Polyunsaturated fatty acid deficiency during neurodevelopment in mice models the prodromal state of schizophrenia through epigenetic changes in nuclear receptor genes

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The risk of schizophrenia is increased in offspring whose mothers experience malnutrition during pregnancy. Polyunsaturated fatty acids (PUFAs) are dietary components that are crucial for the structural and functional integrity of neural cells, and PUFA deficiency has been shown to be a risk factor for schizophrenia. Here, we show that gestational and early postnatal dietary deprivation of two PUFAs—arachidonic acid (AA) and docosahexaenoic acid (DHA)—elicited schizophrenia-like phenotypes in mouse offspring at adulthood. In the PUFA-deprived mouse group, we observed lower motivation and higher sensitivity to a hallucinogenic drug resembling the prodromal symptoms in schizophrenia. Furthermore, a working-memory task-evoked hyper-neuronal activity in the medial prefrontal cortex was also observed, along with the downregulation of genes in the prefrontal cortex involved in oligodendrocyte integrity and the gamma-aminobutyric acid (GABA)-ergic system. Regulation of these genes was mediated by the nuclear receptor genes Rxr and Ppar, whose promoters were hyper-methylated by the deprivation of dietary AA and DHA. In addition, the RXR agonist bexarotene upregulated oligodendrocyte- and GABA-related gene expression and suppressed the sensitivity of mice to the hallucinogenic drug. Notably, the expression of these nuclear receptor genes were also downregulated in hair-follicle cells from schizophrenia patients. These results suggest that PUFA deficiency during the early neurodevelopmental period in mice could model the prodromal state of schizophrenia through changes in the epigenetic regulation of nuclear receptor genes.

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INTRODUCTION

Among the well-established risk factors for schizophrenia that affect early neurodevelopment, nutritional deficiencies are known to have significant effects.1 For example, epidemiological evidence indicates that exposure to famine in early gestation approximately doubles the risk of schizophrenia in offspring, as observed after the Dutch Hunger Winter (1944–1945)2 and the Great Chinese Famine (1959–1961).3,4 Thus, the adverse conditions during gestation may have lasting effects on subsequent health or result in adult-onset diseases due to fetal programming, which is commonly referred to as the Developmental Origin of Health and Disease (DOHaD).5,6 The DOHaD theory has been found to apply to many syndromes and disorders, including schizophrenia.7 In addition, long-lasting epigenetic changes are evident in the offspring of women who experience famine during the first trimester of pregnancy, but are absent in those who either do not experience famine or who experience it at a later gestational period.8 Therefore, the diet of pregnant mothers, especially during early developmental stages, influences epigenetic changes in the fetus, such as DNA methylation, thereby affecting the expression of developmentally regulated genes.8 Deficiencies of several nutrients including folic acids, essential fatty acids, retinoids, vitamin D and iron have been attributed for the schizophrenia risk resulting from gestational famine.1 Among the nutrients, the major polyunsaturated fatty acids (PUFAs) arachidonic acid (AA) (20:4 n – 6) and docosahexaenoic acid (DHA) (22:6 n – 3) have a critical role in brain development.9 DHA and AA are key components of membrane phospholipids, contributing to the structural integrity of neurons, glial cells and endothelial cells in the brain,10 and they also affect neurotransmission, cell survival and neuroinflammation.10 Evidence has begun to suggest links between prenatal DHA deficiency and abnormal neurotransmission and neurocognitive impairments.11,12 Considering the essential role of PUFA, we hypothesized that the compromised availability of dietary PUFA may affect early neurodevelopment and thereby predispose to the development

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of schizophrenia. Therefore, in this study, we sought to determine whether deprivation of the essential fatty acids AA and DHA during early development (gestational and early postnatal) in mice elicits schizophrenia-like phenotypes in the adult offspring. After confirming this phenomenon, we strove to identify its underlying signaling cascades. Our results identified the RXR and PPAR nuclear receptor signaling system as an upstream mechanism that leads PUFA deficiency to result in schizophrenia-like phenotypes. To the best of our knowledge, this is the first report to show that epigenetic modification of nuclear receptor genes has a key role in the prodromal state of schizophrenia, as mediated by PUFA deficiency during early neurodevelopmental stages.

