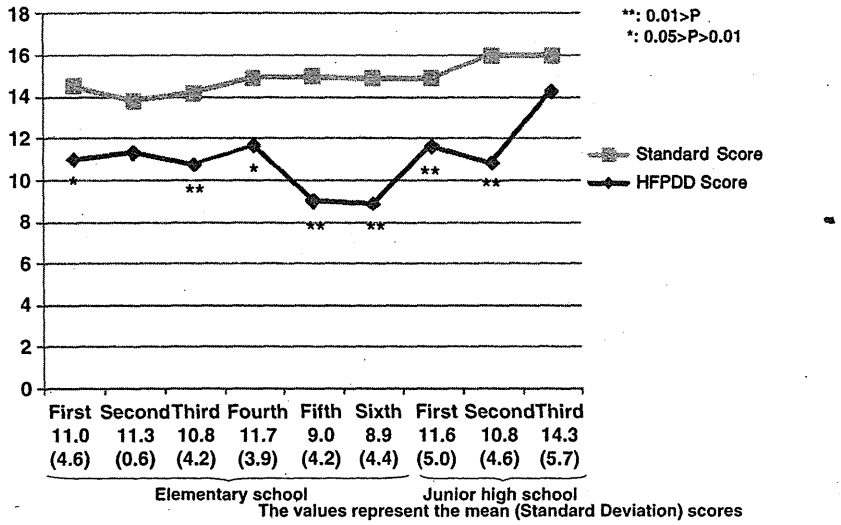


Fig. 3 Mean FM DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

the FM score in the third year of junior high school were not significantly different between the HFPDD boys and population means, because the sample size might have been too small.

Social and communication impairments are the core features of PDD. Criterion C of DCD in the DSM-IV-TR specifies the disturbance does not meet the criteria for PDD (American Psychiatric Association 2000). However, clumsiness in PDD is often clinically recognized by parents or practitioners (Sturm et al. 2004). Movement problems are common in children with autism, Asperger syndrome, or PDD not otherwise specified (Ghaziuddin and Butler 1998). Moreover, a recent meta-analysis

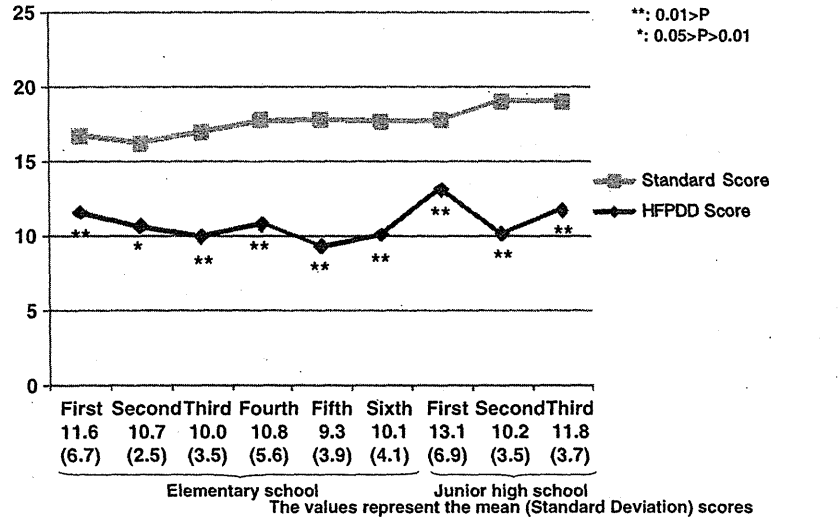


Fig. 4 Mean GC DCDQ-J score at each school level. Values represent mean (SD) (** $P<0.01$, * $0.05>P>0.01$)

Table 2 Correlation between DCDQ-J scores, and WISC-III and ADI-R scores

WISC-III			ADI-R			
VIQ	PIQ	FIQ	Social interaction[a]	Communication[b]	Stereotyped behavior[c]	
[d]CDM	0.07	0.22	0.14	−0.06	−0.32*	−0.27
[e]FM	0.04	0.30*	0.19	−0.09	−0.31*	0.03
[f]GC	−0.18	0.13	−0.03	−0.11	−0.25	−0.02
[g]Total	−0.02	0.23	0.11	−0.09	−0.35*	−0.05

* $0.05>P>0.01$

[a] Qualitative abnormalities in reciprocal social interaction

[b] Qualitative abnormalities in communication

[c] Restricted, repetitive, and stereotyped patterns of behavior

[d] Control during movement

[e] Fine Motor/handwriting

[f] General coordination

[g] Total score

demonstrates motor coordination deficits are a cardinal feature of ASD (Fournier et al. 2010). Indeed, the Australian scale for Asperger syndrome contains 2 items for movement skills such as poor motor coordination, catching a ball, gait, and running (Garnett and Attwood 1998). Furthermore, Gillberg's Criteria for Asperger's Disorder also include motor clumsiness (Gillberg and Gillberg 1989). Children with HFPDD frequently exhibit poor motor coordination from both clinical and scientific perspectives. The present study is the first report using the DCDQ and showing Japanese children with HFPDD frequently have motor coordination impairments. Furthermore, the study provides some evidence supporting the validity of the DCDQ-J for use in Japanese populations, because the results are corroborated by those in non-Japanese populations.

Coordination dysfunction is likely to induce delayed motor development, clumsiness, and poor posture. Children with coordination impairments also tend to exhibit delayed acquisition of skills for performing daily living and school activities (Missiuna et al. 2006; Polatajko and Cantin 2005); therefore, such children tend to be less eager, pay less attention to these activities, and tire more easily. However, such motor coordination problems are likely to be mistakenly attributed to a lack of parental discipline or poor motivation of the child. If parents and teachers continually use inappropriate approaches to such problems, the child may develop emotional difficulties or self-distrust (Piek et al. 2006; Skinner and Piek 2001), which can strain their relationships with the child (Stephanson and Chesson 2008; Rivard et al. 2007). Therefore, support provided to children with HFPDD should include attention to coordination and motor problems in an effort to deliver comprehensive treatment. In addition, the DSM-5 (American Psychiatric Association 2013), which allows the co-occurrence of DCD and ASD, is more clinically applicable in this aspect than the DSM-IV-TR.

The present results show fewer boys with HFPDD had FM difficulties than difficulties in the other DCDQ-J subscales. These findings might be clinically attributable to

the fact that the FM questions in the DCDQ-J are limited to inquiring if a child is able or unable to write and use scissors. Meanwhile, the results of 2 meta-analyses suggest motor coordination deficits such as gait and balance, arm motor function, movement planning, and handwriting are more prevalent in children with PDD than normal children (Fournier et al. 2010; Kushki et al. 2011).

Although children with an IQ<70 are reported to have greater impairment in movement skills than those with an IQ>70, there was no correlation between the subscale or total DCDQ-J scores with FIQ in the WISC-III in boys with HFPDD in the present study. The moderate but significant correlation between PIQ in the WISC-III and the FM score in the DCDQ-J is thought to be attributable to the fact that some subtests in the WISC-III, such as Object Assembly, Mazes, and Picture Arrangement, involve fine motor coordination or manipulation. Meanwhile, the levels of impairment in CDM, FM, and total DCDQ-J scores were significantly correlated with the score of Qualitative Abnormalities in Communication in the ADI-R. Dziuk et al. (2007) report the level of impairment in praxis performance is significantly correlated with total the Autism Diagnosis Observation Schedule-Generic (ADOS-G) score. They state this suggests the impaired performance of skilled gestures may contribute to impaired social interaction and communication in autism. Moreover, dyspraxia may be a core feature of autism or a marker of the neurological deficits that underlie the broad features of the disorder. Some authors state motor coordination is closely related to a child's cognitive and social development; this is because coordination increases a child's ability to explore and manipulate their environment, motivating them to participate in social activities (Missiuna et al. 2006; Piek et al. 2006). The frequent coordination dysfunction observed in children with PDD is believed to be due to impairments in social function and coordination dysfunction caused by the observed coordination dysfunction during development. Haswell et al. (2009) measured generalization patterns as children learned to control a novel tool. Their findings raise the possibility that common problems exist in the brains of people with autism. In addition, they found the brains of children with autism develop a stronger-than-normal association between self-generated motor commands and proprioceptive feedback; furthermore, the greater the reliance on proprioception, the greater the child's social functioning and imitation impairments. Moreover, a recent meta-analysis of structural brain imaging studies revealed the total volume of the brain and volumes of specific regions such as the cerebral hemispheres, caudate nucleus, and cerebellum are greater in people with autism (Stanfield et al. 2008). The cerebellum is considered to play an important role in the modulation of not only motor functions, but also linguistic, cognitive, and empathic functions (Murdoch 2010; Vakalopoulos 2013). Abnormal movement-related potentials in autism, which implicate basal ganglia, thalamus, and supplementary motor area involvement, are a likely source of motor dysfunction in autism (Enticott et al. 2009). Thus, the abovementioned brain regions play crucial roles in the development of motor coordination and social communication in PDD. However, the present and previous results indicate not all patients with PDD suffer from motor impairments and vice versa. Although the reasons for these differences are not well understood, our results help clarify the motor functions and possible heterogeneous neuropathology of PDD in Japan. Indeed, several brain regions are reported to be structurally abnormal in PDD (Fournier et al. 2010). Nevertheless, additional studies are required to clarify the source(s) of motor impairments apparent in PDD.

Limitations

This study has some limitations that should be mentioned. First, few boys with HFPDD were analyzed. Although this study was limited to boys with HFPDD, a previous study reports that girls with ASD also have considerable coordination problems very often (Kopp et al. 2010). Thus, larger studies and comparative studies of boys and girls are needed. Second, it is necessary to examine the factors influencing the motor function of children with HFPDD, such as medication. In the present study, there was no significant difference between children who took medication and those who did not. However, risperidone (Aman et al. 2009), SSRIs (Loubinoux et al. 2005), and methylphenidate (Bart et al. 2013) are reported to have beneficial effects on motor functions. Furthermore, carbamazepine (Braathen et al. 1997) and valproate (Farkas et al. 2010) might be related to motor impairments in children with epilepsy. Third, it is also necessary to examine the relationship between motor coordination and social impairments in normal children. Finally, in the present study, the motor coordination of the boys with HFPDD was only assessed by a questionnaire, i.e., the DCDQ-J, which does not involve neurological examinations.

However, Wilson et al. (2000) confirm the DCDQ is a valid clinical screening tool for DCD; correlations between DCDQ scores, and M-ABC and Test of Visual–Motor Integration scores support concurrent validity. Concordantly, the EACD (2012) states a questionnaire may be useful as an initial diagnostic tool and that the DCDQ is currently the best-evaluated questionnaire. Future studies should investigate the predictive validity of the DCDQ-J and develop a psychometrically sound and culturally appropriate standardized international test (American Psychiatric Association 2013) in Japan. In fact, we are currently developing the Japanese version of the M-ABC2 (Hirata et al. 2014).

Conclusion

This study is the first report indicating Japanese children with HFPDD frequently have motor coordination impairments according to the Japanese version of the DCDQ. The levels of impairment according to the CDM, FM, and total DCDQ-J scores are significantly correlated with the score of Qualitative Abnormalities in Communication in the ADI-R. On the other hand, not all patients with PDD have motor coordination dysfunction. Therefore, additional studies are required to clarify the relationships among the development of motor coordination, cognition, and socialization and between PDD and DCD. Clinicians should consider the screening and assessment of movement impairments as part of the routine investigation for children with PDD. Screening for or assessing motor dysfunctions in HFPDD using tools such as the DCDQ-J could lead to the development of treatments and new pathophysiologic concepts.

