CHAPTER 7

EPIGENETICS IN AUTISM AND OTHER NEURODEVELOPMENTAL DISEASES

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Abstract: Autism was previously thought to be caused by environmental factors. However, genetic factors are now considered to be more contributory to the pathogenesis of autism, based on the recent findings of mutations in the genes which encode synaptic molecules associated with the communication between neurons. Epigenetic is a mechanism that controls gene expression without changing DNA sequence but by changing chromosomal histone modifications and its abnormality is associated with several neurodevelopmental diseases. Since epigenetic modifications are known to be affected by environmental factors such as nutrition, drugs and mental stress, autistic diseases are not only caused by congenital genetic defects, but may also be caused by environmental factors via epigenetic mechanism. In this chapter, we introduce autistic diseases caused by epigenetic failures and discuss epigenetic changes by environmental factors and discuss new treatments for neurodevelopmental diseases based on the recent epigenetic findings.

INTRODUCTION

Autism is a neuroimpairmental disease affecting approximately 30-60 per 10,000 children worldwide including Japan, with the male to female ratio of 2.5:1.[1] Autism has been classified into these three categories: (1) abnormal reciprocal social interactions including reduced interest in peers and difficulty in maintaining social interaction and failure to use eye gaze and facial expressions to communicate efficiently, (2) impaired communication including language delays, deficits in language comprehension and response to voices,

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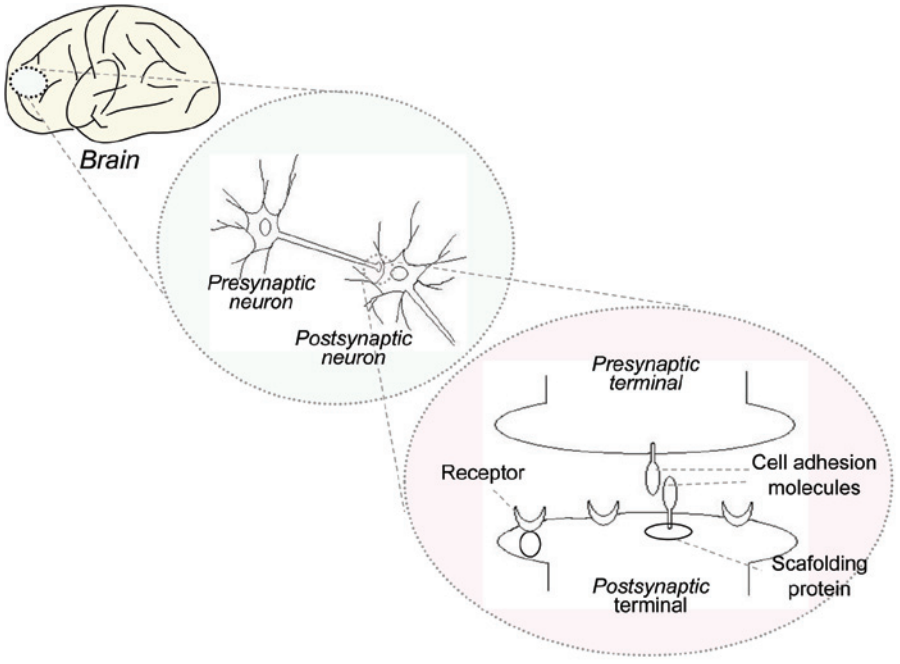

Figure 1. Location of the molecules in the synapse, which are associated with the pathogenesis of autism.

stereotyped use of words and phrases, poor pragmatics and lack of prosody, resulting in monotone and exaggerated speech patterns and (3) repetitive behaviors including motor stereotypes, repetitive use of objects, compulsions and rituals, insistence on sameness, upset to change and unusual or very narrow restricted interests.[2] Autism is diagnosed based purely on behavioral criteria such as DSM-IV99 (the diagnostic manual of the American Psychiatric Association) and ICD-10100 (the diagnostic manual of the World Health Organization.[3,4]

While various environmental factors are thought to contribute to the pathogenesis of autism,[5] recent genetic studies have revealed the involvement of genes in autism, in which mutations in the genes encoding neuronal molecules have been identified in a subset of autistic children. These molecules have been associated with synapse for neuronal connection, including synaptic scaffolding proteins, receptors and transporters on synapses and neuronal cell adhesion molecules.[6] These findings indicate that autism is a synapse disease[7] (Fig. 1).

The number of autistic children has been increasing recent years in Japan, USA and other countries.[8-11] This increase cannot be solely attributed to genetic factors, because it is unlikely that the mutation rate has suddenly increased in recent years. Therefore, environmental factors are more likely to be involved in this increase.[12]

Epigenetic is a DNA and histone-modification based genetic mechanism that is involved in neuronal gene(s) control in cells which are affected by environmental factors.[13] Thus, it is important to understand epigenetic mechanism when to study pathogenesis of autism.

