25kDa (*SNAP25*) were compared in the postmortem brains of individuals with autism and healthy controls. We performed qPCR analysis using the TaqMan method in ABI PRISM 7900HT SDS software. We used *GAPDH* as the endogenous reference. The Ct values of the target gene were normalized (ΔCt) to that of *GAPDH*. Any alteration in gene expression in the autism group was analyzed by relative quantification (ΔΔCt) against the control group. We determined the fold change in gene expression between the autism and control groups by calculating $2^{-\Delta\Delta Ct}$.

Statistical analysis

We examined the difference in age, postmortem interval (PMI) and gene expression between the autism and control groups using a *t* test, and the χ^2 test was used to examine the difference in sex distribution between the 2 groups. Any correlation between the expression of *ZNF804A* and *SNAP25* was examined using the Pearson correlation coefficient.

Results

Genetic association study

Power analysis showed that the overall sample size of 841 families provides 91% power to detect an odds ratio of 1.5 for an allele frequency of 0.1 at an α of 0.05.

In the family-based association test (Table 1), rs7603001 located in intron 2 of *ZNF804A* was nominally associated with autism (z score for risk allele A = 2.362, $p = 0.018$). When individuals with autism were categorized based on verbal abilities, a stronger association of this SNP was found in the Lvrb families (z score for risk allele A = 2.657, $p = 0.008$), whereas no association was observed in the Hvrb families (z score = 0, $p > 0.99$; data not shown). The A allele of rs7603001 was overtransmitted to the individuals with autism (transmission 53% in all families v. 54% in Lvrb families). The genetic association, however, did not withstand multiple testing correction. None of the other SNPs showed any significant association with autism. Genotypic distribution of SNPs were in Hardy–Weinberg equilibrium.

Three LD blocks were identified in *ZNF804A* (Table 2; Appendix, Fig. S1B). The haplotype ACTCATC in the second LD block (rs1038197, rs13026742, rs1987025, rs17509608, rs7603001, rs1344706, rs7593816) showed a significant association with autism in the Lvrb families (z score = 3.103, $p = 0.004$). This haplotype includes the risk allele A of rs7603001. The association remained significant ($p = 0.047$) following multiple testing correction by permutation analysis (100 000 permutations). Interestingly, the haplotype ACTCGTC that includes the protective G allele of rs7603001 showed a tendency toward association with autism in the Lvrb families (z score = –1.907, $p = 0.05$).

Taken together, the A allele of rs7603001 may be considered as a risk allele and the G allele as a protective allele of autism in individuals with verbal defects.

Copy number variation at the ZNF804A *locus*

We observed CNV at the *ZNF804A* locus in the same DNA samples that we used in our genetic association study (Table 3): copy number gain (3 copies) in 6 samples and copy number loss (1 copy) in 2 samples. One of the CNVs (gain)

Table 2: Haplotype association analysis of *ZNF804A* with autism in the low verbal subgroup

Block; haplotype	Frequency	*p* value
Block 1 (SNPs 01–04)		
GCTT	0.377	0.09
AGCT	0.317	0.57
ACCT	0.16	0.06
ACTG	0.135	0.09
Block 2 (SNPs 06–12)		
GTACATC	0.234	0.08
ACTCGGT	0.193	0.69
ACTCGGC	0.178	0.13
ACTCGTC	0.143	0.05
ATTTATC	0.104	0.57
ATTCATC	0.073	0.54
ACTCATC	0.057	0.004
Block 3 (SNPs 14,15)		
AC	0.531	0.73
GC	0.292	0.07
AT	0.177	0.08

SNP = single nucleotide polymorphism; *ZNF804A* = zinc finger protein 804A.

Table 3: Copy number variation at *ZNF804A* locus

Sample ID*	Sex	Age, yr	Affection status	CNV	Gain/loss	De novo/inherited	Lvrb/Hvrb
AU0154302	Male	14	Autism	3	Gain	De novo	Lvrb
AU023803	Male	8	Autism	3	Gain	De novo	Lvrb
AU077304	Male	16	Autism	3	Gain	De novo	Lvrb
AU0871302	Male	7	Autism	1	Loss	De novo	Hvrb
AU1092302	Male	3	Autism	3	Gain	Inherited	Lvrb
AU1466302	Male	10	Autism	1	Loss	De novo	Lvrb
AU1650305	Male	7	Autism	3	Gain	De novo	Lvrb
AU1655301	Male	16	Autism	3	Gain	De novo	Lvrb

CNV = copy number variation; Hvrb = autistic, healthy; Lvrb: autistic, low verbal; *ZNF804A* = zinc finger protein 804A.
*Autism Genetic Resource Exchange (AGRE) identifier.

was inherited from the mother, whereas the other CNVs were caused by de novo events. All the CNVs were observed in boys with autism (age 7–16 yr); all but 1 of them belonged to the Lvrb category. We also observed CNVs in 7 maternal samples (gain in 6 and loss in 1 sample) and in 2 paternal samples (gain in 1 and loss in 1 sample).

ZNF804A *silencing*

Figure 1A shows a significant difference in the expression of *ZNF804A* between the cells electroporated with *ZNF804A*-specific siRNA and the negative control ($p = 0.003$). In qPCR, the expression of *ZNF804A* was knocked down by 77%. *ZNF804A* silencing was confirmed by Western blot (Fig. 1B).

In the *ZNF804A*-knockdown SH-SY5Y cells, the expression of *SNAP25* was significantly reduced compared with the negative controls ($p = 0.009$; Fig. 1C). This was confirmed by Western blot (Fig. 1B). We also found a significant positive correlation between the expression of *ZNF804A* and *SNAP25* (Pearson $r = 0.713$, $p = 0.006$; Fig. 1D). There was no significant alteration in the expression of other genes (data not shown).

Gene expression in postmortem brain

We obtained postmortem brain samples from the ACG (8 autism, 13 control), MC (7 autism, 8 control) and thalamus (8 autism, 9 control). Demographic characteristics of the individuals from whom the samples were obtained are described in Table 4.

There was no significant difference in age, postmortem interval and sex distribution between the control and autism groups (see the Appendix, Table S2). The expression of *ZNF804A* (fold-change $2^{-\Delta\Delta Ct} = 0.277$, $p = 0.009$) and *SNAP25* ($2^{-\Delta\Delta Ct} = 0.258$, $p = 0.009$) were significantly reduced in the ACG of individuals with autism compared with controls (Fig. 2A and B). We also found a strong positive correlation between the expression of *ZNF804A* and *SNAP25* in the ACG (Pearson $r = 0.837$, $p < 0.001$; Fig. 2C). In the MC and thalamus, the expression of *ZNF804A* or *SNAP25* did not differ