Acknowledgments The authors have no conflict of interests to declare. This study was supported in part by the JSPS and Research Grant from the Ministry of Health, Labour, and Welfare, Japan. The authors sincerely thank Dr. Brenda N. Wilson (Alberta Health Services and University of Calgary, Canada) for approving the development and use of the Japanese version of the DCDQ. The authors are also grateful to the children and parents who participated.

References

Aman, M. G., Hollway, J. A., Leone, S., Masty, J., Lindsay, R., Nash, P., et al. (2009). Effects of risperidone on cognitive-motor performance and motor movements in chronically medicated children. *Research in Developmental Disabilities, 30*, 386–396.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders (4th ed., Text Revision) (DSM-IV-TR)*. Washington DC: American Psychiatric Association.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (5th ed.) (DSM-5)*. Washington DC: American Psychiatric Association.

Bárt, O., Daniel, L., Dan, O., & Bar-Haim, Y. (2013). Influence of methylphenidate on motor performance and attention in children with developmental coordination disorder and attention deficit hyperactive disorder. *Research in Developmental Disabilities, 34*, 1922–1927.

Blank, R., Smits-Engelsman, B., Polatajko, H., Wilson, P., & European Academy for Childhood Disability. (2012). European Academy for Childhood Disability (EACD): Recommendations on the definition, diagnosis and intervention of developmental coordination disorder (long version). *Developmental Medicine & Child Neurology, 54*, 54–93.

Braathen, G., von Bahr, L., & Theorell, K. (1997). Motor impairments in children with epilepsy treated with carbamazepine. *Acta Paediatrica, 86*, 372–376.

Dziuk, M. A., Gidley Larson, J. C., Apostu, A., Mahone, E. M., Denckla, M. B., & Mostofsky, S. H. (2007). Dyspraxia in autism: association with motor, social, and communicative deficits. *Developmental Medicine & Child Neurology, 49*, 734–739.

Enticott, P. G., Bradshaw, J. L., Iansek, R., Tonge, B. J., & Rinehart, N. J. (2009). Electrophysiological signs of supplementary-motor area deficits in high-functioning autism but not Asperger syndrome: an examination of internally cued movement-related potentials. *Developmental Medicine & Child Neurology, 51*, 787–791.

Farkas, Z., Gulyás, S., Molnár, R., Szirmai, I., & Kamondi, A. (2010). Quantitative analysis of motor performance in epilepsy patients treated with valproate. *Seizure, 19*, 173–177.

Fournier, K. A., Hass, C. J., Naik, S. K., Lodha, N., & Cauraugh, J. H. (2010). Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. *Journal of Autism and Developmental Disorders, 40*, 1227–1240.

Garnett, M. S., & Attwood, A. J. (1998). The Australian scale for Asperger's syndrome. In T. Attwood (Ed.), *Asperger's syndrome: a guide for parents and professionals* (pp. 13–27). London: Jessica Kingsley Publishers.

Ghaziuddin, M., & Butler, E. (1998). Clumsiness in autism and Asperger syndrome: a further report. *Journal of Intellectual Disability Research, 42*, 43–48.

Gillberg, I. C., & Gillberg, C. (1989). Asperger syndrome: some epidemiological considerations: a research note. *Journal of Child Psychology and Psychiatry, 30*, 631–638.

Green, D., Charman, T., Pickles, A., Chandler, S., Loucas, T., Simonoff, E., et al. (2009). Impairment in movement skills of children with autistic spectrum disorders. *Developmental Medicine & Child Neurology, 51*, 311–316.

Haswell, C. C., Izawa, J., Dowell, L. R., Mostofsky, S. H., & Shadmehr, R. (2009). Representation of internal models of action in the autistic brain. *Nature Neuroscience, 12*, 970–972.

Henderson, S. E., & Sugden, D. A. (1992). *Movement assessment battery for children: manual*. London: The Psychological Corporation Ltd.

Hirata, S., Okuzumi, H., Kitajima, Y., Hosobuchi, T., Nakai, A., Kokubun, M. (2014). Relationship between motor skill and social impairment in children with autism spectrum disorders. International Journal of Developmental Disabilities, *in press*.

Japanese WISC-III Publication Committee. Nihonban WISC-III chinou kensahou (Japanese Wechsler Intelligence Scale for children, 3rd ed.) (in Japanese). (1998). Tokyo: Nihon Bungaku Kagakusha.

Kopp, S., Beckung, E., & Gillberg, C. (2010). Developmental coordination disorder and other motor control problems in girls with autism spectrum disorder and/or attention-deficit/hyperactivity disorder. *Research in Developmental Disabilities, 31*, 350–361.

Kushki, A., Chau, T., & Anagnostou, E. (2011). Handwriting difficulties in children with autism spectrum disorders: a scoping review. *Journal of Autism and Developmental Disorders, 41*, 1706–1716.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism diagnostic interview-revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*, 659–685.

Loubinoux, I., Tombari, D., Pariente, J., Gerdelat-Mas, A., Franceries, X., Cassol, E., et al. (2005). Modulation of behavior and cortical motor activity in healthy subjects by a chronic administration of a serotonin enhancer. *Neuroimage, 27*, 299–313.

Missiuna, C., Moll, S., Law, M., King, S., & King, G. (2006). Mysteries and mazes: parents' experiences of children with developmental coordination disorder. *Canadian Journal of Occupational Therapy, 73*, 7–17.

Murdoch, B. E. (2010). The cerebellum and language: historical perspective and review. *Cortex, 46*, 858–868.

Nakai, A., Miyachi, T., Okada, R., Tani, I., Nakajima, S., Onishi, M., et al. (2011). Evaluation of the Japanese version of the DCDQ as a screening tool for clumsiness of Japanese children. *Research in Developmental Disabilities, 32*, 1615–1622.

Piek, J., Baynam, G. B., & Barret, N. C. (2006). The relationship between fine and gross motor ability, self-perceptions and self-worth in children and adolescents. *Human Movement Science, 25*, 65–75.

Polatajko, H. J., & Cantin, N. (2005). Developmental coordination disorder (dyspraxia): an overview of the state of the art. *Seminars in Pediatric Neurology, 12*, 250–258.

Rivard, L. M., Missiuna, C., Hanna, S., & Wishart, L. (2007). Understanding teachers' perceptions of the motor difficulties of children with developmental coordination disorder (DCD). *British Journal of Educational Psychology, 77*, 633–648.

Skinner, R. A., & Piek, J. P. (2001). Psychosocial implications of poor motor coordination in children and adolescents. *Human Movement Science, 20*, 73–94.

Stanfield, A. C., McIntosh, A. M., Spencer, M. D., Philip, R., Gaur, S., & Lawrie, S. M. (2008). Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *European Psychiatry, 23*, 289–299.

Stephanson, E. A., & Chesson, R. A. (2008). Always the guiding hand: parents' accounts of the long-term implications of developmental co-ordination disorder for their children and families. *Child: Care, Health and Development, 34*, 335–343.

Sturm, H., Fernell, E., & Gillberg, C. (2004). Autism spectrum disorders in children with normal intellectual levels: associated impairments and subgroup. *Developmental Medicine & Child Neurology, 46*, 444–447.

Tsuchiya, K. J., Matsumoto, K., Yagi, A., Inada, N., Kuroda, M., Inokuchi, M., et al. (2013). Reliability and validity of autism diagnostic interview-revised, Japanese version. *Journal of Autism and Developmental Disorders, 43*, 643–662.

Vakalopoulos, C. (2013). The developmental basis of visuomotor capabilities and the causal nature of motor clumsiness to cognitive and empathic dysfunction. *Cerebellum, 12*, 212–223.

Wilson, B. N., Kaplan, B. J., Crawford, S. G., Campbell, A., & Dewey, D. (2000). Reliability and validity of a parent questionnaire on childhood motor skills. *American Journal of Occupational Therapy, 54*, 484–493.

Wilson, B. N., Crawford, S. G., Green, D., Roberts, G., Aylott, A., & Kaplan, B. J. (2009). Psychometric properties of the revised developmental coordination disorder questionnaire. *Physical & Occupational Therapy in Pediatrics, 29*, 182–202.

Archival Report

Biological Psychiatry

Utility of Scalp Hair Follicles as a Novel Source of Biomarker Genes for Psychiatric Illnesses

Motoko Maekawa, Kazuo Yamada, Manabu Toyoshima, Tetsuo Ohnishi, Yoshimi Iwayama, Chie Shimamoto, Tomoko Toyota, Yayoi Nozaki, Shabeesh Balan, Hideo Matsuzaki, Yasuhide Iwata, Katsuaki Suzuki, Mitsuhiro Miyashita, Mitsuru Kikuchi, Motoichiro Kato, Yohei Okada, Wado Akamatsu, Norio Mori, Yuji Owada, Masanari Itokawa, Hideyuki Okano, and Takeo Yoshikawa

ABSTRACT

BACKGROUND: Identifying beneficial surrogate genetic markers in psychiatric disorders is crucial but challenging.

METHODS: Given that scalp hair follicles are easily accessible and, like the brain, are derived from the ectoderm, expressions of messenger RNA (mRNA) and microRNA in the organ were examined between schizophrenia (*n* for first/second = 52/42) and control subjects (*n* = 62/55) in two sets of cohort. Genes of significance were also analyzed using postmortem brains (*n* for case/control = 35/35 in Brodmann area 46, 20/20 in cornu ammonis 1) and induced pluripotent stem cells (*n* = 4/4) and pluripotent stem cell-derived neurospheres (*n* = 12/12) to see their role in the central nervous system. Expression levels of mRNA for autism (*n* for case/control = 18/24) were also examined using scalp hair follicles.

RESULTS: Among mRNA examined, *FABP4* was downregulated in schizophrenia subjects by two independent sample sets. Receiver operating characteristic curve analysis determined that the sensitivity and specificity were 71.8% and 66.7%, respectively. *FABP4* was expressed from the stage of neurosphere. Additionally, microarray-based microRNA analysis showed a trend of increased expression of *hsa-miR-4449* ($p = .0634$) in hair follicles from schizophrenia. *hsa-miR-4449* expression was increased in Brodmann area 46 from schizophrenia ($p = .0007$). Finally, we tested the expression of nine putative autism candidate genes in hair follicles and found decreased *CNTNAP2* expression in the autism cohort.

CONCLUSIONS: Scalp hair follicles could be a beneficial genetic biomarker resource for brain diseases, and further studies of *FABP4* are merited in schizophrenia pathogenesis.

Keywords: Autism, *CNTNAP2*, FABP4, *hsa-miR-4449*, MicroRNA, Schizophrenia

http://dx.doi.org/10.1016/j.biopsych.2014.07.025

The disease mechanisms underlying psychiatric illnesses remain largely undetermined. Great efforts have gone into identifying novel biomarkers that would assist in the development of objective diagnostic tools and novel therapeutic and prophylactic interventions, as well as facilitate the subdivision of disease states, based on pathogenesis, for optimal drug selection. There are, however, major obstacles in the search for novel biomarkers, primarily the difficulty in obtaining brain tissue from living donors and the lack of accurate experimental animal models. Brain is an ectodermal tissue and shares its developmental origins with scalp hair follicles, which are readily accessible miniorgans within the skin. Despite their shared embryonic origins, hair follicles have not previously been utilized as a bio-resource in the hunt for proxy genes in psychiatric diseases. In the current study, we first examined whether schizophrenia-relevant genes, namely those related to the γ-aminobutyric acid (GABA)ergic system (1–3), myelin (3–5), and fatty acids (6–11), are expressed in hair follicles and if expressed whether expression is differential between cases and control subjects, using an exploratory sample set. Next, we attempted to validate any differential expression and examine the effects of potential confounding factors using a second independent sample set. We then analyzed the identified biomarker candidate *FABP4*/fatty acid binding protein 4 (FABP4) expression in serum, postmortem brain samples, induced pluripotent stem cells (iPSCs), and iPSC-derived neurospheres. In addition to messenger RNA (mRNA), we also examined the expression levels of microRNA (miRNA) in hair follicles, postmortem brains, iPSCs, and iPSC-derived neurosphere samples from patients with schizophrenia and control subjects. Lastly, we tested candidate gene expression in hair follicles from patients with autism. Based on the results of our comprehensive analysis, we proposed scalp hair follicles as a beneficial genetic resource for schizophrenia and autism in the search for potential biomarkers.

