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

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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

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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.

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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

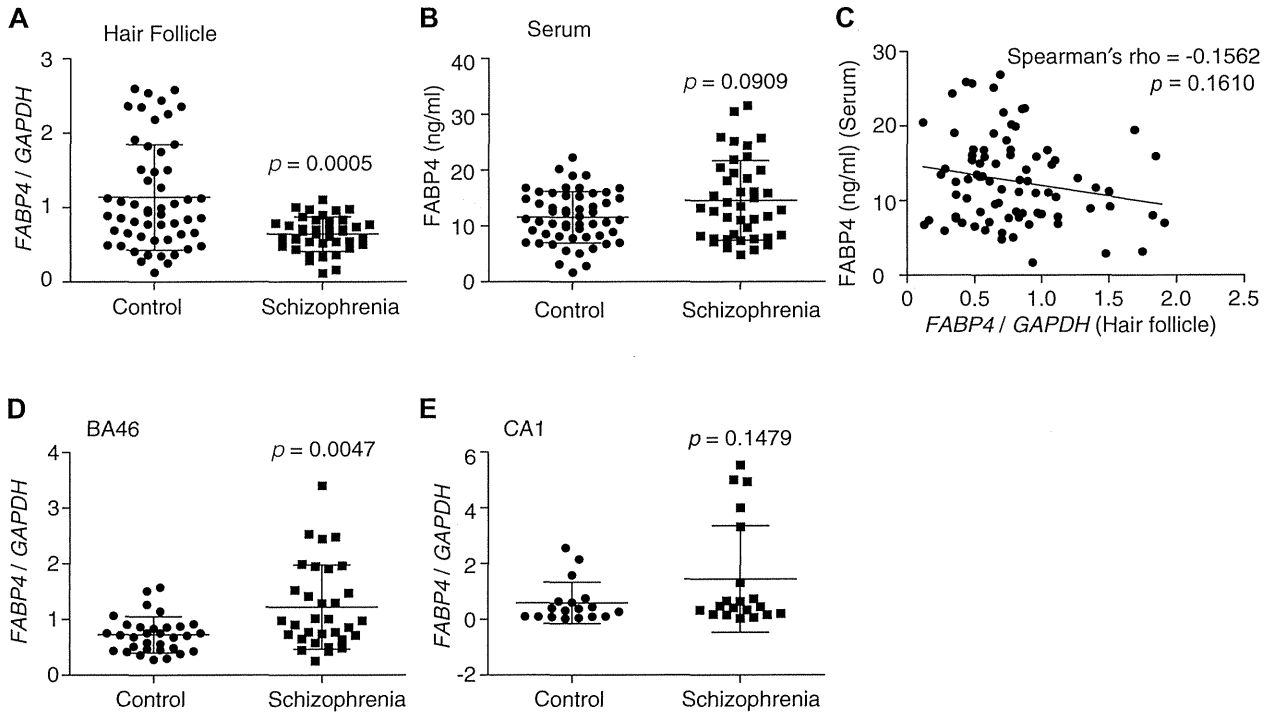

Figure 1. *FABP4*/fatty acid binding protein 4 (FABP4) expression analyses in schizophrenia and control samples. **(A, B, D, E)** Results for hair follicles (the second sample set), serum, and postmortem brain tissue (Brodmann area [BA]46 and cornu ammonis [CA]1) are shown. *GAPDH* was used as an internal 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