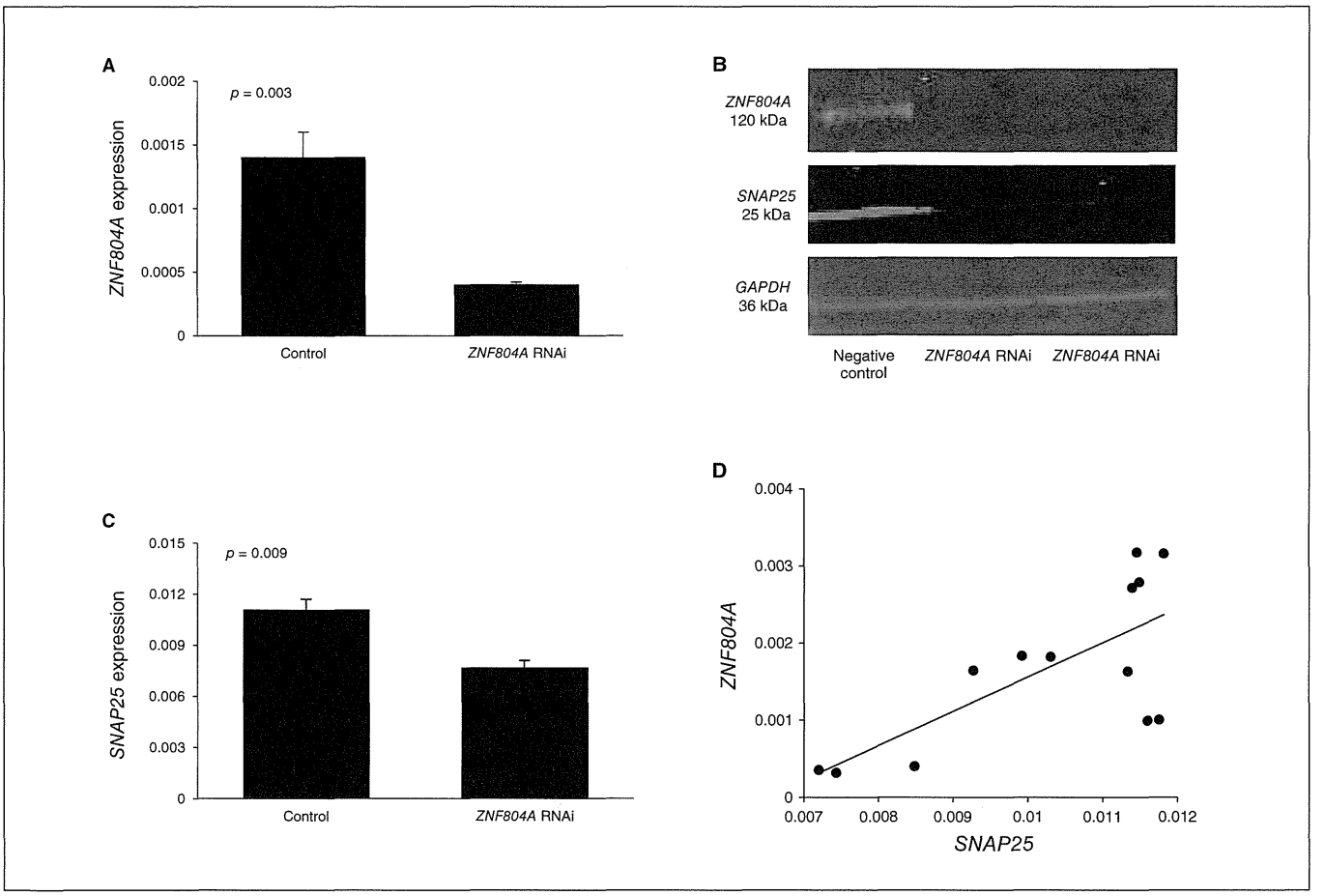

Fig. 1: Zinc finger protein 804A (*ZNF804A*) silencing in SH-SY5Y cells. **(A)** *ZNF804A* expression was knocked down by 77% ($p = 0.003$) in the SH-SY5Y cells electroporated with *ZNF804A*-specific small interfering RNA (siRNA) compared with the negative controls. **(B)** Comparison of the expression of *ZNF804A* and *SNAP25* between *ZNF804A*-silenced SH-SY5Y cells and negative control siRNA-transfected SH-SY5Y cells in Western blot. The expression of *SNAP25* was downregulated in *ZNF804A*-silenced cells. *GAPDH* was used as the loading control. **(C)** *SNAP25* expression was significantly lower in the *ZNF804A*-silenced cells compared with the negative controls ($p = 0.009$). **(D)** Positive correlation between the expression of *ZNF804A* and *SNAP25* in SH-SY5Y cells (Pearson $r = 0.713$; $p = 0.006$).

significantly between the control and autism groups (data not shown).

Discussion

We suggest that *ZNF804A* could be a risk gene mediating the intermediate phenotypes related to verbal skills in individuals with autism. In a GWAS of autism, Anney and colleagues (supplementary data)[7] reported nominal association of several *ZNF804A* SNPs (rs17508877, rs1038197, rs7585738, rs6730122, rs10199843) with the Lvrb subset of individuals with autism. To our knowledge, the present study is the first to confirm the association of *ZNF804A* with a subgroup of individuals with autism characterized by verbal deficits.

The SNP rs7603001, which showed nominal association with autism in all families and in the subset of Lvrb families, is located in intron 2 of *ZNF804A*. Even though this SNP may not have a functional significance, putative regulatory regions have been predicted (FastSNP; http://fastsnp.ibms.sinica.edu.tw/pages/inputSNPListAnalysis.jsp) for the SNPs

Table 4: Postmortem brain tissue information

Sample ID*	Diagnosis	Age, yr	Sex	PMI, h	Race	Cause of death	Brain region†
818	Control	27	M	10	White	Multiple injuries	ACG
1065	Control	15	M	12	White	Multiple injuries	ACG, THL
1297	Control	15	M	16	African American	Multiple injuries	ACG, MC, THL
1407	Control	9	F	20	African American	Asthma	ACG, MC, THL
1541	Control	20	F	19	White	Head injuries	ACG, MC, THL
1649	Control	20	M	22	Hispanic	Multiple injuries	ACG, MC, THL
1708	Control	8	F	20	African American	Asphyxia, multiple injuries	ACG, MC, THL
1790	Control	13	M	18	White	Multiple injuries	ACG
1793	Control	11	M	19	African American	Drowning	ACG, MC, THL
1860	Control	8	M	5	White	Cardiac arrhythmia	ACG
4543	Control	28	M	13	White	Multiple injuries	ACG, MC, THL
4638	Control	15	F	5	White	Chest injuries	ACG
4722	Control	14	M	16	White	Multiple injuries	ACG, MC, THL
797	Autism	9	M	13	White	Drowning	ACG, THL
1638	Autism	20	F	50	White	Seizure	ACG, MC, THL
4231	Autism	8	M	12	African American	Drowning	ACG, MC, THL
4721	Autism	8	M	16	African American	Drowning	ACG, MC, THL
4899	Autism	14	M	9	White	Drowning	ACG, MC, THL
5000	Autism	27	M	8.3	NA	NA	ACG, MC, THL
6294	Autism	16	M	NA	NA	NA	ACG, MC, THL
6640	Autism	29	F	17.83	NA	NA	ACG, MC, THL

ACG = anterior cingulate gyrus; F = female; M = male; MC = motor cortex; NA = not available; PMI = postmortem interval; THL = thalamus.
*Autism Tissue Program (ATP) identifier.
†Brain regions for which each sample was available.

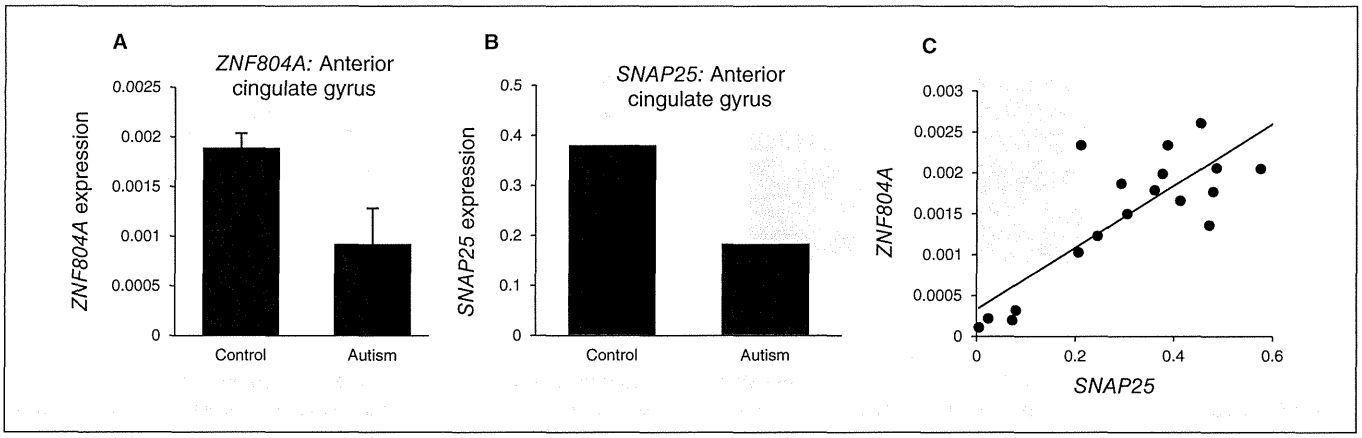

Fig. 2: Gene expression in postmortem brain. The expression of **(A)** zinc finger protein 804A (*ZNF804A*; $p = 0.009$) and **(B)** *SNAP25* ($p = 0.009$) were significantly reduced in the anterior cingulate gyrus (ACG) of individuals with autism compared with healthy controls. **(C)** Positive correlation between the expression of *ZNF804A* and *SNAP25* in the ACG (Pearson $r = 0.837$; $p < 0.001$).

included in the LD bin of rs7603001. The r^2 LD value between rs7603001, the SNP that was associated with autism in our study, and the SNPs that were associated with autism in the GWAS[7] ranged between 0.25 and 0.28. The GWAS finding was thus replicated at the gene level, not at the level of specific SNPs.

