



Pregnant women's cognitive appraisal of a natural disaster affects their children's BMI and central adiposity via DNA methylation: Project Ice Storm



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ARTICLE INFO

Article history:

Received 18 July 2016

Received in revised form 29 August 2016

Accepted 19 September 2016

ABSTRACT

We determined the extent to which DNA methylation mediates the effects of maternal cognitive appraisal of a natural disaster during pregnancy on offspring growth at age 13. Negative maternal cognitive appraisal predicted both lower BMI and central adiposity via DNA methylation of diabetes-related genes, suggesting a protective role of epigenetics.

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1. Introduction

The theory of developmental origins of health and disease (DOHaD) hypothesizes that much of the individual variance in health outcomes depends on factors that impact life in the womb, including “stress” to the pregnant mother. Prenatal maternal stress (PNMS) has been demonstrated to affect offspring health outcomes throughout childhood and into adulthood in both human and animal populations [1,2]. The mother's physical and hormonal reactions are thought to be the main mechanisms underlying the effects of stress during pregnancy on lifelong health outcomes in the exposed fetus. It has been shown that during a stressful event a pregnant woman's cortisol can overwhelm the placenta's natural filter (11β-HSD-2 converts maternal cortisol to inactive cortisone), affecting the developing fetus, with lifelong effects on behavioral, cognitive, motor, and physical development. However, the potential molecular mechanism(s) underlying this phenomenon remain to be clearly elucidated. Epigenetics, especially DNA methylation, has emerged as a candidate mediator of the programming process. A growing body of evidence supports the effects of environmental factors, such as prenatal maternal stress, on epigenetic profiles in both animals and humans. Despite these findings, neither animal nor most human research methodologies are able to capture the different elements involved in the human stress experience. As described by Lazarus and Folkman [3], individuals first experience varying degrees of objective hardship due to an event. Second, there is a two-part cognitive appraisal

element in which the individual perceives the event as a significant threat (or not), and judges (consciously or unconsciously) whether his or her resources are sufficient to meet the threat. In a situation judged to be threatening for which one's resources are perceived to be insufficient, subjective distress will be experienced.

Project Ice Storm provides a unique opportunity to study all of the above factors from an independent stressor due to a natural disaster. Project Ice Storm has been following a cohort of children whose mothers were pregnant during one of the worst natural disasters in Canadian history – the 1998 Quebec ice storm. The strength of this study is that the stressor is “independent” and, thus, not associated with potentially confounding maternal characteristics and socioeconomic status, approximating random assignment to stress conditions. More severe maternal objective hardship, but not subjective distress, predicts greater body mass index (BMI) and risk for obesity at age 5 years [4], an effect that increases in magnitude through childhood and adolescence [5]. Despite the findings on wide-ranging effects of PNMS due to the ice storm [6], the molecular mechanisms underlying these effects need to be fully explored. We have analyzed DNA methylation levels, the best characterized epigenetic mechanism, among a subset of 13-year-old Project Ice Storm children. Our first epigenetic finding demonstrated the effect of mothers' degree of objective PNMS from the ice storm on children's genome-wide DNA methylation levels, showing that maternal objective PNMS, but not subjective distress, was significantly correlated with 1675 CG sites affiliated with 957 genes predominantly related to immune function and metabolism [7]. Subsequently, we have also observed effects of maternal cognitive appraisal on the children's DNA methylation levels. The methylation levels of 2872 CGs differed significantly between children whose mothers had positive or

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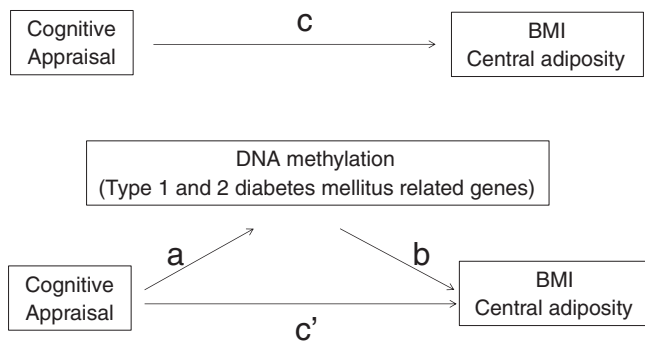