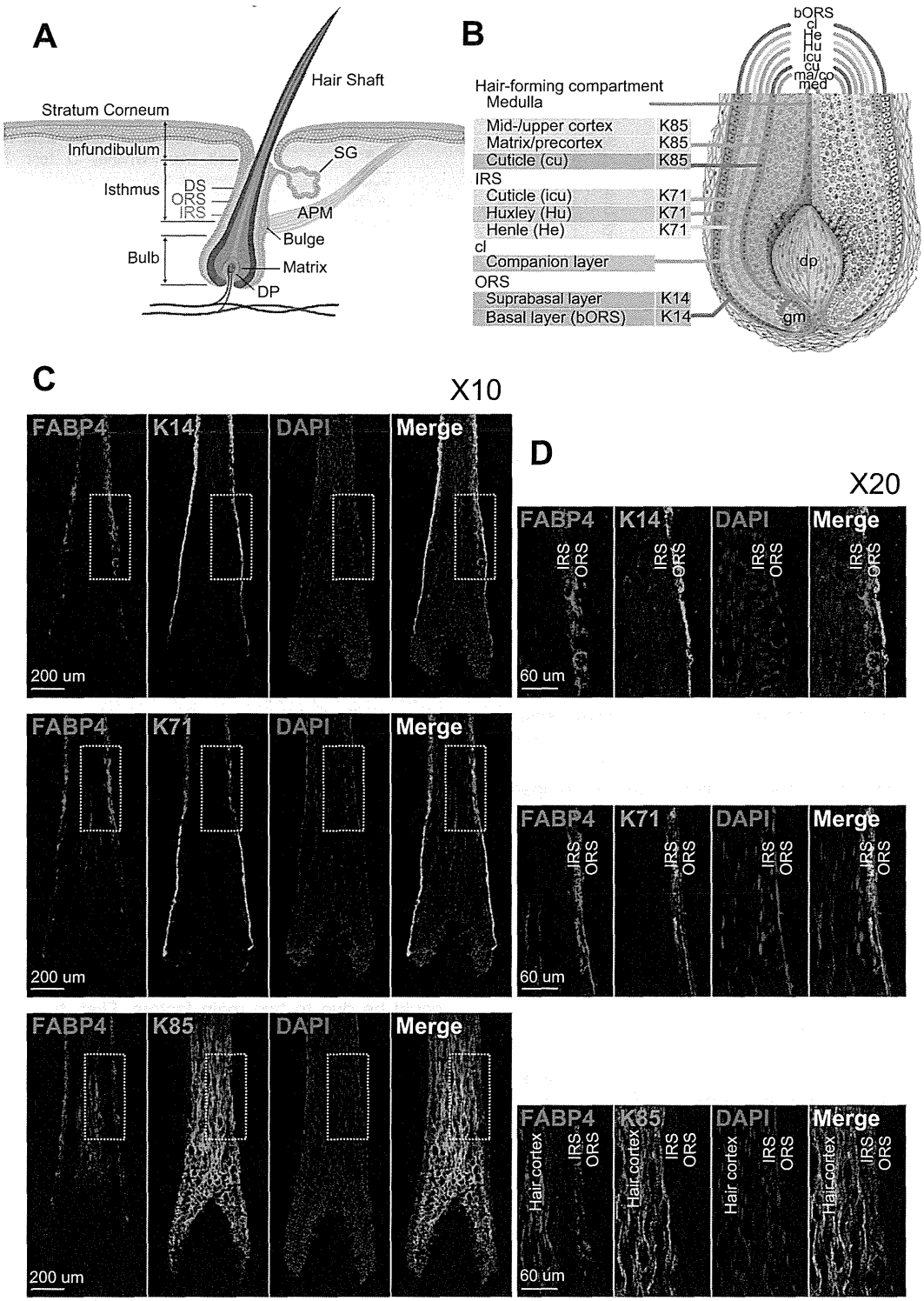

Figure 2. Expression patterns of fatty acid binding protein 4 (FABP4) in scalp hair follicles. **(A)** Schematic illustration showing the structure of hair follicles. **(B)** Schematic presentation of epithelial/hair keratin expression patterns. Keratin K71 is expressed in the three inner root sheath (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.

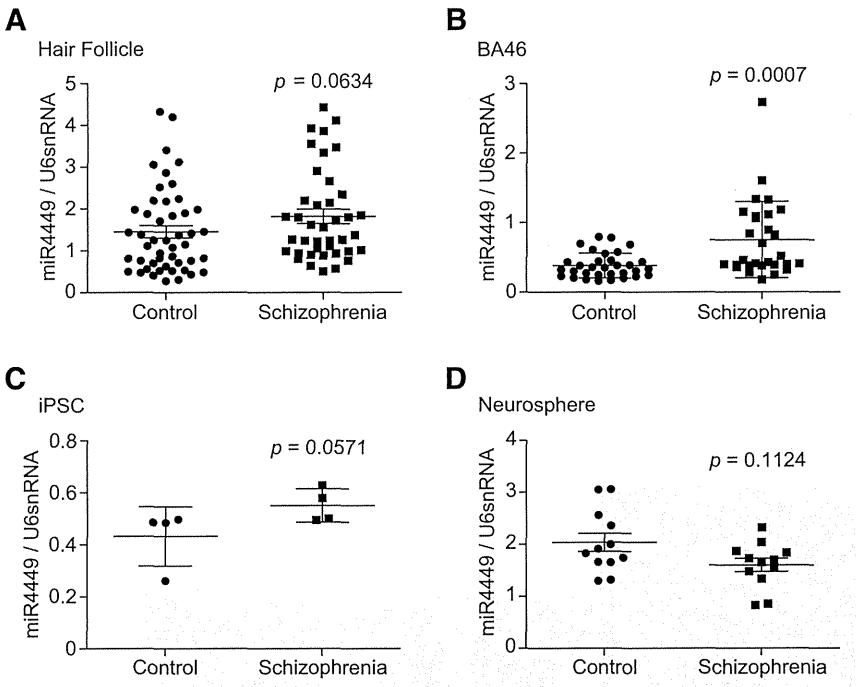

Figure 3. Expression analyses of *hsa-miR-4449* in schizophrenia and control samples. Results from hair follicles (the second sample set) **(A)**, postmortem brains (Brodmann area [BA]46) **(B)**, induced pluripotent stem cells (iPSCs) **(C)**, and neurospheres **(D)** are shown. U6 small nuclear RNA (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

