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Distributional changes in gene-specific methylation associated with temperature

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ABSTRACT

Temperature has been related to mean differences in DNA methylation. However, heterogeneity in these associations may exist across the distribution of methylation outcomes. This study examined whether the association between three-week averaged of temperature and methylation differs across quantiles of the methylation distributions in nine candidate genes. We measured gene-specific blood methylation repeatedly in 777 elderly men participating in the Normative Aging Study (1999–2010). We fit quantile regressions for longitudinal data to investigate whether the associations of temperature on methylation (expressed as %5mC) varied across the distribution of the methylation outcomes. We observed heterogeneity in the associations of temperature across percentiles of methylation in *F3*, *TLR-2*, *CRAT*, *iNOS*, and *ICAM-1* genes. For instance, an increase in three-week temperature exposure was associated with a longer left-tail of the *F3* methylation distribution. A 5 °C increase in temperature was associated with a 0.15%5mC (95% confidence interval (CI): −0.27, −0.04) decrease on the 20th quantile of *F3* methylation, but was not significantly related to the 80th quantile of this distribution (Estimate:0.06%5mC, 95%CI: −0.22, 0.35). Individuals with low values of *F3*, *TLR-2*, *CRAT*, and *iNOS* methylation, as well as a high value of *ICAM-1* methylation, may be more susceptible to temperature effects on systemic inflammation.

1. Introduction

Ambient temperature has been related to increased risk for cardiovascular mortality (Koken et al., 2003). Mechanisms by which temperature causes cardiovascular mortality are not fully elucidated, but include the production of heat-shock proteins and inflammation (Moseley, 1998). Moreover, inflammation has been related to cardiovascular disease (Bucova et al., 2008; Lee et al., 2011; Libby, 2002). Recent research has pointed to epigenetics as a potential mechanism for the adverse effects of temperature (Bind et al., 2014a, 2014b; Marsh and Pasqualone, 2014). We recently conducted a mediation analysis in the Normative Aging Study cohort and found that the association between temperature and the intercellular adhesion molecule 1 (ICAM-1) protein was mediated via a change in *ICAM-1* methylation (Bind et al., 2016). Epigenetics relates to changes in chromatin structure that influence gene expression, but do not modify the genetic code. The most frequently examined epigenetic mechanism is DNA methylation, which involves methylation of cytosine in CpG dinucleotides.

Three-week averaged temperature increase has been associated to increases in mean in DNA methylation measured on the *ICAM-1* and carnitine O-acetyltransferase (*CRAT*) genes and to decrease in mean methylation measured on the toll-like receptor 2 (*TRL-2*) gene (Bind et al., 2014a, 2014b). This existing work used standard regression methods that estimate the change in the mean of an outcome for a given change in exposure. However, focus on the expected value of the response does not fully describe effects of exposure on the shape rather than the location, of the outcome distribution. DNA methylation is measured as the proportion of cells that carry methylated CpG sites at a given location. For several genes, particularly if related to cell signaling such as those on inflammatory pathways, the transition of subset of cells from a non-methylated to a methylated state – or vice versa – might result in the recruitment of a larger proportion of cells resulting in signal amplification. This process has been suggested to determine an apparent threshold effect in methylation dynamics

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as well as in the regulation of gene expression (Liu et al., 2013). Because DNA methylation is a biological mechanism whereby cells control gene expression in this complex manner (Riggs and Xiong, 2004), we hypothesized that mean regression analyses may not capture associations that occur primarily in one extreme of the outcome distribution, and therefore would miss exposure-outcome associations in susceptible groups.

This study investigates whether the association between temperature and DNA methylation varies across nine deciles of the methylation distribution (10th to 90th). Quantile regression is distribution free, thereby allowing one to avoid the assumption of normality of traditional mean regression methods. We focus on gene-specific methylation on nine candidate genes related to heart disease. For genes with low levels of methylation, we hypothesize that temperature decrease (e.g., in winter) or temperature increase (e.g., in summer) may be associated with methylation decrease that could lead to some increased production of cardiovascular-related proteins. However, for gene with medium and high levels methylation, because of the complexity of the methylation machinery, it is less clear how temperature may affect genes methylation. We chose to study an elderly cohort that is susceptible to extreme temperatures (Kim and Joh, 2006).