MATERIALS AND METHODS

Animals and diets

Inbred C57BL/6N(Crl) (B6) mice were obtained from Charles River Laboratories (Tokyo, Japan). Housing conditions have been described elsewhere. Experimental procedures were approved by the RIKEN Animal Ethics Committee. We raised parental mice on four different diets by adding AA and/or DHA (or neither) in AIN-76 food that did not contain either AA or DHA: (1) AA /DHA / (2) AA /DHA , (3) AA /DHA and (4) AA /DHA (Supplementary Table 1). The diets were given from 2 weeks before mating until 3 weeks after pups were born (weaning point). After weaning, mice were raised on a conventional diet (CRF-1) (Charles River formula; purchased from Oriental Yeast, Tokyo, Japan). All food was stored at 4 °C and shielded from light until use to prevent oxidation and denaturation. Food was not treated with gamma rays or autoclaved. We examined male mice except in the milk study.

Fatty acid analysis

The fatty acid composition of mother’s milk (at postnatal day 8) and cortical tissues (at 3 week old and 6 month old) derived from offspring of the four diet groups were examined. Total lipids were extracted according to the method of Bligh and Dyer. Total phospholipid fractions were separated by thin-layer chromatography. The content of each fatty acid was expressed as the percentage area of total fatty acids. The protocols for the four diet groups were examined. Total lipids were extracted according to the method of Bligh and Dyer.14 Total phospholipid fractions were separated by thin-layer chromatography. The content of each fatty acid was expressed as the percentage area of total fatty acids. The protocols for fatty acid analysis are described elsewhere15,16 and in the Supplementary Methods.

Behavioral analysis

Behavior was assessed using the following tests: open-field test, tail-suspension test, Y-maze test, prepulse inhibition (PPI) test, forced swim test, light and dark box test, elevated plus-maze test, home cage activity test, and MK-801 sensitivity test. Differences in continuous variables were evaluated by unpaired t-test (after confirmation of normal distribution), or one way ANOVA or repeated measures ANOVA followed by Dunnett’s test or Mann–Whitney U test as a post hoc test. Bivariate correlation analysis was performed using Spearman’s rank test. P-values < 0.05 were considered significant. The definition of outlier is any data point more than 1.5 interquartile ranges (IQRs) below the first quartile or above the third quartile.

DNA methylation analysis via bisulfite sequencing

We examined the methylation levels of individual CpG sites in the core promoter regions of Rora (300 bp interval from transcriptional start site) and Ppara (280 bp interval from transcriptional start site), by bisulfite sequencing analysis of cortical samples from the AA /DHA and AA /DHA groups at 6 month old. Bisulfite sequencing was performed as described elsewhere. Primer sequences are listed in the Supplementary Methods.

Statistical analysis

Data were analyzed using Prism 5 (GraphPad, La Jolla, CA, USA). Differences in continuous variables were evaluated by unpaired t-test (after confirmation of normal distribution), or one way ANOVA or repeated measures ANOVA followed by Dunnett’s test or Mann–Whitney U test as a post hoc test. Bivariate correlation analysis was performed using Spearman’s rank test. P-values < 0.05 were considered significant. The definition of outlier is any data point more than 1.5 interquartile ranges (IQRs) below the first quartile or above the third quartile.

RESULTS

Behavioral phenotypes of mice deprived of dietary PUFA during gestational and early postnatal period

First, we examined fatty acid composition in the mother’s milk and the pups’ brain (cortex). When compared with the AA /DHA group,
Figure 1. Behavioral analysis of the various arachidonic acid (AA)/docosahexaenoic acid (DHA) diet groups. (a) Experimental design for the administration of the various AA/DHA diets and behavioral tests. The results of the tail-suspension test b, Y-maze test c and open-field test d are shown. (e) (left) Locomotor activity measured before, during and after a single injection of saline or MK-801 (0.15 mg kg\(^{-1}\)). (right) cumulative locomotor activity (for 3 h) after MK-801 injection. In all panels, values are means ± s.e. (b, c) P-values were calculated using two-way repeated measures ANOVA followed by Dunnett’s post hoc test for multiple comparisons [compared with AA\(^{(+)}\)/DHA\(^{(+)}\)]. (d) P-values were calculated using Dunnett’s test for multiple comparisons [compared with AA\(^{(+)}\)/DHA\(^{(+)}\)]. (e) P-values were calculated using two-way repeated measures ANOVA followed by two-tailed post hoc Mann–Whitney U test [AA\(^{(+)}\)/DHA\(^{(+)}\) and MK-801 vs AA\(^{-}\)/DHA\(^{-}\) and MK-801]. \(^{*}\)P < 0.1, \(^{*}^{*}\)P < 0.05, \(^{**}\)P < 0.01. Error bars represent standard error of the mean.
group, AA in the mother’s milk was reduced in the AA(−)/DHA(−) and AA(−)/DHA(+) groups, and DHA was reduced in the AA(−)/DHA(−) and AA(−)/DHA(+) groups (Supplementary Tables 4). These changes were also observed at postnatal day 15 (data not shown).