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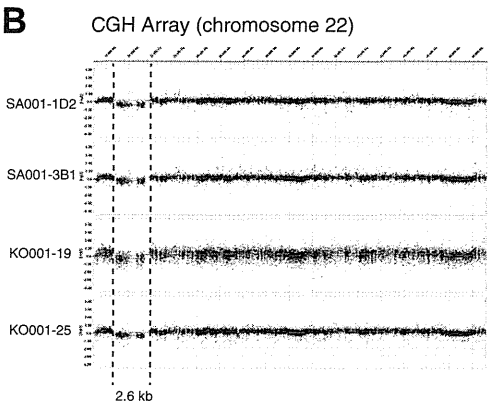
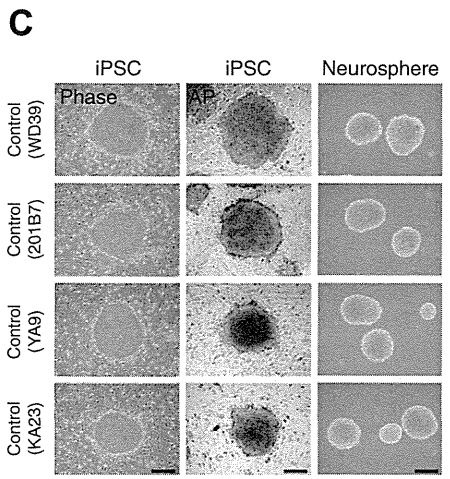
Diagnosis	Sex	Age	Fibroblast	iPSC line No.
Control	F	36	B7	201B7, YA9
Control	F	16	WD	39
Control	M	40	KA	23
Schizophrenia with 22q11del	F	30	KO001	19, 25
Schizophrenia with 22q11del	F	37	SA001	1D2, 3B1





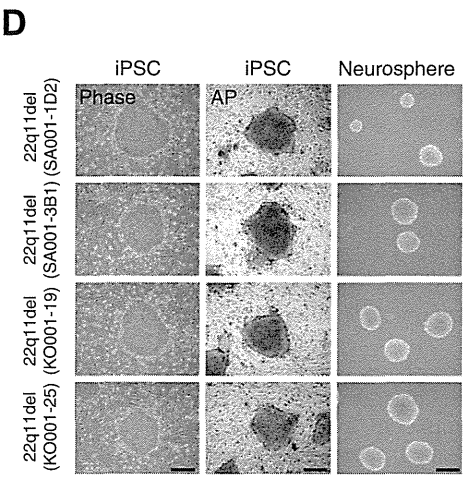


Figure 4. Establishment of iPSCs and iPSC-derived neurospheres from controls and schizophrenia patients with a 22q11.2 deletion (also see ref. 31). **(A)** Demographic data and I.D. information for samples are shown. **(B)** CGH array analysis of chromosome 22 using iPSCs showed that all the iPSC lines 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.

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特集

自閉症の分子基盤

自閉症のPET研究について

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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-

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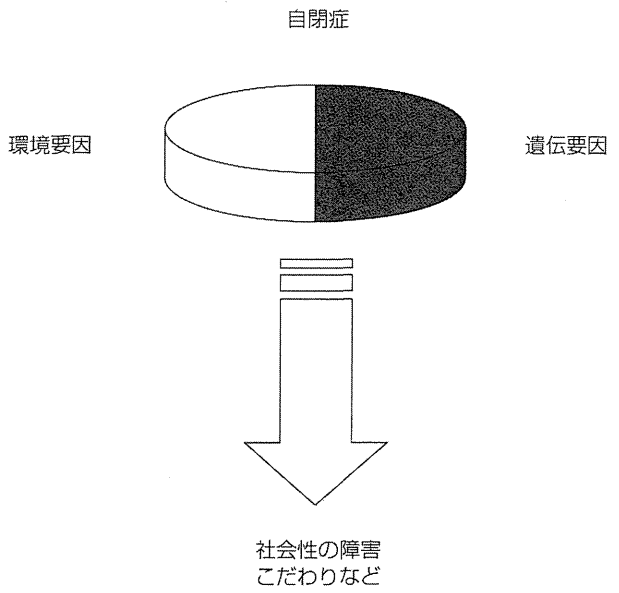

図 1. 自閉症の環境要因と遺伝要因
環境要因：父親の高年齢，体外受精，出生時障害，低体重，多産，妊娠中母体感染症，夏の出産，大気汚染（NO_2，PM2.5，PM10）[3]
＊遺伝的要素が37%，妊娠中・新生児期早期の環境要素が55%のリスクが想定される[2]
遺伝要因：一卵性双生児の一致率．Fragile X mental retardation 1，Neuroligin3，4，Neurexin 1，SHANK3，Contactin-associated protein-like 2，Protocadherin 10 他さまざま（1%程度）．CNV（copy number variation）（7～10%）．Genome-wide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

