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

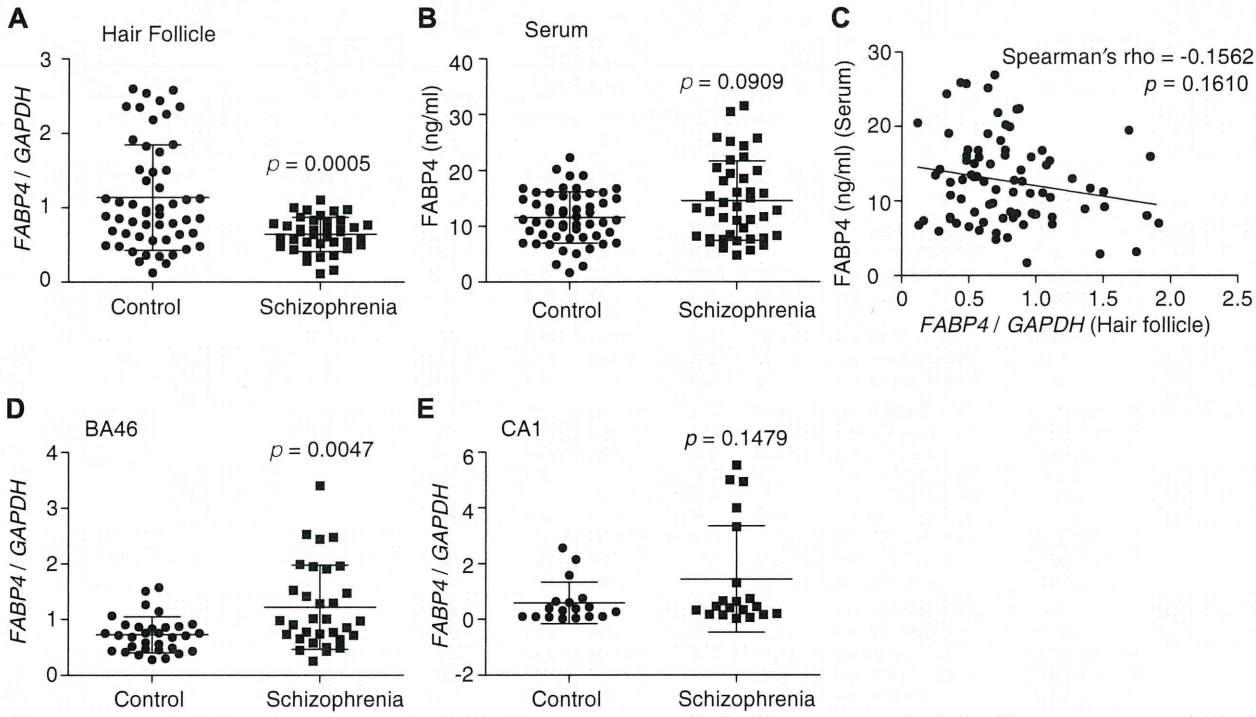

Figure 1. *FABP4*/fatty acid binding protein 4 (FABP4) expression analyses in schizophrenia and control samples. **(A, B, D, E)** Results for hair follicles (the second sample set), serum, and postmortem brain tissue (Brodmann area [BA]46 and cornu ammonis [CA]1) are shown. *GAPDH* was used as an internal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