Fig. 1. Mediation analysis on the relationship between exposure to cognitive appraisal and BMI and central adiposity. Path *a* is the effect of maternal cognitive appraisal (predictor variable) on DNA methylation (mediator); path *b* is the effect of DNA methylation on BMI and central adiposity (outcome variable) controlling for maternal cognitive appraisal; and path *c'* is the direct effect of the maternal cognitive appraisal on growth outcomes controlling for DNA methylation. The path *a**b* indicates the indirect/mediating effect of maternal cognitive appraisal on BMI and central adiposity through DNA methylation (mediator). Path *c* is the total effect of maternal cognitive appraisal on BMI and central adiposity. This model can be represented by the following equation: $c = c' + a \cdot b$ (total effect = direct effect + indirect effect).

negative cognitive appraisals of the ice storm. Once again, these differentially methylated genes were prominently involved in the biological pathways related to immune function, and to a lesser extent metabolic functioning [8].

In a growing body of literature relating to DOHaD, prenatal maternal stress has been found to contribute to metabolic diseases in later life such as diabetes and obesity in both animals and humans [9–15]. Moreover, the association between childhood or adolescent BMI/obesity and DNA methylation has been well studied, as reviewed by Demetriou et al. [16]. Despite many interesting findings, few studies have been able to conduct empirical tests of these effects using mediation analyses. A mediating effect, described by Baron and Kenny, is the possible effect of a third variable, intervening in an existing relationship between a predictor and an outcome [17]. Previously, we performed mediation analyses and demonstrated that genome-wide DNA methylation levels mediate the effects of objective stress on children's BMI and waist to height ratio (WHtR), an index of central adiposity [18] and their cytokine secretion levels [19] during adolescence.

Given that pregnant women's cognitive appraisals of an independent stressor would have widespread effects on their unborn children's DNA methylation levels, we could hypothesize that this aspect of maternal stress could impact children health outcomes in later life through DNA methylation mediation effect. Therefore, the objective of this study was to determine whether a negative cognitive appraisal of pregnant women towards the 1998 Quebec ice storm could impact their children's BMI and central adiposity at age 13 via DNA methylation of genes related to type 1 and 2 diabetes mellitus pathways.

2. Materials and methods

2.1. Participants and assessments

In June 1998, 5 months after the ice storm, we identified women who were 18 years or older, and who were pregnant on January 9, 1998 (the peak of the ice storm), or who became pregnant within 3 months of that date. The 224 women recruited answered postal questionnaires about their ice storm experiences. Of 224 women who responded, 176 returned consented to follow-up and sent back the second questionnaire, "Outcomes of the pregnancy". The complete assessment of mother's objective PNMS, subjective PNMS and cognitive appraisal was described elsewhere [8]. Briefly, to assess cognitive appraisal, women responded to the following item: "Overall, what were the consequences of the ice storm on you and your family?" Response

options were on a five-point scale of "Very negative" (1), "Negative" (2), "Neutral" (3), "Positive" (4), and "Very positive" (5).

In 2011, a subgroup of 34 children from Project Ice Storm agreed to participate in a blood draw that included the epigenetic study. The subgroup cohort and self-excluded cohort did not differ with respect to any maternal variables (psychological functioning, life events, obstetric complications, socioeconomic status, education), child variables (birth weight, birth length, gestational age), or storm-related variables (objective hardship, subjective distress and cognitive appraisal). At the same time as the blood draw, we measured height, weight, and waist circumference following standard anthropometric procedures [20], and computed BMI (kg/m^2) and WHtR. Of the 34 mothers whose children participated in the epigenetic study, none had rated the consequences of the storm as "Very negative", while 12 (35.3%) had given a rating of "Negative", 4 (11.8%) had given a "Neutral" rating, 17 (50.0%) had given a rating of "Positive", and 1 (2.9%) had considered the consequences "Very positive". We identified 3 women (2 with Negative and 1 with Positive appraisal) who had gestational diabetes; the data from their children were excluded in order to avoid confounding effects of the maternal gestational diabetes on DNA methylation. As our interest was the effect of negative cognitive appraisal of the ice storm on child outcomes, we created a "Negative cognitive appraisal group" (recoded as 0) ($n = 10$) and a "Neutral/Positive cognitive appraisal group" (recoded as 1) by combining the participants who had rated the consequences as neutral, positive and very positive ($n = 21$), for a final sample size of 31.

2.2. Ethics statement

All phases of this study were approved by the Research Ethics Board of the Douglas Hospital Research Center in Montreal, Canada. We obtained written informed consent from parents and written assent from children.