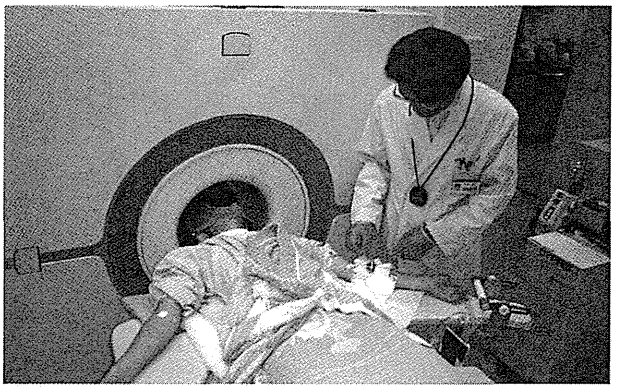

図 2. PETスキャン

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

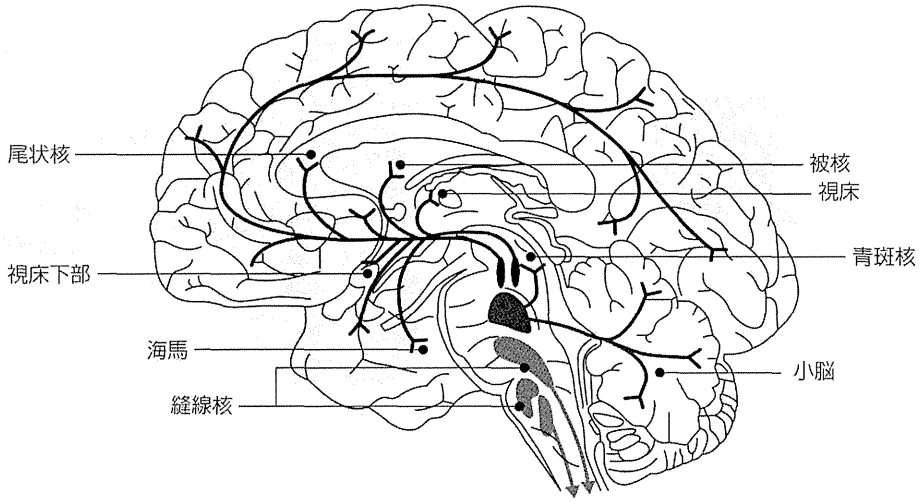

図 3. セロトニン神経系

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らは[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

