Epigenomic-basis of Preemptive Medicine for Neurodevelopmental Disorders

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Abstract: Neurodevelopmental disorders (NDs) are currently thought to be caused by either genetic defects or various environmental factors. Recent studies have demonstrated that congenital NDs can result not only from changes in DNA sequence in neuronal genes but also from changes to the secondary epigenomic modifications of DNA and histone proteins. Thus, epigenomic assays, as well as genomic assays, are currently performed for diagnosis of the congenital NDs. It is recently known that the epigenomic modifications can be altered by various environmental factors, which potentially cause acquired NDs. Furthermore these alterations can potentially be restored taking advantage of use of reversibility in epigenomics. Therefore, epigenome-based early diagnosis and subsequent intervention, by using drugs that restore epigenomic alterations, will open up a new era of preemptive medicine for congenital and acquired NDs.

Keywords: Neurodevelopmental disorder, Autism, Epigenomics, DNA methylation, Acquired, Preemptive medicine.

GENOME-BASED DIAGNOSIS OF CONGENITAL NEURODEVELOPMENTAL DISORDERS

Neurodevelopmental disorders (NDs), which include autism spectrum disorder (ASD), are characterized by genetic and phenotypic heterogeneity. Mutations in the genes, which encode a number of neuronal molecules, can affect the function in cortical interneurons, pyramidal neurons and the medium spiny neurons of the striatum, which is associated with severe intellectual and behavioral ND phenotypes [1, 2]. For example, mutations in *CD38*, which encode an oxytocin activator that is associated with social behavior are though to contribute to the pathogenesis of ASD [3, 4], and mutations in *CAPS2*, which encodes Ca (2+)-dependent activator protein for secretion 2 that is associated with secretion of the brain-derived neurotrophic factor (BDNF), are also though to contribute to the pathogenesis of ASD [5, 6]. Furthermore, mutations in the genes, which encode synaptic molecules such as receptors and transporters, are also associated with ASD [7, 8]. Taken together, autism is currently recognized as a "synapse disease"

Identification of genes associated with NDs has been accelerated by the advent of current next-generation sequencing (NGS) technique [9]. NGS will help not only to elucidate genomic understanding of NDs, but also to establish new diagnostic assays for NDs [10], as well as chronic progressive cerebellar ataxia and neonatal disorders (Fig. **1**, left) [11, 12].
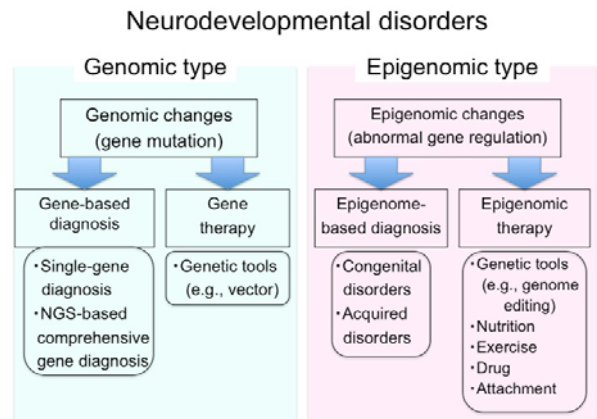

Fig. (1). **Comparison of genomically type and epigenomically based neurodevelopmental disorders.** Left (genomic type): A subset of congenital neurodevelopmental disorders (NDs) are caused by genomic changes (i.e., gene mutations). Gene-based diagnosis and gene therapy can be applied for this type of NDs. Right (epigenomic type): Another subset of congenital NDs and acquired NDs are caused by epigenomic changes (i.e., abnormal gene regulation). Epigenomically-based diagnosis and epigenomic therapies can be applied for these types of NDs.

EPIGENOME-BASED DIAGNOSIS IN CONGENITAL NEURODEVELOPMENTAL DISORDERS

Rett syndrome (RTT) is a representative ND characterized by autistic behavior, seizures and ataxic gait (OMIM #312750). A linkage study has initially shown that the causative gene for RTT is located within the chromosome Xq28 region [13], and a mutation analysis has revealed that most

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RTT patients have mutations in *MECP2* (methyl-CpG binding protein 2) in Xq28 [14]. This discovery surprised pediatric neurologists who see RTT patients, because the gene for RTT does not encode an expected synaptic molecule but encodes an unexpected protein involved in epigenetic gene regulation. Thus, this was a first linkage between ND and epigenetics.