In the 3-week-old cortex, ‘AA content-related indices’ [n = 6 PUFA/ n = 3 PUFA ratio and AA/(n − 6 PUFA + n = 3 PUFA)] were changed according to prediction after the supplementation or deletion of AA in the diet (Supplementary Table 5 and also see Supplementary Table 6). Similar predicted changes were seen in the ‘DHA content-related indices’ [n = 3 PUFA/n = 6 PUFA ratio and DHA/(n − 6 PUFA + n = 3 PUFA)] (Supplementary Table 5). The expected changes were also observed in the DHA/AA ratio (Supplementary Tables 5) and these changes were absent in the cortex at 6 month old (Supplementary Table 7).

To assess whether maternal AA and/or DHA restriction during neurodevelopment evokes behavioral changes indicative of schizophrenia or its prodromal phenotypes in adult offspring, we performed a battery of behavioral tests to evaluate motivation, emotional states and working-memory functions, on mice born to dams which consumed one of four different combinations of diets (upper panel) and GABAergic neuron-related genes (lower panel). Values are means ± s.e. Gapdh was used as an internal control. P-values were calculated using unpaired t-tests. *P < 0.1, **P < 0.05, ***P < 0.01, ****P < 0.001. (d) Quantitative RT-PCR analysis of nucleus accumbens shell-related genes. Values are means ± s.e. Gapdh was used as an internal control. P-values were calculated using unpaired t-tests. *P < 0.1, **P < 0.05, ***P < 0.01. (e) Correlation analyses between relative Rxra or Ppara levels and Olig2 or Gad1 levels. Data were evaluated using Spearman’s rank-correlation tests. (f) Immunohistological analyses. Rxra and Ppara were expressed in the Olig2-positive and Gad67-positive cells.

The AA(−)/DHA(−) group exhibited hyper-neuronal activity upon performing a working-memory task. We next asked whether the abnormal behavioral phenotypes exhibited by the AA(−)/DHA(−) group were associated with altered neuronal activity in brain regions that have been linked to schizophrenia, for example, PFC. We conducted a Mn-enhanced MRI on mice, after performing Y-maze test, which evaluates working-memory capability that is impaired in schizophrenia. The AA(−)/DHA(−) and AA(−)/DHA(+) groups performed slightly worse (in speed) than the AA(−)/DHA(+), group on this test (Figure 1c). In Mn-enhanced MRI, the AA(−)/DHA(−) group was a contrast agent that enters activated neurons through calcium channels and remains there.28 Thus, MRI signals that represent recent neuronal activity can be used to identify brain regions associated with specific tasks.18,29,30 To precisely compare the neuronal activity among groups, we prepared flat maps of each region’s activity by averaging individual regional MRI signals as in the previous study.18 The AA(−)/DHA(−) group exhibited higher Mn-enhanced MRI signals in the medial prefrontal cortex (mPFC) and the nucleus accumbens shell than the AA(−)/DHA(+) group (Figure 2a and Supplementary Figure 1a). The mPFC neurons have been reported to be involved in encoding working memory through increasing firing frequency or synchronization, and the glutamatergic neurons in the mPFC send projections to the nucleus accumbens shell13,32 (Supplementary Figure 1b, relevant neuronal circuits are explained in the legends). The current MRI results suggest the existence of a compensatory mechanism for decreased executive function in the mPFC caused by the AA(−)/DHA(−) diet, which is in agreement with reports of clinical cases of schizophrenia.33–35 We also performed volumetric analyses using the MRI data but detected no changes in the volume of the whole brain, the lateral ventricles, and the hippocampus among the four different diet groups (Supplementary Table 9).