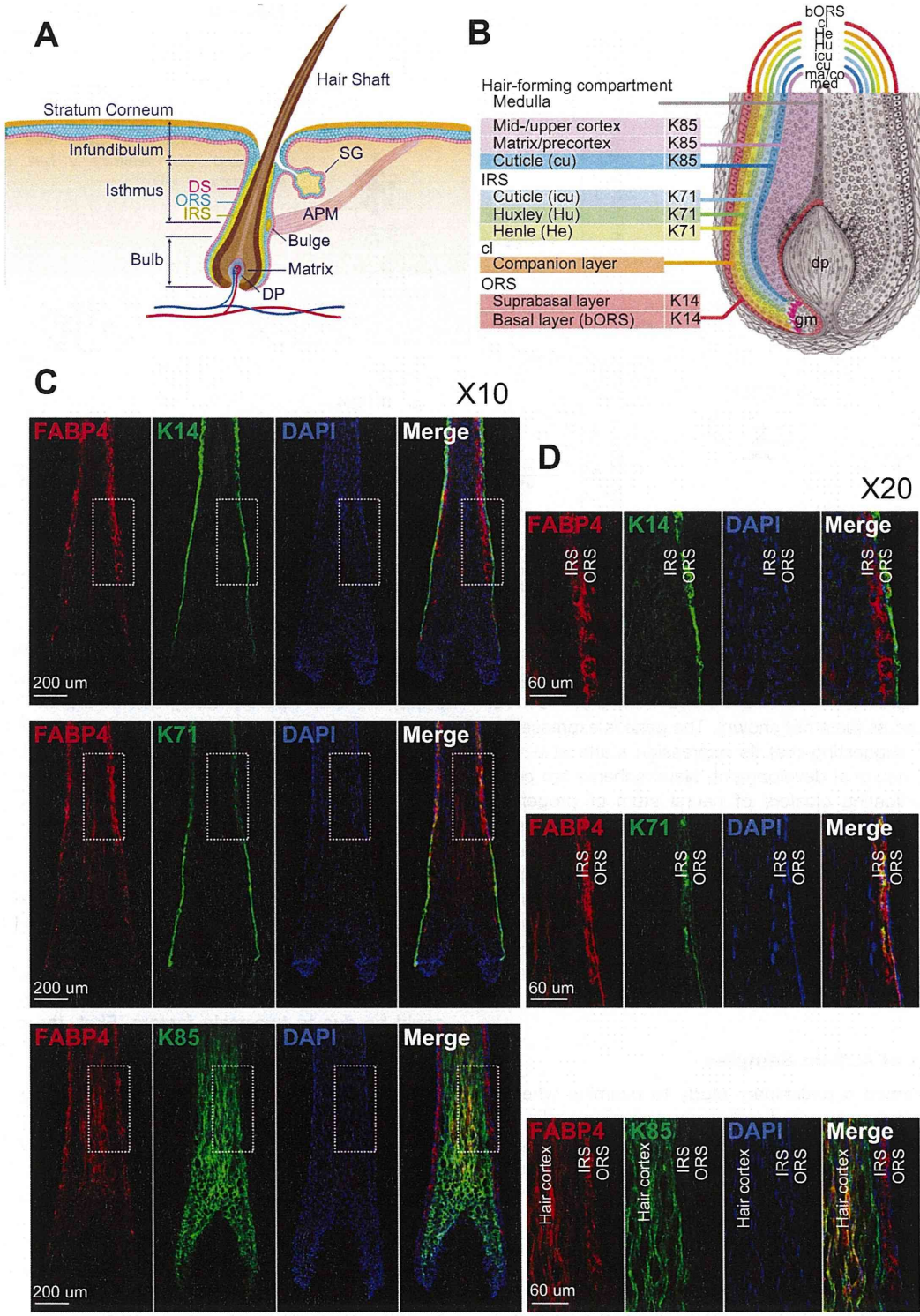

Figure 2. Expression patterns of fatty acid binding protein 4 (FABP4) in scalp hair follicles. **(A)** Schematic illustration showing the structure of hair follicles. **(B)** Schematic presentation of epithelial/hair keratin expression patterns. Keratin K71 is expressed in the three inner root sheath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

