EPIGENETIC FACTORS INVOLVED IN AUTISM SPECTRUM DISORDERS

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S u m m a r y. Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders manifested by impaired social interaction, deficits in verbal and nonverbal communication, and restricted, repetitive patterns of stereotyped behaviors and interests. The investigation of genetic risk associated with ASD has found that 74–93% of ASD risk is heritable. Prospective studies of infant siblings at familial high-risk of ASD provide evidence for regression, and subtle loss of skills in a larger proportion of children with ASD than previously assumed. Despite extensive genetic and biological research, there is no significant understanding of the specific mechanisms of ASD pathogenesis. There is much evidence suggesting the involvement of epigenetic modifications as basic for ASD development. Epigenetic modifications or altered DNA transcription *via* variations in DNA methylation and histone modifications but without alterations in the DNA sequence affect gene regulation which produces altered gene expression manifested as DNA methylation and/or histone modifications. Epigenetic dysregulation may account for a significant proportion of ASD cases. Specific chromosomal regions have been identified in autism susceptibility *loci*, however the results have been inconclusive. There is no single gene which can account for ASD. Genetic research in the nearest future will allow for better understanding of how gene-environmental interactions create autistic symptoms, and should facilitate the development of novel therapeutic targets of gene expression for ASD. Genetic testing has the potential to provide medical explanation for ASD in children. Screening studies can identify concomitant medical problems, improve prognosis, and group families in specific support organizations.

K e y w o r d s: epigenetics, autism spectrum disorders, epigenetic dysregulation

INTRODUCTION

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders manifested by impaired social interactions, deficits in verbal and nonverbal communication, and restricted, repetitive patterns of stereotyped behaviors and interests (Bailey et al., 2012). They are diagnosed by standardized behavioral observation tools and have several comorbidities, including mental retardation and aggression. Moreover, several immune abnormalities are observed, i.e. altered autoantibody and cytokine profiles, neuroinflammation, and changes in cellular populations and function, and ongoing systemic immune activation. The researchers speculate if the immune system malfunctions may be related directly to the biological etiology of autism.

The investigation of genetic risk associated with ASD (Tick, 2016) has found that 74–93% of ASD risk is heritable. The study of siblings indicated that ASD occurs in 7–20% subsequent children after an older child was diagnosed with ASD (Ozonoff, 2011; Sandin, 2014). The prevalence increased in children with two older siblings with ASD. The risk was found to be 3–4-times higher in boys than girls. The evidence for specific genetic risk factors in ASD increased in rare genetic syndromes, e.g. fragile X syndrome 81 and tuberous sclerosis, which included ASD in some children. Fragile X syndrome was detected in less than 2% of children with ASD. Genomic copy-number variants, in which a chromosomal subregion was duplicated or deleted, can be inherited or occur *de novo* meaning it was detected in the child but not in their parents. In ASD, copy-number variants were found to be risk variants as they mostly resulted in ASD in a minority of children, and were found in people with other developmental disorders or undiagnosed individuals. The researchers studied a few variants

such as chromosome 16p11.2 deletions and duplications 83, and maternal 15q11–q13 duplications. Lord et al. (2018) reported that in the past 15 years, recurrent, *de novo*, likely gene-disrupting, single-nucleotide variants have been identified in more than 100 genes, some of which demonstrated inherited single-nucleotide variants, likely to contribute to ASD risk (Lord et al., 2018).

There have been four ASD onset patterns described in infancy: (1) emergence of symptoms in the first year of life, (2) initial attainment of developmental milestones followed by a *plateau* in development, (3) attainment of developmental milestones followed by a regression/loss of skills, and (4) a mixed pattern of early delays followed by later loss. Prospective studies of infant siblings at familial high-risk of ASD cases (Pearson et al., 2018) provided evidence for regression, and subtle loss of skills in a larger proportion of children with ASD than previously assumed; however, there are few reports of clear-cut regressions, such as that involving dramatic loss of language and other skills (Goin-Kochel, Mire, & Dempsey, 2015; Shumway et al., 2011).

Despite extensive genetic and biological research, there is no significant understanding of the specific mechanisms of ASD pathogenesis. There is much evidence suggesting the involvement of epigenetic modifications as bases of ASD development. Epigenetic modifications or altered DNA transcription *via* variations in DNA methylation and histone modifications but without alterations in the DNA sequence affect gene regulation which produces altered gene expression manifested as DNA methylation and/or histone modifications. The researchers agree, it is likely to result from environmental factors, e.g. nutritional deficiencies, medications used, infections, toxins, air pollution, organophosphates, heavy metals, stressors, and immunological factors. These pathologies could be considered as multifactorial and polygenic disorders. They are considered epigenetic regulators modulating the biochemistry and physiology of the individual affected by ASD (Herbert et al., 2010; Lasalle, 2013; Bushnell, 2013; Cheslack-Postava et al., 2013; Siniscalco et al., 2013) .

The term ‘epigenetics’ was first introduced in the 1940s by British embryologist and geneticist Conrad Waddington. He defined it as: ‘the interactions of genes with their environment, which bring the phenotype into being’ (Waddington, 1942). Current knowledge has broadened epigenetics. At present it deals with altered DNA transcription *via* variations in DNA methylation and histone modifications, but without alterations in the DNA sequence. These variants are present in the epigenome, and manifest in the transcriptome. However, the non-coding RNA represents over 90% of the transcripts in most cells. Regions of DNA that do not code for proteins, i.e., intergenic regions can be actively transcribed and participate in the genes regulation, and is known as non-coding RNA (Sinoiscalco et al., 2013).

