

RESEARCH

Open Access

Exon resequencing of H3K9 methyltransferase complex genes, *EHMT1*, *EHMT2* and *WIZ*, in Japanese autism subjects

Shabeesh Balan¹, Yoshimi Iwayama¹, Motoko Maekawa¹, Tomoko Toyota¹, Tetsuo Ohnishi¹, Manabu Toyoshima¹, Chie Shimamoto¹, Kayoko Esaki¹, Kazuo Yamada¹, Yasuhide Iwata², Katsuaki Suzuki², Masayuki Ide³, Motonori Ota⁴, Satoshi Fukuchi⁵, Masatsugu Tsujii^{6,7}, Norio Mori^{2,7}, Yoichi Shinkai^{8,9} and Takeo Yoshikawa^{1,9*}

Abstract

Background: Histone H3 methylation at lysine 9 (H3K9) is a conserved epigenetic signal, mediating heterochromatin formation by trimethylation, and transcriptional silencing by dimethylation. Defective GLP (*Ehmt1*) and G9a (*Ehmt2*) histone lysine methyltransferases, involved in mono and dimethylation of H3K9, confer autistic phenotypes and behavioral abnormalities in animal models. Moreover, *EHMT1* loss of function results in Kleeftstra syndrome, characterized by severe intellectual disability, developmental delays and psychiatric disorders. We examined the possible role of histone methyltransferases in the etiology of autism spectrum disorders (ASD) and suggest that rare functional variants in these genes that regulate H3K9 methylation may be associated with ASD.

Methods: Since G9a-GLP-Wiz forms a heteromeric methyltransferase complex, all the protein-coding regions and exon/intron boundaries of *EHMT1*, *EHMT2* and *WIZ* were sequenced in Japanese ASD subjects. The detected variants were prioritized based on novelty and functionality. The expression levels of these genes were tested in blood cells and postmortem brain samples from ASD and control subjects. Expression of *EHMT1* and *EHMT2* isoforms were determined by digital PCR.

Results: We identified six nonsynonymous variants: three in *EHMT1*, two in *EHMT2* and one in *WIZ*. Two variants, the *EHMT1* ankyrin repeat domain (Lys968Arg) and *EHMT2* SET domain (Thr961Ile) variants were present exclusively in cases, but showed no statistically significant association with ASD. The *EHMT2* transcript expression was significantly elevated in the peripheral blood cells of ASD when compared with control samples; but not for *EHMT1* and *WIZ*. Gene expression levels of *EHMT1*, *EHMT2* and *WIZ* in Brodmann area (BA) 9, BA21, BA40 and the dorsal raphe nucleus (DoRN) regions from postmortem brain samples showed no significant changes between ASD and control subjects. Nor did expression levels of *EHMT1* and *EHMT2* isoforms in the prefrontal cortex differ significantly between ASD and control groups.

Conclusions: We identified two novel rare missense variants in the *EHMT1* and *EHMT2* genes of ASD patients. We surmise that these variants alone may not be sufficient to exert a significant effect on ASD pathogenesis. The elevated expression of *EHMT2* in the peripheral blood cells may support the notion of a restrictive chromatin state in ASD, similar to schizophrenia.

Keywords: Autism, Rare variant, GLP, G9a, Wiz, Histone methyltransferase, H3K9

* Correspondence: takeo@brain.riken.jp

¹Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

⁹CREST (Core Research for Evolutionary Science and Technology), Japan Science and Technology Agency, Kawaguchi, Saitama, Japan

Full list of author information is available at the end of the article

Background

Autism spectrum disorders (ASD), characterized by defects in social reciprocity, impairment in communication and restricted and repetitive stereotyped behavioral patterns, are the most prevalent childhood neurodevelopmental disorders. They affect all racial, ethnic and socioeconomic groups equally, with a worldwide prevalence of approximately 0.6% [1,2]. The genetic influences in the etiology of ASD have been demonstrated in family and twin studies [3,4], along with discoveries of common and rare genetic variants and pronounced chromosomal abnormalities [5]. Recently, *de novo* rare variants with a large effect size were found to increase ASD susceptibility [6,7]. However, generation of the ASD phenotype requires interaction between environmental factors, and inherited and *de novo* genetic variants [8]. Furthermore, the pivotal role of epigenetic regulatory mechanisms involved in the pathogenesis of Rett syndrome, fragile X syndrome and the identification of ASD-associated genetic defects in imprinted regions lends strength to the hypothesis that epigenetic factors are causative in ASD etiology [9].

Epigenetic mechanisms involving post translational modification of histone lysine methylation influence numerous biological processes, including transcription, replication and chromosome maintenance, all of which are tightly regulated by methyltransferases and demethylases [10]. Among them, methylation of lysine 9 in histone H3 (H3K9), marks a conserved epigenetic signal; by heterochromatin formation through trimethylation (H3K9me3) and transcriptional silencing through dimethylation (H3K9me2) [11]. The formation of H3K9me1 and H3K9me2 are mediated by a Suv39h subgroup of histone methyl transferases, namely G9a/KMT1C and GLP/KMT1D, both having Su(var)3-9-Enhancer of zeste-Trithorax (SET) domain, through which they form homomeric and heteromeric complexes [12]. The G9a-GLP heteromeric complex is known to interact with Wiz, a multi-zinc finger-containing molecule, resulting in a stable and dominant intracellular heteromeric methyltransferase complex [13].

Regulation of H3K9 methylation has a powerful impact on neurological function and disease, as exemplified in Kleefstra syndrome. This disease is characterized by severe intellectual disability, developmental delay and psychiatric disorders, and is the result of a 9q34 subtelomeric deletion and loss-of-function mutations in *EHMT1* [14,15]. In *Ehmt1* heterozygous knockout mice, the typical autistic-like features including reduced exploration, increased anxiety, altered social behavior, deficits in fear extinction, and learning and object recognition (novel and spatial) are observed [16,17]. Furthermore, the lack of post-natal and neuron-specific GLP/G9a expression in mouse models dysregulates neuronal transcriptional, resulting in

behavioral abnormalities, such as impaired learning, motivation and environmental adaptation [18].

