Neurotoxicology and Teratology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Developmental dynamics of the epigenome: A longitudinal study of three toddlers

Oxana Yu. Naumova[a,b,c], Mary Dozier[d], Pavel V. Dobrynin[e], Kirill Grigorev[e], Allison Wallin[d], Ida Jeltova[f], Maria Lee[g], Adam Raefski[g], Elena L. Grigorenko[a,c,g,h,*]

[a] Dept. of Psychology, University of Houston, Houston, TX, USA
[b] Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia
[c] Dept. of Psychology, Saint Petersburg State University, St. Petersburg, Russia
[d] Dept. of Psychological and Brain Sciences, University of Delaware, Newark, DE, USA
[e] Theodosius Dobzhansky Center for Genome Bioinformatics, Saint Petersburg State University, St. Petersburg, Russia
[f] Private Psychological Practice, Ridgewood, NJ, USA
[g] Child Study Center, Yale University, New Haven, CT, USA
[h] Office of the Rector, Moscow State University of Psychology and Education, Moscow, Russia

ARTICLE INFO

Keywords:
DNA methylation
MBD-seq
T-lymphocytes
Human
Early development

ABSTRACT

Epigenetic regulation plays an important role in development, at the embryonic stages and later during the lifespan. Some epigenetic marks are highly conserved throughout the lifespan whereas others are closely associated with specific age periods and/or particular environmental factors. Little is known about the dynamics of epigenetic regulation during childhood, especially during the period of rapid early development. Our study was aimed to determine whether the developmental program at the early stages of human development is accompanied by significant changes in the systems of genome regulation, specifically, by genome-wide changes in DNA methylation. Using a sequencing approach (MBD-seq) we investigated genome-wide DNA methylation patterns in the T-lymphocytes of three healthy toddlers at two timepoints within the second year of life. Pairwise comparison of the methylation patterns across the individuals and time points was conducted to determine common longitudinal changes in the DNA methylation patterns. Despite relatively high interindividual variability in their epigenetic profiles and the dynamics of these profiles during the second year of life, all children showed consistent changes in the DNA methylation patterns of genes involved in the control of the immune system and genes related to the development of the CNS. Thereby, we provide evidence that early development might be accompanied by epigenetic changes in specific functional groups of genes; many such epigenetic changes appear to be related to the rapid development of the CNS.

1. Introduction

The epigenome is comprised of modifications made to the genome that do not change its base sequence, but rather change the way in which the genome is read or transcribed. Such modifications include chemical modifications to the DNA and its histone proteins. These epigenetic records result in changes to the structure of chromatin and, as a consequence, lead to changes in the functioning of the genome at distinct loci. One important epigenetic modification is DNA methylation, or the addition of a methyl group at the 5-position of cytosine, which presumably occurs within a cytosine-guanine dinucleotide site (CpG site). CpG methylation may inhibit gene expression by physically impeding the binding of transcriptional proteins to the gene, or by chromatin remodeling via the involvement of methyl-CpG-binding domain proteins (Razin, 1998). Methylation of CpG islands—genomic regions which contain a large number of CpG dinucleotide repeats—plays the most crucial role in gene expression regulation. Most (~70%) human genes have a CpG island in their promoter region. DNA methylation, as a mechanism of gene regulation, is involved in a number of key processes including genomic imprinting, X-chromosome inactivation, the repression of transposable elements in the genome (Fazzari and Greally, 2004), and maintaining the genome activity in a cell- and tissue-specific manner. Thus, the distribution of methylated CpGs in the genome, or the DNA methylation pattern, is cell-specific and substantiates, at least in part, the structural and functional diversity of the cells and tissues of an organism.

* Corresponding author at: University of Houston, TIMES, 4849 Calhoun Rd, Houston, TX 77204-2050, USA.
E-mail address: Elena.Grigorenko@times.uh.edu (E.L. Grigorenko).

https://doi.org/10.1016/j.ntt.2017.12.006
Received 13 June 2017; Received in revised form 20 November 2017; Accepted 8 December 2017

DNA methylation is highly dynamic during early embryogenesis, when DNA methylation patterns are established during a wave of genome-wide demethylation, de novo methylation and re-methylation events, which ultimately underlie the processes of cell and tissue differentiation (Smith et al., 2012). Thereafter, DNA methylation patterns remain relatively stable over the course of the lifespan, transferring via mitotic division through generations of somatic cells. Despite the stability of cell-specific methylation patterns, genomic methylation is subject to systematic and stochastic changes over time. Some epigenomic modifications are important for reprogramming gene activity during development, while others occur in response to environmental stimuli and changes during the lifetime and with aging. This epigenetic dynamism provides plasticity for an organism as it interacts with changing developmental and environmental conditions. Research on the association of DNA methylation with age using a cross-sectional approach has shown that the dynamics of methylation patterns are highly coordinated throughout mammalian development and aging (Numata et al., 2012; Horvath et al., 2012; Hernandez et al., 2011; Alisch et al., 2012) to the point that some epigenetic marks can be used as "molecular clocks," indicating the biological age of a typically developing individual (Horvath, 2013; Koch and Wagner, 2011). Various disturbances of the epigenetic program may have a negative effect, increasing the risk of developing various chronic diseases and developmental disorders. Thus, it has been shown that DNA methylation changes are associated with many common chronic and age-related diseases (Calvanese et al., 2009; Schneider et al., 2015; Zhang and Zhang, 2015; van Otterdijk et al., 2013).