Decreased expression levels of oligodendrocyte- and GABA-related genes were observed in the prefrontal cortex of the AA(−)/DHA(−) group. To characterize the transcriptomic changes that accompany the gestational and early postnatal deprivation of dietary PUFA in mice, we examined gene-expression levels in the PFC of the AA(−)/DHA(+) and AA(−)/DHA(−) groups at 6 months of age, as dysfunc-

tion of the PFC is implicated in schizophrenia. Microarray analysis revealed 174 significantly downregulated genes and 540 upregulated genes (P < 0.01, fold change > 1.3) in the PFC of the AA(−)/DHA(−) group compared with the AA(−)/DHA(+) group (Supplementary Table 10 and Supplementary Figure 2). Using IPA, we queried the differentially regulated genes for the top ‘Diseases and Bio Functions’ annotations, which revealed that genes in the ‘Neurological Disease’, ‘Behavior’ and ‘Nervous System Development and Function’ categories were enriched (Supplementary Table 11). Detailed annotations of these categories showed ‘myelination (remyelination and white matter damage)’, ‘various behaviors related to schizophrenia’ and ‘interneurons’ (Figure 2b and Supplementary Table 12).
Expression levels of genes related to the oligodendrocyte system and the GABA-containing interneuron system have been shown to be decreased compared to normal in the postmortem brains of people with schizophrenia. Quantitative RT-PCR analysis in the PFC validated that the oligodendrocyte-related genes Cldn11, Cspg4, Mbp, Mobp, Mag and the GABA-related gene Sst were expressed at significantly lower levels in the AA⁻/DHA⁻ group than in the AA⁺/DHA⁺ group (Figure 2c). Further examination revealed changes in the expression levels of other genes reported to be expressed at non-normal levels in postmortem schizophrenic brains, which include the neurotransmitter receptor genes Drd1a, Drd2, Htr1a, Htr2a and Cnr1 (Supplementary Table 13).
In addition to downregulation of genes, the mRNA expression of the GABA-receptor subunit GABRA2 has been reported to be 14% higher in layer 2 of the dorsolateral prefrontal cortex of individuals with schizophrenia, which might reflect a compensatory counterpoint in response to a dampened GABAergic system. In our mice, Gabra2 expression was enhanced in the AA/DHA group (Figure 2c), indicating another similarity between the AA/DHA group and people with schizophrenia. Although the GABA content in the cortex did not differ between the two groups (Supplementary Table 14), the results were in agreement with clinical data on GABA concentration in schizophrenia by using magnetic resonance spectroscopy.

Nuclear receptors regulate the expression of oligodendrocyte- and GABA-related genes Next, we utilized the IPA to predict upstream transcriptional regulators of the genes we identified as differentially expressed (Supplementary Table 17), including the enrichment of genes involved in the nuclear receptor-transcription factor family (Figure 3), including CCK, RXRα, and PPARα that have been shown to regulate the expression of oligodendrocyte- and GABAergic interneuron-related genes. Interestingly and importantly, such motifs are also enriched in the nuclear receptor-transcription factor family (Figure 3) in the vehicle- and bexarotene-pretreated mouse groups. For both groups, the last treatment was administered 180 min before MK-801 injection. Values are means ± s.e.

We asked whether the downregulation of these nuclear receptors may directly regulate the expression of these oligodendrocyte- or GABAergic interneuron-related genes (Figure 2c). Interestingly and importantly, such motifs are also enriched in the nuclear receptor-transcription factor family (Figure 3) in the vehicle- and bexarotene-pretreated mouse groups. For both groups, the last treatment was administered 180 min before MK-801 injection. Values are means ± s.e.

As the expression level of nuclear receptors, RXRα and PPARα were highly correlated with the Olig2 and Gad1 expression; we intended to evaluate the effect of nuclear receptor-mediated regulation of downstream genes pharmacologically. We analyzed the effects of the RXR pan-agonist bexarotene and the PPAR pan-agonist bezafibrate on the expression levels of the target genes, by using the oligodendrocyte cell line OLP6—derived from the ventrolateral region of the supracallosal nucleus (rat neuronal cell line)—and GABA-containing KATO-III cells derived from human stomach cancer cells (signet ring cell carcinoma). Bexarotene treatment increased the expression levels of the oligodendrocyte-related genes Olig2, Cldn11, Mal, and Mobp-long in OLP6 cells (Figure 3a). Similarly, bezafibrate increased the expression of Olig2, Mal, and Mobp-long mRNA (Figure 3a). Bexarotene increased CCK and CALB2 mRNA levels at 30 μM, whereas bezafibrate decreased CALB2 mRNA expression at both concentrations (Figure 3b). Treatment did not change expression levels of other oligodendrocyte-related genes or GABA-related genes in either cell line (data not shown). These results suggest that RXR and PPAR regulate the expression of oligodendrocyte- and GABA-related genes in vitro.

