Environment International 121 (2018) 31–40

Contents lists available at ScienceDirect

Environment International

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

PON1 DNA methylation and neurobehavior in Mexican-American children with prenatal organophosphate exposure

Karen Huen[a,*], Olivia Solomon[a], Katherine Kogut[b], Brenda Eskenazi[b], Nina Holland[a]

[a] Children's Environmental Health Laboratory, Global Berkeley Campus, 1301 S. 46th Street, Bldg 112, Richmond, CA 94804, USA
[b] Center for Children's Environmental Health, School of Public Health, University of California, Berkeley, 1995 University Avenue Suite 265, Berkeley, CA 94720, USA

ARTICLE INFO

Handling Editor: Heather Stapleton

Keywords:
Epigenetics
Cognition
IQ

ABSTRACT

PON1 is a multifunctional enzyme involved in oxidative stress and detoxification of some organophosphate (OP) pesticides. It has been associated with nervous system diseases like Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and autism. We previously found that *PON1* susceptible genotypes were associated with lower IQ scores in children. Epigenetic marks, such as DNA methylation, can regulate gene expression. Yet, data on whether DNA methylation may influence the relationship between *PON1* levels and neurobehavior are limited. In this study, we used Illumina 450K and EPIC BeadChip arrays to assess *PON1* DNA methylation in blood specimens collected from children (n = 238) at birth (cord blood) and age 7 years and examined their relationship with cognitive outcomes. The Wechsler Intelligence Scale for Children was used to assess Full Scale IQ and four composite measures (Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed Indexes) in 7-year-old children. We observed a consistent yet nonsignificant inverse relationship of methylation at several CpG sites close to the *PON1* transcription start site with Full Scale IQ and other composite measures of cognition. We also found an inverse relationship between cord blood methylation at cg15887283 with working memory and a positive association of 7-year-old methylation at cg22798737 with processing speed, independent of OP exposure. However, none of the associations remained significant after accounting for multiple comparisons. This study provides some evidence of the role DNA methylation may play in the known relationship between *PON1* and neurobehavior in children, however it appears to be only suggestive and warrants additional research.

1. Introduction

The paraoxonase 1 (*PON1*) gene encodes a multifunctional enzyme that is involved both in detoxification of certain organophosphate (OP) pesticides and oxidative stress pathways (Costa et al., 2013; Li et al., 2003). Studies have implicated oxidative stress as an important mechanism in the pathogenesis of neurodegenerative diseases and developmental programming of neurodevelopmental deficits (Gandhi and Abramov, 2012; Wells et al., 2009). *PON1* genetic polymorphisms and/or enzyme measurements have been associated with a number of diseases of the nervous system, including Alzheimer's disease (Erlich et al., 2006; Leduc and Poirier, 2008; Paragh et al., 2002), amyotrophic lateral sclerosis (Saeed et al., 2006; Slowik et al., 2006), Parkinson's disease (Zintzaras and Hadjigeorgiou, 2004), and brain tumors (Kafadar et al., 2006). In children, PON1 enzyme activity levels were lower in children with autism (Pasca et al., 2010; Pasca et al., 2006). Additionally, in a prospective cohort of children (ages 6–9 years) from New York City, urinary OP metabolite levels were associated with poorer scores in Perceptual Reasoning and Full Scale IQ (FSIQ) only among children whose mothers had the susceptible $PON1_{192\ QQ}$ genotype (Engel et al., 2011). We previously found that maternal urinary OP metabolite levels were associated with poorer Bayley Mental Development Index Scores in 2-year-olds (Eskenazi et al., 2010) and IQ at age 7 (Eskenazi et al., 2014) among Mexican-American children and their mothers from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) birth cohort. Associations between

