EPOCH EPIGENETICS STUDY PROPOSAL:

Epigenetically-regulated BMI growth trajectories in the EPOCH Study cohort

INVESTIGATOR

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PROJECT SUMMARY

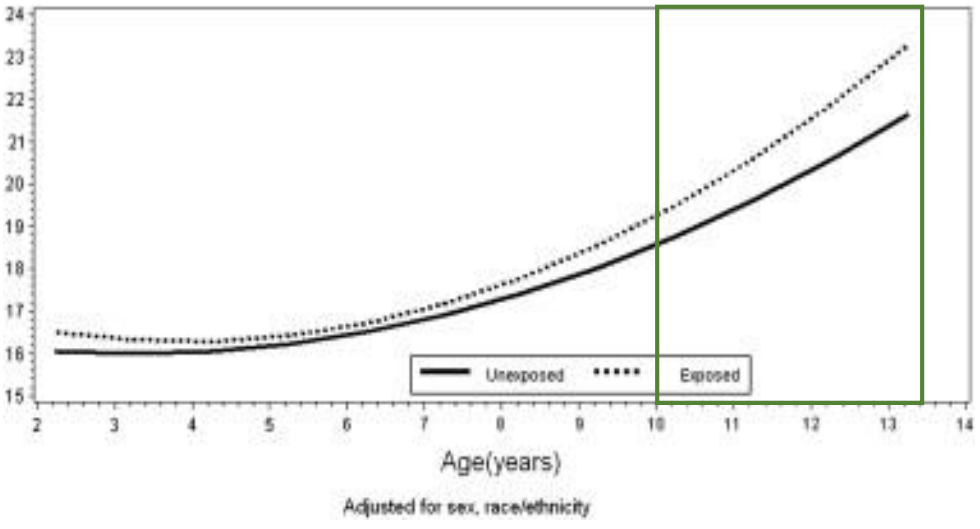
DNA samples obtained from participants in the EPOCH Study will be analyzed to evaluate the relationship between DNA methylation and BMI z-scores measured at the EPOCH I and EPOCH II study visits (T1 and T2, respectively). The proposed analysis was designed to complement the specific aims of the funded EPOCH Epigenetics grant, "Epigenetic marks of in utero exposure to diabetes" (5R01DK100340-03).

BACKGROUND & RATIONALE

The EPOCH Epigenetics Study strives to characterize the epigenetic pathways through which exposure to maternal gestational diabetes (GDM) increases offspring risk for adiposity-related outcomes. One priority of the grant is to understand the role of DNA methylation in mediating the well-demonstrated association between GDM exposure and BMI [1, 2, 3, 4].

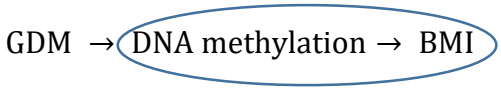
$$\text{GDM} \rightarrow \text{DNA methylation} \rightarrow \text{BMI}$$

In 2011, the EPOCH Study demonstrated that in utero exposure to maternal GDM was associated with higher BMI (P=0.01) and increased BMI growth velocities (P=0.008) between the ages of 27 months and 13 years [3]. The greatest differences between exposed and unexposed participants were observed between the ages of 10 and 14 [3]. The present proposed analysis will evaluate the effects of DNA methylation on BMI z-scores during the same developmental window.



SPECIFIC AIMS

The proposed analysis will evaluate the relationship between quantitative measurements of DNA methylation and BMI z-score obtained at the EPOCH T1 and T2 study visits.



For each of nine CpG sites, specific research questions to be addressed will include:

- Is DNA methylation at T1 associated with BMI z-score at T1?
- Is DNA methylation at T2 associated with BMI z-score at T2?
- Is DNA methylation at T1 associated with change in BMI z-score between T1 and T2?
- Is DNA methylation at T2 associated with change in BMI z-score BMI between T1 and T2?
- Is the change in DNA methylation from T1 to T2 associated with the change in BMI z-score from T1 and T2?

METHODOLOGY & STATISTICAL CONSIDERATIONS

Outcomes

BMI z-scores at T1 and T2 will be included as continuous, longitudinally repeated outcome measures in a generalized linear mixed effects model [5]. BMI will be modeled as continuous, as opposed to as a categorical or dichotomous outcome, in order to maximize statistical power [6, 7, 8, 9]. Estimates of risk for categorical outcomes, such as overweight or obesity, may nevertheless be calculated from the estimates produced by the model of continuous BMI z-score [9, 10].

Predictors

Level of DNA methylation at each of nine CpG sites, measured at T1 and T2, will serve as longitudinally repeated predictors. Quantitative measures of methylation were previously generated by pyrosequencing as part of the EPOCH Epigenetics Study. Methylation Beta values, which may be interpreted as the percentage of methylation at each CpG site, will be normalized via a logit transformation prior to inclusion in the model [11].

Gene ID	No. CpGs	Chromosome
NPHP4	1	1
PTPRN2	1	2
DAPL1	3	2
RNF39	1	6
SH3PXD2A	1	10
ST5	2	11

The nine CpG sites included in this analysis reside within six genes on five chromosomes. As a result, methylation levels at these sites may not be independent. The possibility of spatial or hierarchical correlation will be considered for CpG sites separated by fewer than 1,000 bp [12, 13].

Covariates

Age, sex, and Tanner Stage will be included as covariates. Because the exact age of participants varied within each study visit, age will be modeled as a continuous variable.

Model selection

A general linear mixed effects model will be fit in SAS version 9.4. Models will be fit with and without the inclusion of random effects for subject intercept and slope. Several forms of covariance structures will be considered for both fixed and random effects. All model fits will be compared using the Akaike Information Criterion (AIC). The final selected model will be used for hypothesis testing. Contrast statements will be used to address the research questions outlined above.

References

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