Therefore, the autistic-like features and behavioral abnormalities precipitated by defects in histone methyltransferases provide a powerful case for examining their involvement in ASD pathogenesis. We put forward that rare functional variants in these genes may be associated with ASD. Since G9a-GLP-Wiz forms a stable and dominant heteromeric methyltransferase complex in H3K9 methylation, we set out to resequence the *EHMT1*, *EHMT2* and *WIZ* genes coding for GLP, G9a and WIZ, respectively, in Japanese ASD case and control samples.

Methods

Subjects

A cohort of 315 patients of Japanese descent, with autism (262 males and 53 females, mean age \pm SD =12.09 \pm 5.72 years), comprising 293 independent subjects and affected siblings, were recruited for the resequencing studies. The diagnosis of autism was made using the *Diagnostic and Statistical Manual, Fourth Edition, Text Revision* (DSM-IV-TR: American Psychiatric Association, 2000) criteria. The Autism Diagnostic Interview-Revised (ADI-R) [19] was conducted by experienced child psychiatrists who are licensed to use the Japanese version of the ADI-R. Participants with comorbid psychiatric illnesses were excluded by means of the Structured Clinical Interview for DSM-IV (SCID) [20]. Control subjects (n =1,140, 440 males and 700 females, mean age \pm SD =44.10 \pm 13.63 years) devoid of any past or present psychiatric disorders were recruited from hospital staff and company employees. Samples were also collected from available parents of subjects who harbored novel mutations, in order to determine whether these mutations were *de novo*. All participants were provided with, and received a full explanation of study protocols and objectives, before giving informed, written consent to participate in the study. For patients under the age of 16 years, written informed consent was also obtained from their parents. The study was approved by the Ethics Committees of RIKEN and Hamamatsu University School of Medicine, and conducted according to the principles expressed in the Declaration of Helsinki. DNA was extracted from whole blood according to a standard protocol.

A subset of subjects, 52 ASD (43 males and 9 females, mean age \pm SD =11.98 \pm 2.43) and 32 normal controls (26 males and 6 females, mean age \pm SD =12.31 \pm 2.01), was selected to analyze transcript expression levels in peripheral blood cells from the cohort whose DNA was resequenced for the candidate genes. Postmortem brain tissues from ASD and age-matched control samples were obtained from the National Institute of Child Health and Human Development (NICHD) Brain and Tissue

Bank, University of Maryland School of Medicine (<http://medschool.umaryland.edu/btbank/>), for gene expression analysis (Additional file 1: Table S1). Frozen tissue samples from BA09 (ASD; n =10, control; n =10), BA21 (ASD; n =14, control; n =14), BA40 (ASD; n =14, control; n =13) and DoRN regions (ASD; n =8, control; n =8) were used in this study. Total RNA from peripheral blood cells and brain tissues was extracted using a miRNAeasy Mini kit (QIAGEN GmbH, Hilden, Germany) and single stranded cDNA was synthesized using a SuperScript VIL0 cDNA synthesis kit (Life Technologies Co., Carlsbad, CA, USA), according to the manufacturers' instructions.

Resequencing and variant analysis

Protein-coding regions and exon/intron boundaries of *EHMT1*, *EHMT2* and *WIZ* were screened for variants in ASD case samples by direct sequencing of PCR products, using the BigDye Terminator v3.1 cycle Sequencing Kit (Applied Biosystems (ABI), Foster City, CA, USA), and analyzed on an ABI3730 Genetic Analyzer (ABI), using standard protocols. The primers used for amplification and PCR conditions are listed in Additional file 2: Table S2. The sequences were aligned to the respective reference sequences (*EHMT1* isoform 1: RefSeq NM_024757.4, Isoform 2: RefSeq NM_001145527.1, *EHMT2* isoform a: RefSeq NM_006709.3, isoform b: RefSeq NM_25256.5, and *WIZ*: RefSeq NM_021241.2) and variants were detected using Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA). For the heterozygous variant calls in Sequencher, the height of the secondary peak was set at 35% of the primary peak and all variants were confirmed by bidirectional sequencing of the sample.

Variants were prioritized based on whether they were, (i) located in an important functional domain of the protein, (ii) deemed to be functional, such as a frame shift, stop gain or nonsynonymous mutation, and (iii) novel, that is not documented in the NCBI dbSNP database (Build 137) (<http://www.ncbi.nlm.nih.gov/SNP/>), the 1000 Genomes Project (<http://www.1000genomes.org/>), the Exome Variant Server of NHLBI GO Exome Sequencing Project (ESP6500SI-V2) (<http://evs.gs.washington.edu/EVS/>) or the Human Genetic Variation Database of Japanese genetic variation consortium (<http://www.genome.med.kyoto-u.ac.jp/SnpDB>). The potential functional consequences of variants were evaluated *in silico*, using PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), PROVEAN (<http://provean.jcvi.org/index.php>) and SIFT (<http://sift.jcvi.org/>). In the control samples, we screened only exons coding for functional domains of the candidate genes (Figure 1 and Additional file 3: Figure S1 (A)). Fisher's exact test (two-tailed) was used to compare the differences in allele counts between ASD and control subjects, with statistical significance being defined as $P < 0.05$.

Gene expression analysis

Real-time quantitative RT-PCR analysis was conducted using standard procedures, in an ABI7900HT Fast Real-Time PCR System (ABI, Foster City, CA, USA). TaqMan probes and primers for *EHMT1*, *EHMT2* and *WIZ* and *GAPDH* (internal control) were chosen from TaqMan Gene Expression Assays (ABI, Foster City, CA, USA) (Figure 1 and Additional file 4: Table S3). All real-time quantitative RT-PCR reactions were performed in triplicate, based on the standard curve method. To check for isoform-specific expressional changes between ASD cases and controls (prefrontal cortex), digital PCR was performed using standard procedures for *EHMT1* (variant 1: NM_024757.4 and variant 2: NM_001145527.1) and *EHMT2* (isoform a: NM_006709.3 and isoform b: NM_25256.5) isoforms, using TaqMan Gene Expression Assays in a QuantStudio12K Flex Real-Time PCR System (Life Technologies Co., Carlsbad, CA, USA) (Figure 1 and Additional file 4: Table S3). Significant changes in target gene expression levels between the cases and controls were detected by Mann-Whitney *U*-test (two-tailed) and P values of < 0.05 were considered statistically significant.