2.3. Selection of candidate genes for testing mediation by methylation

In a previous publication, we identified genes from isolated T cells whose methylation signatures were associated with cognitive appraisal levels [8]. In order to investigate the mediation effect of DNA methylation on BMI and central adiposity at age 13½ years, we matched genes from the type 1 and 2 diabetes mellitus pathways, as classified by IPA software (www.ingenuity.com), whose methylation had been significantly correlated with cognitive appraisal. In total, 28 genes (86 CpGs) and 19 genes (29 CpGs) were selected from the type 1 and 2 diabetes mellitus pathway, respectively. After excluding overlapping CpGs, there were 105 unique CpGs corresponding to 41 genes which were used for further analyses (Table S1).

2.4. Statistical analysis

We first analyzed whether BMI and WHtR were significantly different between groups (Negative versus Neutral/Positive). As BMI and WHtR were not normally distributed, nonparametric Mann-Whitney U tests were conducted.

Next, we tested mediation models to determine the extent to which DNA methylation mediates the effect of maternal cognitive appraisal on BMI and WHtR (Fig. 1). The current mediation analysis included BMI or central adiposity as the outcome, cognitive appraisal level as the predictor, and DNA methylation levels of CpG sites associated with type 1 and 2 diabetes pathways (105 CpG sites in 41 genes) as mediators. We used routine bootstrapping methods to determine the significance of mediation effects [21]. Bootstrapping involves randomly resampling with replacement participants from the dataset and then computing the desired statistic in each resample, providing confidence intervals by which the significance of a mediation effect can be determined. To that end, 95% bias-corrected bootstrap confidence intervals were

computed, as explained by Hayes [22]. The PROCESS procedure for SPSS [22] was used to conduct the analyses. Each bootstrap procedure resampled the initial sample 10,000 times. In order to be able to recover the same results, the random seed for all bootstraps was fixed. A mediation effect was considered significant if 0 was not included in the bootstrap confidence interval. All analyses were conducted using SPSS (Version 20, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Cognitive appraisal, BMI and central adiposity (WHtR)

The average BMI scores in the negative and neutral/positive cognitive appraisal groups were 20.86 (SD = 3.73) and 22.84 (SD = 5.19), respectively. The average WHtR in the negative and neutral/positive cognitive appraisal groups were 0.43 (SD = 0.04) and 0.45 (SD = 0.06), respectively. After Mann-Whitney U analyses, neither BMI nor WHtR were significantly different between the groups.

3.2. Mediation analyses

3.2.1. BMI

Five of the 105 CpG sites were found to significantly positively mediate the effect of cognitive appraisal on BMI, that is, negative cognitive appraisal predicted lower BMI through DNA methylation (the values of $a*b$ were positive, and the confidence intervals did not contain 0) (Table S2). Negative cognitive appraisal was associated with higher methylation levels of 4 CpGs (cg11586857 in *LTA*, cg00689225 in *NFKBIA*, cg09254210 and cg17306848 in *PRKCH*; (the values of a were negative)), which in turn, were associated with lower BMI scores (the value of b were negative). Negative cognitive appraisal was associated with lower methylation levels of 1 CpG (cg17316649 in *HLA-DRB1*; (the values of a were positive)), which in turn, were associated with lower BMI scores (the values of b were positive). Together, the combination of cognitive appraisal and DNA methylation of these 5 CpGs explained between 9.05% and 15.82% of the variance in BMI.

3.2.2. WHtR

Three CpGs corresponding to 3 genes showed a significant positive mediation effect on WHtR, that is, negative cognitive appraisal predicted lower WHtR through DNA methylation (the value of $a*b$ was positive, and the confidence interval did not contain 0) (Table S3). Negative cognitive appraisal was associated with higher DNA methylation level of cg11586857 in *LTA* (the values of a were negative), which in turn, were associated with lower WHtR score (the value of b were negative). Negative cognitive appraisal was associated with lower methylation levels of cg17316649 and cg17376015 in *HLA-DRB1*, which in turn, were associated with lower WHtR. Together, the combination of cognitive appraisal and DNA methylation of these 3 CpGs explained between 11.27% and 17.07% of the variance in WHtR.

