays in patients with mental retardation. *Am J Hum Genet*, 2007; 81: 768–779.

36) Hodge JC, Mitchell E, Pillalamarri V, Toler TL, Bartel F, Kearney HM, et al. Disruption of MBD5 contributes to a spectrum of psychopathology and neurodevelopmental abnormalities. *Mol Psychiatry*, 2014; 19: 368–379.