Advance in DNA Methylation Research of Autism Spectrum Disorders

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ABSTRACT

The autism spectrum disorder (ASD) is a common mental disorder in children. The etiology of this disease remains unclear. The studies show that the genetic and environmental factors play an important role in the pathogenesis of the disease. Epigenetic modification plays an important role in the process of gene and environment interaction. As the most common epigenetic modification, DNA methylation is becoming gradually a hot research topic of ASD etiology in recent years.

Key Words: Autism spectrum disorder; Epigenetic modification; DNA methylation

http://ijmf.qingres.com

OPEN ACCESS

DOI: 10.20900/ijmf.20170003

Received: September 24, 2017

Accepted: November 20, 2017

Published: December 20, 2017

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder in children psychiatry. The etiology and pathogenesis of the ASD is still unknown. Studies show that the genetic factors and environmental factors played an important role [1]. Epigenetics, as a mediator of gene and environmental interaction, is gradually becoming a new hot research topic in the study of mental diseases. The epigenetic studies can provide a new perspective to the study of ASD etiology and explore the mechanism of gene and environmental interaction.

1 DNA METHYLATION AND NEURODEVELOPMENT

DNA methylation is a common form of epigenetic modifications and also the most studied epigenetic modification. It refers to that the

methyl is transferred to a specific base under the catalysis of DNA methyltransferase. The most common way is to convert the cytosine at CpG site to 5-methylcytosine. The change of DNA methylation status can affect the chromatin structure, DNA stability, and interactions between DNA and protein, so as to regulate the gene expression.

DNA methylation plays an important role in neurodevelopment process. Along with the neurodevelopment, the DNA methylation status has been changing dynamically, while the aberrant methylation in the key chromosomal region and the abnormal genes associated with the methylation process will lead to severe neurodevelopmental disorders. Studies of human brain samples from different ages showed that the rate of DNA methylation in the brain was the fastest in the embryo period and it was declined significantly after the birth [2]. A rapid change of DNA methylation status in the critical period of brain development suggests that DNA methylation may play an important role in the neurodevelopment process.

Severe neurodevelopmental disorders Prader-Willi syndrome and Angelman syndrome are closely associated with abnormalities of DNA methylation [3]. It is noteworthy that the clinical manifestations of Prader-Willi and Angelman syndromes include the backward speeches and mental development and autism-like symptoms. Gene defect of DNA methylatransferase 3 (DNMT3), a key enzyme in DNA methylation, will lead to the mental retardation [4]. Methyl CpG binding protein 2 genes can bind to the methylated DNA to thus regulate the expression of multiple genes. The mutation of methyl CpG binding protein 2 (MECP2) genes will result in Rett syndrome, and the repetition of MECP2 genes will result in MECP2 syndrome. The clinical manifestations of Rett syndrome and MECP2 syndrome all include mental retardation, epilepsy, and autism-like symptoms [5]. All of these suggest that abnormalities of DNA methylation may be associated with the autism.

2 DNA METHYLATION AND ASD

The studies on abnormalities of DNA methylation of ASD include single-gene association study and genome-wide association study (GWAS). The previous studies were mainly about the abnormal methylation of ASD susceptible genes, which involved in genes such as SHANK3 gene, oxytocin receptor (OXTR) gene, methyl binding protein 2 (MECP2) gene, RELN gene, etc. Li, *et al.* found that the methylation level of SHANK3 gene in ASD patients was significantly higher than the controls, while no correlation was found between the methylation level and the severity of autism clinical manifestations through the further analysis [6]. Gregory, *et al.* found that the methylation level of OXTR genes in bleed and brain tissues of ASD patients was higher than that of the controls, and that the expression level of OXTR genes was negatively correlated with the methylation level of OXTR genes, suggesting that epigenetics may regulate the genetic expression by changing the methylation status of genes. Nagarajan, *et al.* found a high methylation in promoter region of MECP2 genes in ASD patients, and that the methylation level was negatively correlated with the expression of MECP2 protein [7]. By studying the autopsy specimens of 10 autistic patients, Zhubi, *et al.* found a significantly higher DNA methylation level of RELN gene and GAD1 gene promoter regions in ASD patients than the control group and a stronger binding capacity between MECP2 genes and RELN gene and GAD1 genes in ASD patients than the control group. However, this study did not explore the effects of this change of binding status on the genetic expression [8]. The research strategies of candidate genes provide some evidences to show an abnormal DNA methylation status in some genes of the ASD patients.