ISSN: 0006-3223

Biological Psychiatry ▮▮▮, 2014; ▮:▮▮▮–▮▮▮ www.sobp.org/journal

METHODS AND MATERIALS

Scalp Hair Follicle Samples

All samples were collected from ethnic Japanese within Japan. The first set of exploratory scalp hair follicle samples for schizophrenia and control subjects was derived from residents in the northern district of Kanto, while the confirmatory second set came from the Tokyo area. Diagnoses were made by at least two experienced psychiatrists, using DSM-IV criteria. Demographic data for scalp hair follicle samples derived from schizophrenia are described in Table 1. The scalp hair follicle samples from autism participants and control subjects were collected from the Chubu area. The diagnosis of autism spectrum disorder was made using the DSM-IV-TR criteria. We then administered the Autism Diagnostic Interview-Revised (ADI-R) (12) to 14 of 18 cases and made a confirmed diagnosis of autism for those 14 cases. Interviews for the ADI-R were conducted by experienced child psychiatrists who are licensed to use the Japanese version of the ADI-R (13). Demographic data relating to scalp hair follicle samples for autism are described in Table 1.

RNA Extraction and Quantification

Ten hairs were plucked from the scalp of each subject using forceps. The hairs were checked for the presence of a sheath. Hairs were trimmed to approximately 1.5 cm in length, containing the bulb region, and dropped into a 1.5 mL microfuge tube (BM Equipment, Tokyo, Japan) containing RNAlater solution (Ambion, Grand Island, New York). Total RNA was extracted using the RNAqueous-Micro kit (Ambion). Single-stranded complementary DNA (cDNA) was synthesized using SuperScript VILO Master Mix (Invitrogen, Grand Island, New York). Quantitative reverse-transcription PCR (qRT-PCR) analysis of mRNAs was conducted using an ABI7900HT Fast Real-Time PCR System (Applied Biosystems, Grand Island, New York). TaqMan probes were TaqMan Gene Expression Assays products (Applied Biosystems). All qRT-PCR data were captured using the SDS v2.4 (Applied Biosystems). The ratios of relative concentrations of target molecules to the *GAPDH* gene (target molecule/*GAPDH* gene) were calculated. All reactions were performed in triplicate based on the standard curve method.

Statistical Analysis

We used the interquartile range to find outliers. The differences between the 25th (quartile 1) and 75th percentiles (quartile 3) were used to identify extreme values (outliers) in the tails of the distribution. Statistical evaluation was performed by Mann-Whitney *U* test for means between patient and control groups and by Spearman's R test for correlation using SPSS software version 19 (IBM, Tokyo, Japan).

Analyses of miRNA Expressions and Potential Targets of miRNAs

For microarray-based miRNA analysis, we used the miRBase Rel. 18.0 platform (Agilent Technologies, Santa Clara California), capable of measuring 1919 human mature miRNAs in the age-/sex-matched subset of the first hair follicle sample set (Table S1 in Supplement 1). The miRNAs were labeled using the miRNA Complete Labeling Reagent and Hyb Kit (Agilent Technologies) and hybridized to the arrays. Images were scanned with a High-Resolution C scanner (Agilent Technologies) and analyzed using GeneSpring GX (Agilent Technologies). Comparisons of miRNA expression values between schizophrenia and control groups were performed using GeneSpring 12.6 (Agilent Technologies). To normalize the intermicroarray range of expression intensities, the percentile shift method (90th percentile) was used. The genes whose expression data were available in more than 50% of hybridizations were statistically evaluated between schizophrenia and control groups using the two-tailed Mann-Whitney *U* test. For quantification of individual miRNAs, we performed TaqMan-based miRNA qRT-PCR (Applied Biosystems, Grand Island, New York) according to the manufacturer's instructions, using *U6 snRNA* as a control probe. All reactions for miRNA quantification were also performed in triplicate, based on the standard curve method. Statistical evaluation methods were the same as those for mRNA.

Table 1. Demographic Characteristics of Hair Follicle Sample Sets

	Control Subjects	Patients	*p* Value
First Sample Set for Schizophrenia			
n	62	52	
Sex (female/male)	41 / 21	25 / 27	.0518[a]
Age (mean ± SD)	41.26 ± 12.26	50.98 ± 10.86	<.0001[b]
Second Sample Set for Schizophrenia			
n	55	42	
Sex (female/male)	26 / 29	20 / 22	.973[a]
Age (mean ± SD)	46.87 ± 13.56	49.93 ± 12.97	.2777[b]
Duration of illness (mean ± SD)		22.79 ± 14.66	
Autism Sample Set			
n	24	18	
Sex (female/male)	24 / 0	16 / 2	.1777[a]
Age (mean ± SD)	32.60 ± 3.91	25.61 ± 4.95	<.0001[b]

[a]Evaluated by chi-square test.
[b]Evaluated by two-tailed *t* test.

To identify the potential targets of a specific miRNA, we performed in silico analysis using web-based miRNA target prediction methods, TargetScan (http://www.targetscan.org, Release 6.2; Whitehead Institute for Biomedical Research, Cambridge, Massachusetts) and miRDB (http://mirdb.org/miRDB/; Washington University School of Medicine, St. Louis, Missouri).

Immunohistochemistry

The plucked hairs were rinsed briefly in phosphate-buffered saline and dropped into a 1.5 mL microfuge tube containing 1 mL of 10% neutral-buffered formalin (4°C, 1 hour). The fixed hairs were pre-embedded in 4% agarose (Sigma-Aldrich, St Louis, Missouri) in phosphate-buffered saline, pH 7.4. At this point, it was possible to orientate the hairs into their desired position for either longitudinal or transverse sectioning. Blocks were embedded in capsules, which were filled with O.C.T. compound (Sakura Finetek, Tokyo, Japan). Cryostat sections (8 μm thick) of plucked hair follicles were processed for immunohistochemistry. The sections were blocked with 10% goat serum in .05 mol/L Tris buffered saline plus .05% Tween 20 (TBST), followed by three rinses in TBST (20 min each). The primary antibodies were applied for overnight at 4°C. After three washes in TBST (20 min each), secondary antibodies were applied to sections at room temperature (1 hour). Slides were counterstained with 4',6-diamidino-2-phenylindole to highlight nuclei. After washing in TBST, the slides were mounted in PermaFluor Aqueous Mounting Medium (Thermo Fisher Scientific, Waltham, Massachusetts). Fluorescent signals were detected using a confocal laser-scanning microscope FV1000 (Olympus, Tokyo, Japan).

Antibodies

See Supplementary Methods and Materials in Supplement 1.

Analysis of FABP4 Protein Levels in Serum

See Supplementary Methods and Materials in Supplement 1.

Postmortem Brain Analysis

See Supplementary Methods and Materials in Supplement 1.

Establishment of iPSC Lines

Dermal fibroblasts (human dermal fibroblasts) from the facial dermis of a 36-year-old Caucasian female subject (Cell Applications, Inc., San Diego, California) were used to establish control iPSCs 201B7 and YA9 (14). The remaining control iPSCs, WD39 and KA23, were generated from a 16-year-old Japanese female subject (15) and a 40-year-old Japanese male subject (Matsumoto, Ph.D., *et al.*, personal communication, 2013), respectively. The 201B7 iPSCs were kindly provided by Yamanaka, M.D., Ph.D., Kyoto University (14). The iPSCs YA9, WD39, and KA23 have been described in a previous report (15). The schizophrenia derived iPSCs from patients with 22q11.2 deletions SA001 and KO001 were generated from Japanese female subjects aged 37 and 30 years old, respectively (see Clinical History in Supplement 1).

The maintenance of human dermal fibroblasts, lentiviral production, retroviral production, infection, stem cell culture, and characterization were performed as described previously (15).

In Vitro Neural Differentiation of Induced Pluripotent Stem Cells

The iPSCs were plated in T75 flasks after dissociation into single cells and cultured for 14 days in neural culture medium supplemented with leukemia inhibitory factor (Merck Millipore, Darmstadt, Germany) and basic fibroblast growth factor (Peprotech, Rocky Hill, New Jersey). Neurospheres were passaged repeatedly by culturing in the same manner (16,17).

Comparative Genomic Hybridization Array Analysis

See Supplementary Methods and Materials in Supplement 1.

Ethical Issues

This study was approved by the Ethics Committees of RIKEN and all participating institutes, including the Keio University School of Medicine, an ethical committee for skin biopsy and iPSC production (approval No. 20080016), and conducted according to the principles expressed in the Declaration of Helsinki. All control subjects and patients gave informed, written consent to participate in the study after being provided with and receiving an explanation of study protocols and objectives.

RESULTS

Expression of mRNA in Scalp Hair Follicles from Schizophrenia and Control Subjects

Gene expression profiles of schizophrenia postmortem brains have been well studied. However, studies have been hampered by uncontrollable confounding factors associated with postmortem brains and an inaccessibility of brain tissue from living donors. Therefore, we set out to analyze gene expression in hair follicles. Previous studies provide substantial support for reduced expression of genes related to oligodendrocyte and GABAergic systems in schizophrenia pathology (1–4). In addition, our (6,7,9) and other studies (8,10,11) on *FABPs* (genes for fatty acid binding proteins) raise the possibility of disturbed lipid metabolism in the susceptibility to this disease. Based on these findings, we selected 22 genes: 8 from the GABAergic system, 9 with myelin relevance, and 5 with lipid relevance (Table 2). The amount of mRNA from an individual subject's hair follicles was not enough for a systemic cDNA microarray. We used *GAPDH* as an internal control. An exploratory scalp hair follicle sample panel (the first sample set) consisted of samples from 52 patients with schizophrenia and 62 control subjects (Table 1). qRT-PCR analysis showed that seven genes, namely *CALB2*, *SST*, *CNP*, *PMP22*, *FABP4*, *FABP7,* and *FAAH* were differentially expressed ($p < .05$) in samples from schizophrenia compared with control subjects (Table 2; Figure S1 in Supplement 1).