In this chapter we show examples of neurodevelopmental diseases associated with epigenetics and environmental factors that affect epigenetic gene regulation and discuss future medical directions for autistic children based on recent epigenetic understanding.

CONGENITAL NEURODEVELOPMENTAL DISEASES ASSOCIATED WITH EPIGENETIC MECHANISM

Epigenetic gene control is an intrinsic mechanism for normal brain development[14] and abnormalities in the molecules associated with this mechanism are known to cause congenital neurodevelopmental diseases including autistism.[15-23] Genomic imprinting is the epigenetic phenomenon initially discovered in human diseases. In an imprinted gene, out of the two parental alleles, one allele is active and the other is inactive due to epigenetic mechanism such as DNA methylation. Therefore, defect in the active allele of the imprinted gene results in the loss of expression. This has been found in autistic diseases, Angelman syndrome and Prader-Willi syndrome.[15]

Since the number of genes in X chromosome is higher than those in Y chromosome, females (XX) have more genes than males (XY). To minimize this sex imbalance, one of the two X chromosomes in females is inactivated by epigenetic mechanism.[16] If X-inactivation does not properly occur in a female, such fetuses are believed to be aborted. This hypothesis is supported by the recent findings in cloned animals produced by somatic nuclear transfer in which failure of X-chromosome inactivation induces embryonic abortion.[17,18] Even if one of the X chromosomes is extremely small due to a large terminal deletion, so that over dosage effect of X-linked genes is small, the female show a severe congenital neurodevelopmental delay,[19] indicating that proper gene suppression by epigenetic mechanism is essential for the development.

DNA methylation is a fundamental step in epigenetic gene control and it is achieved by an addition of the methyl group (CH_3) to CpG dinucleotides mediated by DNA methyltransferase. Defect in this enzyme, DNMT3B, causes an ICF syndrome characterized by Immunodeficiency, centromere instability, facial abnormalities and mild mental retardation.[20-22]

Methyl-CpG binding proteins, which bind to the methylated DNA regions, are also important to control the gene expression. Mutations in one of the Methyl-CpG binding proteins, methyl CpG binding protein 2 (MeCP2), causes Rett syndrome, which is characterized by seizures, ataxic gait, language dysfunction and autistic behavior.[23-24] Therefore, it has been suggested that MeCP2 dysfunction, due to a mutation, leads to aberrant gene expression in the brain, which are associated with features in Rett syndrome including autism. Recent studies have shown that MeCP2 controls some neuronal genes, such as brain derived neurotrophic factor (BDNF), distal-less homeobox 5 (DLX5) and insulin-like growth factor binding protein 3 (IFGBP3).[25-28] These findings suggest that not only mutations,[6] but also epigenetic deregulation of genes that encode synaptic molecules may be attributed to autism.[29-31] Our recent findings, in which MeCP2 regulate a synaptic scaffolding protein and neuronal cell adhesion molecules and deficiency of MeCP2 leading aberrant expression of these genes, may support this hypothesis (our unpublished data).

ENVIRONMENTAL FACTORS WHICH AFFECT BRAIN FUNCTION VIA EPIGENETIC MECHANISM

In autism, both environmental factors (e.g., toxins, infections, ways of child rearing) and genetic factors (e.g., mutations in genes involved in synaptic system) have been discussed.[32-34] However, the biological mechanism that links these two factors has not been identified. Epigenetics may bridge these two[13] (see Fig. 2). Besides the intrinsic

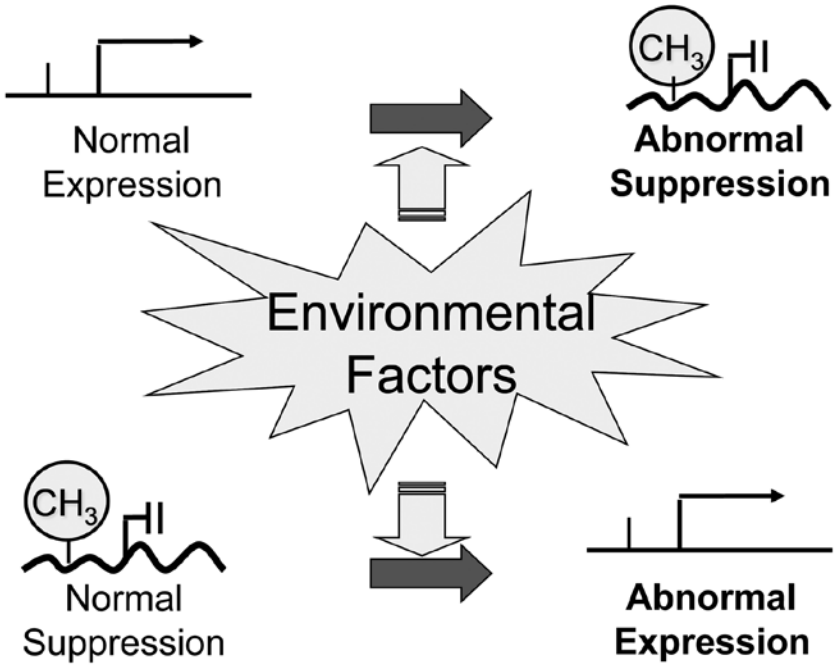

Figure 2. Epigenetic mechanism, which is affected by environmental factors.