Abbreviations: ASMN, All Sample Mean Normalization; AREase, arylesterase; BMIQ, beta mixture quantile; CDC, Centers for Disease Control and Prevention; CHAMACOS, Center for the Health Assessment of Mothers and Children of Salinas; CV, coefficient of variation; DAP, dialkyl phosphate; DE, diethyl; DM, dimethyl; FSIQ, full-scale IQ; LOD, limit of detection; OP, organophosphate; *PON1*, paraoxonase 1; PPVT, Peabody Picture Vocabulary Test; PRI, Perceptual Reasoning Index; PSI, Processing Speed Index; TSS, transcription start site; VCI, Verbal Comprehension Index; WISC, Wechsler Intelligence Scale for Children; WMI, Working Memory Index

* Corresponding author at: School of Public Health, UC Berkeley, 50 University Hall, CA 94720-7360, USA.
E-mail address: khuen@berkeley.edu (K. Huen).

https://doi.org/10.1016/j.envint.2018.08.044
Received 19 April 2018; Received in revised form 17 August 2018; Accepted 18 August 2018

maternal OP exposure and MDI were strongest in children with the *PON1*$_{-108T}$ allele. Furthermore, the relationship between OPs and FSIQ was strongest in children of mothers with the lowest tertile of arylesterase (AREase) activity, a measure of PON1 enzyme quantity. We also found a direct relationship of *PON1* with neurodevelopment, demonstrating associations of the child *PON1*$_{-108T}$ allele (the allele linked to lower PON1 activity) with lower MDI scores at age 2 and maternal pregnancy AREase levels with lower WISC scores at age 7. The relationship between PON1 and adverse neurodevelopment likely involves both its ability to detoxify OP pesticides and its role in oxidative stress.

We have reported a broad variability (> 100-fold range) of PON1 levels and substrate-specific activities among CHAMACOS children and mothers (Furlong et al., 2006; Holland et al., 2006) that could affect differential susceptibility among individuals. Although genetic variants, particularly *PON1* promoter polymorphism (*PON1*$_{-108}$; rs705379), influence *PON1* gene expression and protein levels, they explain < 25% of the variability of PON1 protein levels (Huen et al., 2010). Epigenetic marks, like DNA methylation, can affect gene expression without changes in DNA sequence. We previously showed that DNA methylation in the *PON1* gene can mediate the effect of the *PON1*$_{-108}$ genotype on PON1 protein levels (Huen et al., 2015). One recent study reported inverse associations between *PON1* DNA methylation in cord blood and cognition in young children aged 2 to 5 years exposed to prenatal mercury (Cardenas et al., 2017) but there is no research examining relationships of *PON1* DNA methylation with cognition in older school-aged children when PON1 enzyme levels approach that of adults (Gonzalez et al., 2012; Huen et al., 2010). We hypothesize that increased *PON1* DNA methylation levels may detrimentally influence children's cognitive function. In this study, we examined associations of *PON1* DNA methylation in blood assessed at birth and age 7 with children's cognitive abilities as measured by Wechsler Intelligence Scale for Children (WISC) at age 7.

2. Materials and methods

2.1. Study subjects

CHAMACOS is a longitudinal birth cohort study of mothers and their children living in the agricultural region of Salinas Valley, California. Pregnant women enrolled in the study (1999–2000) were at least 18 years of age, < 20 weeks gestation, Spanish- or English-speaking, eligible for low-income health insurance, receiving prenatal care at one of the participating community clinics, and planning to deliver at the local public hospital. Six hundred and one pregnant women were enrolled and 526 remained in the study at delivery of live, singleton newborns (Eskenazi et al., 2003). In this study, we restricted analysis to children who had blood samples available for analysis at birth (cord blood) and/or age 7 years and who also completed neurobehavioral assessments at age 7 years. In total, 238 children were included in the analysis. Of these, 185 had methylation data at both time points and 53 children had methylation data available at birth only. Children included in the study did not differ from all children in the cohort by other demographic and exposure variables (e.g. poverty level, maternal marital status, or maternal prenatal farm work status, maternal OP urinary metabolite levels, or use of alcohol or tobacco).