To replicate the finding, we examined the expression levels of these seven genes using an age-/sex-matched, independent confirmatory set (a second sample set) composed of 42 patients with schizophrenia and 55 control subjects (Table 1). Of the seven genes, only *FABP4* showed significantly decreased expression (an average reduction of 43% compared with a reduction of 40% in the first set of samples) in schizophrenia samples (Figure 1A; Table 2). Correlation analyses demonstrated no significant effects for age, dose of

Table 2. List of Examined Genes and Their Expression in the First and Second Scalp Hair Follicle Sample Sets from Schizophrenia

			First Sample Set			Second Sample Set		
			Mean ± SD of Corresponding Gene / *GAPDH*			Mean ± SD of Corresponding Gene / *GAPDH*		
Gene Category	Gene Symbol	Assay ID[a]	Control (n = 62)	Schizophrenia (n = 49)	p Value[b]	Control (n = 62)	Schizophrenia (n = 49)	p Value[b]
GABAergic System	*GAD1*	Hs01065893_m1	.881 ± .598	1.119 ± .707	.118			
	GAD2	Hs00609534_m1	Not detectable					
	GABRA1	Hs00168058_m1	2.347 ± 2.761	.832 ± .964	.378			
	GABRD	Hs00181309_m1	1.055 ± .758	.945 ± .618	.666			
	SLC6A1	Hs01104475_m1	1.047 ± .830	.985 ± .555	.682			
	PVALB	Hs00161045_m1	1.067 ± .569	1.074 ± .669	.87			
	CALB2	Hs00418693_m1	1.024 ± .355	1.163 ± .303	.037[d]	.715 ± .373	.857 ± .300	.095[c]
	SST	Hs00356144_m1	.626 ± .549	1.052 ± .923	.028[d]	.910 ± .683	1.812 ± 1.802	.151[c]
Myelin Relevance	*APC*	Hs01568269_m1	1.001 ± .243	.939 ± .233	.131			
	CLDN11	Hs00194440_m1	.860 ± .605	.984 ± .854	.862			
	CNP	Hs00263981_m1	1.148 ± .336	.985 ± .186	.002[d]	.928 ± .415	1.052 ± .210	.456
	CSPG4	Hs00361541_g1	.976 ± .536	1.050 ± .364	.252			
	MAG	Hs01114387_m1	Not detectable					
	NES	Hs00707120_s1	1.018 ± .496	1.013 ± .403	.98			
	OLG2	Hs00300164_s1	Not detectable					
	PMP22	Hs00165556_m1	1.006 ± .370	.804 ± .261	.003[d]	.807 ± .410	.844 ± .400	.987
	SOX10	Hs00366918_m1	1.072 ± .748	.984 ± .508	.99			
Lipid Relevance	*FABP3*	Hs00997360_m1	.763 ± .486	.807 ± .372	.292			
	FABP4	Hs01086177_m1	1.050 ± .470	.653 ± .251	<.0001[d]	1.138 ± .708	.650 ± .232	**<.001**
	FABP5	Hs02339439_g1	1.118 ± .215	1.084 ± .179	.312			
	FABP7	Hs00361426_m1	.562 ± .332	1.018 ± .744	.003[d]	.519 ± .372	.530 ± .355	.754
	FAAH	Hs01038660_m1	1.008 ± .344	.857 ± .221	.013[d]	.836 ± .303	.753 ± .281	.180[c]
Control	*GAPDH*	Hs02758991_g1						

GABA, gamma-aminobutyric acid.

[a]Probe ID in TaqMan Gene Expression Assay system.

[b]Evaluated by two-tailed Mann-Whitney *U* test.

[c]For these analyses, only 49 control and 36 schizophrenia samples were available.

[d]Significant changes.

antipsychotics [haloperidol equivalent (18,19)], or duration of illness on the expression levels of *FABP4* (Figure S2A–C in Supplement 1). Since serum levels of Fabp4 were reported to be affected by nutritional fluctuations in mice (that is, suppressed by feeding) (20), we examined the effect of sampling time after the last meal on *FABP4* expression in hair follicles and found no significant change (Figure S2D in Supplement 1). Nor did we detect an effect for sex on *FABP4* levels: male control versus female control subjects, p = .950; male schizophrenia versus female schizophrenia subjects, p = .360; male (control + schizophrenia subjects) versus female (control + schizophrenia subjects), p = .387; all evaluated by the Mann-Whitney *U* test.

Circulating FABP4 is known to be associated with metabolic markers (21,22), so we examined the effects of weight, height, body mass index, and body fat percentage on *FABP4* expression in the second hair follicle sample set (Figure S3 in Supplement 1). None of these factors affected the expression ratios of *FABP4/GAPDH* in hair follicles. Despite the fact that olanzapine alters lipid metabolism (23,24), we detected no significant correlation between *FABP4* expression levels in hair follicles and olanzapine dose (mg/day) in the second set of schizophrenia samples (Spearman's rho = −.2289; 95% confidence interval = −.5258 to .1178; p = .180).

From these results, *FABP4* expression levels in hair follicles would appear to be a robust marker for schizophrenia. Receiver operating characteristic curve analysis determined an optimal cutoff level of .769, based on the minimum distance from the curve to upper left corner (= .191) and area under the curve = .713 (95% confidence interval = .609–.817) (Figure S4 in Supplement 1). With this cutoff level for the *FABP4/GAPDH* mRNA ratio, the sensitivity, specificity, and positive and negative predictive values were 71.8%, 66.7%, 60.9%, and 76.6%, respectively.

Immunohistochemical Analysis of FABP4 in Scalp Hair Follicles

Figure 2A shows the structure of a hair follicle (25,26). Moving inward, a plucked scalp hair consists of the following components: the outer root sheath, companion layer, inner root sheath (IRS), the cortex, and medullar. Each of these components has an epidermal origin and each compartment expresses specific genes from the keratin family (26) (Figure 2B). FABP4 is co-expressed with K71 in the IRS cuticle layer and displays partially overlapping expression with K85 in the cuticle, matrix/precortex, and mid/upper cortex (27). However, FABP4 shows scant co-expression with K14 in the outer root sheath layer (Figure 2C, D). These results indicate that FABP4 is expressed in the IRS and part of the hair cortex.

Expression of FABP4 in Serum and Postmortem Brains

We measured FABP4 protein levels in the same cohort as the second hair follicle sample, using an enzyme-linked immunosorbent assay kit, to see whether serum levels of FABP4 could

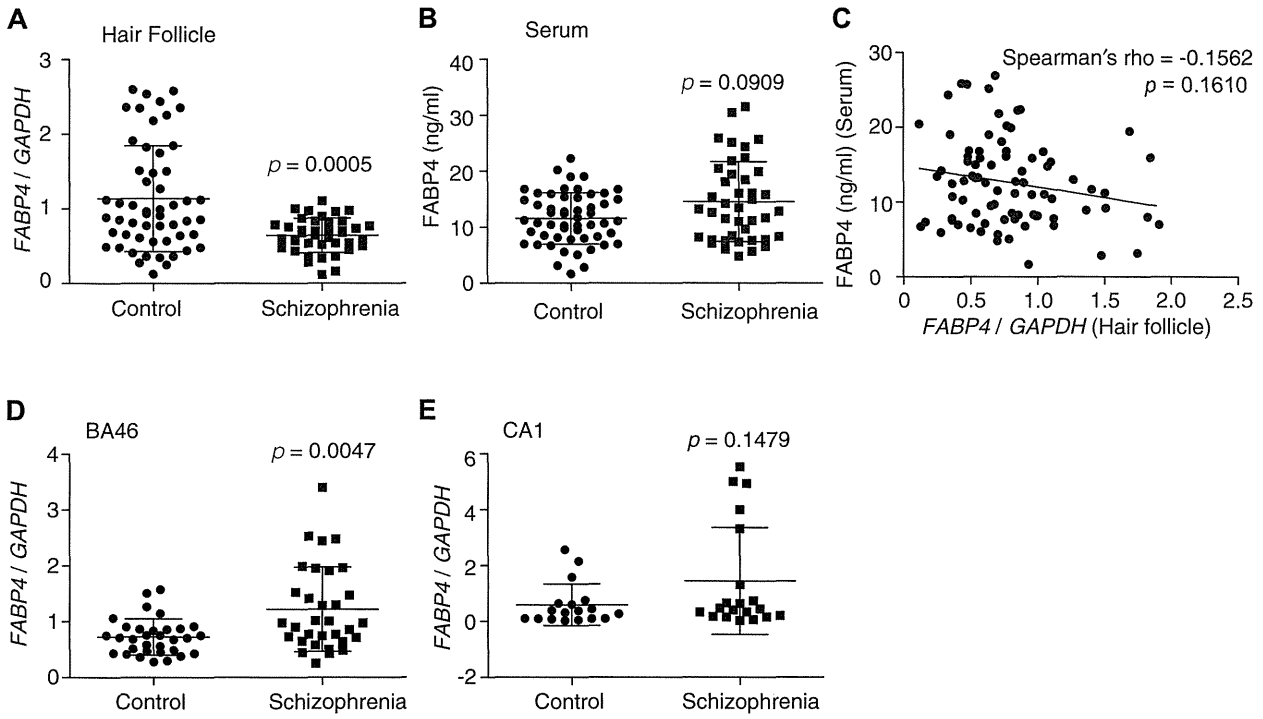


Figure 1. *FABP4*/fatty acid binding protein 4 (FABP4) expression analyses in schizophrenia and control samples. **(A, B, D, E)** Results for hair follicles (the second sample set), serum, and postmortem brain tissue (Brodmann area [BA]46 and cornu ammonis [CA]1) are shown. *GAPDH* was used as an internal control. *p* values were calculated using two-tailed Mann-Whitney *U* test. Horizontal bars show mean ± SD. **(C)** Correlations between relative *FABP4* expression levels in scalp hair follicles and FABP4 levels in serum are also shown. Statistical evaluations were performed using Spearman's rank correlation test.

also be a proxy for schizophrenia. However, the measure did not differ significantly between schizophrenia and control samples, although a trend of increase was seen in schizophrenia (Figure 1B). In addition, using the second sample cohort, there was no significant correlation between serum FABP4 and *FABP4* mRNA levels in hair follicles (Figure 1C). Interestingly, in contrast to findings in mice (20), serum FABP4 levels were not affected by time elapsed after the last meal in either disease or control groups (Figure S5 in Supplement 1).

In postmortem brains, *FABP4* transcript expression was significantly elevated in the frontal cortex (Brodmann area [BA]46) of schizophrenia compared with control samples (p = .0047) (Figure 1D), suggesting its role in schizophrenia pathophysiology. Expression of *FABP4* in hippocampus cornu ammonis 1 remained unchanged between schizophrenia and control samples (Figure 1E), implicating region specificity for the function of *FABP4* in schizophrenia. Both of these brain regions showed particularly high expression levels in four schizophrenia samples derived from patients not recorded to have taken particular therapeutic drugs (Table S3 in Supplement 1), although the possibility of drug effects cannot be excluded.

Expression Analysis of miRNAs in Scalp Hair Follicles and Postmortem Brains

We further performed microarray-based miRNA analysis and measured the expression levels of 1919 human mature miRNAs using the miRBase Release 18.0 platform (Agilent) in an age- and sex-matched subset of the first hair follicle sample set (Table S1 in Supplement 1). We detected three miRNAs, which satisfied our criteria of an absolute fold change (FC) (schizophrenia group/control group) ≥ 2 and $p < .05$ (by Mann-Whitney *U* test, two-tailed). These were *hsa-miR-4449* (FC = 3.45, p = .0032), *hsa-miR-1237* (FC = 2.55, p = .028), and *hsa-miR-4769-3p* (FC = 2.03, p = .028). In the next step, we tested these three miRNAs in the second hair follicle sample set (Table 1), using qRT-PCR, with U6 small nuclear RNA as a control probe. *hsa-miR-4449* showed a top hit with upregulation, although not to significant levels, in schizophrenia (FC = 1.25, p = .063) (Figure 3A).

In postmortem brains (BA46), *hsa-miR-4449* showed increased expression (p = .0007) in schizophrenia samples (Figure 3B), suggesting possible contribution of this gene also to schizophrenia.