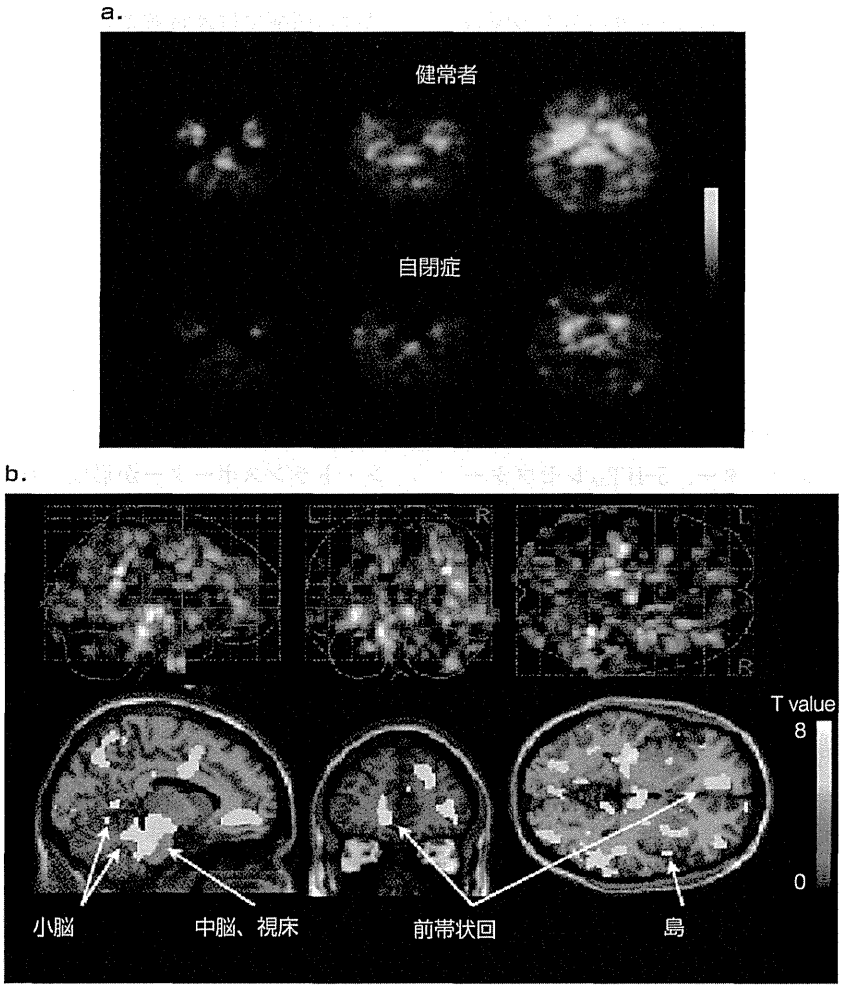

図 4. 自閉症では脳内セロトニン・トランスポーター密度が低下している
a：代表的画像
b：全脳解析

相関が認められ，視床においてセロトニン神経機能が低いほど強迫症状が強いことが明らかになった（**図 5**）．視床は強迫性障害の治療薬である SSRI が集積する重要な部位である．自閉症において強迫症状に対する SSRI の効果については有効な人と有効でない人がある．強迫症状の責任部位が複数あると考えられるが，SSRI は主に視床に分布されるので，強迫症状に対する SSRI の薬物効果には限界があると推察される．

今回の結果により，自閉症のセロトニン機能の障害は，出生後から始まっていると推測される．ゆえに，生後の脳の発達時期にセロトニン神経伝達を正常化するような治療法開発の参考になると考えられる．そして療育的観点からは療育により別の経路，側副路でセロトニン機能障害を補う考え方がある．たとえば，脳梗塞の人がリハビリをするように，自閉症の人は療育を受けながら別の神経経路で能力を補っていく必要がある．療育指導は，指導する側の固定観念にとらわれずに工夫して，個々にあった療育指導の方策を築き上げる必要がある．今後更なる研究や療育方法の開発が必要である．

4．免疫系に着目した PET 研究

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．遺伝学的研究からは *HLA* 遺伝子や *MHC class Ⅲ* 遺伝子が自閉症と関連[25)~27)]しているとの報告があり，血液学的研究からは tumor necrosis factor-α (TNF-α)，インターロイキン (IL)-6，macrophage chemoattrac-

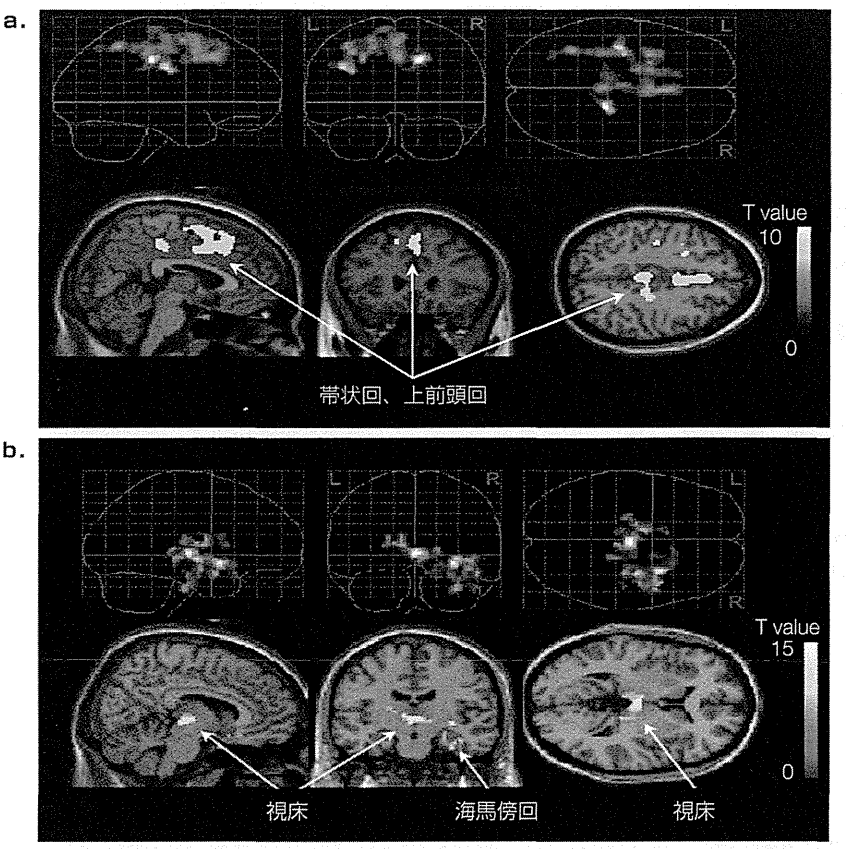

図 5. セロトニン・トランスポーター密度の低下は 2 つの中核症状と関係している

a：「心の理論の獲得障害」は帯状回，上前頭回のセロトニン・トランスポーター密度の低下と関係していた．

b：「強迫症状（こだわり）」は視床，海馬傍回のセロトニン・トランスポーター密度の低下と関係していた．

tant protein-1（MCP-1）の量が自閉症者では増大しているとの報告がある[28)~32)]．また自閉症の子どもをもつ母親は自閉症の脳に対する抗体をもつ[33)~36)]との報告や，自閉症死後脳研究からは脳実質中および脳脊髄液中の TNF-α，IL-6，MCP-1 の増加が報告されている[37)]．