Recent large-scale NGS sequencing studies have revealed that the mutations can be identified in the proteins for transcriptional regulation, WNT pathways and chromatin remodeling, which include enzymes that mediate post-transcriptional histones modifications [15-17]. These findings indicate that autism can be recognized as a " chromatin and WNT pathway disease".

Prior to the realization of the link between ND and epigenetics, abnormal development of the epigenomic patterns have been shown to result in abnormal embryonic development. For example, mice with knockout mutations of the genes for DNA methyltransferases (DNMTs) are embryonic lethal [18]. Also, epigenetic modifications are demonstrated to be essential for neuronal and hematopoietic differentiations of the stem cells [19, 20]. These results indicate that failure of the epigenetic mechanism in early development can potentially be associated with congenital NDs.

Subsequent clinical studies have revealed that mutations of a DNA methyltransferase (DNMT) gene, *DNMT3B*, causes ICF syndrome, which is characterized by immunodeficiency, centromere instability, facial anomalies and mild ND [21], and mutation of *DNMT3A* causes an overgrowth syndrome with mild ND (Fig. **2A**) [22]. These suggest that dysregulation of genes due to abnormal establishment of DNA methylation pattern is associated with congenital NDs.

As mentioned above, dysregulation of genes due to mal-suppression by a mutant methylated-DNA binding protein (e.g., MeCP2) is associated with RTT, a representative ND (Fig. **2B**) [14, 23-26].

The CGG repeat sequences in the genome are unstable and can be expanded in the upstream region of *FMR1* (fragile X mental retardation syndrome 1) on the X chromosome, and expanded CGG repeat sequences are subject to DNA methylation, which results in Fragile X syndrome, another typical ND. A recent study revealed that the suppression was achieved by hybridization between the transcribed CGG-repeat tract in the expanded 5′ untranslated region and the complementary CGG-repeat portion by forming an RNA·DNA duplex (Fig. **2C**) [27]. Since FMR1 protein (FMRP), encoded by *FMR1*, suppresses various neuronal genes, suppression of *FMR1* due to DNA methylation can result in over-production of neuronal molecules, which can be a cause of schizophrenia [28]. Taken together, genetic changes followed by epigenomic changes can a ND and adult a mental disorder.

Genomic imprinting is a chromosome-level epigenetic mechanism that determines parent-of-origin specific gene expression. The established maternal or paternal expression pattern is maintained throughout the lifetime.

At the stage of gametogenesis, however, the patterns are once thoroughly erased and are newly established patterns according to the sex of the individual. For example, an im-

printed *Igf2* (the insulin-like growth factor 2) is differentially methylated between the maternal and paternal alleles, but it is demethylated in primordial germ cells by day 13.5, and parent-of-origin specific DNA methylation patterns are created in the germ cells [29, 30]. It is also known that defects in genomic imprinting lead to inappropriate gene expression, resulting in congenital NDs, such as Prader-Willi and Angelman syndromes (Fig. **2D**) [31]. Based on the findings of altered DNA methylation pattern in these syndromes, an epigenetic diagnostic assay has been developed [31], which now provide early diagnosis and early treatment with growth hormone to prevent Prader-Willi syndrome patients from obesity and subsequent diabetes mellitus [32] (Fig. **1**, right).