Although the important role of DNA methylation in development and aging is well-established in general, there is insufficient specific knowledge concerning the developmental DNA methylation changes that may occur within an individual. Especially little is known about the dynamics of DNA methylation during early childhood—a critical developmental period for establishing the epigenomic status, which may have a profound impact on health and well-being in later life (Waterland and Michels, 2007). Thus, most longitudinal research has been carried out with adults while studying longevity, cancer, aging, and cell senescence (Bollati et al., 2009; Bjornsson et al., 2008; Wu et al., 2012; Joyce et al., 2015; Zhang et al., 2015). Relatively few studies have examined epigenomic profiles and their longitudinal dynamics at early stages of typical development, i.e., infancy, toddlerhood, and childhood (Murphy et al., 2012; Acevedo et al., 2015; Wang et al., 2012; Martino et al., 2013; Herbstman et al., 2013; Simpkin et al., 2015). Studies of epigenomic changes in early childhood have been carried out with DNA derived from buccal cells (Murphy et al., 2012; Martino et al., 2013; Wong et al., 2010) and cord and/or peripheral blood (Murphy et al., 2012; Acevedo et al., 2015; Wang et al., 2012; Herbstman et al., 2013; Simpkin et al., 2015) using, with few exceptions (Murphy et al., 2012; Simpkin et al., 2015), a two time-point DNA-methylation design distributed across a large age period, 0–5 years of age. Main findings from these studies have indicated that, along with individual modifications of the methylome driven by non-shared environmental conditions, age-related DNA methylation changes occur in correspondence with developmental programs related, primarily, to anatomical development, cell differentiation and morphogenesis, as well as to the maturation of the immune system and the CNS. Altogether studies of age-related dynamics of the epigenome reveal that most changes in DNA methylation occur during the first years of life and then stabilize during adolescence. Therefore, detailed investigations of the implementation of epigenetic programs during early development are needed to uncover the most crucial periods of epigenomic remodeling that underlie the different developmental periods. Understanding this remodeling, in turn, will shed light on the degree of epigenetic plasticity within developmental programs.

Here we investigate whether human development in toddlerhood is accompanied by rapid and significant changes in the epigenetic regulation of gene activity. To address this issue, we identified longitudinal changes in the genome-wide DNA methylation patterns in the peripheral T-lymphocytes of three toddlers aged between 1.5 and 2 years and examined these changes in the context of the biological processes and pathways that might be subject of age-related dynamics in epigenetic regulation.

Table 1
Participants of the study.

Participant ID	Gender	Age, months	
		Time point 1	Time point 2
MDO101	Male	18	22
MDO102	Male	19	25
MDO200	Male	14	18

2. Materials and methods

2.1. Participants

The participants in this study were 3 healthy, typically developing toddlers of European (Northern Slavic) descent. Blood samples from the children were collected two times, at ages 14–19 months and then 4–6 months later (Table 1). Primary caregivers provided written consent for the blood sampling and children's participation in the study; ethical approval was obtained from the proper authorities.

2.2. DNA isolation and processing, MBD-seq

Given the high cell-specificity of DNA methylation patterns and differential white blood cell counts among individuals, we focused on a particular cell type involved in the main immune response, T-lymphocytes. Peripheral T-lymphocytes were isolated from blood samples using the Dynabeads FlowComp Human CD3 kit, and genomic DNA was isolated using the Dynabeads Silane genomic DNA kit according the manufacturer's (Invitrogen) protocols.

For genome-wide DNA methylation profiling, Methyl-CpG binding domain sequencing (MBD-seq) was utilized, in which methylated DNA is captured using the MBD2, with subsequent next generation sequencing of the eluted DNA (Aberg et al., 2012; Serre et al., 2010). Genomic DNA was fragmented using a Biorupter ultrasonicator (fragment length 400 $\pm$ 100 bp). A recombinant form of the human MBD2 and Ni NTA agarose beads (Qiagen) were applied for binding and the precipitation of DNA fragments enriched with methylated CpGs (ME-DNA). The effectiveness of the enrichment procedure was assessed by assaying the recovery of two control regions with known methylation statuses, CpGD2 (unmethylated) and PDE9A (fully methylated), using qPCR of the input genomic DNA and ME-DNA fractions. ME-DNA quality was assessed using Bioanalyzer. Paired-end sequencing libraries were constructed using Illumina's adapters, and were sequenced as 2×75 bp sequencing tags on the Illumina HiSeq 2000. Library construction and sequencing were performed according to the manufacturer's (Illumina) protocols at the Yale Center for Genome Analysis (http://ycga.yale.edu). Sequencing data have been submitted to the NCBI database—-SequenceRead Archive (SRA) and are available through accession number SRP077757 (http://www.ncbi.nlm.nih.gov/sra/SRP077757).

2.3. Sequencing data processing and gene annotation

Pre-alignment quality control of the sequencing reads was assessed using FASTQC. The sequencing reads were trimmed using Trimmomatic to remove low quality bases (Bolger et al., 2014), and were aligned to the genome (hg19) using Bowtie2 software (Langmead and Salzberg, 2012). A genome-wide methylation profile was obtained using a window calling technique implemented in a custom Python script. A DNA methylation measurements matrix was generated as the number of