Results

Resequencing and genetic association analyses

Resequencing of the coding regions and exon/intron boundaries of the three genes, yielded several novel and previously reported variants in the ASD cohort, with varying minor allele frequencies (Additional file 5: Table S4). Filtering of variants based on functionality (nonsynonymous and frameshift) and novelty, revealed three nonsynonymous variants in *EHMT1*, two nonsynonymous variants in *EHMT2* and one nonsynonymous variant in *WIZ* (Table 1). All variants showed low minor allele frequencies (MAF < 0.01) and were deemed to be inherited from the parents, although this could not be confirmed in cases bearing the *EHMT1* variant, Lys968Arg, due to a lack of parental samples for testing (Figure 2).

Since histone methylation is effected through the formation of multimeric complexes of histone methyltransferases, which in turn are mediated by interaction of functional domains, we focused our interests on these regions. Results revealed that rare variants in the *EHMT1* ankyrin repeat domain (Lys968Arg) and *EHMT2* SET domain (Thr961Ile) were present in ASD cases but not in any of the 1,140 screened control subjects. Examining the cases, we observed no variations in the functional domains of *WIZ*. The case-control comparison showed no statistically significant association of any identified variants with ASD (Table 2). In addition, we also identified *EHMT1* and *EHMT2* variants that were present only in the control population (Additional file 4: Table S4).

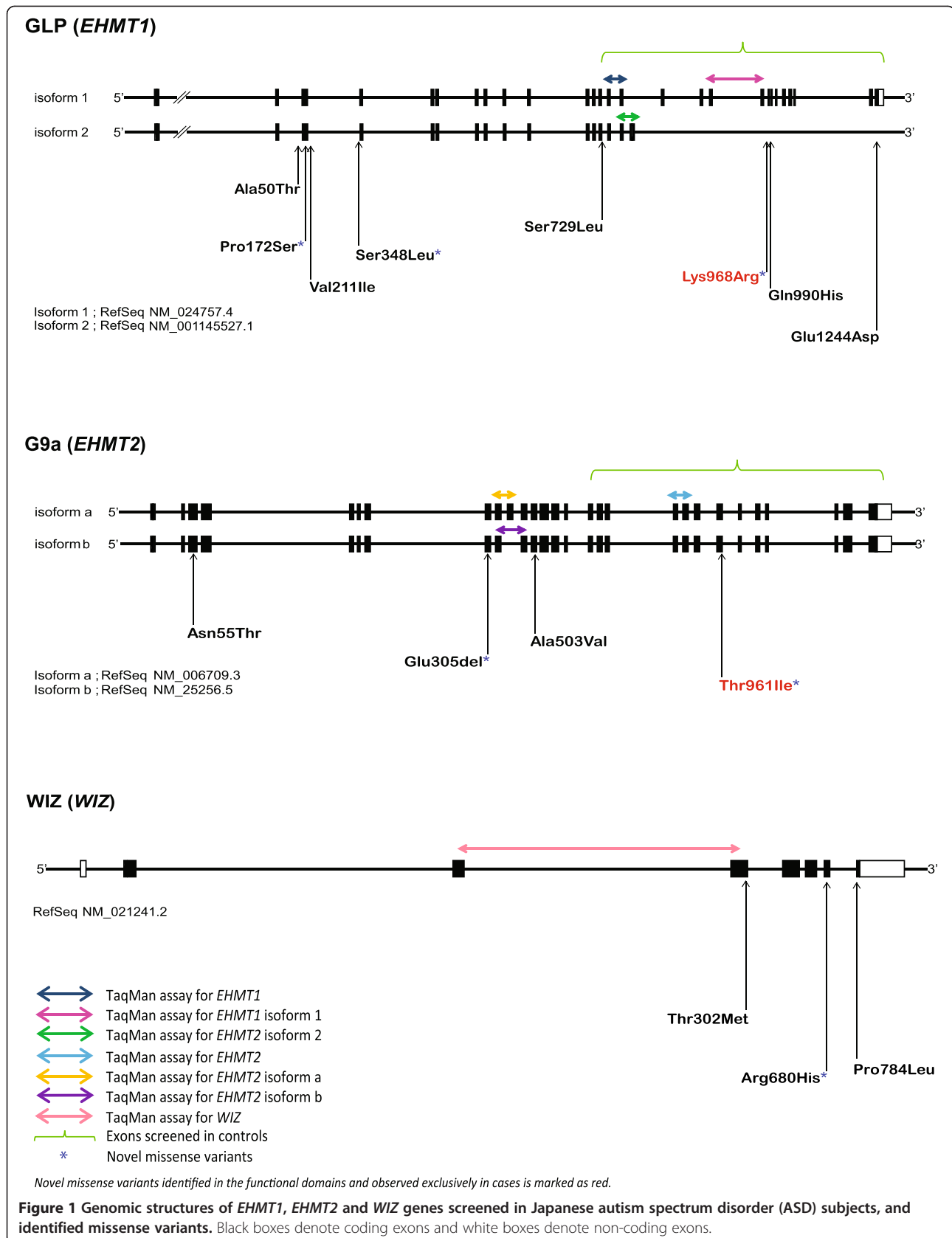


Table 1 Novel missense variants identified in *EHMT1*, *EHMT2* and *WIZ* genes from autism spectrum disorders (ASD) cases and controls