In addition to genetic association, CNVs (gain and loss), mostly de novo, were observed at the *ZNF804A* locus of boys with autism who had a verbal deficit. Griswold and colleagues[8] and Talkowski and colleagues[9] have also reported CNVs at the *ZNF804A* locus in individuals with autism. Since the penetrance of CNVs is variable, it is not possible to predict the effect of these CNVs in the pathogenesis of autism. Copy number gain and loss were observed in autistic individuals, and similar CNVs were observed in unaffected parents. Furthermore, similar CNVs have also been observed in patients with other neuropsychiatric disorders,[32] suggesting pleiotropic effects. Future studies to correlate specific CNVs with detailed clinical characteristics and to assess their effects on neurodevelopment are warranted.

Impaired linguistic/verbal ability is a key cognitive defect in individuals with autism.[33,34] Based on our results, we suggest that ZNF804A could be a modulator of verbal traits in individuals with autism. There is ample evidence of the involvement of ZNF804A in the development of ToM,[10] which in turn, is closely intertwined with the development of linguistic/verbal abilities from infancy.[15–17]

Genetic, neuropsychological and neuroimaging studies have suggested that ZNF804A is involved in higher-order cognitive processes such as ToM,[10] working memory[35] and executive control of attention.[36] It has been found to play a pivotal role in the maintenance of functional connectivity in the brain.[37,38] We observed a reduced expression of *ZNF804A* in the ACG of individuals with autism compared with controls. The ACG, a brain region vital for cognitive and behavioural abilities, is involved in emotion formation and processing, learning and memory.[39,40] Downregulated expression of *ZNF804A* could lead to adverse effects on the cognitive processes associated with this gene.

Even though the previous studies on *ZNF804A* were focused on schizophrenia, overwhelming evidence suggests that the risk variants of this gene may be involved in the modulation of intermediate cognitive phenotypes associated with the disorder rather than the disorder itself.[10,35,36,38] Adult-onset schizophrenia and early-onset autism, despite being 2 clinically distinct, complex neurodevelopmental disorders, share several deficits in cognitive functioning.[41–43] A deficient ToM has been identified as a potential contributor to the social cognitive dysfunction in individuals with schizophrenia and autism,[44,45] and it could be a common factor mediating ToM-related key intermediate phenotypes in people with these disorders. Several studies have shown the association of *ZNF804A* variants with cognitive dysfunction in individuals with schizophrenia.[46–48] Interestingly, we observed a stronger association of *ZNF804A* in individuals with an autism subtype characterized by verbal deficits.

The protein sequence of ZNF804A shows a C2H2-type zinc-finger domain at its N-terminal end, suggesting that it may bind DNA and have a role in regulating gene expression.[18] ZNF804A has been found to modulate the expression of several genes implicated in the pathogenesis of schizophrenia.[18,49]

We examined the possible role of ZNF80A as a regulator of the expression of genes previously reported to be associated with verbal/linguistic abilities and/or social cognition. The expression of *SNAP25* was downregulated in *ZNF804A*-silenced cells compared with control cells. Furthermore, the expression of *SNAP25* was significantly reduced in the ACG of individuals with autism, and a strong positive correlation was observed between the expression of *ZNF804A* and *SNAP25* in the ACG.

SNAP25 is a presynaptic plasma membrane protein that is specifically and abundantly expressed in nerve cells. It participates in synaptic vesicle exocytosis through the formation of a soluble NSF attachment protein receptor complex[50] and plays a pivotal role in modulating calcium homeostasis.[51] SNAP25 is important for axonal growth and synaptic plasticity, 2 essential steps in the wiring of the central nervous system.[50,52] *SNAP25* variants have been found to modulate cognitive performances.[29,53,54] *SNAP25* is located in a chromosomal region (20p12–p11.2) with a previously suggested linkage to intelligence.[55] Moreover, polymorphisms in *SNAP25* have been associated with hyperactivity in individuals with autism.[56] However, at present, there is no literature linking ZNF804A and SNAP25.

Limitations

A replication study in a larger cohort of verbally deficient individuals with autism from different racial backgrounds would have been more informative. Further studies on the functional implications of *ZNF804A* CNVs and on the nature of the interaction between ZNF804A and SNAP25 in the pathogenesis of autism are warranted. The small number of postmortem brain samples used is another limitation of our study.

Conclusion

We suggest that *ZNF804A* could have a pivotal role in mediating the intermediate phenotypes associated with verbal traits in individuals with autism. It could be a common factor modulating the ToM-related intermediate phenotypes in individuals with schizophrenia and autism.

Acknowledgements: We gratefully acknowledge the resources provided by the AGRE Consortium and the participating AGRE families. The AGRE is a program of Autism Speaks and is supported in part by grant 1U24MH081810 from the National Institute of Mental Health to Clara M. Lajonchere (P.I.). We thank Dr. Jane Pickett, Director of Brain Resources and Data, ATP, for facilitating brain tissue collection. Human tissue was obtained from the NICHD BTB for Developmental Disorders at the University of Maryland. Tissue samples were also provided by the Harvard Brain Tissue Resource Center, which is supported in part by PHS grant number R24 MH 068855. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (A.A., K.N.), the Takeda Science Foundation, Japan (K.S.), and partly by the Strategic Research Program for Brain Sciences (Integrated research on neuropsychiatric disorders). We thank Tae Takahashi and Mika Oyaizu for technical assistance.

Competing interests: None declared.

Contributors: A. Anitha, I. Thanseem and K. Nakamura designed the study, acquired and analyzed the data and wrote the article. M.M. Vasu, K. Yamada, T. Ueki, Y. Iwayama and T. Toyota acquired and analyzed the data and reviewed the article. K.J. Tsuchiya, Y. Iwata and K. Suzuki analyzed the data and reviewed the article. T. Sugiyama, M. Tsujii, T. Yoshikawa and N. Mori designed the study and reviewed the article. All authors approved the final version for publication.