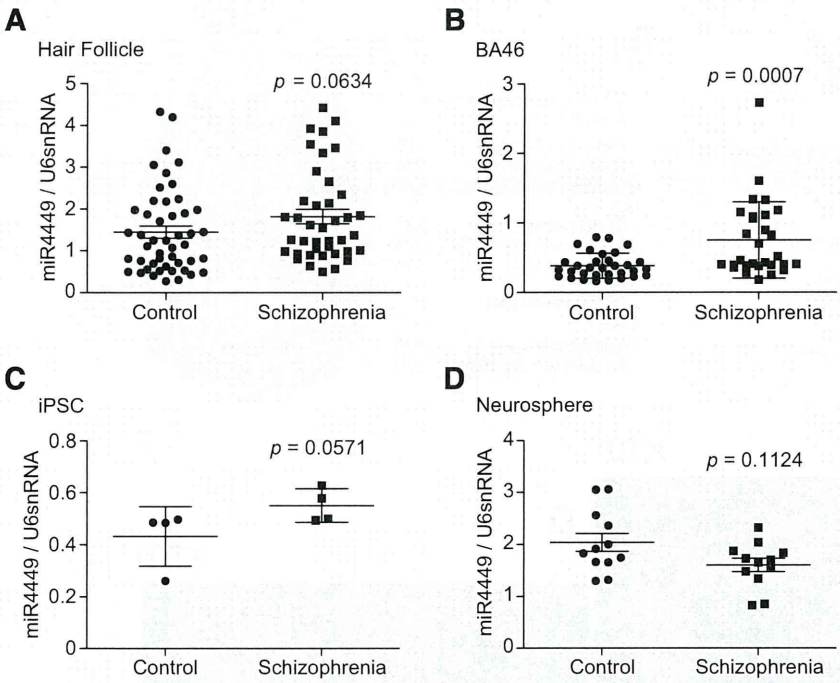

Figure 3. Expression analyses of *hsa-miR-4449* in schizophrenia and control samples. Results from hair follicles (the second sample set) **(A)**, postmortem brains (Brodmann area [BA]46) **(B)**, induced pluripotent stem cells (iPSCs) **(C)**, and neurospheres **(D)** are shown. U6 small nuclear RNA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