Gene	Chromosome position	Exon	cDNA position	Amino acid change	Protein domain	Autism count	Control count*	PolyPhen2	Provean	SIFT
<i>EHMT1</i>	9,140611506,C,T	Exon3	c.514C > T	p.Pro172Ser	-	2	-	Possibly damaging	Neutral	Damaging
<i>EHMT1</i>	9,140638415,C,T	Exon6	c.1,043C > T	p.Ser348Leu	-	2	-	Possibly damaging	Deleterious	Damaging
<i>EHMT1</i>	9,140707493,A,G	Exon20	c.2,903A > G	p.Lys968Arg	ANK repeat domain	1	0	Possibly damaging	Neutral	Tolerated
<i>EHMT2</i>	6,31857330,C,-	Exon8	c.913_915delGGA	p.Glu305del	-	1	-	NA	NA	NA
<i>EHMT2</i>	6,31851617,G,A	Exon22	c.2,882C > T	p.Thr961Ile	SET domain	1	0	Possibly damaging	Neutral	Tolerated
<i>WIZ</i>	19,15535180,C,T	Exon7	c.2,039G > A	p.Arg680His	-	1	-	Probably damaging	Neutral	Damaging

Legend: '-' denotes that the corresponding variant was not examined in control samples because it was located outside of a functional domain; ANK, ankyrin repeat domain; SET, Su(var)3-9-Enhancer of zeste-Trithorax domain.

Gene expression study

The *EHMT2* transcript expression was significantly elevated in the peripheral blood cells of ASD when compared with control samples ($P = 0.02$) (Figure 3B). But the *EHMT1* and *WIZ* levels were not significantly different between the ASD and control groups (Figure 3A, C). The gene expression analysis of *EHMT1*, *EHMT2* and *WIZ* in BA09, BA21, BA40 and DoRN regions from postmortem samples, showed no significant changes in expression levels between ASD and control groups (Figure 4A, B, C). We further examined the expression of *EHMT1* and *EHMT2* isoforms in the prefrontal cortex (BA09) of ASD patients. The *EHMT1* variant 1 (NM_024757.4) and *EHMT2* isoform a (NM_006709.3) were highly expressed compared to alternative isoforms. However, there was no significant difference in expression levels of these isoforms in the prefrontal

cortex, when the ASD cases were compared to controls (Figure 4D).

Discussion

Disruption of histone lysine methylation plays an important role in the pathogenesis of neurological disorders and cancer, as evidenced by the reports of genomic aberrations in histone methyltransferases in these diseases [10]. Since defective G9a and GLP histone lysine methyltransferases, give rise to autistic phenotypes [21], we searched for loss of function variants in the genes involved in H3K9 methylation, concentrating on rare mutations that show enrichment in ASD subjects. We focused on the variants located in the functional domains that are important in the formation of multimeric enzyme complex, and we identified the *EHMT1* ankyrin repeat domain variant (Lys968Arg) and *EHMT2* SET

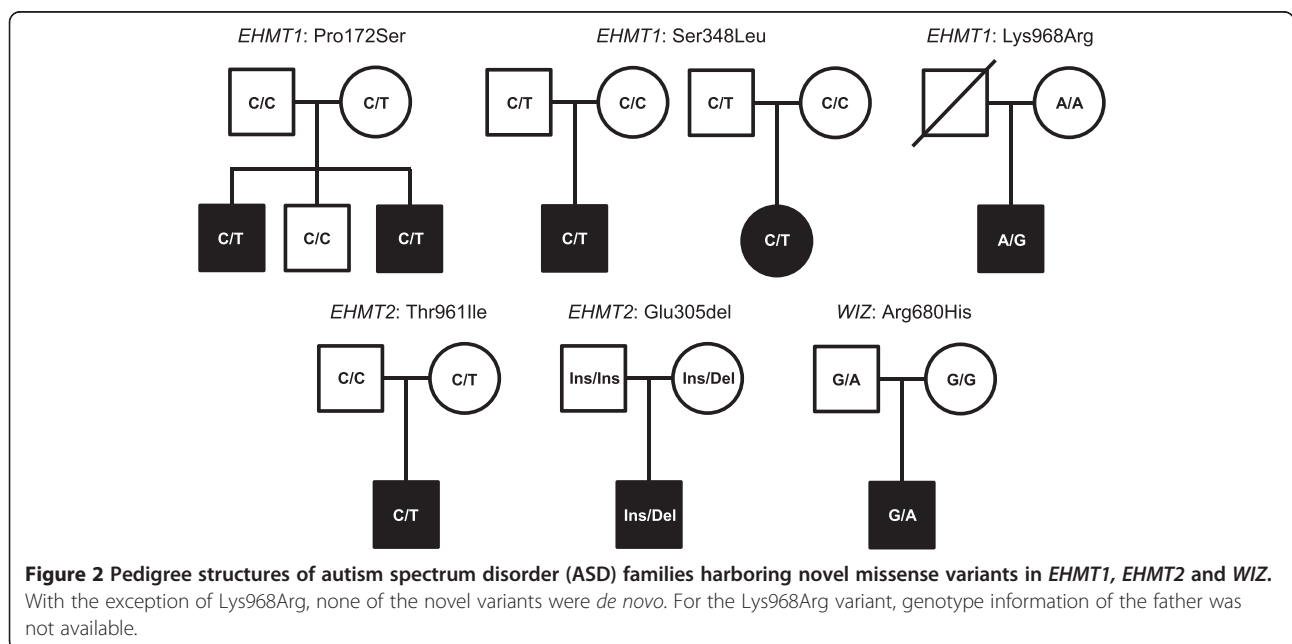


Table 2 Comparison of genotype and allele frequencies of *EHMT1* and *EHMT2* missense variants between autism spectrum disorder (ASD) cases and controls

Gene	Variant	Subject	Genotype			P-value	Allele		P-value	MAF ^a (%)
<i>EHMT1</i>	c.2903A > G		A/A	A/G	G/G		A	G		G
	Lys968Arg	Autism	292	1	0	0.14	585	1	0.46	0.170
	(ANK repeat domain)	Control	1,139	0	0		2,278	0		0
<i>EHMT2</i>	c.2882C > T		C/C	C/T	T/T		C	T		T
	Thr961Ile	Autism	292	1	0	0.14	585	1	0.46	0.170
	(SET domain)	Control	1,139	0	0		2,278	0		0

^aMAF: minor allele frequency. ANK, ankyrin repeat domain; SET, Su(var)3-9-Enhancer of zeste-Trithorax domain.