The X chromosome is larger than the Y chromosome, and thus the X chromosome carries more genes than the Y chromosome. Therefore, XX females (karyotype) carries have more genes than XY males. This gene imbalance between the two sexes is compensated by DNA methylation-based inactivation of one of the two X chromosomes in females [33]. It has been known that failure of the X-chromosome inactivation (XCI) leads to abortion, which has been found in aborted mice produced by somatic cloning (Fig. **2E**) [34], suggesting that establishment of the proper XCI pattern in early development is essential to normal birth. However, if one of the two X chromosomes was extremely small (e.g., a small ring X chromosome), failure of XCI did not lead to abortion, but such case showed severe congenital ND [33]. Taken together, a proper XCI pattern established by epigenetic mechanism is essential for normal embryonic development.
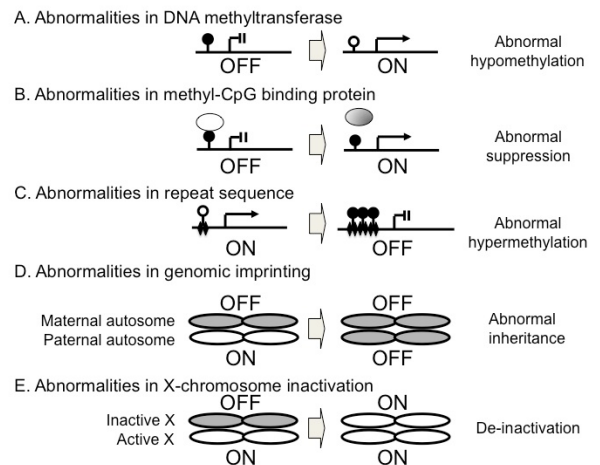

Fig. (2). Epigenomic abnormalities related to NDs. (A) Mutations in the genes encoding DNA methyltransferases produce enzymatic deficiencies, which result in reduced DNA methylation status that leads to aberrant expression of the target genes. **(B)** Mutations in the genes encoding proteins with methyl-CpG binding domains (MBDs) cause abnormal regulation of the target genes. **(C)** Expansion of CGG repetitive sequences in the promoter regions induce DNA hypermethylation that leads to suppress the downstream genes, which can be associated with neurodevelopmental disorders. **(D)** Abnormal suppression of the active allele of imprinted genes are associated with genomic imprinting disorders. **(E)** Abnormal activation of the inactive X chromosome in females can be associated with neurodevelopmental disorders.

ACQUIRED EPIGENOMIC CHANGES ASSOCIATED WITH NEURODEVELOPMENTAL DISORDERS

It has been demonstrated that a short-term mental stress alters epigenomic status in the brain [35]. In this study, maternal separation during neonatal period increased DNA methylation at the promoter of the glucocorticoid receptor gene (*GR*, also known as the *NR3C1*) in the hippocampal region of the brain, which suppressed *GR* expression (Fig. **3**). On the other hand, high maternal care during the same period resulted in a decrease of DNA methylation that up-regulates *GR* expression [35]. The environmental effect on rat development can be viewed as a possible animal model for childhood neglect and maltreatment in humans, and this viewpoint has been supported by postmortem analyses of the hippocampus in the brains of suicide victims with a history of childhood abuse, in which the brains of these individuals showed hypermethylation of a neuron-specific promoter of *NR3C1* in combination with a decreased in *NR3C1* expression [36]. These findings suggest that mental stress can adversely alter DNA methylation status in early development, and that the altered DNA methylation pattern can potentially be associated with NDs and adult mental disorders [37].
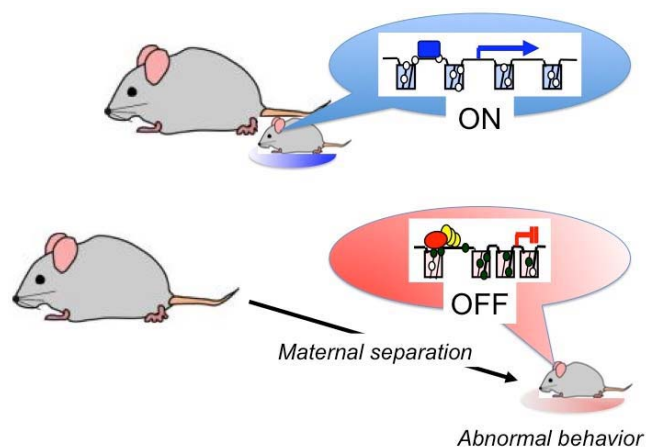

Fig. (3). Epigenomic ND rodent model for the induction of mental stress during the early postnatal development. Environmental factors (e.g., maternal separation-stress during the first week of life) can alter the epigenetic status (e.g., DNA methylation) in a neuronal gene (e.g., the glucocorticoid receptor gene promoter) in the rat brain. These epigenetic changes can lead to persistent gene expression changes, resulting in abnormal behavior throughout the individual's lifespan.