Expression Analysis of *FABP4* and *hsa-miR-4449* in iPSCs and iPSC-Derived Neurospheres

Recently, iPSCs have been used for human disease modeling, particularly in neurological disorders (28–30). We have established iPSCs from control subjects (one line each from four subjects) and schizophrenia patients carrying a 22q11.2 microdeletion (two lines each from two patients) (31) (Figure 4). Then, we established three neurosphere lines from each iPSC line. We chose 22q11.2 deletion carriers for analysis (for comparative genomic hybridization array analysis using the iPSCs, see Supplementary Methods and Materials in Supplement 1), since the 22q11.2 deletion is a well-defined

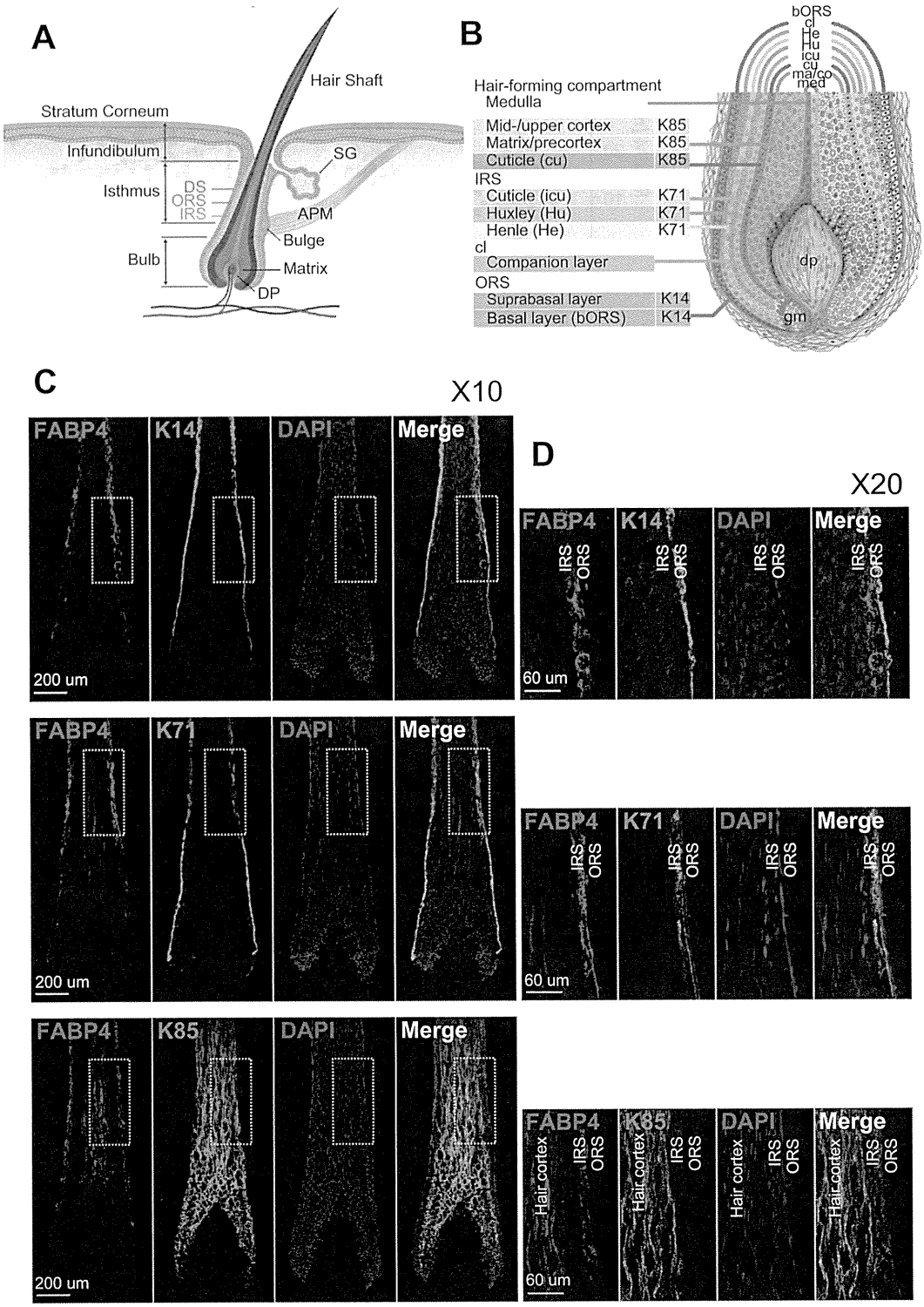


Figure 2. Expression patterns of fatty acid binding protein 4 (FABP4) in scalp hair follicles. **(A)** Schematic illustration showing the structure of hair follicles. **(B)** Schematic presentation of epithelial/hair keratin expression patterns. Keratin K71 is expressed in the three inner root sheath (IRS) layers, while K14 is known as outer root sheath (ORS) keratin. Keratin K85 is present in the hair-forming compartment. **(C)** Immunofluorescent labeling of FABP4 and hair keratins (K14, K71, and K85) in scalp hair follicles. K14 is uniformly expressed throughout the widely stratified follicular ORS. K71 is expressed in all compartments of the hair IRS. Keratin K85 expression extends from the hair matrix to the upper cortex and the hair cuticle. FABP4 is seen in the IRS and part of the hair cortex (merged green and red). 4',6-diamidino-2-phenylindole (DAPI) was used for nuclear staining. **(D)** Magnified picture of **(C)**. APM, arrector pili muscle; cl, companion layer; DP, dermal papilla; DS, dermal sheath; gm, germinative matrix; ma/co, matrix/precortex; med, medulla; SG, sebaceous gland.

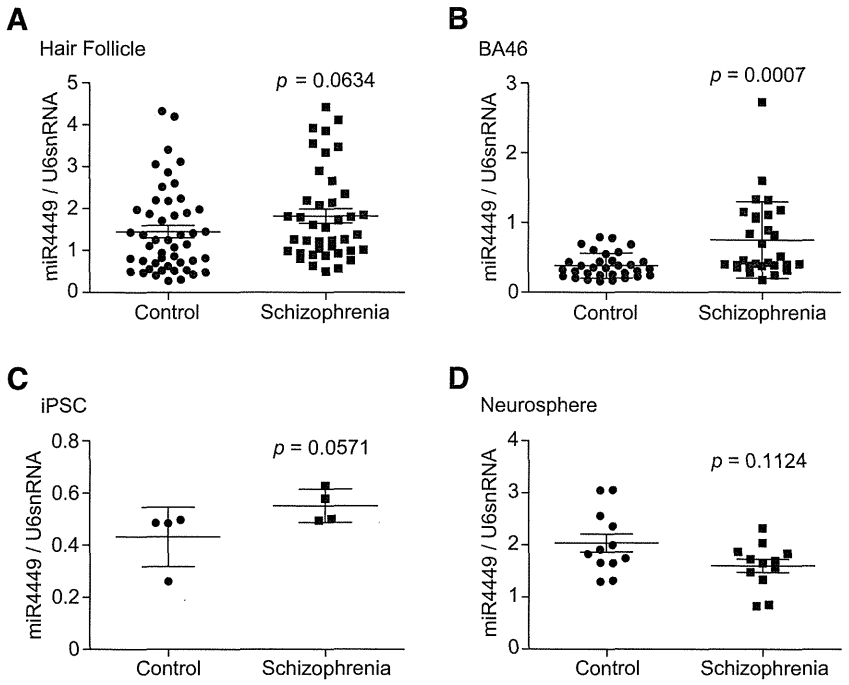


Figure 3. Expression analyses of *hsa-miR-4449* in schizophrenia and control samples. Results from hair follicles (the second sample set) **(A)**, postmortem brains (Brodmann area [BA]46) **(B)**, induced pluripotent stem cells (iPSCs) **(C)**, and neurospheres **(D)** are shown. U6 small nuclear RNA (snRNA) was used as an internal control. *p* values were calculated using two-tailed Mann-Whitney *U* test. Horizontal bars show mean ± SD.

genetic feature with the highest risk for schizophrenia, affecting around .3% of schizophrenia patients (32). The *FABP4* gene shows little expression in iPSCs derived from either control subjects or patients (data not shown). The gene is expressed in neurospheres, suggesting that its expression starts at a very early stage of neuronal development. Neurospheres are composed of free-floating clusters of neural stem or progenitor cells, differentiated from iPSCs. However, *FABP4* expression levels were not significantly different between control subjects and cases (Figure S6 in Supplement 1; expressional variance in the control group was large). Expression of *hsa-miR-4449* showed a trend of upregulation in iPSCs from patients (p = .0571) (Figure 3C); however, there was no differential expression between neurospheres derived from control subjects and cases (Figure 3D).

Examination of Autism Samples

We also performed a preliminary study to examine whether expression patterns of putative autism genes in scalp hair follicles could discriminate between autism and control samples. The sample cohort is shown in Table 1. We selected genes from candidates for autism susceptibility and included *FABP4*, due to the genetic overlap between schizophrenia and autism (33). The remaining genes were *FABP7* (9), *NHE6* (34), *NHE9* (34), *A2BP1* (35), *CADPS2* (36), *AH1* (35), *CNTNAP2* (35), and *SLC25A12* (35). Of the nine genes, only *CADPS2* (p = .0401) and *CNTNAP2* (p = .0212) showed significantly decreased expression in autism-derived samples compared with control follicles (Figure S7 in Supplement 1). It should be noted that the average age of autism subjects was significantly lower than that of control subjects (Table 1) and that *CADPS2* levels showed a positive correlation with age in autism and control + autism groups (Figure S8 in Supplement 1). Therefore, we can only safely nominate *CNTNAP2* level as a potentially valid marker for autism in this study (Figure S9 in Supplement 1). Approximately half of the examined patients were medicated. However, these patients were not outliers in terms of *CNTNAP2* expression in hair follicles; that is, they fell within the mean ± 2SD (detailed data not shown).

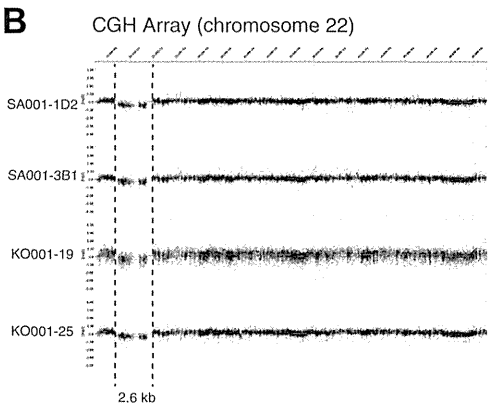
DISCUSSION

We examined and attempted to validate expression levels of schizophrenia and autism candidate genes using scalp hair follicles as a surrogate source of disease markers. Of the protein-coding genes tested that are putative schizophrenia genes, *FABP4* was confirmed to be downregulated in disease samples in our two-stage analyses. Our low rate of replication could be due to two main factors. First, the current sample size is insufficient, which may represent one of the limitations in this study. Another potential reason might be that stable detection of expression levels is dependent on where a particular gene is expressed in the hair follicle. For instance, *FABP4* is expressed in more central portions (IRS and cortex) of the hair follicle and the integrity of these areas may be well maintained during the plucking process, leading to more consistent results.