Because more cointeracting in vitro effects were seen in the oligodendrocyte and GABAergic systems after treatment with bexarotene than with bezafibrate, we next examined the effect of bexarotene in vivo. We administrated vehicle or bexarotene (30 or 100 mg kg⁻¹) to the mice for 3 weeks. Then we examined the expression of oligodendrocyte- and GABA-related genes in the PFC of the mice 24 h after the last injection. The 30 mg kg⁻¹ bexarotene treatment elicited a trend toward increased expression of Olig2 and Gad1 (Figure 3c and Supplementary Table 21). Behavioral results thus supported the roles of nuclear receptor systems in schizophrenia-related pathophysiology.
at Rxra and Ppara gene promoters. To this end, we examined the methylation levels of individual CpG sites in the core promoter regions of Rxra and Ppara, by bisulfite sequencing analysis of cortical samples from the AA(−)/DHA(−) and AA(−)/DHA(−) groups (Supplementary Figure 4).

In the case of the Rxra promoter, the CpG-3 site showed significantly higher methylation levels in the AA(−)/DHA(−) group than in the AA(−)/DHA(−) group, and the CpG-10 site showed a similar trend (Figure 4a). The mean methylation levels of Rxra over the entire interval exhibited a higher trend in the AA(−)/DHA(−) group (3.34%) than in the AA(−)/DHA(−) group (1.35%) (Figure 4b).

For the Ppara promoter, the CpG-20 site displayed significantly higher methylation levels in the AA(−)/DHA(−) group than in the AA(−)/DHA(−) group, and the CpG-4, -9 and -13 sites exhibited similar trends (Figure 4c). The mean methylation levels of the whole Ppara promoter region did not differ between the two groups (Figure 4d). Most of the differentially methylated sites in both genes were within putative transcriptional factor-binding
motifs (Supplementary Figure 5), suggesting that alterations in the level of methylation of specific CpGs in the Rxra or Ppara promoters may induce negative relationships with their mRNA expression through the change of the response to the binding motifs.

As DNA methyltransferases mediate promoter DNA methylation,\textsuperscript{51,52} we also compared expression levels of Dnmt1 [DNA (cytosine-5)-methyltransferase 1], Dnmt3a-1, Dnmt3a-2 and Dnmt3b between the AA\textsuperscript{+/-}/DHA\textsuperscript{+/-} and AA\textsuperscript{-/-}/DHA\textsuperscript{-/-} groups in 3-week-old mice to complement the differential methylation levels observed in the Rxra and Ppara promoters. Dnmt3b was expressed significantly more in the AA\textsuperscript{-/-}/DHA\textsuperscript{-/-} group than in the AA\textsuperscript{+/-}/DHA\textsuperscript{+/-} group, Dnmt3a-2 expression followed a similar trend (Figure 4e). DNA methylation may therefore be one of the mechanisms that links dietary nutrition (in this case, the presence or absence of PUFAs in the diet) to long-lasting changes in gene-expression levels.

Low expression of nuclear receptor genes in hair-follicle cells from individuals with schizophrenia

To test whether expression levels of nuclear receptor genes are associated with the pathophysiology of schizophrenia in humans, we probed the transcript-expression levels of nuclear receptors (RXRs and PPARs) in hair-follicle cells from two cohorts of Japanese people with schizophrenia and in those without schizophrenia (controls). These samples were the same that was used in our prior study.\textsuperscript{19} In the first cohort of samples (control, n = 62; schizophrenia, n = 52), RXRA, PPARA and PPARB/D were significantly downregulated (P < 0.05) in individuals with schizophrenia compared with the control subjects (Table 1). In an additional independent sample set (control, n = 55; schizophrenia, n = 42), the findings for PPARA and PPARB/D were replicated (Table 1), thus suggesting that these nuclear receptor genes are involved in the pathophysiology of schizophrenia. We also examined expression levels of myelin- and GABA-related genes in hair follicles, and correlations between the expression levels of nuclear receptor genes and GABA- or oligodendrocyte-related genes in the combined samples (n = 211) (Supplementary Table 22). These analyses revealed that the expression levels of specific nuclear receptor genes were correlated to the expression levels of target genes. For example, the expression level of RXRA was highly correlated with that of the oligodendrocyte-related gene CSPG4 (Spearman’s ρ = 0.54770, P < 0.0001). These data also suggest that those nuclear receptors control gene expression of GABA- or oligodendrocyte-related genes.