References

1. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: Both shared and disorder-specific pathogenesis via perinatal inflammation? *Pediatr Res* 2011;69:26R-33R.
2. Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. *Genome Med* 2009;1:102.
3. O'Donovan MC, Craddock N, Norton N, et al. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 2008;40:1053-5.
4. Riley B, Thiselton D, Maher BS, et al. Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. *Mol Psychiatry* 2010;15:29-37.
5. Li M, Luo XJ, Xiao X, et al. Allelic differences between Han Chinese and Europeans for functional variants in ZNF804A and their association with schizophrenia. *Am J Psychiatry* 2011;168:1318-25.
6. Williams HJ, Norton N, Dwyer S, et al. Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. *Mol Psychiatry* 2011;16:429-41.
7. Anney R, Klei L, Pinto D, et al. A genome-wide scan for common alleles affecting risk for autism. *Hum Mol Genet* 2010;19:4072-82.
8. Griswold AJ, Ma D, Cukier HN, et al. Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. *Hum Mol Genet* 2012;21:3513-23.
9. Talkowski ME, Rosenfeld JA, Blumenthal I, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell* 2012;149:525-37.
10. Walter H, Schnell K, Erk S, et al. Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. *Mol Psychiatry* 2011;16:462-70.
11. Baron-Cohen S. The autistic child's theory of mind: a case of specific developmental delay. *J Child Psychol Psychiatry* 1989;30:285-97.
12. Yirmiya N, Erel O, Shaked M, et al. Meta-analyses comparing theory of mind abilities of individuals with autism, individuals with mental retardation, and normally developing individuals. *Psychol Bull* 1998;124:283-307.
13. Bora E, Yucel M, Pantelis C. Theory of mind impairment in schizophrenia: meta-analysis. *Schizophr Res* 2009;109:1-9.
14. Frith CD, Frith U. Interacting minds — a biological basis. *Science* 1999;286:1692-5.
15. Miller CA. Developmental relationships between language and theory of mind. *Am J Speech Lang Pathol* 2006;15:142-54.
16. Ruffman T, Slade L, Crowe E. The relation between children's and mothers' mental state language and theory-of-mind understanding. *Child Dev* 2002;73:734-51.
17. Dahlgren S, Dahlgren Sandberg A, Larsson M. Theory of mind in children with severe speech and physical impairments. *Res Dev Disabil* 2010;31:617-24.
18. Girgenti MJ, Loturco JJ, Maher BJ. ZNF804a regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2. *PLoS ONE* 2012;7:e32404.
19. Geschwind DH, Sowinski J, Lord C, et al. The autism genetic resource exchange: a resource for the study of autism and related neuropsychiatric conditions. *Am J Hum Genet* 2001;69:463-6.
20. 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;24:659-85.
21. Goldberg TE, Iudicello J, Russo C, et al. BDNF Val66Met polymorphism significantly affects d' in verbal recognition memory at short and long delays. *Biol Psychol* 2008;77:20-4.
22. Vernes SC, Newbury DF, Abrahams BS, et al. A functional genetic link between distinct developmental language disorders. *N Engl J Med* 2008;359:2337-45.
23. Palo OM, Antila M, Silander K, et al. Association of distinct allelic haplotypes of Drosoph Inf ServC1 with psychotic and bipolar spectrum disorders and with underlying cognitive impairments. *Hum Mol Genet* 2007;16:2517-28.
24. Beaver KM, Delisi M, Vaughn MG, et al. Association between the A1 allele of the DRD2 gene and reduced verbal abilities in adolescence and early adulthood. *J Neural Transm* 2010;117:827-30.
25. Balter M. Genetics. First gene linked to speech identified. *Science* 2001;294:32.
26. Kircher T, Krug A, Markov V, et al. Genetic variation in the schizophrenia-risk gene neuregulin 1 correlates with brain activation and impaired speech production in a verbal fluency task in healthy individuals. *Hum Brain Mapp* 2009;30:3406-16.
27. Park J, Willmott M, Vetuz G, et al. Evidence that genetic variation in the oxytocin receptor (OXTR) gene influences social cognition in ADHD. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:697-702.
28. Waga C, Okamoto N, Ondo Y, et al. Novel variants of the SHANK3 gene in Japanese autistic patients with severe delayed speech development. *Psychiatr Genet* 2011;21:208-11.
29. Cagliani R, Riva S, Marino C, et al. Variants in SNAP25 are targets of natural selection and influence verbal performances in women. *Cell Mol Life Sci* 2012;69:1705-15.
30. Roll P, Vernes SC, Bruneau N, et al. Molecular networks implicated in speech-related disorders: FOXP2 regulates the SRPX2/uPAR complex. *Hum Mol Genet* 2010;19:4848-60.
31. Lennertz L, Rujescu D, Wagner M, et al. Novel schizophrenia risk gene TCF4 influences verbal learning and memory functioning in schizophrenia patients. *Neuropsychobiology* 2011;63:131-6.
32. Steinberg S, Mors O, Borglum AD, et al. Expanding the range of ZNF804A variants conferring risk of psychosis. *Mol Psychiatry* 2011;16:59-66.
33. Turner MA. Generating novel ideas: fluency performance in high-functioning and learning disabled individuals with autism. *J Child Psychol Psychiatry* 1999;40:189-201.
34. Yirmiya N, Gamliel I, Shaked M, et al. Cognitive and verbal abilities of 24- to 36-month-old siblings of children with autism. *J Autism Dev Disord* 2007;37:218-29.
35. Linden DE, Lancaster TM, Wolf C, et al. ZNF804A genotype modulates neural activity during working memory for faces. *Neuropsychobiology* 2013;67:84-92.
36. Balog Z, Kiss I, Keri S. ZNF804A may be associated with executive control of attention. *Genes Brain Behav* 2011;10:223-7.
37. Esslinger C, Kirsch P, Haddad L, et al. Cognitive state and connectivity effects of the genome-wide significant psychosis variant in ZNF804A. *Neuroimage* 2011;54:2514-23.
38. Rasetti R, Sambataro F, Chen Q, et al. Altered cortical network dynamics: a potential intermediate phenotype for schizophrenia and association with ZNF804A. *Arch Gen Psychiatry* 2011;68:1207-17.
39. Takenouchi K, Nishijo H, Uwano T, et al. Emotional and behavioral correlates of the anterior cingulate cortex during associative learning in rats. *Neuroscience* 1999;93:1271-87.
40. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci* 2000;4:215-22.
41. Frith CD, Corcoran R. Exploring 'theory of mind' in people with schizophrenia. *Psychol Med* 1996;26:521-30.
42. Baron-Cohen S, Wheelwright S, Hill J, et al. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry* 2001;42:241-51.
43. Couture SM, Penn DL, Losh M, et al. Comparison of social cognitive functioning in schizophrenia and high functioning autism: more convergence than divergence. *Psychol Med* 2010;40:569-79.
44. Muris P, Steerneman P, Meesters C, et al. The ToM test: a new instrument for assessing theory of mind in normal children and children with pervasive developmental disorders. *J Autism Dev Disord* 1999;29:67-80.
45. Couture SM, Penn DL, Roberts DL. The functional significance of social cognition in schizophrenia: a review. *Schizophr Bull* 2006;32(Suppl 1):S44-63.
46. Walters JT, Corvin A, Owen MJ, et al. Psychosis susceptibility gene ZNF804A and cognitive performance in schizophrenia. *Arch Gen Psychiatry* 2010;67:692-700.

47. Chen M, Xu Z, Zhai J, et al. Evidence of IQ-modulated association between ZNF804A gene polymorphism and cognitive function in schizophrenia patients. *Neuropsychopharmacology* 2012;37:1572-8.
48. Hashimoto R, Ohi K, Yasuda Y, et al. The impact of a genome-wide supported psychosis variant in the ZNF804A gene on memory function in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:1459-64.
49. Umeda-Yano S, Hashimoto R, Yamamori H, et al. The regulation of gene expression involved in TGF-beta signaling by ZNF804A, a risk gene for schizophrenia. *Schizophr Res* 2013;146:273-8.
50. Oyler GA, Higgins GA, Hart RA, et al. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *J Cell Biol* 1989;109:3039-52.
51. Pozzi D, Condliffe S, Bozzi Y, et al. Activity-dependent phosphorylation of Ser187 is required for SNAP-25-negative modulation of neuronal voltage-gated calcium channels. *Proc Natl Acad Sci U S A* 2008;105:323-8.
52. Osen-Sand A, Catsicas M, Staple JK, et al. Inhibition of axonal growth by SNAP-25 antisense oligonucleotides in vitro and in vivo. *Nature* 1993;364:445-8.
53. Gosso MF, de Geus EJ, van Belzen MJ, et al. The SNAP-25 gene is associated with cognitive ability: evidence from a family-based study in two independent Dutch cohorts. *Mol Psychiatry* 2006; 11:878-86.
54. Söderqvist S, McNab F, Peyrard-Janvid M, et al. The SNAP25 gene is linked to working memory capacity and maturation of the posterior cingulate cortex during childhood. *Biol Psychiatry* 2010; 68:1120-5.
55. Posthuma D, Luciano M, Geus EJ, et al. A genome-wide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 2005;77:318-26.
56. Guerini FR, Bolognesi E, Chiappedi M, et al. SNAP-25 single nucleotide polymorphisms are associated with hyperactivity in autism spectrum disorders. *Pharmacol Res* 2011;64:283-8.

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http://www.molecularautism.com/content/5/1/49

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Exon resequencing of H3K9 methyltransferase complex genes, *EHMT1*, *EHTM2* and *WIZ*, in Japanese autism subjects

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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 Kleefstra 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

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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 postnatal 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 ± SD =12.09 ± 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 ± SD =44.10 ± 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 ± SD =11.98 ± 2.43) and 32 normal controls (26 males and 6 females, mean age ± SD =12.31 ± 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 VILO 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_025256.5) 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).