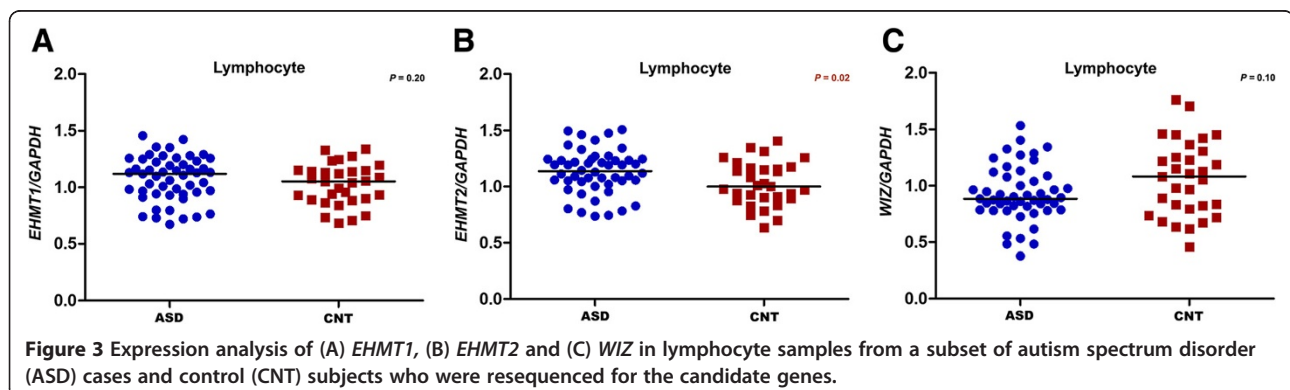
domain variant (Thr961Ile), which were present only in ASD cases and not in 1,140 control subjects. Although these two mutations were found exclusively in cases, case-control comparisons found no statistically significant association. Thus, our results did not support a role for these rare variants in ASD. This is in keeping with *in silico* analyses which predicted that the effects for both the *EHMT1* (Lys968Arg) and *EHMT2* (Thr961Ile) mutations would be 'neutral' and 'tolerated' by Proven and SIFT, respectively, although PolyPhen2 predicted a 'possibly damaging' phenotype.

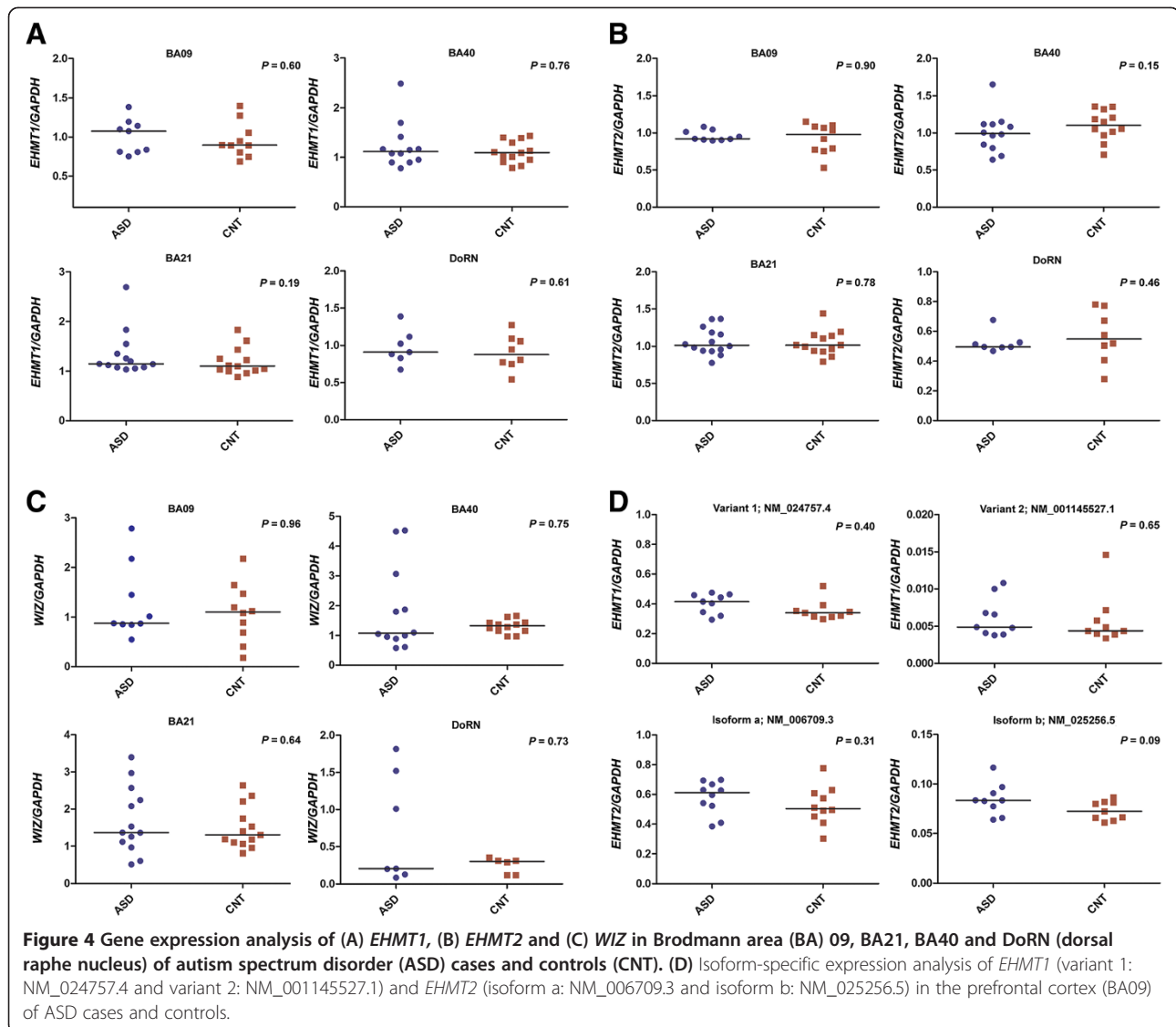
Since a large number of 'loss of function' variants are present in healthy human genomes [22], we speculate that the variants we identified may be private, owing to their lack of 'predicted functional defects,' consistent through the three algorithms. On the other hand, balanced chromosomal abnormalities seen in ASD and related neurodevelopmental disorders are reported to disrupt the *EHMT1* gene [23]. In addition, a *de novo* deletion and rare inherited loss of function mutation in *EHMT1* were observed in a sporadic ASD trio sample [24] and in ASD families [25], respectively. It is clear that to understand the exact role of our identified variants, it will be necessary to examine them using much larger sample sets and more sophisticated functional assessments.