**DISCUSSION**

In the current study, we demonstrated that gestational and early postnatal dietary deprivation of PUFAs (AA and DHA) in mice elicited behavioral signs reminiscent of early psychosis (the
Table 1. List of examined genes and their expression levels in the first and second scalp hair-follicle sample sets

| Gene category | Gene symbol | Assay IDa | First sample set | Second sample set |
|---------------|-------------|-----------|------------------|-------------------|
|               |             |           | Control (n = 62) | Schizophrenia (n = 52) | Control (n = 55) | Schizophrenia (n = 42) |
|               |             | Mean ± s.d. of corresponding gene/ GAPDH | P-valueb | Mean ± s.d. of corresponding gene/ GAPDH | P-valueb |
| Nuclear receptor | RXRA | Hs01067640_m1 | 1.127 ± 0.334 | 0.962 ± 0.236 | 0.006 | 0.506 ± 0.191 | 0.545 ± 0.169 | 0.491 |
|               | RXRB | Hs00232774_m1 | 1.121 ± 0.231 | 1.094 ± 0.215 | 0.444 | 0.506 ± 0.191 | 0.545 ± 0.169 | 0.491 |
|               | RXRG | Hs00199455_m1 | 0.946 ± 0.608 | 1.116 ± 0.587 | 0.132 | 0.506 ± 0.191 | 0.545 ± 0.169 | 0.491 |
|               | PPARA | Hs00197539_m1 | 1.158 ± 0.416 | 0.863 ± 0.246 | 0.0001 | 0.838 ± 0.206 | 0.699 ± 0.190 | 0.0005 |
|               | PPARB/D | Hs04187066_g1 | 1.238 ± 0.661 | 0.832 ± 0.325 | 0.0004 | 0.714 ± 0.460 | 0.458 ± 0.263 | 0.0024 |
|               | PPARG | Hs01115513_m1 | 1.146 ± 0.881 | 0.873 ± 0.586 | 0.224 | 0.506 ± 0.191 | 0.545 ± 0.169 | 0.491 |
| Control | GAPDH | Hs02758991_g1 |           |           |           |           |           |

aProbe ID in TaqMan Gene Expression Assay system (Thermo Fisher Scientific, Waltham, MA, USA). bEvaluated by two-tailed Mann–Whitney U test. Significant P-values are shown in boldface.

This study also revealed that the epigenetic silencing of nuclear receptor genes Rxr and Ppar is a molecular event upstream from the dysregulated expression of oligodendrocyte and GABAergic system genes. Our results indicate up-regulation of de novo DNA (cytosine-5)-methyltransferases, Dnmt3a-2 and Dnmt3b, in the AA(−)/DHA(+) group relative to the AA(−)/DHA(+) group are consistent with a scenario in which increased de novo methylation of nuclear receptor genes was induced by changes in the mother’s diet.

Supplementation of PUFAs during the gestation and/or lactation period is known to influence DNA methylation. It was also reported that prostaglandin E2 (PGE2), a metabolite of AA, alters DNA methylation status. PUFAs and their metabolites might have a role in the epigenetic changes of nuclear receptor genes observed between the different diets. In parallel, the preformed AA and DHA are substrates for pro-inflammatory (for example, AA-PGE2) and anti-inflammatory (for example, DHA-D-resolvins) processes, respectively. Thus, the involvement of pro- and anti-inflammatory mechanisms in the current model mice should be examined in a future study.

Mouse genetic studies have supported the potential roles of RXR or PPAR genes in the susceptibility to schizophrenia. The current findings showing that expression of RXRA, PPARA and PPARB/D is abnormally reduced in the hair-follicle cells of patients with schizophrenia not only support their putative role in schizophrenia pathogenesis but also suggest that this measure is a potential biomarker for schizophrenia. For example, scrutinizing the expression levels of genes for nuclear receptors in hair follicles would be useful to stratify complex schizophrenia pathophysiology. In addition, the combination of drugs and companion diagnostics by using hair follicles may bring potential value for patients.