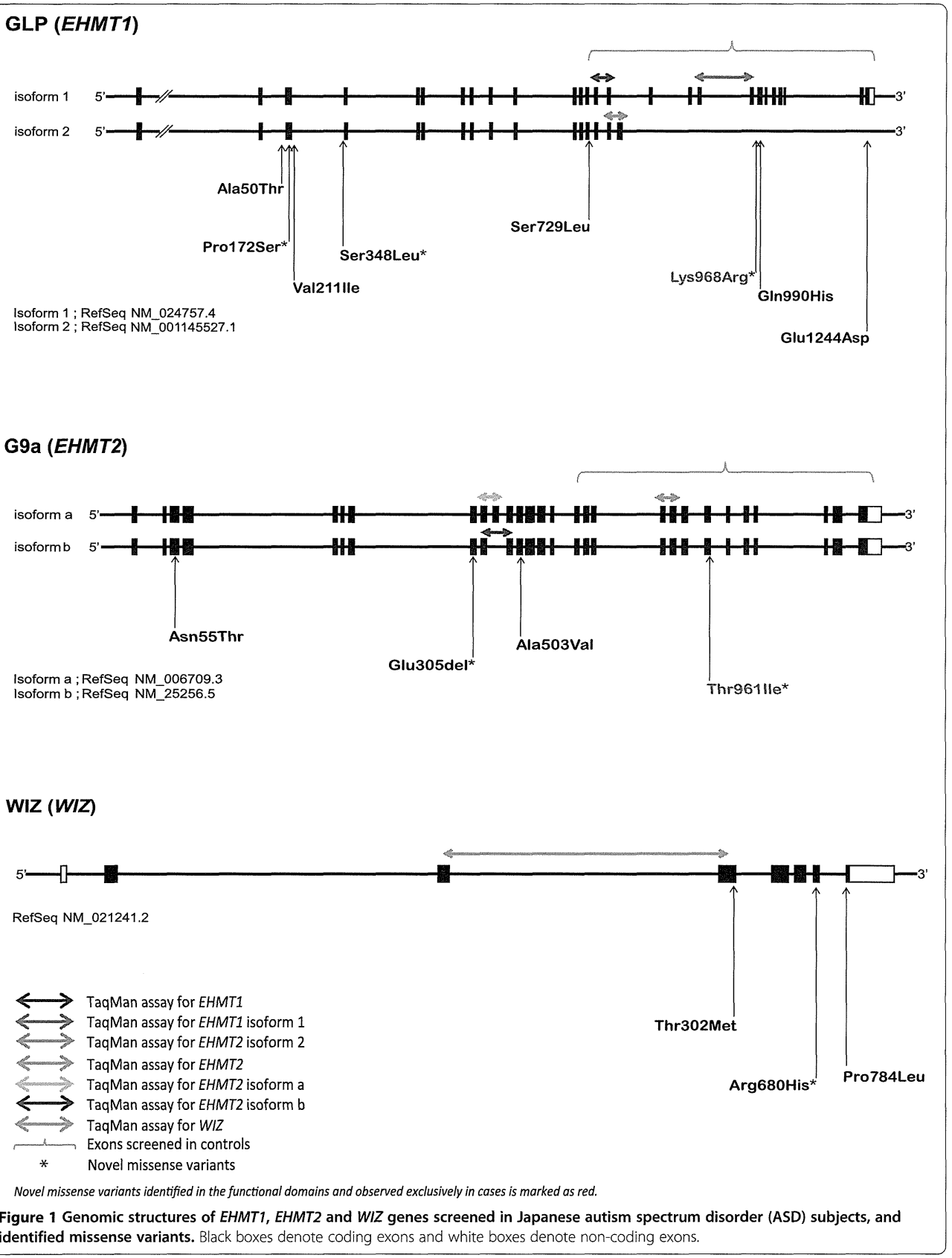

Figure 1 Genomic structures of *EHMT1*, *EHMT2* and *WIZ* genes screened in Japanese autism spectrum disorder (ASD) subjects, and identified missense variants. Black boxes denote coding exons and white boxes denote non-coding exons.

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
EHMT1	9,140611506,C,T	Exon3	c.514C > T	p.Pro172Ser	-	2	-	Possibly damaging	Neutral	Damaging
EHMT1	9,140638415,C,T	Exon6	c.1,043C > T	p.Ser348Leu	-	2	-	Possibly damaging	Deleterious	Damaging
EHMT1	9,140707493,A,G	Exon20	c.2,903A > G	p.Lys968Arg	ANK repeat domain	1	0	Possibly damaging	Neutral	Tolerated
EHMT2	6,31857330,C,-	Exon8	c.913_915delGGA	p.Glu305del	-	1	-	NA	NA	NA
EHMT2	6,31851617,G,A	Exon22	c.2,882C > T	p.Thr961Ile	SET domain	1	0	Possibly damaging	Neutral	Tolerated
WIZ	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

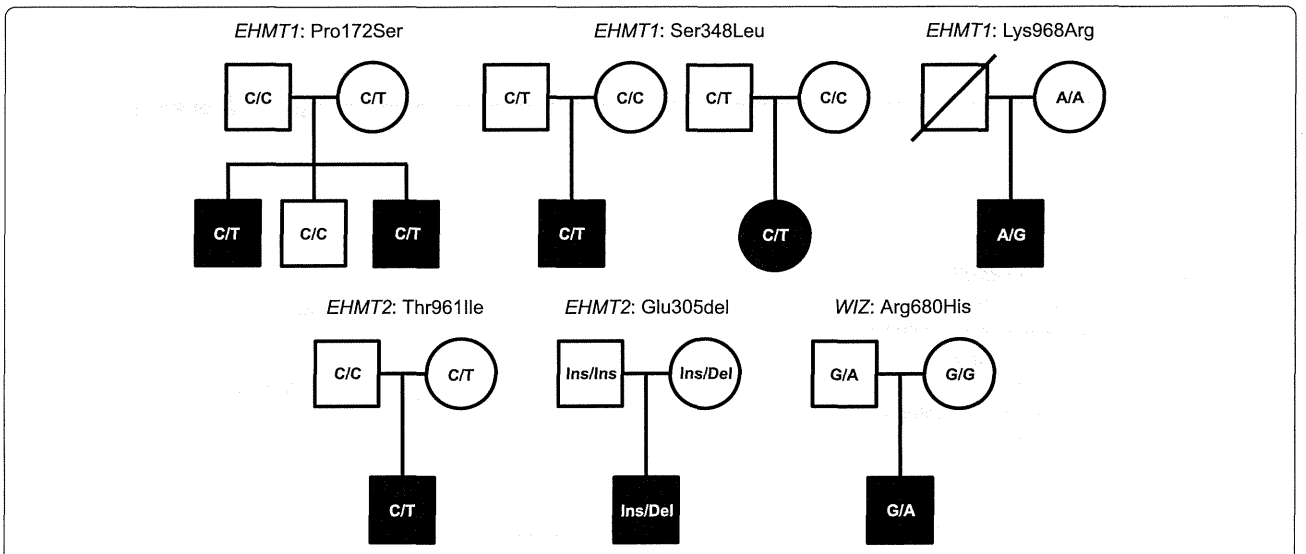

Figure 2 Pedigree structures of autism spectrum disorder (ASD) families harboring novel missense variants in *EHMT1, EHMT2* and *WIZ*. With the exception of Lys968Arg, none of the novel variants were *de novo*. For the Lys968Arg variant, genotype information of the father was not available.