Interestingly, we observed an overexpression of the *EHMT2* gene in peripheral blood cells from ASD patients

pointing towards a role of restricted chromatin state in ASD pathogenesis. A recent study showed increased expression of the *EHMT2* gene in lymphocytes and the *EHMT1* gene in both postmortem parietal cortex and lymphocyte samples, from patients with schizophrenia [26]. The study also found that a diagnosis of schizophrenia was a significant predictor for increased expression of histone methyltransferases. Therefore, the present results are interesting, given the genetic overlap between schizophrenia and ASD [27]. However, no significant changes in the expression levels of *EHMT1*, *EHMT2* or *WIZ* were observed in the postmortem brain samples from BA09, BA21, BA40 and DoRN region, between ASD subjects and controls. Additionally, we detected no differential expression of *EHMT1* and *EHMT2* isoforms in the prefrontal cortex (BA09) between the two subject groups. The results suggest an absence of common variants in the regulatory genomic elements of these genes associated with ASD.

Mutations in the chromatin remodeling enzymes have been reported in psychiatric diseases, which disrupt the chromatin regulation leading to altered neuronal function and behavioral abnormalities [28]. But in our study, such a loss of function mutation was not observed. Moreover, the identified mutations did not have a cogent effect in ASD pathogenesis, either through functional deficits or changes in expression levels. Therefore, it





can be concluded that the loss of function mutations in histone methyltransferases may constitute a rare event in ASD pathogenesis, which is supported by the fact that H3K9 modifying enzymes have fewer reported mutations, when compared to other chromatin regulators [29].

Since *EHMT2* overexpression correlates with the increased H3K9me2 levels [30], it could result in the repressed transcription of the genes/genetic network relevant to ASD pathogenesis. However, the results from expression analysis of peripheral blood cells should be interpreted cautiously because peripheral blood chromatin may not essentially provide information specific to a brain region or neuronal phenotype [31]. Future studies are warranted to profile the global H3K9 (mono and di) methylation status in ASD brain to delineate the genetic networks, which are dysregulated in ASD.

Although the present study did not show statistically significant enrichment of variants in ASD, their possible contribution to disease cannot be ruled out, due to the relatively small sample size restricting the statistical power of this study and also the absence of identified patient-specific mutations in global databases for the control population. From the available three-dimensional structures, it would appear that both mutations are located on the surface of the proteins (Additional file 3: Figure S1 (B and C)), implying a potential role for the variants in complex formation. Recent whole genome and exome sequencing studies have clearly shown a heterogeneous genetic basis for ASD and have identified a large number of candidate genes, converging on functional pathways of neuronal signaling and development, synapse function and chromatin regulation [32]. It is also known that SETDB1 and Suv39h1

co-exist in the H3K9 methylation multimeric complex, with interdependent functionality [33]. Therefore, the polygenic burden of ASD may mask the effects of single rare variants, obscuring their individual contribution to disease pathogenesis [34].

Conclusion

In summary, we identified two novel, rare missense variants in the *EHMT1* and *EHMT2* genes from ASD patients. We surmise that these variants alone may not be sufficient to exert a significant effect on ASD pathogenesis and that a concerted interaction with additional genetic or epigenetic effects may be needed to manifest the disease phenotype. The elevated expression of *EHMT2* observed in peripheral blood cells from ASD patients may support the notion of a restrictive chromatin state in ASD pathogenesis, similar to schizophrenia. Future studies with larger sample sizes and sophisticated functional assessments are warranted to define the precise role of *EHMT1* and *EHMT2* in ASD pathogenesis.

Additional files

Additional file 1: Table S1. Demographic details of autism spectrum disorder (ASD) and control brain samples from the NICHD Brain and Tissue Bank, University of Maryland School of Medicine (<http://medschool.umaryland.edu/btbank/>).

Additional file 2: Table S2. PCR amplification primers and conditions.

Additional file 3: Figure S1. (A) Domain structure of EHMT1 (GLP) and EHMT2 (G9a), indicating mutated and their conserved positions, (B) three-dimensional structure of EHMT1 (GLP), and (C) three-dimensional structure of EHMT2 (G9a). The structural data were obtained from Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>) and visualized using the UCSF Chimera package (<http://www.cgl.ucsf.edu/chimera/>) for determining the position of identified variants. The EHMT1/GLP complex (PDB entry: 3B95) contains three peptide chains, where the A and B chains are from GLP, and the P chain is a histone H3 N-terminal peptide. The B chain (blue), P chain (green) and the variant (red) are shown in figure (B). The mutation is located on the surface of the protein. The EHMT2/G9a complex (PDB entry: 3K5K) contains two SET domains from G9a (A and B chains). The A chain is shown here in (C) with ligands DXQ (7-[3-(dimethylamino) propoxy]-6-methoxy-2-(4-methyl-1,4-diazepan-1-yl)-N-(1-methylpiperidin-4-yl)quinazolin-4-amine) and S-adenosyl-L-homocysteine marked in green and cyan, respectively. The variant position (red) is located on the surface of the protein, away from substrate binding sites.