Bexarotene (Targretin, LC Laboratories) is a third-generation retinoid drug that functions via activation of RXR receptors and is approved for the treatment of cutaneous T-cell lymphoma by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In addition, bexarotene was evaluated as an anti-psychotic-augmenting agent in a phase III clinical trial (NCT00535574). It was reported that add-on oral bexarotene to antipsychotic treatment in schizophrenia patients induced significant improvements in positive and negative symptoms with moderate effect size. On the basis of our results, we speculate that bexarotene’s effects might be partly exerted by rectifying oligodendrocyte- and/or GABA- gene expression through nuclear receptor stimulation. Therefore, the current study could be a proof-of-concept (POC) for the use of bexarotene in clinical practice. Our results further suggest that the drug could be useful for subjects at risk for the schizophrenia mental state to prevent progression into evident psychosis.
the ‘AA content- and DHA content-related indices’ were changed in an expected manner in the pups’ cortex at 3 weeks old according to the diet formula. Therefore, supplementation or deletion of AA and DHA in the diet is thought to have a role in the alterations of behavioral outcomes, gene expression and DNA methylation status observed among mice fed the different diets. Given that the amount of preformed AA or DHA in the standard rodent chow (CRF-1) is small (Supplementary Table 1), the current results support the hypothesis of beneficial effects of supplementation of AA and DHA in neurodevelopmental stages. However, the limitations of this study include the importance of future experiments to examine the contents of PUFAs, in particular AA and DHA, in earlier embryonic brain developmental stages. In our study, the embryonic cortex was too small to obtain reliable data but future experiments using new methods may better examine this prediction. Other unsolved issues include: (1) the distinct and/or combinatorial roles of AA and DHA, along with their critical doses, should be further clarified, and (2) the timing of the ‘epigenetic window’, a critical period for the generation of epigenetic changes,9,88 remains an important unanswered question.