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] (%)
EHMT1	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
EHMT2	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

[a]MAF: 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 to be 'neutral' and 'tolerated' by Provean 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

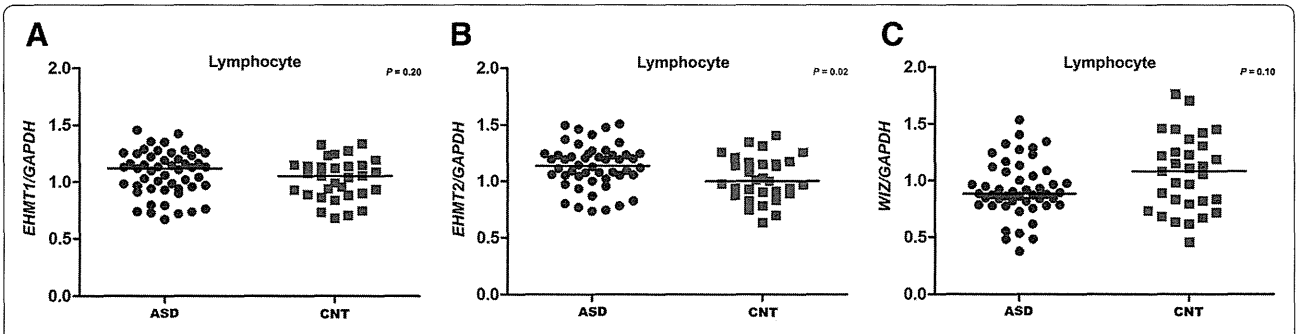

Figure 3 Expression analysis of (A) *EHMT1*, (B) *EHMT2* and (C) *WIZ* in lymphocyte samples from a subset of autism spectrum disorder (ASD) cases and control (CNT) subjects who were resequenced for the candidate genes.

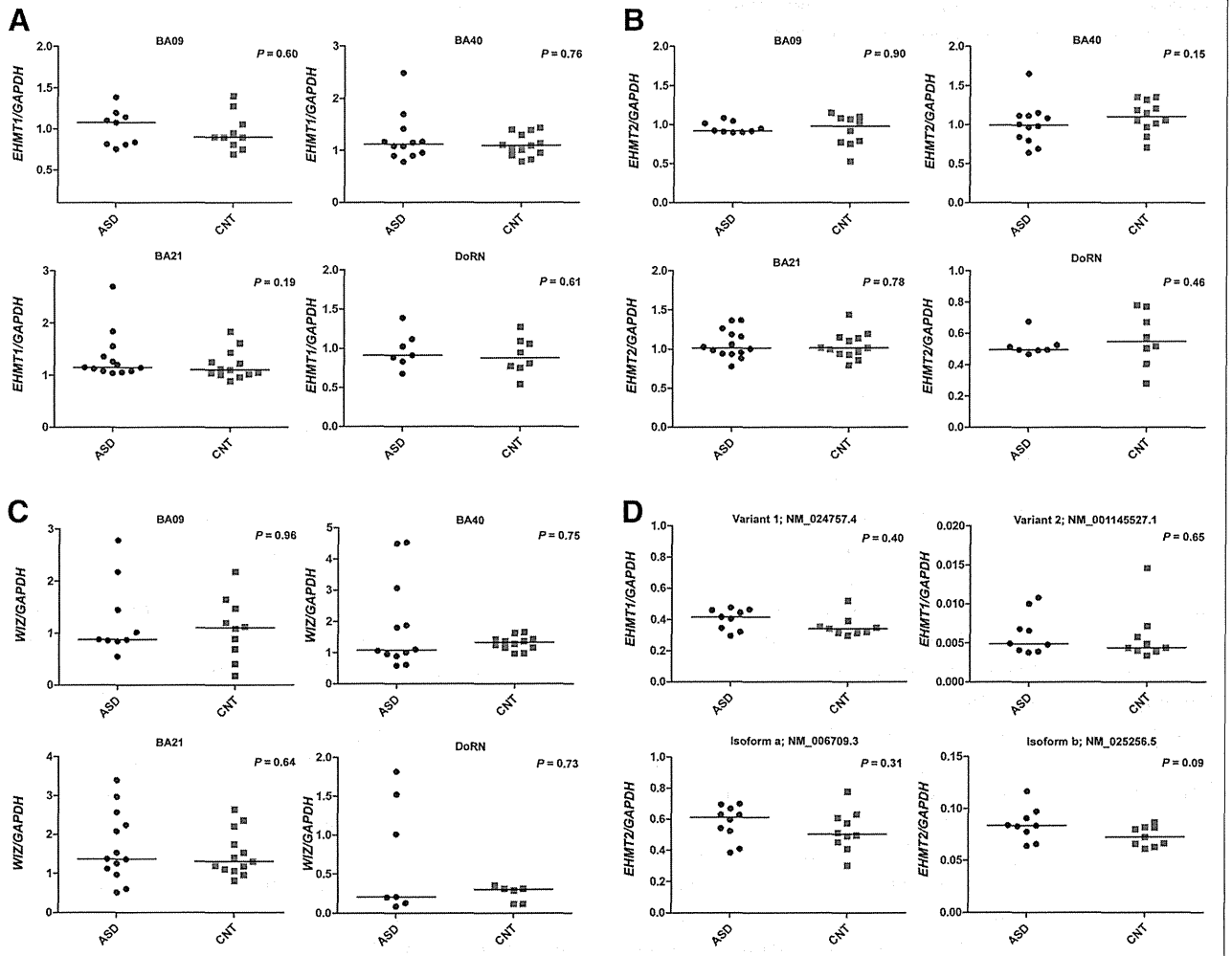

Figure 4 Gene expression analysis of (A) *EHMT1*, (B) *EHMT2* and (C) *WIZ* in Brodmann area (BA) 09, BA21, BA40 and DoRN (dorsal raphe nucleus) of autism spectrum disorder (ASD) cases and controls (CNT). (D) Isoform-specific expression analysis of *EHMT1* (variant 1: NM_024757.4 and variant 2: NM_001145527.1) and *EHMT2* (isoform a: NM_006709.3 and isoform b: NM_025256.5) in the prefrontal cortex (BA09) of ASD cases and controls.

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. YaI 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.

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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.

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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, Whetstine 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 < i > euchromatin histone methyl transferase 1 (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, Tarakhovsky 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, Whetstine 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, Komiyama 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.

連載

適応行動としてのソーシャルスキル Part 1

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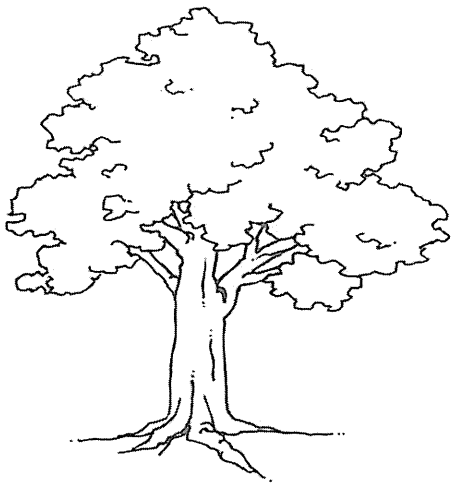
前回まで2回にわたって適応行動の全般を述べてきました。前号から黒田先生が日本版Vineland（ヴァインランド）-II適応行動尺度のことに関する記事を連載されています。Vineland-IIは、これまで日本には存在しなかった、適応行動レベルを包括的に測定できる標準化尺度です。標準化尺度の代表的なものは、WISC-IVなどのウェクスラー知能検査シリーズですが、Vineland-IIによって、WISC-IVのような分かりやすい標準スコアで適応行動の特徴が検査できることになります。Vineland-IIのことは黒田先生が詳しく説明してくださいますのでここで述べることはしませんが、Vineland-IIで測定できる領域の一つに「社会性スキル」があります。これまでの稿でも述べましたが、適応行動は、社会で「生き抜く」ために必要なスキル、そして社会に「適応して」生きるためのスキルと、大雑把に捉えることができます。つまり、必然的に社会性スキルは適応行動を構成する重要な領域とされなければならないのです。適応行動の領域を語る一番手として、ソーシャルスキルを考えてみます。ASDの社会性やソーシャルスキルに関しては実にたくさんの研究や著書があり、いまさら僕が改めて述べるほどではないと思うのですが、僕なりに捉えていることをまとめてみたいと思います。



適応行動とソーシャルスキル

現在、ASDのある人々にとっての課題の一つは、個人の適応行動特性の把握とその特性に合わせた支援だと言われています。ASDにおける最大の特性が社会性の困難であることから、適応行動がASD支援の重要なポイントとなるのもごく自然だと思われますが、適応行動に対して支援の焦点が絞られるようになったのは最近のことです。これは、日本にVineland-IIのような適応行動尺度がなかったからというわけではありません。英語で出版されている論文を読んでも、このような動きは国際的に見ても最近であることが分かります。これはつまり、高機能であろうが知的障害があろうがASDというスペクトラムで特性が捉えられ、その基準に合わせて診断を受けた人々が成人期を迎えるようになった現在の実態が浮き彫りになったからだと思います。