(congenital) epigenetic defects (associated diseases described above), several lines of evidence suggest that extrinsic (environmental) factors, such as malnutrition, drugs, mental stress, maternal care and neuronal stimulation can alter the epigenetic status thereby affecting brain functions.[35-39] Therefore, it is intriguing to note that acquired neurodevelopmental diseases, including autistic diseases, may be the result of epigenetic deregulation caused by environmental factors. One example is drug addiction in which the gene expression in the dopaminergic and glutamatergic systems is mediated by epigenetic mechanism and, cocaine and alcohol alter the epigenetic state (chromatin structure) on a subset of genes, inducing drug addiction state[40,41] (Fig. 2).

Environmental factors via epigenetic mechanism are not always harmful. Imipramine, a major antidepressant, has recently been found to have the effect of restoring depressive state by alteration of epigenetic state (histone modification) of the *Bdnf* gene, leading to up-regulation of BDNF (brain-derived nutrition factor) in the hippocampus.[38] Valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, is another example that alters the epigenetic state. VPA normalizes histone acetylation in the genes in the hippocampus, which lead to suppress seizure-induced cognitive impairment by blocking seizure-induced aberrant neurogenesis.[36] These indicate that chemicals that alter epigenetic gene expression, such as HDAC inhibitors, may become the candidates as new drugs for neurodevelopmental diseases including autism.[42]

The findings above are mainly obtained from animal experiments and there is little indication of its existence in humans. However, epigenetic difference is larger in monozygotic twins with older age than those with younger age,[43] suggesting that in humans epigenetic status may be altering during aging by environmental factors in humans.

EPIGENETIC TREATMENTS FOR PATIENTS WITH NEURODEVELOPMENTAL DISEASES

Because epigenetic mechanism is reversible and variable unlike most mutational changes, epigenetic information can be potentially used as a disease-condition marker and a target for the treatment of the diseases.

Folic acid is the substrate for supplying methyl-residues to cytosine when is converted into methylated cytosine in DNA. Therefore, in order to maintain DNA methylation, proper intake of folic acid is important. In Japan young women who do not have sufficient amount of folic acid during pregnancy are now increasing and this increases the risk of having babies with neural tube defects.[44] Inappropriate supply of nutrients from mother to the fetus also increases the susceptibility of the fetus to develop diabetes mellitus by changing their epigenetic status.[45] These hypotheses are supported by experiments on rats, that protein restriction during pregnancy induces mal-nutrition and hyperlipedemia in the fetus and that supplementation of folic acid during pregnancy relieves these abnormal conditions, increases DNA methylation of the promoter regions of PPAR-alpha and glucocorticoid receptor genes in liver and leads to proper suppression of the genes.[35] These findings indicate that specific nutrient intakes may alter the phenotype of the offspring through epigenetic changes.

Royal jelly is known to have an effect of changing the phenotype from genetically identical female honeybees to a fertile queen; thus an effect of epigenetic changes. A recent study has revealed that royal jelly has effect of removing global DNA methylation, because silencing the expression of Dnmt3, a DNA methyltransferase, lead to a royal jelly-like effect on the larval development.[46]

Since 1980s folic acid has empirically been used for the treatment of autism and these studies have shown that it is effective only to a subset of the patients.[47-49] However, it is unknown whether folic acid leads epigenetic changes in autistic patients. Therefore, it is important to find if DNA methylation is altered by the treatment of folic acid. From the recent advancement in genome analysis technology, it has been possible to identify "folate-responsible genomic region (gene)" where DNA methylation is altered by administration of folic acid in the autistic children.[50,51] Such identified gene will be a therapeutic marker for folic acid treatment and the folic acid-sensitive (treatable) patients can be distinguished from the nonsensitive (nontreatable) patients (Fig. 3).