In a study, the levels of DNA methylation in Alu repetitive sequences in human genome were measured in serum DNA (cell-free DNA) of US military service personnel who had been deployed in Iraq and Afghanistan, and increased methylation was found in the repetitive sequences in post-traumatic stress disorder (PTSD) cases after deployment [38], suggesting that Alu hypermethylation may represent a response to psychological stress [39]. Increased DNA methylation was also found in L1 (LINE-1) repetitive sequences in personnel who had not suffered PTSD after deployment, suggesting that mental stress sustained during deployment may change the methylation status of L1 to protect against PTSD [38, 39].

To date, there is limited evidence of environmentally induced-epigenomic changes in relation to mental disorders in humans. However, since epigenomic differences are greater between older monozygotic twins than younger twins, epigenomic patterns can potentially be changed by environmental factors during the lifetime (Fig. **4**) [40]. Likewise, discordant monozygotic twins for severity of ND have been shown to differ in their epigenomic patterns and expression of neuronal genes [41]. These findings indicate that environmental factors may influence the human epigenome and this may modify ND phenotypes.
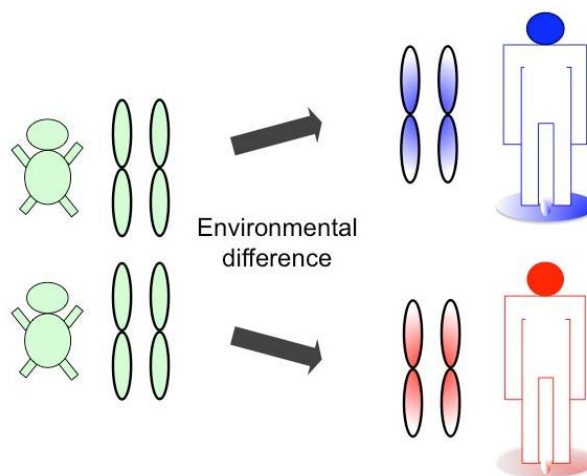

Fig. (4). Genome-wide epigenetic phenomena in humans. Epigenomic differences have been identified between older monozygotic twins, suggesting that either aging or environmental stresses induce epigenetic changes in humans.

EPIGENOMIC REVERSIBILITY IN ACQUIRED NEURODEVELOPMENTAL DISORDERS

Folic acid administrated to pregnant rats can pass to the fetuses via the placenta and can alter the DNA methylation status of the fetuses [42]. Supplementation of the diet of pregnant rats with folic acid under malnutrition conditions prevents hypomethylation of a hepatic gene in their offspring [43]. Furthermore, other environmental factors, such as royal jelly [44], drugs for mental disorders [45-47], environmental chemicals [48, 49], and external stimuli (electro-convulsive treatment for psychiatric diseases) [50], are known to change epigenetic status in model animal species.

As described above, mental stress in the first week of life can cause epigenetic abnormalities in the brains of rats [35]. Recent studies have shown that appropriate nurturing conditions can ameliorate behavioral abnormalities in mice. For example, environmental enrichment (larger-sized cages with a variety of objects including running wheels) improved the motor coordination and decreased anxiety-related behavior in RTT model (*Mecp2* heterozygous knockout female) mice [51, 52]. Environmental enrichment also improved locomotor activity with reduced ventricular volume, and restored the expression of synaptic markers in the brain of hemizygous *Mecp2*-/Y male mice [53, 54].

Although it is difficult to cure congenital NDs caused by mutations that encode neuronal molecules because there is