5．ミクログリアについて

われわれはミクログリアに注目した．ミクログリアは脳の中に均一に分布する中胚葉由来の免疫担当細胞である．脳内でのミクログリアの役割は 1 つ目として，感染，出血，虚血で急速に活性化し，活性型ミクログリアになり異物を貪食する．2 つ目として逆の作用として保護作用のある抗炎症性サイトカインを産生し脳細胞を保護する．3 つ目として興味深いことに，脳における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．ミクログリアと自閉症の関連については自閉症の死後脳研究で，中前頭回，前帯状回，小脳において活性型ミクログリアやアストログリアの増加が認められている[38)39)]．

この PET 研究はセロトニン系の研究と同様に NPO 法人アスペ・エルデの会の当事者の人で ADI-R（Autism Diagnostic Interview-Revised），ADOS（Autism Diagnostic Observation Schedule）で自閉症スペクトラム（autism spectrum disorder：ASD）と診断し，薬物療法を受けている者，慢性の炎症性疾患を有する者，神経疾患（てんかんを含む）を有する者，IQ が 80 未満の者は除外し，研究の目的と内容について十分説明し，本人および保護者の同意が得られた者のみを対象とし，定型発達の男性を対照とした．

結果は ASD の脳内では，活性型ミクログリアが広汎な部位で増加していた（**図 6**）．活性型ミクログリアの

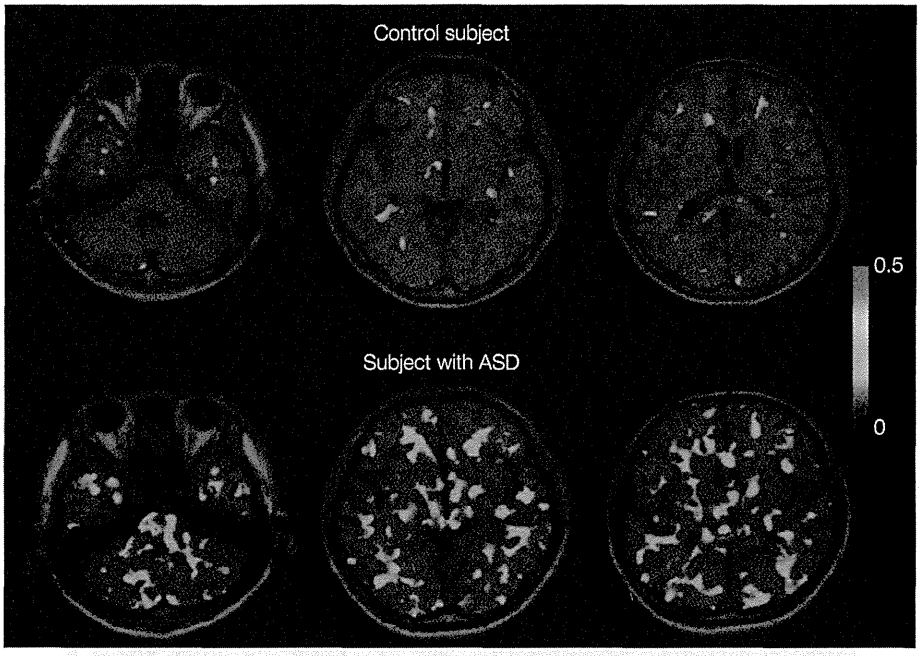

図 6. 活性型ミクログリアの広汎な部位での増加
(Suzuki K *et al*, 2013[4] より引用)