4. Discussion

Cognitive appraisal (subjective assessment of situational demands and available resources) of a stressful event has been considered as a predictor variable in stress responses. The main goal of this study was to determine whether negative maternal cognitive appraisal of the 1998 Quebec ice storm during pregnancy could impact children's BMI and central adiposity at age 13 via DNA methylation of genes related to type 1 and 2 diabetes mellitus pathways. We observed significant positive mediation effects on both BMI and central adiposity, that is, negative maternal cognitive appraisal predicted both lower BMI and central adiposity via DNA methylation, suggesting a protective role of the selected CpGs/genes related to type 1 and 2 diabetes mellitus pathways.

Five of the 105 CpG sites (4.7%) showed positive mediation effects on children's BMI and three (2.8%) on central adiposity. These eight CpGs are located on 4 genes (*HLA-DRB1*, *LTA*, *NFKBIA* and *PRKCH*). Therefore, negative maternal cognitive appraisal could predict lower BMI or central adiposity through either lower (*HLA-DRB1*) or higher (*LTA*, *NFKBIA* and *PRKCH*) methylated CpGs, depending on the genes. Thus, it seems that the methylation of the selected type 1 (*HLA-DRB1*, *LTA*, *NFKBIA*) and type 2 (*NFKBIA* and *PRKCH*) diabetes-related genes protects offspring from higher BMI and central adiposity levels. Despite the small number of significant findings (8 of 105 CpGs), the direction of the present findings replicate our previous Project Ice Storm finding that type 1 and 2 diabetes-related CpGs/genes mediated the association between objective PNMS and children's BMI and central adiposity at age 13 [18], that is, higher objective PNMS predicted lower BMI and central adiposity through DNA methylation, also suggesting a protective effect of DNA methylation from the selected genes. Interestingly, cg11586857 corresponding to *LTA* and cg00689225 corresponding to *NFKBIA* are of particular interest, as they were also found to significantly mediate the effect of objective PNMS on BMI and central adiposity even though objective hardship and cognitive appraisal are not highly correlated with each other ($r = 0.296$). *LTA* is involved in regulating the innate and adaptive immune systems [23] and is a well-identified pro-inflammatory cytokine produced by lymphocytes. Polymorphisms of *LTA* have been reported to be associated with metabolic traits such as diabetes [24–26]. Similarly, *NFKBIA*, involved in encoding cellular proteins that function to inhibit NF-kappa-B, is associated with diabetes mellitus and obesity-induced insulin resistance [27,28]. Together, our findings suggest that common CpGs/genes related to type 1 and 2 diabetes mellitus could play a protective role by mediating effects of both disaster-related objective PNMS and negative maternal cognitive appraisal on BMI and central adiposity outcomes.

Cognitive appraisal has been implicated in the cortisol stress response. For example, two independent research groups both reported that cognitive appraisal of the stressor predicted cortisol reactivity using acute stress from the Trier Social Stress Test [29,30]. Based on the DOHaD theory, we could speculate that maternal cognitive appraisal of the ice storm altered maternal cortisol secretion, which then altered fetal development resulting in life-long consequences. This process is believed to work through epigenetic programming, such as DNA methylation.

This study is limited by the small sample size. Given that a total of 105 CpGs were selected for mediation analyses for each outcome, the five CpGs showing mediation effects on BMI and three on central adiposity are possibly due to chance. As only 31 children were involved in this epigenetic study, we could not use the full range of 5 cognitive appraisal ratings (very negative through very positive) in the mediation analyses. Moreover, combining the neutral and positive groups possibly reduced the effect of positive cognitive appraisal; this combination, however, increased our sample size and isolated the effects of negative appraisal. Furthermore, gene expression was not able to be assessed since RNA was not obtained from this cohort. Thus, an independent replication with a larger sample is warranted to address these issues in the future.

Despite these limitations, this is the first study investigating the mediation effect of DNA methylation on the association between prenatal maternal cognitive appraisal from a natural disaster and children's health outcomes.

In conclusion, we observed positive mediation effects of DNA methylation on both BMI and central adiposity, that is, the pregnant woman's negative cognitive appraisal predicted both lower BMI and central adiposity via DNA methylation, suggesting a protective role of the selected CpGs/genes related to type 1 and 2 diabetes mellitus pathways. Our finding suggests that women's negative cognitive appraisals of an independent stressor during their pregnancy might plausibly be associated with child metabolic outcomes through DNA methylation.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgement

We are grateful to families for their continued participation in Project Ice Storm. This research was supported by a grant (MOP-1150067) from the Canadian Institute of Health Research (CIHR).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.earlhumdev.2016.09.013>.

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