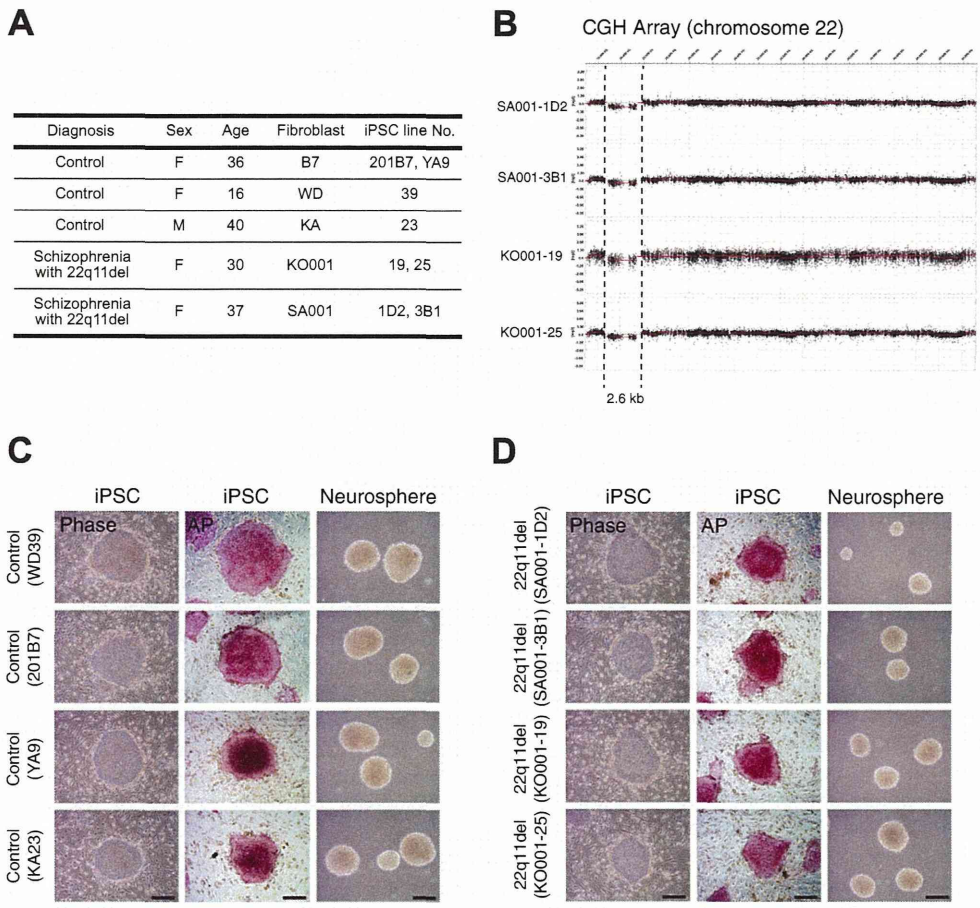

Figure 4. Establishment of iPSCs and iPSC-derived neurospheres from controls and schizophrenia patients with a 22q11.2 deletion (also see ref. 31). **(A)** Demographic data and I.D. information for samples are shown. **(B)** CGH array analysis of chromosome 22 using iPSCs showed that all the iPSC lines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

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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.

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iPS細胞創薬への期待と課題

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はじめに

患者から採取した体細胞から作製する多能性幹細胞（pluripotent stem cells），すなわち疾患特異的iPS細胞（induced pluripotent stem cells）は，その疾患で障害を受けるとされる細胞（疾患感受性細胞）へと分化誘導することで，患者由来細胞を用いた新たな疾患モデルとなり得るため，新たな疾患解析のパラダイムを生み出す技術として期待されてきた。本稿では，疾患特異的iPS細胞を創薬へと発展させるための課題と今後の期待について考察したい。

従来の疾患解析の問題点

従来の疾患解析では，患者剖検サンプルを用いた解析や，原因遺伝子の導入やノックアウト/ノックダウンにより作製された培養細胞や疾患モデル動物を用いた解析が広く行われてきた。しかし，剖検サンプルは限られているうえに，病態が進行した結果についての情報しか得られず，疾患の発症や進行に関して解析するのは難しい。また，原因遺伝子が明らかになっている遺伝性疾患はごく一部であり，多くの疾患が孤発性であることを考慮すると，先に述べた方法で作成できる疾患モデル細胞・動物は限られている。さらに，原因遺伝子が明らかな疾患であっても，モデル動物と患者との間での原因遺伝子の発現量の違いや，ヒトとマウスなどのモデル動物における生物学的，遺伝学的な違いのために，患者の病態を正確に再現できないことも多い。特に，種間での保存性が低い非翻訳領域の変異が病態と関連している場合や，薬剤に対する感受性（効果や毒性）が種間で異なる場合には，種の相違が大きな問題となる[1]。そのため，近年では，少しでもヒトに近いモデルとして，霊長類モデルの開発が進められているが[2]，いまだ霊長類の疾患モデルの作製と使用には，技術的，時間的，経済的な問題点が残されている。また霊長類モデルといえど，ヒトとは異なるために，厳密な意味でヒト疾患を反映するモデルを作製するのは困難である。

ヒトiPS細胞を用いた疾患解析の利点

2007年に山中伸弥先生（京都大学iPS細胞研究所所長）らにより開発されたヒトiPS細胞の技術を用いることで，患者の体細胞から多能性幹細胞を作製できるようになった[3]。この技術を用いて作製された疾患特異的iPS細胞は，患者自身の疾患感受性細胞へと分化誘導することができるため，病態を忠実に再現する疾患モデルとなり得る。したがって，遺伝的な多様性が表現型とどのように結びついているのか，すなわちゲノム情報と疾患表現型との関連を明らかにできるうえに，遺伝的背景が明らかでない場合でも疾患モデルを作製することができる。また，健常者由来細胞との比較により，疾患のある時点を輪切りで解析する横断的解析のみならず，その分化誘導過程を解析することで疾患の発症や進行過程を縦断的に解析することが可能である。特に*in vivo*では表現型の表出に時間がかかる場合でも，iPS細胞を用いた*in vitro*モデルでは，神経変性などの表現型を比較的短期間で得られる場合もある。さらに表現型の解析のみならず，生化学的解析，分子生物学的解析が行いやすく，また近年次々に開発されているゲノム編集技術（ZFN nuclease, Talen, Crisper Cas 9など）[1]を用いることで，比較的容