これまでは、ソーシャルスキルという一つの分野として支援が考えられてきました。しかしこれからは、ソーシャルスキルは適応行動の一部として捉えていくと、個人の支援がより包括的に計画、実践できるような気がします。例えば、小学校で友達に物を借りるときに奪い取ってしまう子どもに対しては、「借りてもいいか相手に尋ねる」「相手の許可を得てから借りる」「返すときにはお礼

を言う」などのスキルを教えます。支援手段はストーリーやロールプレイング、ルールブックなど、その子や環境に適したものを選ぶことが可能です。しかし、これらのスキルはその場限りのものではないはずです。つまり、その子どもが大人になっても使っていけるようなスキルに発展させていかなければなりません。これは、「社会でうまくやっていくスキル」、つまり適応行動の観点になります。

何がソーシャルスキル獲得の妨げになるのか？

社会性は確かにASDにとっては最大の課題ではあるのですが、この困難性のメカニズムは単純なものではありません。また、必ず効くという方法もないのです。それはASDのある個人の特性が実に多様であること、社会性、また大きくは適応行動というものが、実にあいまいな、境界線のはっきりしていないものであることなどが影響しています。このことについてはこれまでの稿をご覧ください。

「心の理論」の困難性は、確かにASDのソーシャルスキル獲得の妨げになると思います。他者の気持ちを推測する、自分のとる行動がどのような影響を相手に及ぼすのかを予測する、などという心の理論に関するさまざまなスキルは、ASDのある人々は苦手であるとされています。しかし一方で、考える時間が十分にある、社会的場面における刺激がある程度制限されている、または、シミュレーションであるような環境においては、ある程度の訓練は必要であるけれども、ASDのある人々は比較的うまく心の理論に関連する課題をこなすことができることが研究で分かっています。

これは、家庭や学校などの普段の場面でもよく見ることができます。例えば、小学5年生のA君が複数のクラスメートとけんかをしました。明らかに、グループで談笑していたクラスメートの輪に入ろうとしたA君のアプローチに問題があるので、先生はA君を個別に呼んで、話をしました。先生は、けんかに至るまでの場面の推移を、具体的に事実のみ（つまり、感情を含まずに）に絞り、また、マンガチックな絵を描いたりして、A君が社会的場面を理解しやすいように工夫しながら、二人で振り返ってみました。先生が、「さて、A君。君がどのようにしたら、みんなの話に入ることができたと思う？」と質問すると、A君の回答は、ソーシャルスキルトレーニング（SST）で習うような、適切な行動でした。思わず、「おまえ、分かってるんだったら、そうすればいいじゃん」と言いたくなってしまう先生の気持ちはとても良くわかります。でも、ざわざわした教室で、楽しく話しているグループ、鍵盤ハーモニカを練習している子どもたち、次の授業の準備をしている子どもなど、さまざまなことが同時に進行している環境で、自分が加わりたい場所を探してそこにうまく入り込もうとする時に、冷静に場面を社会的にアセスメントして、自分のとるべき行動を選択し実行に移すというプロセスを瞬間的にやるということは、多くのASDのある子どもにとっては苦手なようです。

子どもにとってのSST、就労訓練などでやる問題解決技法などは、整備された環境で、落ち着いてソーシャルスキルのプロセスを学ぶことができるようになっています。ですから、本人に合った形（これが重要です）でこれらの支援を行うと、さまざまなソーシャルスキルを獲得することが可能になります。しかし、訓練時に獲得できたと思っていたスキルも、実際の場面で「今だ！ここで使うんだ～！」と支援者が心で叫んでも、本人は相変わらず独特のアプローチをして他者からネガティブな反応をされてしまうということは、決して少なくありません。なぜこのようなことが起きるのかと考えますと、ASDのある人々は、定型発達の人々がやっているようなプロセスおよびスピードで社会的場面を読み取って、自分がとるべき行動を実行していくことが難しいのではないかと思うのです。ですから、ソーシャルスキルを「獲得」したと評価されるように実際の場面で使えるようになるためには、統制された環境でスキルを学んだあと、実際場面においては、支援者のプロンプト（ここでは声かけやサイン）とスキルを実行したあとの振り返り（例えば、ビデオ録画して支援者と一緒に見るなど）が必要になります。ソーシャルスキルの訓練では、ここの部分が抜けてしまっていることがあります。このような支援については次回述べることにします。

暗黙のルール

さて、これまで述べたように、適切な行動をとるプロセスには、社会的場面の読み取りと、適切な行動を選択するという段階があります。ASDのある人々がこれらの段階をクリアするのが苦手な理由の一つとして、「暗黙のルール」を知らないということがあります。暗黙のルールとは、みんな知っているけれども直接教えられることが少なく、また、通常文字などで明確に表されていない社会的ルールのことです。暗黙のルールの多くは、その社会が存在する地域の文化や習慣であることが多く、言い換えれば、適応行動は地域の文化に大きく影響されるということになります。

暗黙のルールには、絶対的な倫理などから程遠いものも多く含まれます。例えば、エスカレーターに乗るときは、乗ってから歩かない場合、左右どちらかに寄って立つ習慣があります。歩く、または走る人はその横を通っていきます。一般に、関東から東の人は左側、関西の人は右側に立つそうですね。これらのルールは駅の構内などに書いてあるわけではありません。以前テレビのニュース番組で見たことがあるのですが、実は、このようにエスカレーター上で一端に立つのは、エスカレーターの構造上良くないのだそうです。つまり、この習慣が始まってから、エスカレーターのメンテナンスはより頻繁に行わなければならず、また、使用中の事故発生も懸念されるわけです。それでも、この暗黙のルールは90年代初頭に始まってから、根強く続いていますね。真ん中に立っているお年寄りが、エスカレーターを駆け上がるサラリーマンに突き飛ばされていることを何回か見たことがあります。そういうときに怒っているのは、たいてい駆け上がっている人々です。つまり、エスカレーターの真ん中に立つという安全面でも機械的構造面でも正しいことをしている人が、危険で機械にも良くない行為をしている人に負けちゃっているのです。暗黙のルールには、このような理不尽なものがたくさんあります。

特に、学校には理不尽な暗黙のルールがいっぱいです。部活動などはそのもっとも顕著な例ですね。集合時間が決まっていても、後輩は必ずその1時間前には集合すること、試合の移動中は先輩の荷物は後輩が運ぶ、スタメンしか練習しないときでもその他の生徒はグラウンドで待機…、このようなルールは書いてあるわけでもないし、部外者が考えれば理不尽なものばかりです。しかし、このような暗黙のルールがその集団形成に役立っていることは事実です。それらが極端なものではなく、またいじめなどに直接つながるものでなければあっても良いのではないでしょうか。

ASDのある人々はなぜ、このような暗黙のルールを知る、理解することが苦手なのでしょう。暗黙のルールの獲得には、社会的場面での経験が不可欠になります。つまり、そのルールが適用される場面で、周りの人々のふるまいを見る、また、ルールに従った場合と従わなかった場合の結果を体験することなどでルールの存在を知ります。また、ルールを他人からこっそり耳打ちされることもあるでしょう。社会的コミュニケーションをとる機会が定型発達の人々よりも少ないと言われているASD特性のある人々にとっては、これらのような体験を逸してしまっていることが多いのかもしれません。さらに、「社会的手がかり」を見つけることが苦手だという特性もこれらに関係してきます。つまり、ASDのある人々は、暗黙のルールがわからないというよりは、それらのルールを知る機会が少ないと言えるのです。