Folic acid-based treatment is relatively safe, since it is a nutrient. However, its effect is global, not gene specific. One ideal therapeutic way would be to target the specific gene(s) that can alter epigenetic status only in the genomic region (gene) associated with autistic pathogenesis. This treatment is now under development using PI polyamide that is designed on DNA sequence of a target gene.[52]

It has recently been discovered that DNA sequence is different in each neuron,[53] and epigenetic change underlies the somatic change.[54] This phenomenon is based on retrotransposition, in which a repetitive L1 sequence is inserted into various genomic regions when it is hypomethylated, which could alter the adjacent gene expression. Interestingly, the retrotransposition is activated by voluntary exercise (for example running).[55] Taken together, adequate intake of folic acid will ensure proper DNA methylation status and prevent from aberrant genomic change in neurons and mal-nutrition possibly induce hypomethylation, which leads to genomic changes in neurons, resulting in mental diseases including autism.

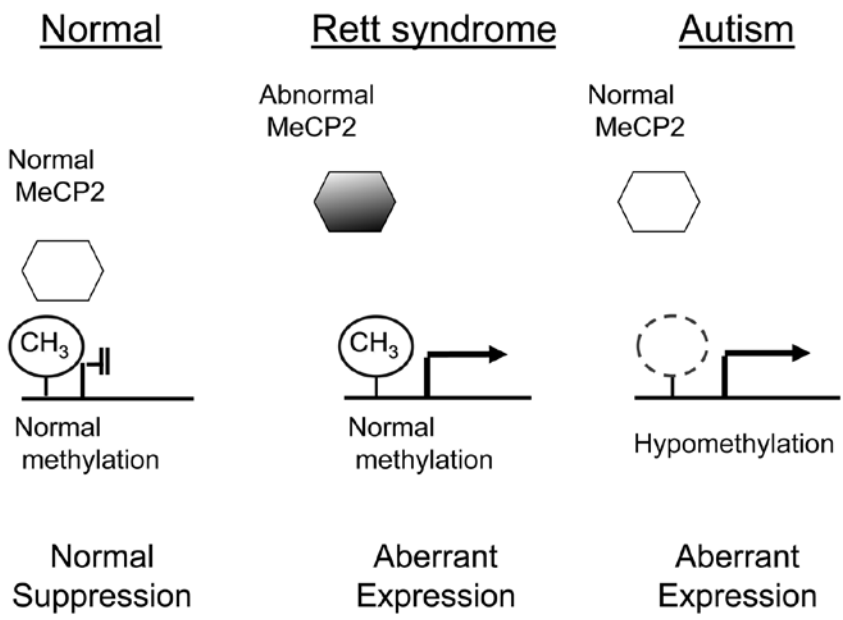

Figure 3. Pathogenesis of Rett syndrome and putative pathogenesis of autistic children to whom folic acid is effective. The gene with hypomethylation may be changed into normal hypermethylated status by administration of folic acid.

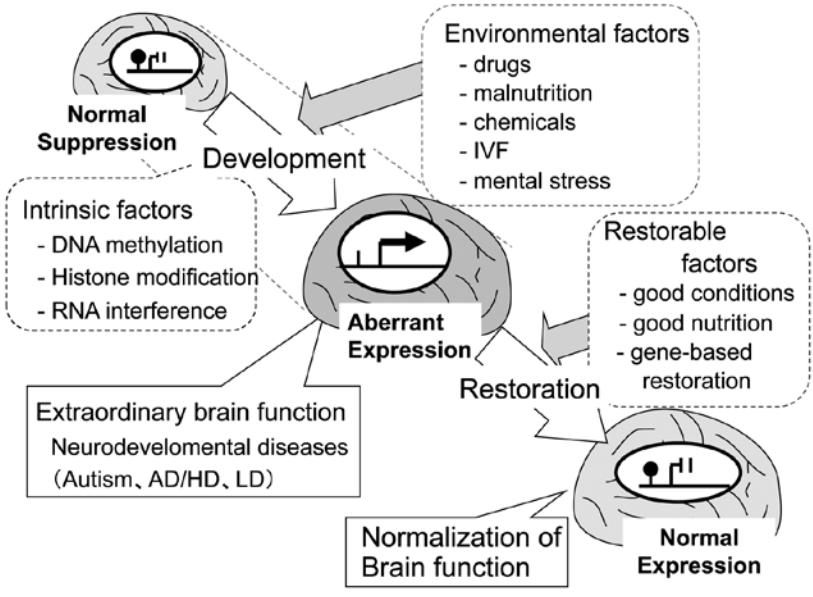

Figure 4. Overview of the epigenetic change and environmental factors. Note: epigenetic mechanism is reversible and restrable.

CONCLUSION

In summary, various environmental factors potentially rewrite epigenetic codes. However, epigenetic mechanism is reversible which is different from that of changes in genetic codes. Thus epigenetics are treatable and preventable when we will understand further as to which environmental factor(s) induce epigenetic changes and which gene is affected in this case (Fig. 4). In this context, epigenetic research is now important.

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