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Multiple epigenetic factors predict the attention deficit/hyperactivity disorder among the Chinese Han children

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ABSTRACT

Attention deficit/hyperactivity disorder (ADHD) is one of the most common psychiatric disorders of childhood. Despite its prevalence, the critical factors involved in its development remain to be identified. It was recently suggested that epigenetic mechanisms probably contribute to the etiology of ADHD. The present study was designed to examine the associations of epigenetic markers with ADHD among Chinese Han children, aiming to establish the prediction model for this syndrome from the epigenetic perspective. We conducted a pair-matching case–control study, and the ADHD children were systematically evaluated via structured diagnostic interviews, including caregiver interviews, based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, revised criteria (DSM-IV-R). The expression levels of risk genes *DAT1, DRD4, DRD5,* as well as their promoter methylation, were determined respectively, followed by the expression profiles of histone-modifying genes *p300, MYST4, HDAC1, MeCP2*. The multivariate logistic regressions were performed to establish ADHD prediction models. All of the seven genes tested were identified as risk factors for ADHD. The methylation of one critical CpG site located upstream of *DRD4* was shown to affect its transcription, suggesting a role in ADHD's development. Aberrant DNA methylation and histone acetylation were indicated in ADHD patients. In addition, a prediction model was established using the combination of *p300*, *MYST4* and *HDAC1*, with the accuracy of 0.9338. This is, to our knowledge, the first study to clearly demonstrate the associations between epigenetic markers and ADHD, shedding light on the preliminary diagnosis and etiological studies of this widespread disorder.

1. Background

ADHD is a common childhood psychiatric disorder defined by symptoms of inattention, impulsivity and hyperactivity. Its worldwide prevalence could reach up to 5.29%, making it one of the biggest threats towards children's health (Eubig et al., 2010; Mill and Petronis, 2008; Sharma and Couture, 2014). Despite its prevalence, the etiology of ADHD has never been fully understood. On one hand, ADHD was generally regarded as a highly heritable psychiatric disorder, with an estimated heritability of 76% (Faraone et al., 2005); on the other hand, there is mounting evidence that ADHD result from complex interplay of genetic and environmental factors (Eubig et al., 2010). Actually, it has been established that exposure to environmental insults at early age could induce a range of neurobehavioral disorders, a process mediated by epigenetics, which was receiving a lot of interest recently within the psychiatric genetics community (Mill and Petronis, 2008).

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Epigenetics refers to heritable changes in gene function without a change in the nucleotide sequence, and it does not alter the function of gene product, but provides information as to when and where gene could be expressed (Callaway, 2014; Clark et al., 2006). Generally regarded as the integration of intrinsic and environmental signals into genome (Murgatroyd et al., 2009), epigenome was shaped over the life course by environmental factors (Docherty et al., 2009). Epigenetic process, primarily including DNA methylation and histone modification, was intrinsically linked to its regulation of gene expression, for instance, DNA methylation of promoter CpG islands could directly exert negative effects on the expression of objective genes. In light of this, it was well known that DNA methylation patterns could be disrupted in a growing number of pathological processes, thus aberrant methylation of promoter CpG islands was frequently considered as stable signatures for some complex diseases (Clark et al., 2006; Docherty et al., 2009; Murgatroyd et al., 2009; Philibert et al., 2013; Santoro et al., 2014). Actually, epigenetic mechanisms have been shown to contribute to several psychiatric disorders (Archer et al., 2011; Kubota et al., 2012; van Mil et al., 2014). However, the association between DNA methylation level and disease itself was quite inconsistent (Bromberg et al., 2008; Carrard et al., 2011), with both increased and unchanged methylation reported in schizophrenia patients.