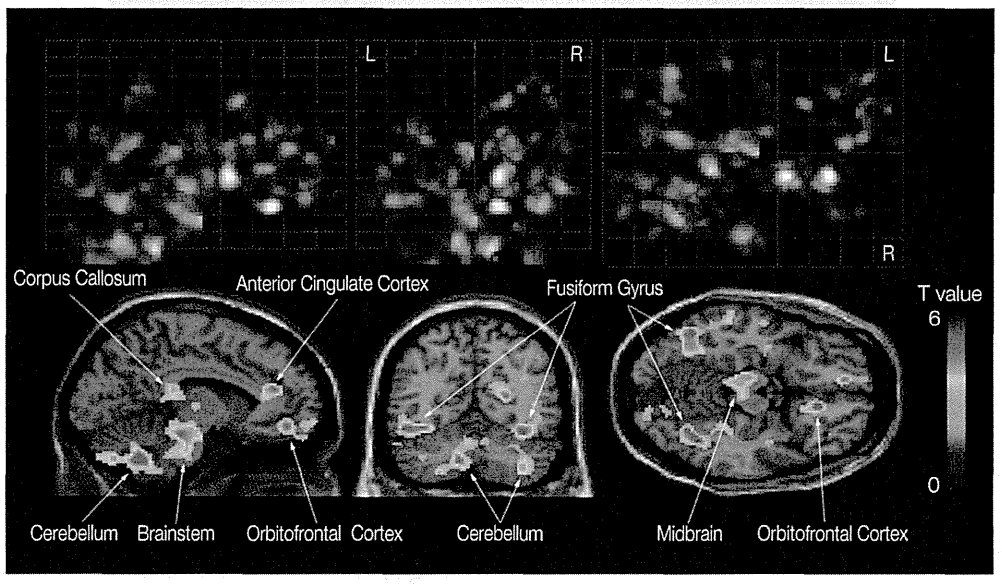

図 7. 活性型ミクログリアの帯状回，眼窩前頭回，紡錘状回などでの増加
(Suzuki K *et al*, 2013[4] より引用)

増加は，小脳と脳幹（中脳，橋）で最も顕著であった．これに加え，ASD の病態との関係が指摘されている脳前部帯状回，眼窩前頭回，紡錘状回にも顕著な増加が認められた（**図 7**）．これらの脳部位における活性型ミクログリアは，ASD 群でも対照群でも，互いに有意に正相関していた（**図 8**）．さらに疾患内比較で，ASD 群の中で活性型ミクログリアが多い群（High-BP）と低い群（Not-High-BP）の 2 群に分けお互いの臨床症状を比較した．多い群については ADI-R による社会性の障害（ADI-R Social Score），これは子どものころの社会性の

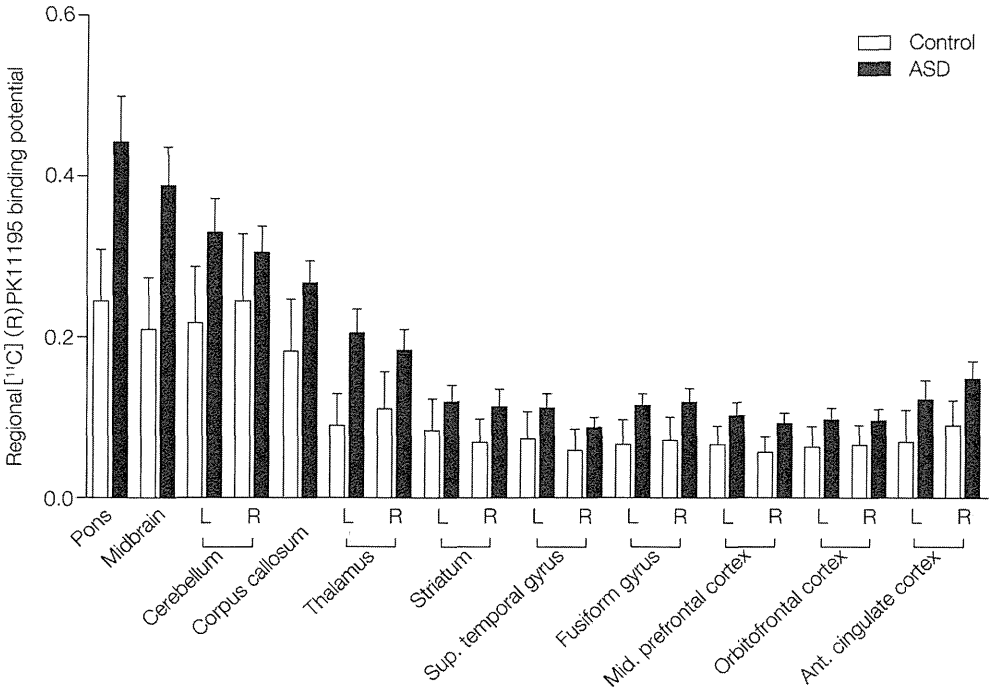

図 8. 活性型ミクログリアのすべての脳部位での増加
(Suzuki K *et al*, 2013[4]より引用)