易に遺伝子改変を行うことができるため，疾患の背後にある分子遺伝学的な要因の探索，遺伝子治療に向けた詳細な解析や分子病態を標的とした治療法の開発においてきわめて有用なツールとなり得る。

iPS細胞の応用における問題点とその解決に向けた取り組み

培養細胞株であるiPS細胞は，一見，動物モデルに比べて実験が容易で短時間，低コストで解析できるようにみえる。しかしながら，実際に実験を進めてみると，多くの問題点に直面する。

まず，ヒトの細胞であるだけに培養や分化誘導に比較的時間がかかるうえに（ヒトの発生には受精から出生まで10ヵ月かかることを考慮する必要がある），培養細胞であるがゆえに日々の培養には手間がかかり，また未分化iPS細胞をよい状態で維持するためには，十分な技術を必要とする。高価な培地や組換え蛋白，サイトカインなどを用いる場合も多く，コストの問題も無視できない。このような問題を解決するために，分化誘導期間を短くするための工夫や，組換え蛋白ではなく低分子化合物を用いるプロトコルが開発されてきている。例えば，神経分化誘導では，Dual Smad inhibition（BMP阻害剤であるNoggin，dorsomorphinやLDN-193189などと，TGF-β阻害剤であるSB431542を併用する方法）が報告されており[4]，また筆者らのグループでも，この方法にGSK3β-inhibitor（BIO，CHIR99021）をあわせて用いることで，短期間で高効率に神経系前駆細胞を誘導する方法を報告している[5]。

また，ヒト多能性幹細胞から特定の細胞への分化誘導効率は一般的にはそれほど高くなく，誘導された分化細胞は不均一な集団であることが多い。同じ種類の細胞へと分化誘導していても，誘導されたすべての細胞の分化の度合いを同期させることはほとんど不可能であり，細胞の成熟度と発症が関与する多くの疾患において解析上の問題点となっている。さらに，クローン間，培養バッチ間での分化傾向の違いやばらつきも大きいため[6][7]，コンスタントに同じ品質の分化細胞を作製するのがなかなか難しい。ヒト多能性幹細胞が，マウス多能性幹細胞（ES/iPS細胞）のような分化全能性をもつnaïve typeの多能性幹細胞ではなく，もう少し分化が進んだepiblast stem cellに近い細胞（primed type）であることも，このような問題の一因となっていると考えられる[8][9]。このような問題点を解決するために，より分化誘導効率を改善するための工夫や，表面抗原，細胞種特異的レポーター遺伝子を用いた細胞の純化，iPS細胞の樹立方法や品質の均一化，naïve typeのiPS細胞の作製方法の開発などが重要となってくる。

また，多能性幹細胞の分化誘導は，個体発生を*in vitro*で再現するのが基本である。そのため，胚発生の早い時期の細胞に相当するiPS細胞から分化誘導した細胞は，まだ十分に成熟した細胞とはいえない。すなわち，発達期の疾患のモデルとしては大いに期待できるが，成人発症の疾患，特に遅発性疾患では，発症に足る十分な老化を経ていないため，基本的な培養条件では十分に病態を再現できない，すなわち「発症しない」可能性がある。そのため，老化遺伝子である*Progerin*の導入などが試みられている[10]。また筆者らは逆に，どのようなストレスが発症に必要なのかを解析することで，どのような老化関連シグナルが疾患の発症とかかわっているのかを明らかにすることができると考えている。

さらに，すべてのヒトは何らかの遺伝的多様性をもっているため，異なる患者・健常者から作製されたヒトiPS細胞は，当然のことながら個人差を有していることになる。したがって，得られた解析結果が個人差の範囲を出ないものなのか，疾患に特徴的な所見といえるのかの判断を下すには，通常の臨床研究と同様に多数のサンプルを解析する必要がある。このような問題を解消するためには，先に述べたゲノム編集技術を用いて，正常細胞へ疾患関連遺伝子を導入する，あるいは患者細胞における遺伝子変異を修復する，などの方法により，同じ遺伝子情報をもつ疾患・対照iPS細胞（isogenic iPS cells）を作製して比較することが求められている[1]。この方法により，クローン間のばらつきや解析のノイズの軽減が期待できる。いうまでもなく，多数のサンプルの収集，