no proper method to deliver a gene product to the appropriate brain regions at the appropriate time during brain development, there is some hope that RTT might be curable. This hope is based on the fact that *MECP2* does not encode a protein associated with brain structure, but encodes a "lubricant" that acts at a comparatively late stage of brain development. This hypothesis has recently been demonstrated that introduction of *MECP2* into severe RTT model (*Mecp2* hemizygous knockout male) mice successfully rescued the RTT-like neurological symptoms [55, 56]. Furthermore, restoration of MeCP2 function in astrocytes substantially improves main RTT symptoms, such as abnormal locomotion, anxiety, and respiratory abnormalities in *Mecp2* null mice, as well as restoring dendritic morphology [57]. These results indicate that administration of drugs that up-regulate *MECP2* expression potentially improves brain function in patients with RTT. Various drugs can increase *MECP2* expression, for example, valproic acid (a popular drug for epileptic seizure and a known HDAC inhibitor), fluoxetine (a drug for mental disorders) and cocaine [58-60]; these drugs are now under considerations as candidates for treating RTT. Since NGS recently revealed that a subset of ND patients have mutations in the genes encode histone modification enzymes [15], the chemicals that effect to these proteins, such as HDAC inhibitor, are potential candidates for the treatment of NDs [45, 61].

EPIGENOMIC-BASED PREEMPTIVE MEDICINE FOR NEURODEVELOPMENTAL DISORDERS

A recent study demonstrated that dieting among young women in Japan minimized weight gain during pregnancy, which might be associated with a decline in birth weights in recent 20 years [62]. The previous epidemiological studies at the time of famines in the Netherlands and China have shown that hyponutrition in the fetal period due to hunger of the pregnant mothers increases the risk of diabetes mellitus and mental disorders [63, 64]. These results suggest that the current generation with the history of lower birth weight in Japan is expected to have metabolic and mental disorders in adulthood [62]. This concept refers to "Developmental Origin of Health and Diseases (DOHaD)" [65].

Recent animal studies have demonstrated that malnutrition during the fetal period decreased the level of DNA methylation on *PPARa* (peroxisome proliferator-activated receptor alpha) gene in the liver [66]. Similar methylation changes have been confirmed in the peripheral blood tissue of the individuals who suffered malnutrition during a period of famine in the Netherlands [67].

It has been reported that assisted reproductive technologies (ART) used for women of advanced maternal age, such as *in vitro* fertilization and intracytoplasmic sperm injection, decreases DNA methylation at multiple maternally methylated-imprinted genes [68, 69]. Therefore, it is intriguing to think that current two social factors, DOHaD and ART, may be associated with the recent increase of children and adult with mild NDs in advanced nations [70-73] and that epigenomic changes may underlie these social phenomena.

The goal of clinical epigenomics is to identify "epigenetic signatures", which are genomic loci where epigenetic changes are introduced by environmental factors. Such epigenetic signature-based medicine has already applied to a subset congenital NDs, such as Prader-Willi syndrome and Fragile X syndrome [31, 74]. One means to identify the epigenetic signatures, which can possibly be used as early diagnostic markers of acquired NDs, is to utilize methylation-
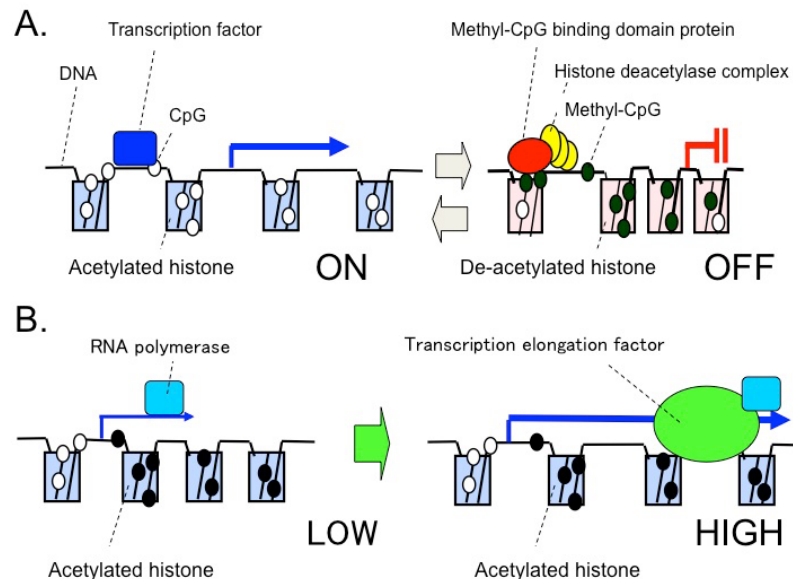