2. Materials and methods

2.1. Study population

Participants included in this analysis came from the Normative Aging Study (NAS), a longitudinal investigation established in Boston in 1963 by the U.S. Veterans Administration. (Bell et al., 1966) We measured DNA methylation on a total of 777 individuals one to five times with intervals of three to five years ($n_{observations} = 1798$). We assessed DNA methylation on blood samples collected after smoking abstinence and an overnight fast over the 1999–2010 period. Participants with levels of C-reactive protein over 10 mg/L were excluded so that results are not confounded by infection state (Simon et al., 2004).

2.2. Temperature

The time window of temperature exposure most relevant for changes in DNA methylation is unclear. A recent marine study suggested an association spread over four weeks (Marsh and Pasqualone, 2014). Adult worms were cultured for four weeks at two temperatures (-1.5 °C as ambient control and $+4$ °C as warm treatment). About 3000 sites evidenced a change in methylation state and among them 85% involved a gain of a 5-methyl group on a CpG site (net increase in methylation). In the same cohort investigated in this study (i.e., Normative Aging Study), we observed associations between temperature averaged up to three weeks preceding the medical visit and the mean of gene-specific methylation distributions (Bind et al., 2014a, 2014b). Therefore, we chose *a priori* to explore the same intermediate-term exposure window and focused on temperature averaged over the three-week period preceding each participant's methylation assessment. We examined only one exposure window to limit the number of tests.

We obtained measurements of ambient temperature from Boston Logan Airport weather station, which is located 8 km from the medical center. Since most of the participants live throughout the metropolitan area, we assumed that the monitored temperature can serve as a surrogate of their exposures.

2.3. DNA methylation

We collected participant's blood at every visit and isolated DNA to assess gene-specific DNA methylation using highly quantitative methods based on bisulfite polymerase chain reaction pyrosequencing (Baccarelli et al., 2010a). Nine candidate genes that were expressed in leukocytes and plausibly related to cardiovascular disease were chosen for high precision pyrosequencing analysis *a priori* as part of a previous study. We previously examined the mean association between temperature and methylation on the same set of genes (Bind et al., 2014a, 2014b).

We measured methylation levels in tissue factor (*F3*), intercellular adhesion molecule 1 (*ICAM-1*), toll-like receptor 2 (*TRL-2*), carnitine O-acetyltransferase (*CRAT*), 8-oxoguanosine DNA glycosylase-1 (*OGG1*), interferon gamma (*IFN-γ*), inducible nitric oxide synthase (*iNOS*), and glucocorticoid receptor (*GCR*) genes, at two to five CpG positions within each gene's promoter region (see more details in Table 1). The degree of methylation was expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines at position 5 (%5mC), and each gene-specific outcome was calculated as the mean values of the position-specific measurements. Interleukin 6 (*IL-6*) methylation was quantified outside the gene's promoter region.

2.4. Statistical methods

After ruling out any deviation from a linear relationship between temperature and mean methylation (using cubic splines), we investigated whether temperature levels averaged over the three-week period before assessment was linearly associated with nine deciles (10th to 90th) of the methylation distribution. In our regression models, the dependent variable was gene-specific DNA methylation. Because 70% of the participants had repeated methylation measures, we fit quantile regressions for longitudinal data and report the associations on the additive scale (Koenker, 2004). Briefly, this approach fits fixed-effects and correlated-random-effects quantile regression models and relies on Bootstrap inference. Median regression chooses regression coefficients that minimize the sum of the absolute values of the residuals instead of the sum of squared residuals. The result is an estimate of covariate effects on the median, instead of the mean, of the outcome distribution. Quantile regression generalizes this approach by weighting the positive and negative residuals differently, which forces the regression line through other percentile of the distribution, with the exact percentile depending on the exact weighting of the residuals. We use the “rqpd” R package that allows for positive correlation between the repeated measures of outcomes (Koenker, 2004).

We reported estimated quantile regression coefficients, which correspond to differences in methylation (expressed in %5mC) at that quantile associated with a 5 °C increase in temperature. We adjusted for the following potential confounders: relative humidity, sine and cosine terms as a function of day of the season, and batch of methylation measurement. We also controlled for the following factors likely to influence methylation but not exposure: age, diabetes, body mass index, smoking status, statin use, as well as percentages of neutrophils, lymphocytes, monocytes, and basophils in differential blood count (Baccarelli et al., 2010b).

We conducted sensitivity analyses that 1) examined different exposure time windows (24 h-mean and 7-day moving average) and 2) adjusted for 3-week moving average of $PM_{2.5}$ concentrations.