Study protocols were approved by the University of California, Berkeley and the Centers for Disease Control and Prevention (CDC) Committees for the Protection of Human Subjects. Written informed consent was obtained from all mothers, and children provided verbal assent at age 7.

2.2. Blood collection and processing

Blood specimens from CHAMACOS children were collected from umbilical cords after delivery and by venipuncture when children were approximately 7 years old. Heparinized whole blood was collected in BD vacutainers® (Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were then centrifuged, separated into aliquots of plasma, buffy coats and red blood cells, and then stored at −80 °C at the School of Public Health Biorepository, University of California, Berkeley. Whole blood was also collected in BD vacutainers® (Becton, Dickinson and Company, Franklin Lakes, NJ) containing no anticoagulant. These samples were centrifuged, divided into serum and clot, and then stored at −80 °C.

2.3. Bisulfite treatment and DNA methylation analyses

DNA isolation from clots was performed using a QIAamp Blood DNA Maxi kit (Qiagen, Inc., Santa Clarita, CA) as previously described (Holland et al., 2006). DNA was normalized to 55 μg/ml and bisulfite conversion was performed on 1 μg aliquots of DNA using Zymo Bisulfite conversion Kits (Zymo Research, Orange, CA). Methylation levels at 18 *PON1* CpG sites in cord blood were analyzed as part of a genome-wide methylation assessment using the Illumina Infinium 450K DNA methylation BeadChip as previously described (Huen et al., 2015). The EPIC BeadChip is the most recently released version of Illumina's methylation array and replaces the previous 450K BeadChip. It covers > 90% of the CpG sites assessed by 450K and extends coverage to an additional > 400,000 sites. The EPIC BeadChip, was used to assess DNA methylation in samples collected from 7-year-old children because the 450K BeadChip was no longer available at the time of the experiment. EPIC includes 19 *PON1* CpG sites, 15 of which were also included in the 450K BeadChip. All *PON1* CpG sites assessed by either platform are shown graphically in Fig. 1 and described in Supplemental Table 1. Pidsley et al. (2016) reported that methylation levels assessed by EPIC and 450K BeadChip arrays were very highly correlated with Spearman Rank Correlation coefficients of 0.99 and it was suggested that data from the two platforms could easily be integrated for analysis. We recently reported similar findings in CHAMACOS children (Solomon et al., 2018) finding a strong correlation of overall methylation between both platforms. However, correlations at individual CpG sites, including those located in the *PON1* gene, tended to be weaker (Pearson's *r*: 0.02–0.18) at the extreme levels of methylation (highly methylated or highly unmethylated) compared to moderately methylated CpG sites (Pearson's *r*: 0.27–0.59). When we assessed differential methylation by sex using data from both assays, we confirmed good reproducibility, replicating the majority of significant hits with correlated effect sizes between platforms.

DNA samples were whole genome amplified, enzymatically fragmented, purified, and applied to the Infinium BeadChips according to the Illumina methylation protocol (Bibikova et al., 2011; Pidsley et al., 2016; Sandoval et al., 2011). BeadChip processing was performed using robotics and the Illumina Hi-Scan system was used for analysis. Samples included in the analysis had detection *p*-values below 0.01 for 95% of CpG sites. Three *PON1* CpG sites (2 in 450K and 3 in EPIC) with common SNPs (minor allele frequency > 5%) within 50 bp of the target identified in the MXL (Mexican ancestry in Los Angeles, California) HapMap population were excluded, resulting in a total of 16 450K and 16 EPIC *PON1* CpG sites included in the subsequent analyses. Raw signal intensities were background corrected and then normalized for color-channel bias using the All Sample Mean Normalization (ASMN) method as described previously by Yousefi et al. (2013). We also applied beta mixture quantile (BMIQ) normalization to make interpretation between type I and type II probes comparable (Teschendorff et al., 2013). Methylation data were expressed as M-values, which are calculated as the $\log_2$ ratio of the intensities of methylated to unmethylated probes (Du et al., 2010). Quality assurance procedures included use of repeats and internal standards to minimize technical variability.