また、先に述べたように暗黙のルールには、理にかなっていないものや、中には法律などに反しているものもあります。「テクニカルに正直な」（何となくこんな表現が合っていると思いました）ASDのある人にとっては，このようなルールは理解するのが難しく、また、従う気にもならないかもしれません。以前、障害者職業センターの職員さんたちの研修会で話す機会がありました。そこには当事者の方が一人いらっしゃいました。僕が、発達障害のある人々の中には職場の慣習など（つまり、暗黙のルールですね）に気づくのが遅かったり、またその慣習に従うことに反発してしまったりする人もいるという話をしたところ、自分の体験を話してくださいました。その方は入社して間もない時、退社時間になったので更衣室で着替えて帰ろうとしたら、「あんたなにやってんの」のようなことを周りに言われたそうなのです。その方がキョトンとしていたら、「新入社員は先輩

たちが着替え終わるまでは退社しちゃいけないって知らないの」と言われたそうです。このようなことは、当然就労規定には書いてありません。その方はまず、そのような暗黙のルールが就労時の契約に反していることに腹を立てたそうです。退社時まで先輩たちの行動を気にして自分のふるまいを制御しなければならないなんて、やっていられないという感じがしたのでしょう。

学校でも、このような場面は多くあります。帰りの会のときに、今日一日学校のきまりを守らなかったクラスメートを一人一人名指しで発表してしまう子がいました。クラスメートたちは、学校のきまりを守らなかった子を見たとしても、多くの場合は黙っているという暗黙のルールを知っています。しかし、学校のきまりは守るべきであり、帰りの会のときに担任の先生が「今日は、ちゃんときまりを守っていましたか」などと訊いてしまったら、「テクニカルに正直」な子どもは自分の知っている限りのことを話してしまうでしょう。

「裸の王様」というお話は、王様が裸だということを言ってはいけないという暗黙のルールをかたくなに守り続ける人々のなかで、ある子どもが正直に見たままを言ってしまうというオチになっています。ここでは、暗黙のルールを必死で守り続ける人々が滑稽なわけですが、ASDのある人々が暮らしている現実では、浮き上がってしまうのは暗黙のルールに従っている周りの人ではなく、当事者本人であることがほとんどです。

ASD当事者の「社会的失敗」が目立つ環境

社会的環境における問題発生や不適応は、ASD特性だけが原因となるわけではありません。以前書いた「ASDと適応行動　Part1」でも触れましたが、社会的期待の影響はとても大きいと思います。「中学生なのに…」や「もう大人でしょ」などのセリフは、「社会的期待に従え」と暗に言っているわけです。つまり、社会では、「こうあるべき」というふるまいの型枠が常に存在しており、人々にはその型枠にある程度はまっていることが求められます。このような要求は、乳幼児期から始まっているのです。

幼稚園や保育園のコンサルテーションをしていると、「園児らしくない」という尺度で子どもを見てしまっているケースに当たることがあります。このような見方についての善し悪しをここで述べるつもりはありません。しかし、もしここで「園児らしい」という型枠がかなりきっちりとしている場合、また、非常に主観的である場合などは、注意が必要です。乳幼児期は、年齢が同じでも発達にかなりばらつきがあり、そのばらつきを了解した上での支援が求められます。ですから、例えば4歳児の集団がきっちり同じふるまいをすることには無理がありますし、またそれを強要するものではないと思います。ある保育園で、先生がすべて笛の音で子どもたちの行動を制御しているのを見たことがありますが…。

大人になるに従って、社会的期待の型枠は厳しいものになってきます。さらにまずいことに、この型枠にはまるためにどのようにふるまわなければならないかを、具体的に教えてくれる機会も年齢を重ねていくごとに減っていくのです。ですから、「高校生なのにこんなことも知らないの」などと言われると、暗黙のルールを理解することが苦手な人は戸惑ってしまいます。「ふつうそうでしょ」とか、「ありえない」などというコメントは、発達障害に対する理解が少ない環境ではよく聞かれます。つまり、周りの人々にとっては、当たり前のことからかけ離れたふるまいを理解することが難しいのです。暗黙のルールを理解することが苦手な立場と、当たり前のふるまい以外を理解することができない立場が同じ環境にいたら、問題が生じるのは当然のことです。

学校の先生方に対する研修などで、僕はよく「『ありえない』という結論に直結しないようにしてください」とお願いしています。通常学級の一斉指導や集団行動の場においては、社会的期待と学校のきまり、そして暗黙のルールに従って行動することが当たり前です。ASD特性をもった人がその中で浮いた存在になってしまう、さらには攻撃を受けてしまうということは、そのような環境では仕方のないことなのかもしれません。しかし、その集団行動を見守る先生も集団から浮いた存在になっている子どもを排他的に扱ってしまっているとしたら、その子どもが適応行動を学ぶ機会は

なくなってしまいます。ですから、とりあえず「こういったふるまいも有りなのかな」程度で良いので、先生が子どもを柔軟に見る余裕をつくってもらうことは、学校環境ではとても重要なことだと思います。

意味が見えない、または省略されている社会的やりとり

社会的場面では、一見、ブロックサインのような、暗号のような発信を読み取らなければならないことが多くあります。一般的に比喩や暗喩、省略された表現などがそれに当たります。

僕の家庭のネタで恐縮ですが、妻と子どものやりとりで笑ってしまったことがあります。夕食の時、お皿とか箸とかをテーブルに並べるのは子どもたちの役割になっており、妻は「もうすぐご飯よ」と言って、子どもたちの行動を促していました。しかしテレビをぼけーっと見ている兄弟2人は全く動きません。2分くらいして、妻がもう一度「もうすぐご飯だからね」と言いました。2人とも「うん」と返事しましたが動きません。さらに数分経って、妻が「何回も言ってるでしょ。さっさとご飯の支度しなさい」と叱りました。子どもたちはキョトンとして、ようやく動き始めました。僕は黙ってビールを飲みながらそれを眺めていたのですが、子どもに「『もうすぐご飯』ってどういう意味だと思う」と訊いてみました。そうすると、子どもは「あと少しでご飯だということでしょ」と答えたのです。お分かりだと思いますが、妻は「もうすぐご飯だから支度してちょうだいね」という気持ちで言ったのですが、この「支度してちょうだいね」の部分は曖昧にして、子どもたちがこの意図を解釈してくれるという前提の上で言っていたのです。夕飯をつくりながらプンプンしている妻にこの解釈を述べたら、「この忙しいときに…！」と今度は僕が怒られました。子どもたちには、この機会に「もうすぐご飯」は「支度をしなさい」という意味が含まれているんだぞという暗黙のルールをこっそり伝授しました。これと同じような例はたくさんありますよね。「お風呂見てきて」には、「お湯の量を見て、いっぱいになっていたらお湯を止めてね」という指示が含まれているように。

僕がアメリカで個別支援をしていた少年は、イディオムを覚えるのが趣味でした。いわゆる故事成語などを普通の人が知らないようなものまで、まるで彼自身が辞書のように記憶していました。でも、彼はイディオムを覚えることだけが趣味であり、自分の記憶している膨大なイディオムを日常会話で使うことはほとんどありませんでした。突然僕に聞いたこともないイディオムを言ってきて、戸惑っている僕にニヤニヤしながら意味を教えてくれることはしょっちゅうありましたが。

日本語と同じように、英語の世界でも、ASDのある人々は慣用句などを字義通りにとらえてしまうことがあります。例えば、「What's Up?」は挨拶のはじめによく使われる言葉ですが、「最近どう？」などの意味で、相手に本気で訊くという意図もなく、とりあえずこれを言うという感じで使います。ASDのある少年は、「What's Up?」と訊かれて上を見てしまったというケースをアメリカの大学で働いてきたときの同僚に聞いたことがあります。このような、本当の意味や意図が隠されている表現が満載の日常会話は、多くのASDのある人々にとってわかりにくいものなのでしょう。このことは特に、異性（同性の場合もありますが）との恋愛関係を継続していくときに問題となり、思春期以降の支援において僕が頭を悩ませていることの一つです。映画「モーツァルトとくじら」は、フィクションということでちょっとやり過ぎの感はありますが、このことをよく描写しています。

適応行動としてのソーシャルスキル支援へ

ソーシャルスキル支援に関する著作物はたくさんあります。それぞれが独特のアプローチをとっていますが、実は共通している点は結構あります。例えば、暗黙のルールを視覚的に提示したり、社会的やり取りを具体的に理解しやすいように系統的にまとめたり。次回は、このようなソーシャルスキル支援の基盤のようなものを述べていきたいと思います。