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An Epigenetic Pilot Study Investigating Biomarkers in Maternal-Infant Pairs

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Rationale:

Particulate matter (PM) is a measurable component of air pollution that has been associated with adverse cardiovascular and respiratory outcomes. Research indicates environmental factors such as air pollution are involved in changes through epigenetic mechanisms during development that may persist into adulthood and even span multiple generations of inheritance. Epigenetics is the study of heritable changes of gene expression that do not alter the actual DNA sequence. One epigenetic mechanism is DNA methylation. Long Interspersed Nuclear Element (LINE-1) is a DNA repetitive element that can be used as a proxy measurement of DNA global methylation.

The purpose of this pilot study was to compare epigenetic biomarkers across different sample matrices (i.e. blood and buccal) and across related subjects (i.e. maternal and infant).

Methods:

Informed consent was provided by pregnant women (n=23) who were recruited through Women, Infants, and Children (WIC), hospital birthing classes, or flyers in obstetrician's offices. Demographic and medical data was collected from hospital records for both mothers and newborns after birth. Follow-up health surveys were administered by telephone that were designed to collect indicators of pre-asthmatic respiratory symptoms or conditions.

Biological samples were collected before or shortly after time of birth at Community Medical Center of Missoula, MT. The samples collected were maternal blood (n=15), umbilical cord blood (n=15), and maternal (n=23) and newborn (n=23) buccal (cheek) cells. Buccal cells were collected and processed according to the Gentra Puregene Kit (Qiagen, Germantown, MD). These biologically accessible tissues serve as surrogates to study gene methylation associated with respiratory health.

Samples were stored at -80°C until DNA extraction and subsequent bisulfite treatment. The samples were amplified in duplicates with polymerase chain reaction (PCR). LINE-1 methylation was analyzed with pyrosequencing on a Pyromark Q96 MD (Qiagen, Germantown, MD). All statistical analysis was performed in Statistical Analysis Software (SAS, version 9.3).

Results:

The mean (standard deviation (sd)) of LINE-1 methylation percentage for mother and infant buccal cell derived DNA were 58.75 (3.89) and 57.16 (2.54), respectively. Percent methylation maximum for mother and infant buccal samples were 70.22 and 64.25, respectively, and minimum were 54.86 and 52.94, respectively. Paired t-test indicated that LINE-1 methylation percentages in maternal buccal samples were higher than

methylation percentages in the paired infant samples (mean difference (95%CL) = 4.4 (2.3, 6.6)).

The mean (sd) of LINE-1 methylation percentage for mother and infant/cord blood derived DNA were 75.19 (3.17) and 75.86 (3.05), respectively. Percent methylation maximum for mother and infant blood samples were 79.42 and 79.50, respectively, and minimum were 70.39 and 69.31, respectively. Paired t-test indicated that LINE-1 methylation percentages in maternal blood samples were similar to methylation percentages in infant blood samples (mean difference (95% CL) = 0.66 (-2.0,3.3)).

Conclusions:

LINE-1 methylation percentages between sample matrices (i.e. blood and buccal) and subjects (i.e. maternal and infant) were not correlated. The percent methylation of LINE-1 in DNA from blood was consistently greater than for DNA from buccal tissue for both mother and newborn samples. It was expected that LINE-1 measurements for blood DNA would differ from buccal DNA because circulating blood represents a more diverse cell population. Gene-specific methylation of the promoter region for interferon- γ , a cytokine associated with asthma, will be studied with the remaining samples of bisulfite-treated DNA from this study. Epigenetic changes may serve as useful biomarkers for predicting asthma risk in children exposed to biomass smoke. These methods can be applied to future studies to investigate the epigenetic relationship of prenatal asthma risk and PM wood smoke exposure.