In summary, we showed that PUFAs deficiency during the early neurodevelopmental period in mice could model the prodromal state of schizophrenia most likely through epigenetic silencing of nuclear receptor genes, thereby dysregulating downstream neural gene expression. Our mouse model also provides an example strategy for elucidating how early-stage environmental insults are intertwined with the risk for schizophrenia.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES
1 Brown AS, Susser ES. Prenatal nutritional deficiency and risk of adult schizophrenia. Schizophr Bull 2008; 34: 1054–1063.
2 Maekawa M, Owada Y, Yoshikawa T. Role of polyunsaturated fatty acids and fatty acid binding protein in the pathogenesis of schizophrenia. Curr Pharm Des 2011; 17: 168–175.
3 Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D et al. Schizophrenia after prenatal famine. Further evidence. Arch Gen Psychiatry 1996; 53: 25–31.
4 St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F et al. Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. JAMA 2005; 294: 557–562.
5 Barker DJ. The origins of the developmental origins theory. J Intern Med 2007; 261: 412–417.
6 Fukudo H. DOHaD (developmental origins of health and disease) and birth cohort research. J Nutr Sci Vitaminol 2015; 61: 52–54.
7 Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci USA 2008; 105: 17046–17049.
8 Kirkbride JB, Susser E, Kundakovic M, Kresovich JK, Davey Smith G, Relton CL. Prenatal nutrition, epigenetics and schizophrenia risk: can we test causal effects? Epigenomics 2012; 4: 303–315.
9 Marszalek JR, Lodish HF. Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. Annu Rev Cell Dev Biol 2005; 21: 633–657.
10 Bazinet RP, Laye S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 2014; 15: 771–785.
11 Massahbe N, Schloegelhofer M, Schaefer MR, Fusari-Poli P, Smesny S, McGorry P et al. Polyunsaturated fatty acids in emerging psychosis. Curr Pharm Des 2012; 18: 576–591.
12 Liu JJ, Green P, John Mann J, Rapoport SI, Sublette ME. Pathways of polyunsaturated fatty acid utilization: Implications for brain function in neuropsychiatric health and disease. Brain Res 2015; 1597: 220–246.
13 Shimomoto C, Ohnishi T, Maekawa M, Watanabe A, Ohba H, Arai R et al. Functional characterization of FABP3, 5 and 7 gene variants identified in schizophrenia and autism spectrum disorder and mouse behavioral studies. Hum Mol Genet 2014; 23: 6495–6511.
14 Bhig EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37: 911–917.
15 Hamazaki K, Maekawa M, Toyota T, Dean B, Hamazaki T, Yoshikawa T. Fatty acid composition of the postmortem prefrontal cortex of patients with schizophrenia, bipolar disorder, and major depressive disorder. Psychiatry Res 2015; 227: 353–359.
16 Hamazaki K, Maekawa M, Toyota T, Dean B, Hamazaki T, Yoshikawa T. Fatty acid composition of the postmortem corpus callosum of patients with schizophrenia, bipolar disorder, or major depressive disorder. Eur Psychiatry 2016; 39: 51–56.
17 Ohnishi T, Murata T, Watanabe A, Hida A, Ohba H, Iwayama Y et al. Defective craniofacial development and brain function in a mouse model for depletion of intracellular asinosol synthase. J Biol Chem 2014; 289: 10785–10796.
18 Kimura T, Yamashita S, Murata T, Park JM, Murayama M, Mizoroki T et al. Hyperphosphorylated tau in parahippocampal cortex impairs place learning in aged mice expressing wild-type human tau. EMBO J 2007; 26: 5143–5152.
19 Maekawa M, Yamada K, Toyoshima K, Ohnishi T, Iwayama Y, Shimomoto C et al. Utility of scalp hair follicles as a novel source of biomarker genes for psychiatric illnesses. Biol Psychiatry 2015; 78: 116–125.
20 Maekawa M, Takashima N, Matsumata M, Ikegami S, Kontani M, Harra Y et al. Arachidonic acid drives postnatal neurogenesis and elicits a beneficial effect on prepulse inhibition, a biological trait of psychiatric illnesses. PLoS ONE 2009; 4: e5085.
21 Nagai T, Takata N, Shinohara Y, Hirase H. Adaptive changes of extrafollicular amino acid concentrations in mouse dorsal striatum by 4-AP-induced cortical seizures. Neuroscience 2015; 295: 229–236.
22 Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE et al. ApoE-directed therapeutics rapidly clear beta-amyloid and reverse deficits in AD mouse models. Science 2012; 335: 1503–1506.
23 Iwamoto K, Bundo M, Ueda J, Oldham MC, Ukai W, Hashimoto E et al. Neurons show distinctive DNA methylation profile and higher interindividual variations compared with non-neurons. Genome Res 2011; 21: 688–696.
24 Addington J, Liu L, Buchy L, Cadenhead KS, Cannon TD, Corbetta BA et al. North American prodrome longitudinal study (NAPLS 2): the prodromal symptoms. J Neuropsychiatry Clin Neurosci 2015; 2015; 203: 328–335.
25 Valmaggia LR, Stahl D, Yung AR, Nelson B, Fusari-Poli P, McGorry PD et al. Negative psychotic symptoms and impaired role function predicting transition outcomes in the at-risk mental state: a latent class cluster analysis study. Psychol Med 2013; 43: 2317–2325.
26 Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMIDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999; 20: 201–225.
27 Lakhan SE, Caro M, Hazd himichalis N. NMIDA receptor activity in neuropsychiatric disorders. Front Psychiatry 2013; 4: 52.
28 Malheiro JM, Paiva FF, Longo BM, Hamani C, Covolan L. Manganese-enhanced MRI: biological applications in neuroscience. Front Neurol 2015; 6: 161.
29 Wadghiri YZ, Blind JA, Duan X, Moreno C, Yu X, Joyner AL et al. Manganese-enhanced magnetic resonance imaging (MEMRI) of mouse brain development. NMR Biomed 2004; 17: 613–619.
30 Yu X, Wadghiri YZ, Sanes DH, Turnbull DH. In vivo auditory brain mapping in mice with Mn-enhanced MRI. Nat Neurosci 2005; 8: 961–968.
31 Bossert JM, Stern AL, Theberge FR, Marchant NJ, Wana HL, Morales M et al. Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. J Neurosci 2012; 32: 4982–4991.
32 Kalivas PW, Volkow ND. New medications for drug addiction hiding in glutamatergic neuroplasticity. Mol Psychiatry 2011; 16: 974–986.
33 Paz RD, Tardito S, Atzori M, Tseng KY. Glutamatergic dysfunction in schizophrenia: from basic neuroscience to clinical psychopharmacology. Eur Neuropsychopharmacol 2008; 18: 773–786.
34 Manoach DS, Press DZ, Thangaraj V, Searl MM, Goff DC, Halpern E et al. Schizophrenic subjects activate dorsolateral prefrontal cortex during a working memory task, as measured by fMRI. Biol Psychiatry 1999; 45: 1128–1137.
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