FABP4, also known as adipocyte-specific fatty acid-binding protein, belongs to the fatty acid-binding protein super family, whose members have molecular masses of approximately 15,000. FABPs are highly conserved cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. It is thought that FABPs are active in fatty acid uptake, transport, and metabolism. In the periphery, FABP4 is highly expressed in adipose tissue and moderately expressed in macrophages, endothelial cells, and bone marrow (37). The protein has been intensively studied in terms of systemic insulin sensitivity and

A

Diagnosis	Sex	Age	Fibroblast	iPSC line No.
Control	F	36	B7	201B7, YA9
Control	F	16	WD	39
Control	M	40	KA	23
Schizophrenia with 22q11del	F	30	KO001	19, 25
Schizophrenia with 22q11del	F	37	SA001	1D2, 3B1



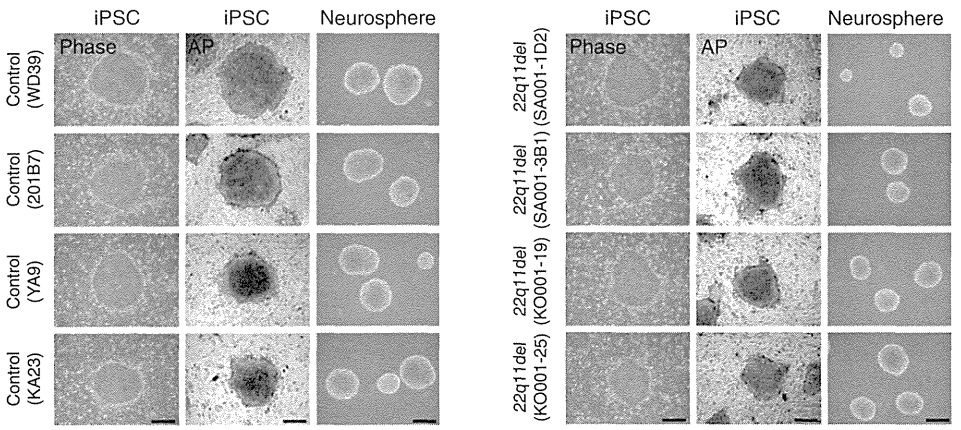


Figure 4. Establishment of iPSCs and iPSC-derived neurospheres from controls and schizophrenia patients with a 22q11.2 deletion (also see ref. 31). **(A)** Demographic data and I.D. information for samples are shown. **(B)** CGH array analysis of chromosome 22 using iPSCs showed that all the iPSC lines derived from the patients carried a 2.6 Mb hemizygous deletion at chromosome 22q11.2. **(C)** Alkaline phosphatase (AP) staining of iPSCs from controls (WD39, 201B7, YA9 and KA23). AP activity was detected using an Alkaline Phosphatase Staining kit (Miltenyi Biotec, Bergisch Gladbach, Germany). **(D)** Those from patients with a 22q11.2 deletion (SA001-1D2, SA001-3B1, KO001-19 and KO001-25). All the iPSC clones were AP-positive showing the pluripotency. Scale bars: phase contrast and AP staining, 400 μm; neurospheres, 150 μm. iPSC, induced pluripotent stem cells.

lipid and glucose metabolism, both of which correlate with inflammatory mechanisms (21). Since the results showing downregulation of *FABP4* in scalp hair follicles from schizophrenia subjects are robust against confounding factors, including those related to metabolic state, our findings are unlikely to represent either metabolic or inflammatory conditions. In addition, our patients had been treated with second-generation antipsychotics, including olanzapine, which often induce metabolic syndrome, but *FABP4* levels in hair follicles were independent of drug dose and duration of illness. Conformingly, there was no significant correlation between serum FABP4 and *FABP4* transcript levels in hair follicles. Therefore, elevated *FABP4* expression in hair follicles may point toward a pathophysiological step in schizophrenia.

In our protocol, all cells in neurospheres expressed the neural markers Nestin, or β3-tubulin, suggesting that our neurospheres consist almost entirely of neural stem or progenitor cells (38). The fact that *FABP4* is expressed in neurospheres may suggest a potential role in neuronal stem cell maintenance or neuronal differentiation or both processes. Although iPSC-derived neurospheres showed no significant differences in *FABP4* expression levels between control and schizophrenia cohorts, before a conclusion can be made, it would be necessary to examine a much larger cohort. According to the Human Protein Atlas database (Knut and Alice Wallenberg Foundation, Stockholm, Sweden; http://www.proteinatlas.org/), *FABP4* transcripts are expressed in neuronal cells (35%) and glial and endothelial cells (65%) of the adult cerebral cortex.

To evaluate whether common genetic variants of *FABP4* determine a predisposition to schizophrenia, we performed a genetic association study using approximately 2000 schizophrenia cases and 2000 age- and sex-matched control subjects with six tag single nucleotide polymorphisms (Supplementary Methods and Materials in Supplement 1). This analysis found no significant allelic or genotypic association (Table S4 in Supplement 1). The *FABP4* gene is composed of two haplotype blocks, based on Gabriel's confidence intervals (39) (Figure S10 in Supplement 1). Haplotype analysis also failed to reveal any significant signals. The exact reasons for the different directional changes seen in hair follicles, serum, postmortem brains, and neurospheres between control and schizophrenia subjects remain unknown. All *FABP* family genes contain a canonical TATA box, followed by a conserved gene structure. The tissue-specific and developmental regulation of *FABP* subtype expression, including that of *FABP4*, is thought to be controlled by unidentified genomic regulatory elements (6,40).

Mechanistically speaking, although not yet confirmed, the *FABP4* may be more central to schizophrenia pathophysiology beyond being a mere biomarker for disease. This is based on the following observations: 1) *FABP4* is expressed in the early neuronal lineage (a current finding); 2) other *FABP* genes are

reported to be associated with schizophrenia (6,7,9,11); and 3) there is evidence linking polyunsaturated fatty acids (endogenous ligands for FABPs) with schizophrenia etiology (41) and brain development (42).

Regarding miRNA, we detected *hsa-miR-4449* from a total of 1919 human mature miRNAs in this study. Although its expression in hair follicles was not significantly altered, expression did show significant upregulation in postmortem brains (BA46) and a trend of increase in iPSCs from schizophrenia samples. Web-based target predictions for *hsa-miR-4449* hit 18 protein-coding genes using TargetScan (Whitehead Institute for Biomedical Research, Release 6.2) (Table S5 in Supplement 1) and 10 protein-coding genes using miRDB (Washington University School of Medicine; http://mirdb.org/miRDB/) (Table S6 in Supplement 1). Between the two programs, the following three genes overlapped: 1) *HIC1*; 2) *RBM4*; and 3) *TOMM40*. Although the predicted roles for these three genes in schizophrenia pathogenesis are not known, *hsa-miR-4449* would make an interesting candidate in future studies, since this miRNA is expressed in early human neurodevelopmental stages such as iPSCs and iPSC-derived neurospheres.

In the analysis of autism-derived scalp hair follicles, we found significant downregulation of *CNTNAP2* in sufferers compared with control subjects and that the results are not affected by age. *CNTNAP2*, which encodes the contactin associated protein-like 2, is one of the strongest autism susceptibility genes with convergent evidence from several independent studies (43).

In the case of schizophrenia, biomarkers are an essential tool, particularly in the early phase of disease onset, such as the prodromal phase or at-risk mental state (44). It would be important to confirm whether *FABP4* expression levels in scalp hair follicles constitute a valid measure for discriminating between those individuals in at-risk mental state who will spontaneously recover and those who will need therapeutic treatment. As a starting point, it is interesting that the decreased *FABP4* levels in schizophrenia-derived hair follicles are not influenced by duration of illness.

In summary, our results provide an original concept for identifying novel disease markers, with potential benefits for the clinical practice of psychiatric medicine, as well as possible applications to other brain disorders. The development of methods that enable the analysis of a transcriptome using hair follicles (~10 samples) would be highly desirable. At the moment, approximately 40 ng of total RNA is extractable from a single hair follicle, but this amount is not enough for currently available cDNA microarray analysis, a technique which needs roughly 1 μg of total RNA.

ACKNOWLEDGMENTS AND DISCLOSURES

This study was supported, in part, by grants-in-aid for Scientific Research and by grant-in-aid for Scientific Research on Innovative Areas from the Japan Society for the Promotion of Science, Japan. In addition, this study was supported by RIKEN Brain Science Institute Funds, and a part of this study is the result of the "Development of biomarker candidates for social behavior" and "Integrated research on neuropsychiatric disorders" projects, carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

We thank Dr. Tomoe Ichikawa, Dr. Kazuya Toriumi, and Ms. Akiko Kobori at Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, for their help with collecting scalp hair follicle samples. We also thank Drs. Kenji J. Tsuchiya and Kaori Matsumoto at Research Center for Child Mental Development, Hamamatsu University School of Medicine, who have an established reliability of diagnosing autism with the Japanese version of Autism Diagnostic Interview-Revised and conducted the diagnostic tool.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Laboratory of Molecular Psychiatry (MMa, KY, MT, TO, Ylway, CS, TT, YN, SB, TY), RIKEN Brain Science Institute, Saitama; Graduate School of Humanities and Sciences (CS), Department of Life Science, Ochanomizu University, Tokyo; Research Center for Child Mental Development (HM), University of Fukui, Fukui; Department of Psychiatry and Neurology (Ylway, KS, NM), Hamamatsu University School of Medicine, Shizuoka; Department of Psychiatry and Behavioral Sciences (MMi, MI), Tokyo Metropolitan Institute of Medical Science, Tokyo; Department of Psychiatry and Neurobiology (MKi), Kanazawa University Graduate School of Medicine, Kanazawa; Department of Neuropsychiatry (MKi), Keio University School of Medicine, Tokyo; Physiology (YOk, WA, HO), Keio University School of Medicine, Tokyo; Department of Neurology (YOk), School of Medicine, Aichi Medical University, Aichi; Center for Genomic and Regenerative Medicine (WA), Juntendo University School of Medicine, Tokyo; and Department of Organ Anatomy (YOw), Yamaguchi University Graduate School of Medicine, Ube, Japan.

Address correspondence to Takeo Yoshikawa, M.D., Ph.D., Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan; E-mail: takeo@brain.riken.jp.

Received Mar 17, 2014; revised Jul 24, 2014; accepted Jul 25, 2014.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.07.025.

REFERENCES

1. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA (2008): Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. Am J Psychiatry 165:479–489.
2. Benes FM, Berretta S (2001): GABAergic interneurons: Implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology 25:1–27.
3. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, *et al.* (2003): Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet 362:798–805.
4. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, *et al.* (2001): Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A 98:4746–4751.