DNA methylation plays roles in disease development mainly through transcriptional regulation of target genes. As for ADHD, dopaminergic system received a significant amount of attention due to its role in its function in motor control and cognition, as well as its sensitivity to environmental alterations (Gong et al., 2011; Martel et al., 2011; Zhou et al., 2010). Specifically, genetic polymorphisms concerning both dopamine transporter (DAT1) and dopamine receptor D4 (DRD4) are risk factors for ADHD (Brown, 2003; Oak et al., 2000; Xu et al., 2009). Consequently, *DAT1, DRD4, DRD5* have been widely accepted as genetically candidate genes for ADHD research (Archer et al., 2011; Faraone et al., 2005; Thapar et al., 2007). However, their changes were quite inconsistent for different studies, partly due to the recruitment of non-drug-free patients, as it is known that psychostimulant's medication is responsible for significant changes of gene expression levels.

Besides DNA methylation, histone modification is usually viewed as another layer of epigenetic regulation. Up to date, there were very few reports concerning the histone acetylation underlying ADHD, except for the recent animal trials (Kim et al., 2014; Luo et al., 2014). It was known that alterations of type I histone deacetylase (HDAC1-3, 8) were involved in epigenetic regulation among depressed patients, along with changes of some histone acetyltransferases (HATs) like p300/CBP (Sun et al., 2013). In our previous work on the animal experiments, the mRNA level of p300 showed changes for the hyperactivity led by lead exposure (Luo et al., 2014). Moreover, in a previous study, another HAT, namely MYST4, was demonstrated to play roles in pathogenesis of Noonan syndrome (Kraft et al., 2011), which was frequently accompanied by the symptoms of ADHD (Pierpont et al., 2014). Therefore, HDAC1, p300, MYST4 could be recognized as eligible candidate epigenetic markers for ADHD study.

Blood gene expression have been frequently used to study neurological disorders, due that gene expression was considered moderately correlated between central nervous system and blood lymphocytes (Cai et al., 2010). This assumption was strongly supported by a comparative transcriptome study (Cai et al., 2010), which also indicated that chromosome modifying genes were highly conserved between the tested systems: brain and blood. This research aids in other efforts to identify peripheral blood markers for neurological diseases, particularly for schizophrenia (Ilani et al., 2001; Zvara et al., 2005).

In this study, an epidemiological survey was conducted using Chinese Han children as subjects, the expression levels of both genetic and epigenetic markers were determined, analyzed and integrated, various confounding factors such as age and gender were analyzed and a significantly potent prediction model was established for ADHD. This model was constituted by histone acetylation module, which could be considered as a new step towards understanding the mystery of ADHD's development, as well as its preliminary diagnosis.

2. Results

2.1. Sample characteristics

Descriptive statistics for the examined samples were summarized in Table 1. 50 patients with ADHD and 50 non-ADHD control children took part in this investigation, with relatively equal samples for age and gender distributed into each group. A previous report showed some social-environmental factors, including preterm birth, pregnancy frequency and parental smoking were highly associated with ADHD (Archer et al., 2011), which, therefore, were also addressed in this study. As shown in Table 1, 6.25% of the mothers gave birth to their children prior to due date, 16.7% of the fathers had a long history of tobacco usage, and about 12.5% of the participants were not their parents' first child. To our regret, no associations between these factors and ADHD's development and severity were observed, partly due to the limited sample size. Besides, the contaminants representative of lead exposure were also examined. And the results showed that ADHD patients had a

Table 1
Descriptive characteristics of the study sample.

Demographic	Healthy controls, N (%)	Patients with ADHD, N (%)
Sex		
Males	40 (80.0)	40 (80.0)
Females	10 (20.0)	10 (20.0)
Total	50	50
Age (Mean ± SD)	8.3 ± 2.3	8.1 ± 2.5
Caregiver characteristics		
Premature birth	ND[a]	3 (6.25)
Not first pregnancy	ND	6 (12.5)
Paternal smoking	ND	8 (16.7)
Middle SES[b]	49 (98)	48 (96)
Contaminants		
Lead (μg/dL) (Mean ± SD)	14.4 ± 9.2	17.1 ± 9.6

[a] Not determined.
[b] SES is socioeconomic status, and middle SES is defined as the yearly income exceeding 50,000 yuan and education reaching college.