Modifications of DNA seem to represent an interface between changing environment and fixed genome. Many environmental factors that have epidemiological association with ASDs affect physiological process within cells, tissues and organs *via* altered gene regulation. They include chemicals that affect the function of endocrine glands, hormones, receptors and signaling pathways. They are natural compounds, e.g. genistein, and/or synthetic compounds, such as the plasticizing agent bisphenol A (BPA), fluorosurfactants (perfluoro- octanesulfonic acid and perfluorooctanoic acid), herbicides (atrazine) and phthalate plasticizers [bis-[2-ethylhexyl] phthalate or di-2-ethylhexyl phthalate (DEHP)], lead, arsenic, dioxins, benzene, toluene. The exposure to them is hardly avoidable since they are widespread in the environment, e.g. drinking water, household dust, several consumer products, like food and beverage containers (Siniscalco et al., 2013).

DNA METHYLATION AND ASD

Studies determined a specific methylation pattern associated with ASD severity (Mamrut et al., 2013). In addition, they found significant correlations between DNA methylation and quantitatively measured autistic trait scores. The authors analyzed quantitative relationship between the severity of the autistic phenotype and epigenetic variation at several multiple *loci* previously implicated in the pathogenesis of ASDs, including *AFF2*, *AUTS2*, *GABRB3*, *NLGN3*, *NRXN1*, *SLC6A4* and *UBE3A*. However, systemic changes in epigenetic programming were not related to ASDs, whereas considerable variability was found in DNA methylation at individual CpG sites within ASD-discordant monozygotic twin pairs.

Dysregulation of DNA methylation and pro-oxidant environmental stressors were found to modulate autism development. A link between epigenetic regulation and antioxidant/detoxification capacity was observed in many children with

autism that showed genome-wide DNA hypomethylation and oxidative protein/DNA damage. The authors conclude that deficient antioxidant and methylation capacity could promote cellular damage and alter epigenetic gene expression (Melnyk et al., 2012).

HISTONE MODIFICATIONS AND ASD

Histone modifications are involved in ASD development (Akbarian et al., 2009; Biron et al, 2001). They include lysine acetylation, methylation, SUMOylation, and ubiquitinylation; arginine methylation; serine phosphorylation; proline isomerization are the covalent modifications of histone proteins. The researchers identified them in the amino- and carboxy-terminal histone tails, and a few in the histone globular domains. They suggested lysine methylation of histone H3 could be involved in autism development. The amino-acid lysine can carry up to three methyl groups. Each methylation could represent a distinct functional state of the cell. Histone methylation mechanism is involved in brain function and development and thus influence ASD pathology.

The investigations by Schuha et al. (2012) found altered methylation of H3K4 sequences in genes and *loci* implicated in regulating neuronal connectivity, social behaviors, and cognition. In the pre-frontal cortex of autistics mutations in the X-linked gene *SMCX* which encodes a histone 3 lysine 4 (H3K4)me3-specific demethylase was established. This gene regulates in turn other genes, i.e., *SCN2A*, *CACNA1H*, *BDNF*, *SLC18A1*, associated with autism and cognitive dysfunction. Moreover, possible connection between epigenetic changes in ASD behaviors and gene expression alterations were observed. Histone deacetylase inhibitors, i.e. sodium butyrate and trichostatin A were found to enhance up-regulation of oxytocin receptor and vasopressin V1a receptor. The genes encoding those receptors are strongly associated with ASD-like behaviors.

ENVIRONMENTAL FACTORS IMPLICATED IN ASD

Animal studies found that environmental factors involved in epigenetic changes in epitestosterone synthesis could contribute to the development of autistic-like behaviors in rodents administered with a postnatal dose of citalopram, estradiol or valproic acid. Dietary vitamin D could regulate epigenetic events. Maternal vitamin D deficiency was considered as a risk factor for infantile autism. Vitamin D and its receptor (VDR) are involved in the regulation of several genes controlling inflammation, immunity, cellular proliferation, differentiation, and apoptosis. The researchers found nuclear VDR activated by a metabolite of vitamin D, the 1,25-dihydroxyvitamin D(3), cooperates with some chromatin modification enzymes (i.e. histone acetyltransferases and histone deacetylases), taking a role in complex epigenetic events (Whitehouse et al., 2013; Grant et al., 2009).

CONCLUSIONS

Epigenetic dysregulation may account for a significant proportion of ASD cases. Specific chromosomal regions have been identified in autism-susceptibility *loci*, however the results have been inconclusive. There is no single gene which can account for ASD. Treatment studies are underway in some children with genetically defined syndromes, such as fragile X syndrome. Genetic research in the nearest future will allow the development of new treatments. Better understanding of how gene-environmental interactions create autistic symptoms should facilitate the development of novel therapeutic targets of gene expression for ASD. Genetic testing has the potential to provide medical explanation for ASD in children. Screening studies can identify concomitant medical problems, improve prognosis, and group families in specific support organizations.

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