5. Jitoku D, Hattori E, Iwayama Y, Yamada K, Toyota T, Kikuchi M, *et al.* (2011): Association study of Nogo-related genes with schizophrenia in a Japanese case-control sample. Am J Med Genet B Neuropsychiatr Genet 156B:581–592.
6. Watanabe A, Toyota T, Owada Y, Hayashi T, Iwayama Y, Matsumata M, *et al.* (2007): Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype. PLoS Biol 5:e297.
7. Iwayama Y, Hattori E, Maekawa M, Yamada K, Toyota T, Ohnishi T, *et al.* (2010): Association analyses between brain-expressed fatty-acid binding protein (FABP) genes and schizophrenia and bipolar disorder. Am J Med Genet B Neuropsychiatr Genet 153B:484–493.
8. Maekawa M, Takashima N, Matsumata M, Ikegami S, Kontani M, Hara Y, *et al.* (2009): Arachidonic acid drives postnatal neurogenesis and elicits a beneficial effect on prepulse inhibition, a biological trait of psychiatric illnesses. PLoS One 4:e5085.
9. Maekawa M, Iwayama Y, Arai R, Nakamura K, Ohnishi T, Toyota T, *et al.* (2010): Polymorphism screening of brain-expressed FABP7, 5 and 3 genes and association studies in autism and schizophrenia in Japanese subjects. J Hum Genet 55:127–130.
10. Freeman MP, Hibbeln JR, Wisner KL, Davis JM, Mischoulon D, Peet M, *et al.* (2006): Omega-3 fatty acids: Evidence basis for treatment and future research in psychiatry. J Clin Psychiatry 67:1954–1967.
11. Shimamoto C, Ohnishi T, Maekawa M, Watanabe A, Ohba H, Arai R, *et al.* (2014): Functional characterization of FABP3, 5 and 7 gene variants identified in schizophrenia and autism spectrum disorder and mouse behavioral studies [published online ahead of print July 15]. Hum Mol Genet.
12. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, *et al.* (2000): The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30:205–223.
13. Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M, Takebayashi K, *et al.* (2013): Microglial activation in young adults with autism spectrum disorder. JAMA Psychiatry 70:49–58.
14. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007): Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872.
15. Imaizumi Y, Okada Y, Akamatsu W, Koike M, Kuzumaki N, Hayakawa H, *et al.* (2012): Mitochondrial dysfunction associated with increased oxidative stress and alpha-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. Mol Brain 5:35.
16. Nori S, Okada Y, Yasuda A, Tsuji O, Takahashi Y, Kobayashi Y, *et al.* (2011): Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. Proc Natl Acad Sci U S A 108:16825–16830.
17. Yagi T, Ito D, Okada Y, Akamatsu W, Nihei Y, Yoshizaki T, *et al.* (2011): Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum Mol Genet 20:4530–4539.
18. Andreasen NC, Pressler M, Nopoulos P, Miller D, Ho BC (2010): Antipsychotic dose equivalents and dose-years: A standardized method for comparing exposure to different drugs. Biol Psychiatry 67:255–262.
19. Inagaki A, Inada T (2006): Dose equivalence of psychotropic drugs. Part 18: Dose equivalence of psychotropic drugs: 2006-version. Jpn J Clin Psychopharmacol 9:1443–1447.
20. Cao H, Sekiya M, Ertunc ME, Burak MF, Mayers JR, White A, *et al.* (2013): Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production. Cell Metab 17:768–778.
21. Kralisch S, Fasshauer M (2013): Adipocyte fatty acid binding protein: A novel adipokine involved in the pathogenesis of metabolic and vascular disease? Diabetologia 56:10–21.
22. Ishimura S, Furuhashi M, Watanabe Y, Hoshina K, Fuseya T, Mita T, *et al.* (2013): Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. PLoS One 8: e81318.
23. Daly EJ, Kent JM, Janssens L, Newcomer JW, Husken G, De Boer P, *et al.* (2013): Metabolic and body mass parameters after treatment with JNJ-37822681, a novel fast-dissociating D2 receptor antagonist, vs olanzapine in patients with schizophrenia. Ann Clin Psychiatry 25: 173–183.
24. Hahn MK, Wolever TM, Arenovich T, Teo C, Giacca A, Powell V, *et al.* (2013): Acute effects of single-dose olanzapine on metabolic, endocrine, and inflammatory markers in healthy controls. J Clin Psychopharmacol 33:740–746.
25. Mistriotis P, Andreadis ST (2013): Hair follicle: A novel source of multipotent stem cells for tissue engineering and regenerative medicine. Tissue Eng Part B Rev 19:265–278.
26. Langbein L, Rogers MA, Praetzel-Wunder S, Helmke B, Schirmacher P, Schweizer J (2006): K25 (K25irs1), K26 (K25irs2), K27 (K25irs3), and K28 (K25irs4) represent the type I inner root sheath keratins of the human hair follicle. J Invest Dermatol 126:2377–2386.
27. Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, *et al.* (2006): New consensus nomenclature for mammalian keratins. J Cell Biol 174:169–174.
28. Imaizumi Y, Okano H (2014): Modeling human neurological disorders with induced pluripotent stem cells. J Neurochem 129:388–399.
29. Okano H, Yamanaka S (2014): iPS cell technologies: Significance and applications to CNS regeneration and disease. Mol Brain 7:22.
30. Horiuchi Y, Kano S, Ishizuka K, Cascella NG, Ishii S, Talbot CC Jr, *et al.* (2013): Olfactory cells via nasal biopsy reflect the developing brain in gene expression profiles: Utility and limitation of the surrogate tissues in research for brain disorders. Neurosci Res 77:247–250.
31. Bundo M, Toyoshima M, Okada Y, Akamatsu W, Ueda J, Nemoto-Miyauchi T, *et al.* (2014): Increased l1 retrotransposition in the neuronal genome in schizophrenia. Neuron 81:306–313.
32. Rees E, Walters JT, Georgieva L, Isles AR, Chambert KD, Richards AL, *et al.* (2014): Analysis of copy number variations at 15 schizophrenia-associated loci. Br J Psychiatry 204:108–114.
33. Cross-Disorder Group of the Psychiatric Genomics Consortium, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, *et al.* (2013): Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 45:984–994.
34. Schwede M, Garbett K, Mirnics K, Geschwind DH, Morrow EM (2014): Genes for endosomal NHE6 and NHE9 are misregulated in autism brains. Mol Psychiatry 19:277–279.
35. Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, *et al.* (2011): Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature 474:380–384.
36. Sadakata T, Furuichi T (2009): Developmentally regulated Ca2+-dependent activator protein for secretion 2 (CAPS2) is involved in BDNF secretion and is associated with autism susceptibility. Cerebellum 8:312–322.
37. Boord JB, Fazio S, Linton MF (2002): Cytoplasmic fatty acid-binding proteins: Emerging roles in metabolism and atherosclerosis. Curr Opin Lipidol 13:141–147.
38. Matsui T, Takano M, Yoshida K, Ono S, Fujisaki C, Matsuzaki Y, *et al.* (2012): Neural stem cells directly differentiated from partially reprogrammed fibroblasts rapidly acquire gliogenic competency. Stem Cells 30:1109–1119.
39. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, *et al.* (2002): The structure of haplotype blocks in the human genome. Science 296:2225–2229.
40. Haunerland NH, Spener F (2004): Fatty acid-binding proteins—insights from genetic manipulations. Prog Lipid Res 43:328–349.
41. Maekawa M, Owada Y, Yoshikawa T (2011): Role of polyunsaturated fatty acids and fatty acid binding protein in the pathogenesis of schizophrenia. Curr Pharm Des 17:168–175.
42. Basak S, Das MK, Duttaroy AK (2013): Fatty acid-induced angiogenesis in first trimester placental trophoblast cells: Possible roles of cellular fatty acid-binding proteins. Life Sci 93:755–762.
43. Penagarikano O, Geschwind DH (2012): What does CNTNAP2 reveal about autism spectrum disorder? Trends Mol Med 18:156–163.
44. Schultze-Lutter F, Schimmelmann BG, Ruhrmann S (2011): The near Babylonian speech confusion in early detection of psychosis. Schizophr Bull 37:653–655.

特集 自閉症の分子基盤

自閉症のPET研究について

中村和彦* 鈴木勝昭** 尾内康臣*** 辻井正次§ 森 則夫**

KEY WORDS

・PET
・セロトニン・トランスポーター
・活性型ミクログリア
・末梢マクロファージ

SUMMARY

自閉症は脳内セロトニンの機能異常によるというセロトニン仮説が最も有力視された．われわれはPETにより，自閉症脳内の広汎な脳部位におけるセロトニン・トランスポーター脳内密度の低下を明らかにした．さらにその低下は自閉症の中核症状である社会性の障害やこだわり症状と関係していることを見出した．自閉症に関する免疫系の異常については各種の研究があるが，われわれはミクログリアに注目した．ミクログリアは，感染，出血，虚血での貪食，脳細胞の保護，神経回路形成や神経伝達の恒常性を維持する役割をもつ．PETにより，自閉症脳内の広汎な脳部位における，活性型ミクログリア増加を明らかにした．そして活性型ミクログリアが多いほど，社会性の障害が子どものころも現時点においても強いことを見出した．自閉症は遺伝的要因と環境的要因によって胎生期において骨髄系などに影響を受け血管脳関門が閉じられる前に末梢マクロファージが脳内に移行する量が増え，胎生期から活性型ミクログリアによって脳内のセロトニン系などのシナプス形成障害を起こし自閉症の病因となると推測した．

はじめに —自閉症発症の遺伝要因と環境要因について—

自閉症は高血圧症，糖尿病などと同様で遺伝要因，胎生期での環境要因他，多くの事柄が関与する多因子疾患であることが定説となった．自閉症は家族性の疾患や遺伝病ではない．自閉症の発症は，約400個の遺伝子が関与し，人によって遺伝子の組み合わせが違い，特徴としては孤発例（家系の中に他に自閉症の人がいない）が多いと報告されている．**図1**で示すように遺伝要因としては，さまざまな遺伝子が報告されている．自閉症では，一卵性双生児の一致率が70～90%であり，従来遺伝的な関与が強いと考えられ，さまざまな自閉症関連遺伝子が報告されている[1]．環境要因としても，各種報告があり，最近は遺伝要因よりは環境要因のほうが強いと報告され，新しい双生児研究よると自閉症の環境要因は55%と報告された[2]．環境要因としては，父親の高年齢，体外受精，出生時低体重，多産，妊娠中母体感染症などが報告され，最近では妊娠中や，生まれてから1年における大気汚染のPM2.5，PM10が自閉症と関連しているとの報告がある[3]．

1．自閉症の画像研究について

脳科学の最近の進歩，検査機器の向上に伴い，自閉症に関する精神医学研究は日々進んでいる．さまざまな研究手法の中で，画像研究は直接脳の様子がわかることより注目されている．自閉症の画像研究に関しては，MRI（magnetic resonance imaging），SPECT（single-pho-

NAKAMURA Kazuhiko, SUZUKI Katsuaki, OUCHI Yasuomi, TSUJII Masatsugu, MORI Norio/＊弘前大学大学院医学研究科神経精神医学講座，＊＊浜松医科大学精神医学講座，＊＊＊浜松医科大学メディカルフォトニクス研究センター，§中京大学現代社会学部

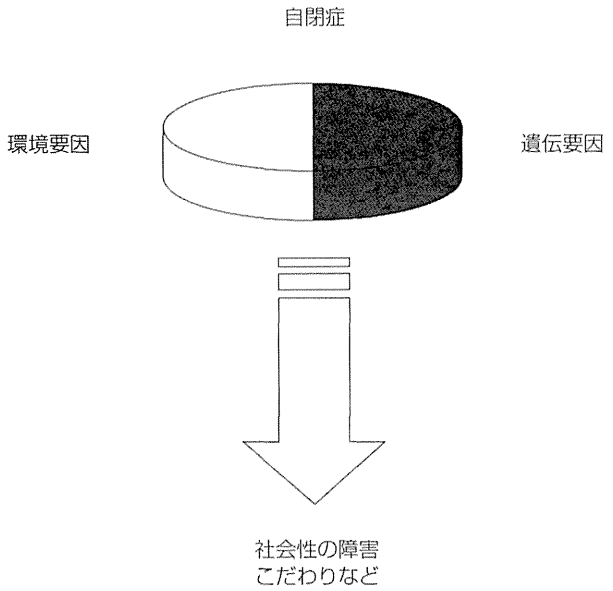


図 1. 自閉症の環境要因と遺伝要因
環境要因：父親の高年齢，体外受精，出生時障害，低体重，多産，妊娠中母体感染症，夏の出産，大気汚染（NO_2，PM2.5，PM10）[3]
＊遺伝的要素が37%，妊娠中・新生児期早期の環境要素が55%のリスクが想定される[2]
遺伝要因：一卵性双生児の一致率．Fragile X mental retardation 1，Neuroligin3，4，Neurexin 1，SHANK3，Contactin-associated protein-like 2，Protocadherin 10 他さまざま（1%程度）．CNV（copy number variation）（7～10%）．Genome-wide association study（GWAS）．OR（odd ratio）1.1～1.3倍．epigenetics.

ton emission computed tomography），PET（positron emission tomography）を用いた数多くの研究が報告されている．また研究内容についても，神経伝達系の異常，脳の構造や大きさの異常，表情認知や追視の異常などさまざまな報告がある．筆者らはその中で，PETを用いた自閉症研究を行っている[4)~6)]．PETは脳内における神経伝達機能の測定が可能である．ドパミン系，セロトニン系，アセチルコリン系，ベンゾジアゼピン系，ヒスタミン系などさまざまなトレーサー（分子プローブ）があり，それらを用いることで脳内の生化学的過程の画像化や定量的解析ができる．