With the popularization of methylated chip technology, the changes of methylation status within the genome-wide range of ASD patients were studied in recent years. Nguyen, *et al.* found that the genes with different methylation status in ASD patients were mainly related to neurodevelopment, gene transcription and cell apoptosis, such as RORA gene and BCL2 gene [9]. 50 pairs of identical twins were included in another study. The study showed a higher methylation level of NFYC genes and a lower methylation level of DUSP2 genes in ASD patients than that in unaffected siblings, and this study also showed that the methylation level was associated with the severity of the autism [10]. After the comparison of methylation difference of brain tissues between 19 ASD patients and 21 controls, Ladd, *et al.* found that there were methylation level abnormalities near PRRT1 genes, TSPAN32 genes, ZNF57 genes and SDHAP3 genes, and a low methylation status was found at sites near PRRT1 genes and TSPAN32 genes, while a hypermethylation status was found at sites near ZNF57 genes and SDHAP3 genes [11]. Nardone, *et al.* found that genes with differences in DNA methylation are concentrated in the pathways involved in immune function, synaptic membrane, synaptic cleavage and glial cell differentiation. By the comparison of published expression database and real-time PCR

method, it was found that a low DNA methylation status would result in a high gene expression, and the involved genes included C1Q, C3, ITGB2 (C3R), TNF-α, IRF8, SPI1, HDAC4, C11orf21/TSPAN32, and the like [12]. Taking oral mucosal epithelial cells as the subjects, Berko, *et al.* found that the majority of methylation differentially expressed regions were associated with the synaptic transmission [13]. Wang, *et al.* found a higher methylation level of ENO2 genes in ASD patients after the study of methylation level difference in peripheral blood of 131 ASD patients and normal controls, and meanwhile, the expression level of ENO2 protein in patients with a higher methylation level of ENO2 genes was significantly reduced [14]. The ADI-R was used as the diagnostic criteria in the above studies except for the study of Wang Y, *et al.*

Table 1. Summary of Genome-wide Research Results on DNA Methylation Status of ASD

References	Sample source	Sample size	Abnormal methylated genes
Wong, *et al.* 2014	Peripheral blood cells	50pairs of male identical twins (patients and their unaffected siblings)	NFYC genes, DUSP2 genes
Nardone, *et al.* 2014	BA10 (prefrontal lobe), BA24 (anterior cingutate) cortex	13 patients and 12 controls	Genes involved in immune function, synaptic membrane, synaptic cleavage and glial cell differentiation
Ladd, *et al.* 2014	Frontal lobe, prefrontal lobe, cerebellar cortex	19 patients and 21 controls	PRRT1 genes, TSPAN32 genes, ZNF57 genes, SDHAP3 genes
Berko, *et al.* 2014	Oral mucosal epithelial cells	47 patients and 48 controls	Genes related to synaptic transmission
Wang, *et al.* 2014	Peripheral blood cells	131 patients and 131 controls	ENO2 genes
Nguyen, *et al.* 2010	Lymphoblastoid cell lines	3 pairs of male identical twins (patients and their unaffected siblings)	BCL-2 genes, RORA genes

3 PROSPECTS

The etiology and pathogenesis of autism spectrum disorder (ASD) remains unknown. The previous studies showed that the genetic factors and environmental factors played an equally important role in the pathogenesis of the ASD. The mechanism of gene-environment interaction remains unclear. We hypothesized that the possible mechanism was that the genetic factors made the individuals susceptible to the disease. Under the influence of environmental factors, including environmental factors during pregnancy and postnatal periods, they were interacted to cause the neurodevelopmental abnormalities so as to result in the clinical phenotype of the autism. As a mediator of gene and environmental interaction, epigenetic modification is gradually becoming a hot research topic in the etiology study of autism, which provides a new candidate gene for future genetic research. The genome-wide study showed that the methylation difference was found in genes associated with immune functions in ASD patients, suggesting that the immune factors might play a role

in pathogenesis of the ASD, which has also provided indirect evidences for the hypothesis of ASD maternal immunization activation. Future studies can further explore the effect of maternal immunization activation on DNA methylation status at embryonic stage.

The following three issues shall be considered for the future studies on the DNA methylation of ASD Patients: firstly, specimens or samples. The specimens used in previous studies included brain tissues, lymphoblastoid cell lines, peripheral blood cells, oral mucosal epithelial cells. Different tissues of the same individual may have different DNA methylation status due to their different biological functions. The brain tissue samples can the best reflect the methylation status of the brain, but it is difficult to obtain the samples. If they are taken as the research specimens, the sample size is usually small and it may be difficult to reach the statistical power of genome-wide study. However, whether the DNA methylation status of peripheral tissues can reflect the methylation of the brain will be a problem to be solved in future studies. Secondly, the epigenetic modification is influenced by the environment, and the DNA methylation status is in a dynamic process during the neurodevelopment. Therefore, it is difficult to deduce whether the DNA methylation difference of ASD patients found in the studies is the cause of ASD or the result of the symptoms. Finally, the future studies on DNA methylation shall contain the data of gene expressions to explore the effect of DNA methylation status of the same sample on the gene expressions. DNA methylation may be used as a biomarker for disease diagnosis in the future [15]. At present, there are still few studies on relationship between DNA methylation and ASD, and a large number of samples are needed for the studies in different populations.

FUNDING SUPPORT

This paper was supported by the National Natural Science Foundation of China (grant No. 81541115).

CONFLICT OF INTERESTS

Authors claim no conflict of interests.

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