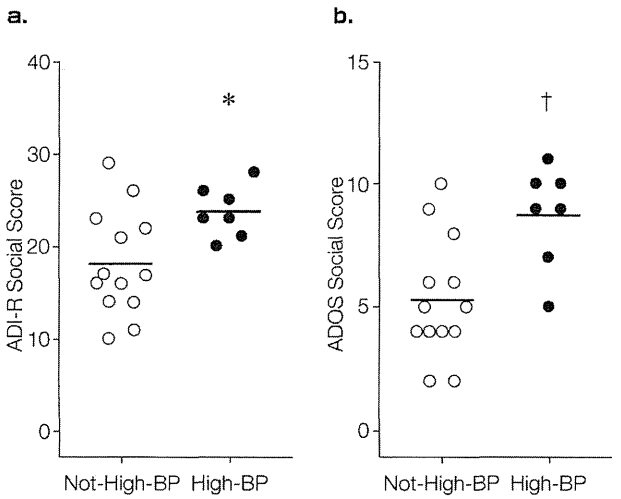

図 9. 活性型ミクログリアの増加と社会性の障害
(Suzuki K *et al*, 2013[4]より引用)

障害の程度を示すがこの指標が高く，ADOS による社会性の障害（ADOS Social Score）つまり現時点における社会性の障害の程度を示すがこの指標が高いことが明らかになった（**図 9**）．活性型ミクログリアが多いほど，自閉症の障害の程度が子どものころも現時点においても強いことが明らかになった．以上の結果から，成人 ASD の脳内では過剰なミクログリア活性化がおきていることが示唆された．つまり，ミクログリアの活性化は，ASD 脳内で小児期から成人期まで継続している現象であると考えられる．ASD 群では，対照群と同様に，すべての脳部位のミクログリア活性が互いに正相関していたという所見から（**図 6**），脳内のすべてのミクログリアが一様に活性化していることが示唆された．すなわち，局所の炎症や神経傷害を反映したミクログリアの活性化ではなく，ASD 脳内のほぼすべてのミクログリアが過剰な反応性を有することを示している．脳のミクログリアの由来は胎生期に血液脳関門が形成される以前に，末梢のマクロファージが脳内に沈着したことによる．ASD では，ミクログリア活性化により出生前に正常なシナプス形成が阻害される可能性（toxic），またはシナプス形成が不整になり，その結果としてミクログリアが活性化する（protective）考え方があるが，われわれは，過剰なミクログリア活性化によって出生前における正常なシナプス形成が阻害されると考えた．ゆえにわれわれが報告したセロトニン系の異常はセカンダリーと

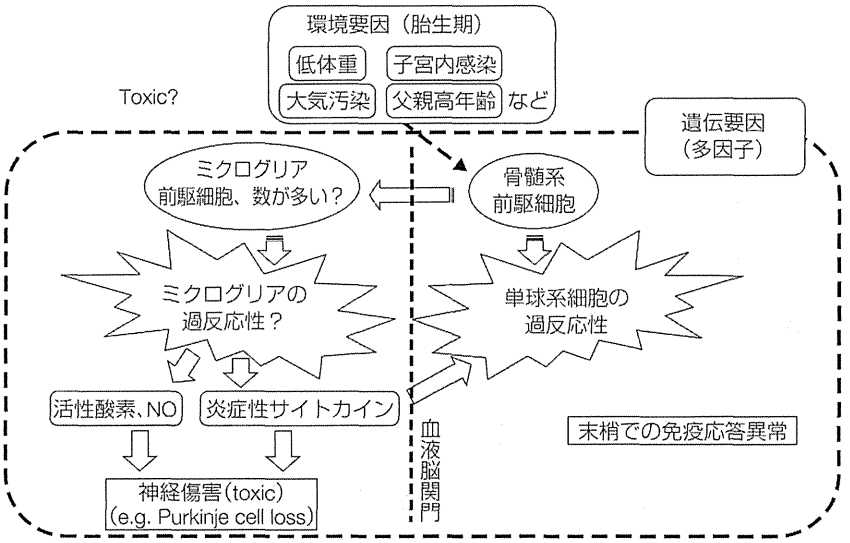

図 10. 自閉症の病態仮説

考えた．**図 10** で自閉症の病態仮説を示した．自閉症は各種の遺伝的要因とさまざまな環境的要因によって胎生期に骨髄系などに影響し末梢のマクロファージが脳内に移行する量が増え，胎生期に増えている活性型ミクログリアによって脳内のシナプス形成障害をおこし ASD の病因となると考えた．

おわりに

われわれは自閉症の PET 研究を各種行い自閉症の脳内での障害を，PET 脳画像研究によって明らかにすることができた．自閉症は近年ではその近縁状態も含めれば 100 人に 1 人とも言われる発現頻度の高い障害であるが，身体障害のような「見える障害」ではないために，社会的な理解が遅れ，今まで，親の育て方が悪い，あるいは本人のわがままだなどといった偏見に満ちた間違った理解をされていたが，今回の研究においても，自閉症が間違いなく脳機能の障害によって生じていることが明らかになり，自閉症の社会的理解を推し進めることにつながると考えられる．なお，この研究は，NPO 法人アスペ・エルデの会の成人当事者が積極的に研究への協力を行い，継続的な自助活動やそれらを背景とする長期に渡る発達支援によって可能になった．

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