2．PETについて

PETについて少し解説をする．PETとはpositron emission tomography（陽電子放出型断層撮影）の略称

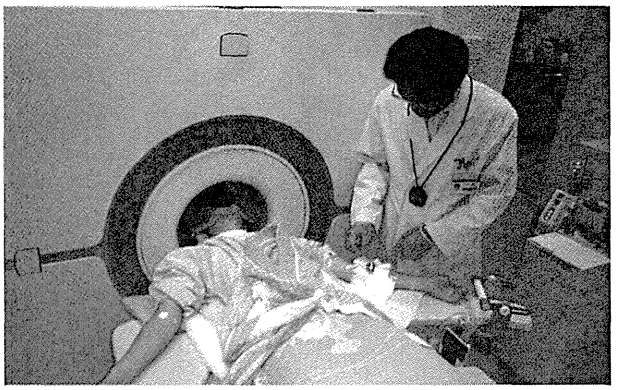


図 2. PETスキャン

で，陽電子（ポジトロン）の体内分布を画像化する撮影法である．ポジトロンは，電子と同じ質量をもち，電子とは正反対のプラスの電荷をもっている．PETはサイクロトロン，薬剤合成標識装置，PET装置という一連のシステムによって支えられた統合技術であるとともに，工学，物理学，化学，薬学，医学知識の産物である．PETでは，生体内の生理的・生化学的情報をとらえることが可能である．さらに，生体内のいろいろな機能を調べることによって，病気の早期診断や治療後の経過を知ることができる．特に，脳疾患，心臓病，腫瘍に対しては，個々の機能的異常を正確かつ事前にみつけられるという点で優れた検査法である．このように，PETは科学および社会に貢献する先端技術である．われわれのPETは頭部専用PETスキャナ（SHR12000，Hamamatsu Photonics KK，Hamamatsu，Japan）を用いた（**図2**）．最近はPETによるがん検診が日本各地で行われており，PETの機械が普及した．われわれの用いたPETスキャンは，頭を入れるためだけの穴（ガントリー）がある．がん検診用だとこの穴は体が入るぐらい大きさであるが，これは頭部用につくられた特別なPETで，穴が小さいので，ここに頭を入れると，センサーとの距離が近く精度の良い値を取ることができる．サイクロトロンは隣の施設にあり，トレーサーを研究所で化学者が生成の具合をチェックしながら合成する．PETは，イメージングの専門家である浜松医科大学・尾内康臣教授，放射線技師，画像解析のコンピューター専門技師，トレーサー合成担当，浜松医科大学のスタッフなどいろいろな方が共同で行う．1日に2人しか撮影

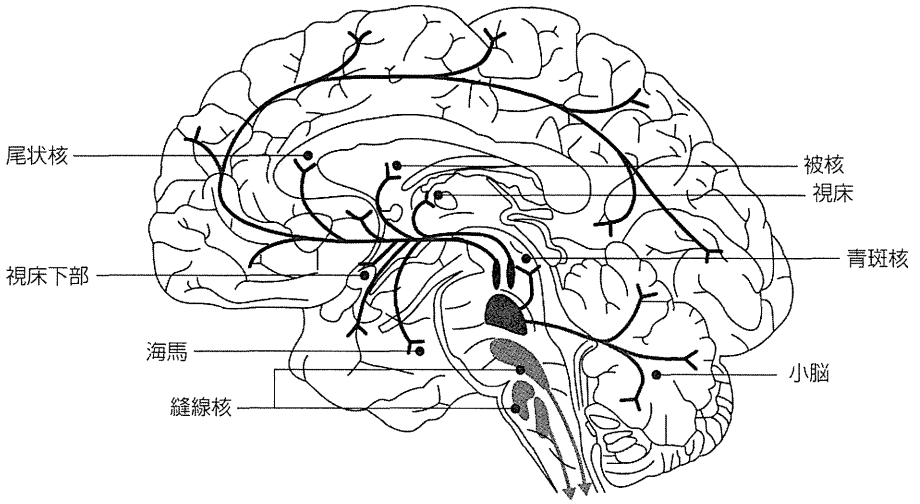


図 3. セロトニン神経系

できず，1回のPETを行うのに約100万円の実費がかかる．そしてPET研究をしていくうえで，脳が薬物の影響を受けていない状態の人を対象にすることが重要である．NPO法人アスペ・エルデの会の自閉症の当事者の人は薬を服用せずに，療育指導によって補っている人が多いので，そういう人に協力して頂いた．

3．セロトニン系に着目したPET研究

神経伝達のプロセスには，神経伝達物質の生合成・貯蔵・放出，受容体への結合・代謝・再取り込みなど多くの機能が関与している．したがって，神経伝達機能を対象としたPETの分子イメージングには，神経伝達物質の神経細胞内の合成酵素やシナプス間隙中に存在する分解酵素などの酵素，シナプス前膜や後膜に存在する受容体，再取り込み部位やシナプス小胞膜に存在するトランスポーターなどが対象となる[7]．自閉症においてはさまざまな神経伝達系の研究が報告されている．自閉症の病態に関する最初の研究は，セロトニン神経系である（**図3**）．1977年から自閉症の血液中や血小板においてセロトニン値の上昇が報告された[8][9]．さらに，セロトニンの前駆体であるトリプトファンの欠乏食を与えるとこだわり症状が強くなり，不安や不幸せ感が上昇すると報告された[10]．そしてうつ病の治療薬である選択的セロトニン再取り込み阻害薬（selective serotonin reuptake inhibitor：SSRI）の使用によって自閉症のこだわり症状や強迫症状に効果があるとの報告がある[11]．さらにセロトニン系の重要な因子であるセロトニン・トランスポーターに関する自閉症研究では，セロトニン・トランスポーター遺伝子の制御部分であるプロモーター領域の機能に関連するポリモルフィズムのshort allele（S）とlong allele（L）と自閉症との相関が報告された[12]．そして，2歳から4歳の44名の男子の自閉症対象群の各脳部位の体積とこのポリモルフィズムとの相関について，short alleleはcorticalやfrontalの部位の灰白質の体積の10～16%に関連していた[13]．さらに自閉症のゲノムスキャン解析を行うと，セロトニン・トランスポーターのある17番染色体の部位に有意差があると報告された[14]．Chuganiら[15]～[17]，脳におけるセロトニン合成能についてmethyl-L-tryptophanをトレーサーとして用いたPET研究を行った．自閉症の子ども30名（2～15歳）とてんかんの子ども16名（3ヵ月～13歳）を比較した（正常の子どもは倫理的にPETが施行できないゆえ，てんかん群を正常群としている）．正常群では5歳までは，大人の200%以上のセロトニン合成能があり，その後次第に大人のレベルまで減少する．一方自閉症の子ども群においては，セロトニン合成能は2歳から15歳まで次第に上昇し大人の150%までしかならなかった．子どもの早い段階ではセロトニン合成能が正常群では高いが，自閉症群では何らかの障害を受けセロトニン合成能が低いと考えられる．このように自閉症児におい

ては，成長過程においてセロトニン系メカニズムの障害が派生していることが推測される．そしてSPECTによってセロトニン・トランスポーターの脳内分布が報告されている[18]．使用したトレーサーは123I nor-β-CITである．15名の自閉症の子どもと10名の正常対象群を比較したところmedial frontal cortexにおいてセロトニン・トランスポーターが低下していた．medial frontal cortexは心の理論や，他人の考えや意思を理解するための重要な領域である．現在のところセロトニン神経系としてPETで現在測定できるのは，セロトニン・トランスポーター，5-HT_{1A}レセプター，5-HT_{2A}レセプターである．そこでわれわれはPETを用いて，セロトニン神経終末の構成要素であるセロトニン・トランスポーター脳内密度を定量した．そして，自閉症のセロトニン神経系の状態を健常者と比較検討し，同疾患のセロトニン神経系の異常の有無を検索し，臨床症状との関連を研究した[19]．対象は自閉症20名（すべて男性；年齢：18～26歳），および，性別，年齢の合致した健康健常者20名（すべて男性）である．ADI-R（Autism Diagnostic Interview-Revised）で自閉症の診断基準を満たし，Wechsler Adult Intelligence Scale（WAIS）で総合IQが70以上である．自閉症のうち，他の精神疾患，脳の器質的異常を有する者，重篤な身体疾患（甲状腺機能障害，免疫疾患などを含む），および，精神科薬物療法を受けた既往のある者は除外した．これは，Structured Clinical Interview for Diagnostic and Statistical Manual Ⅳ（SCID）に準じた問診を本人およびその家族に施行することにより決定した．

PETには頭部専用PETスキャナ（SHR12000，Hamamatsu Photonics KK，Hamamatsu，Japan）を用いた．トレーサーにはセロトニン・トランスポーターへの選択性の高い［11C］（＋）McN5652を用いた．臨床スコアとの相関について，自閉症に対する臨床症状は，社会性障害についてはFaux Pas Test［fou-pa：］を用いた．これは成人の自閉症の心の理論の障害を計るテストとして考案されたものである（合計20問）[20]．こだわり症状に対しては強迫症状スケールであるYale-Brown Obsessive Compulsive Scale（Y-BOCS）を用いた．それらの臨床スコアとPET画像との相関を検討した．結果は自閉症では健常者と比較して，大脳皮質全般，基底核，中脳，小脳に渡る広範囲の部位でセロトニン・トランスポーターが有意に低下していた（図4）．Faux Pas Testで測定した自閉症の心の理論の障害の程度と帯状回におけるセロトニン・トランスポーターの低下は相関していた（図5）．われわれは自閉症群が，健常者群にくらべ，大脳皮質全般，基底核，中脳，小脳などの脳部位でセロトニン・トランスポーターが有意に低下していることを見出した．自閉症に関して，重要な所見は，血液におけるセロトニンの上昇である．これは，セロトニン・トランスポーターが形成される，発達段階のときにセロトニン終末の脱落によって引き起こされると考えられている[21][22]．おそらく脳においても，同様であろうと推測されている．それゆえ，脳のさまざまな部位におけるセロトニン・トランスポーターの低下は，発達段階においてセロトニン神経伝達系が変化していることに起因すると推測される．本研究では，Faux Pas Testで測定した自閉症の心の理論の障害の程度と帯状回におけるセロトニン・トランスポーターの低下は相関していた．心の理論と帯状回の関連については，先行研究により支持されているところである．たとえば，rCBF（regional cerebral blood flow）をみるSPECT研究[23]や，18F-deoxyglucoseを用いたPET研究[24]ではCARS（Childhood Autism Rating Scale）やADI-Rでスコア化した社会性の障害，心の理論に関与していると考えられるコミュニケーション障害が，帯状回の血流量や代謝と関与していると報告されている．これらのことから帯状回は心の理論を制御する重要なメカニズムであることが示唆された．次に自閉症のこだわりの指標としての強迫症状とセロトニン神経系の関係については，セロトニン・トランスポーターの低下と強迫症状の強度との有意な相関が認められた．自閉症の主な臨床症状の1つとして強迫的で繰り返される行動があげられ，具体的には，行動，興味および活動が限定され，反復的で常同的で強度で，異常なほど，1つまたはいくつかの興味だけに熱中することや特定の無意味な習慣や儀式にかたくなにこだわることがあげられる．強迫症状の責任部位について，自閉症の各脳部位でのセロトニン・トランスポーターの低下と強迫症状の疾患内相関を検討したところ視床において