Additional file 4: Table S3. List of TaqMan assay IDs used for gene expression studies.

Additional file 5: Table S4. Novel and previously reported variants in the ASD cohort and variants specific to the control population.

Abbreviations

ADI-R: Autism Diagnostic Interview-Revised; ASD: autism spectrum disorders; BA: Brodmann's area; CNT: control; DoRN: dorsal raphe nucleus; DSM-IV-TR: *Diagnostic and Statistical Manual, Fourth Edition, Text Revision*; MAF: minor allele frequency; NICHD: National Institute of Child Health and Human Development; RT-PCR: reverse transcription polymerase chain reaction; SCID: Structured Clinical Interview for DSM-IV; SET: Su(var)3-9-Enhancer of zeste-Trithorax domain.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SB participated in the study design, performed the experiments, data analysis, interpreted the data and drafted the manuscript. YoI performed the experiments and data analysis. MM recruited participants, undertook the clinical evaluation and collected DNA samples. TT recruited participants, undertook the clinical evaluation and collected DNA samples. MTo recruited participants and collected DNA samples. CS recruited participants and collected DNA samples. KY recruited participants, undertook the clinical evaluation and collected DNA samples. Yal recruited participants, undertook the clinical evaluation and collected DNA samples. KS recruited participants, undertook the clinical evaluation and collected DNA samples. MTs recruited participants, undertook the clinical evaluation and collected DNA samples. MO performed *in silico* protein structure analysis. SF performed *in silico* protein structure analysis. TO analyzed and interpreted the data. KE analyzed and interpreted the data. MI interpreted the data. NM participated in the study design. YS conceived the study and participated in the study design. TY conceived the study and participated in the study design, interpreted the data and prepared the manuscript. All authors read and approved the manuscript.

Authors' information

Kayoko Esaki: Research Fellow of Japan Society for the Promotion of Science.

Acknowledgments

This study was supported in part by Grant-in-Aid for Scientific Research on Innovative Areas (TY) from the Japan Society for the Promotion of Science (JSPS), Japan, and by CREST (Core Research for Evolutionary Science and Technology) (YS and TY) from the Japan Science and Technology Agency (JST), Japan. In addition, this study was supported by RIKEN Brain Science Institute Funds (TY). Sections of this study was conducted as part of the 'Development of biomarker candidates for social behavior' (TY) and 'Integrated research on neuropsychiatric disorders' (NM) projects, carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan. A part of this work was also supported by a grant 'Platform for Drug Discovery, Informatics, and Structural Life Science' (MO and SF) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Author details

¹Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. ²Department of Psychiatry and Neurology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan. ³Department of Psychiatry, Division of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan. ⁴Graduate School of Information Science, Nagoya University, Nagoya, Aichi, Japan. ⁵Faculty of Engineering, Maebashi Institute of Technology, Maebashi, Japan. ⁶Faculty of Sociology, Chukyo University, Chukyo, Aichi, Japan. ⁷Research Center for Child Mental Development, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan. ⁸Cellular Memory Laboratory, RIKEN, Wako, Saitama, Japan. ⁹CREST (Core Research for Evolutionary Science and Technology), Japan Science and Technology Agency, Kawaguchi, Saitama, Japan.

Received: 20 June 2014 Accepted: 23 September 2014

Published: 6 October 2014

References

1. Volkmar FR, Pauls D: **Autism.** *Lancet* 2003, **362**:1133–1141.
2. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, Montiel-Nava C, Patel V, Paula CS, Wang C: **Global prevalence of autism and other pervasive developmental disorders.** *Autism Res* 2012, **5**:160–179.
3. Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L, Bryson S, Carver LJ, Constantino JN, Dobkins K: **Recurrence risk for autism spectrum disorders: a baby siblings research consortium study.** *Pediatrics* 2011, **128**:e488–e495.
4. Ronald A, Hoekstra RA: **Autism spectrum disorders and autistic traits: a decade of new twin studies.** *Am J Med Genet B Neuropsychiatr Genet* 2011, **156**:255–274.
5. Murdoch JD: **Recent developments in the genetics of autism spectrum disorders.** *Current Opinion Genet Dev* 2013, **23**:310–315.
6. Muers M: **Human genetics: fruits of exome sequencing for autism.** *Nat Rev Genet* 2012, **13**:377–377.
7. 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.