Fig. (5). Conventional and new epigenetic gene regulation models. (**A**) DNA that is methylated by DNA methyltranferase at a promoter region is recognized by MBD proteins that bind to the methylated CpG dinucleotides. This binding stimulates the recruitment of histone deacetylase complexes, leading to chromatin condensation by deacetylation of histones, and results in suppression of the relevant gene. (**B**) Environmental factors (e.g., hyperglycemia induced by excess of nutrition) can activate a transcription elongation factor (e.g., BRD4), leads to a higher level of transcription of metabolism-related genes, resulting in metabolic disorders such as diabetes mellitus. This model can be applied to acquired NDs.
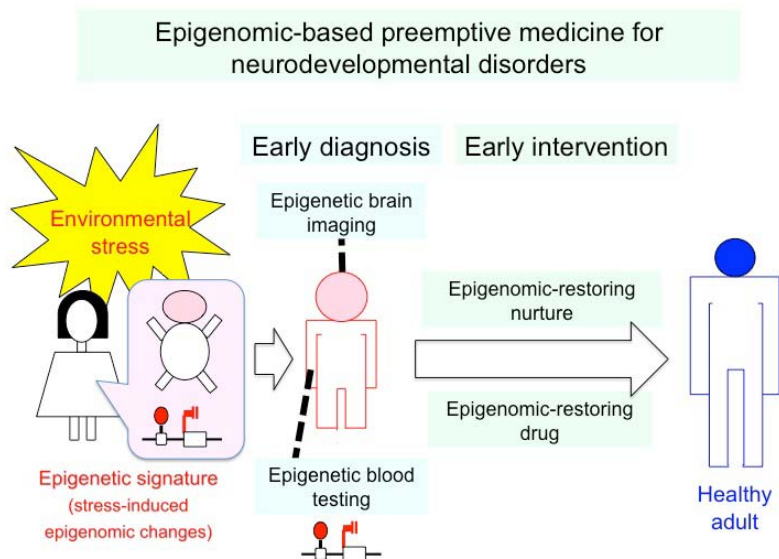
Fig. (6). Model of epigenomically-based preemptive medicine for NDs. Analysis of blood or neuroimaging examinations may permit early detection of epigenomic alterations induced by environmental stresses during fetal and postnatal periods. Early intervention, for example, appropriate environmental enhancement, nutrition or drugs, may help to prevent development of NDs or to rescue features of NDs after they have developed.

specific microarray technologies, which became a standard method to identify genomic loci where epigenetic changes are introduced by various environmental chemicals in recent public health studies [75, 76].

Although the epigenetic patterns in the peripheral blood and the brain cells are not completely identical, recent studies have demonstrated that DNA methylation status are similar between these tissues in a subset genomic regions, such as *BDNF* promoter region [77], and that the DNA methylation pattern in the peripheral blood can be used for assessment for mental disorders [78]. However, it is necessary to establish a non-invasive method that detects epigenetic abnormalities in the brains of patients for achieving real-time epigenetic assessment of mental disorders. In fact, recent brain imaging systems successfully detect activities of histone acetylation-related enzymes in the brains of ND patients, although resolution is not high [79, 80].

It has recently been shown that environmental factors not only change the gene expression status by an ON / OFF epigenetic switching mechanism (Fig. **5A**) but also change it in a LOW / HIGH modification mechanism (Fig. **5B**). For example, a given environmental factor, such as hyperglycemia induced by nutritional excess, is known to activate a transcription elongation factor, such as a BET bromodomain protein (e.g., BRD4), which is bound to acetylated histones [81]. This activation might increase the transcription of fat metabolism-related genes in the liver, resulting in metabolic disorders such as obesity and diabetes mellitus [82, 83]. However, such aberrant expression due to environmentally induced hyperacetylation is possibly restored by JQ1, a BRD4 inhibitor [84, 85]. Furthermore, this model can be applied to environmentally induced acquired NDs.

Future epigenomic studies allow us to conduct "Personalized and preemptive medicine" with early diagnosis based on

detection of "epigenetic signatures" and with early intervention by supplying "epigenetic restoration factors", such as appropriate nutrition and nurture (Fig. **6**) [86, 87].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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