8. Gratten J, Visscher PM, Mowry BJ, Wray NR: **Interpreting the role of *de novo* protein-coding mutations in neuropsychiatric disease.** *Nat Genet* 2013, **45**:234–238.
9. Schanen NC: **Epigenetics of autism spectrum disorders.** *Hum Mol Genet* 2006, **15**:R138–R150.
10. Black JC, Van Rechem C, Whetstone JR: **Histone lysine methylation dynamics: establishment, regulation, and biological impact.** *Mol Cell* 2012, **48**:491–507.
11. Martin C, Zhang Y: **The diverse functions of histone lysine methylation.** *Nat Rev Mol Cell Biol* 2005, **6**:838–849.
12. Tachibana M, Ueda J, Fukuda M, Takeda N, Ohta T, Iwanari H, Sakihama T, Kodama T, Hamakubo T, Shinkai Y: **Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9.** *Genes Dev* 2005, **19**:815–826.
13. Ueda J, Tachibana M, Ikura T, Shinkai Y: **Zinc finger protein Wiz links G9a/GLP histone methyltransferases to the co-repressor molecule CtBP.** *J Biol Chem* 2006, **281**:20120–20128.
14. Kleefstra T, Brunner HG, Amiel J, Oudakker AR, Nillesen WM, Magee A, Geneviève D, Cormier-Daire V, Van Esch H, Fryns J-P: **Loss-of-function mutations in *EHMT1* cause the 9q34 subtelomeric deletion syndrome.** *Am J Hum Genet* 2006, **79**:370–377.
15. Kleefstra T, van Zelst-Stams WA, Nillesen WM, Cormier-Daire V, Houge G, Foulds N, van Dooren M, Willemsen MH, Pfundt R, Turner A: **Further clinical and molecular delineation of the 9q subtelomeric deletion syndrome supports a major contribution of *EHMT1* haploinsufficiency to the core phenotype.** *J Med Genet* 2009, **46**:598–606.
16. Balemans M, Huibers MM, Eikelenboom NW, Kuipers AJ, van Summeren RC, Pijpers MM, Tachibana M, Shinkai Y, van Bokhoven H, Van der Zee CE: **Reduced exploration, increased anxiety, and altered social behavior: autistic-like features of euchromatin histone methyltransferase 1 heterozygous knockout mice.** *Behav Brain Res* 2010, **208**:47–55.
17. Balemans MC, Kasri NN, Kopanitsa MV, Afinowi NO, Ramakers G, Peters TA, Beynon AJ, Janssen SM, van Summeren RC, Eeftens JM: **Hippocampal dysfunction in the euchromatin histone methyltransferase 1 heterozygous knockout mouse model for Kleefstra syndrome.** *Hum Mol Genet* 2013, **22**:852–866.
18. Schaefer A, Sampath SC, Intrator A, Min A, Gertler TS, Surmeier DJ, Tarakhovskiy A, Greengard P: **Control of cognition and adaptive behavior by the GLP/G9a epigenetic suppressor complex.** *Neuron* 2009, **64**:678–691.
19. Lord C, Rutter M, Le Couteur A: **Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders.** *J Autism Dev Disord* 1994, **24**:659–685.
20. First MB, Spitzer RL, Gibbon M, Williams JB: *Structured Clinical Interview for DSM-IV® Axis I Disorders (SCID-I), Clinician Version, User's Guide.* Washington, DC: American Psychiatric Press; 1997.
21. Shinkai Y, Tachibana M: **H3K9 methyltransferase G9a and the related molecule GLP.** *Genes Dev* 2011, **25**:781–788.
22. MacArthur DG, Balasubramanian S, Frankish A, Huang N, Morris J, Walter K, Jostins L, Habegger L, Pickrell JK, Montgomery SB: **A systematic survey of loss-of-function variants in human protein-coding genes.** *Science* 2012, **335**:823–828.
23. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, Ernst C, Hanscom C, Rossin E, Lindgren AM: **Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries.** *Cell* 2012, **149**:525–537.
24. O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Levy R, Ko A, Lee C, Smith JD: **Sporadic autism exomes reveal a highly interconnected protein network of *de novo* mutations.** *Nature* 2012, **485**:246–250.
25. Jiang Y-H, Yuen RK, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M: **Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing.** *Am J Human Genet* 2013, **93**:249–263.
26. Chase KA, Gavin DP, Guidotti A, Sharma RP: **Histone methylation at H3K9: evidence for a restrictive epigenome in schizophrenia.** *Schizophrenia Res* 2013, **149**:15–20.
27. Vorstman JS, Burbach JP: **Autism and Schizophrenia: Genetic and Phenotypic Relationships.** In *Comprehensive Guide to Autism.* Edited by Patel VB, Preedy VR, Martin CR. New York: Springer; 2014:1645–1662.
28. Renthal W, Nestler EJ: **Chromatin regulation in drug addiction and depression.** *Dialogues Clin Neurosci* 2009, **11**:257.
29. Van Rechem C, Whetstone JR: **Examining the impact of gene variants on histone lysine methylation.** *Biochimica et Biophysica Acta (BBA)-Gene Regulat Mechanisms* 2014.
30. Maze I, Covington HE, Dietz DM, LaPlant Q, Renthal W, Russo SJ, Mechanic M, Mouzon E, Neve RL, Haggarty SJ: **Essential role of the histone methyltransferase G9a in cocaine-induced plasticity.** *Science* 2010, **327**:213–216.
31. Sharma RP: **Blood chromatin as a biosensor of the epigenetic milieu: a tool for studies in living psychiatric patients.** *Epigenomics* 2012, **4**:551–559.
32. Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z, Vorstman JA, Thompson A, Regan R, Pilorge M, Pellecchia G, Pagnamenta AT, Oliveira B, Marshall CR, Magalhaes TR, Lowe JK, Howe JL, Griswold AJ, Gilbert J, Duketis E, Dombroski BA, De Jonge MV, Cuccaro M, Crawford EL, Correia CT, Conroy J, et al: **Convergence of genes and cellular pathways dysregulated in autism spectrum disorders.** *Am J Hum Genet* 2014, **94**:677–694.
33. Fritsch L, Robin P, Mathieu JR, Souidi M, Hinaux H, Rougeulle C, Harel-Bellan A, Ameyar-Zazoua M, Ait-Si-Ali S: **A subset of the histone H3 lysine 9 methyltransferases Suv39h1, G9a, GLP, and SETDB1 participate in a multimeric complex.** *Mol Cell* 2010, **37**:46–56.
34. Purcell SM, Moran JL, Fromer M, Ruderfer D, Solovieff N, Roussos P, O'Dushlaine C, Chambert K, Bergen SE, Kahler A, Duncan L, Stahl E, Genovese G, Fernandez E, Collins MO, Komiya NH, Choudhary JS, Magnusson PK, Banks E, Shakir K, Grimella K, Fennell T, DePristo M, Grant SG, Haggarty SJ, Gabriel S, Scolnick EM, Lander ES, Hultman CM, Sullican PF, et al: **A polygenic burden of rare disruptive mutations in schizophrenia.** *Nature* 2014, **506**:185–190.

doi:10.1186/2040-2392-5-49

Cite this article as: Balan et al.: Exon resequencing of H3K9 methyltransferase complex genes, *EHMT1*, *EHTM2* and *WIZ*, in Japanese autism subjects. *Molecular Autism* 2014 **5**:49.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

