Maternal smoking in pregnancy is a major risk factor for adverse health outcomes in children including reduced birth weight, sudden infant death syndrome, reduced lung function and early respiratory illnesses. Evidence for a causal relationship between prenatal smoking and asthma development remains incomplete. The diverse effects of prenatal smoking on offspring may involve epigenetic modifications, such as DNA methylation, but data are few. We have evidence from the Norwegian Mother and Child Birth Cohort (MoBa) that methylation patterns in offspring can serve as a biomarker of maternal smoking in pregnancy. We evaluated the relationship between maternal smoking (assessed by cotinine measured in plasma from week 18 of pregnancy) and DNA methylation in 1,062 infant cord blood samples using the Illumina HumanMethylation450 Beadchip (Methyl 450K) which interrogates methylation at ~485,000 CpG sites across the genome. We observed statistically significant differential methylation in relation to maternal cotinine for 26 CpGs mapped to 10 genes. We replicated our findings in an independent US birth cohort. This agnostic screen identified two genes that mediate the detoxification of components of tobacco smoke and novel genes not previously implicated in response to tobacco smoke. To determine whether these methylation differences seen at birth persist into childhood as a biomarker of exposure to maternal smoking during pregnancy and whether they can serve as a biomarker of risk of childhood asthma due to maternal smoking, we propose to address the following specific aims using the MoBa study:

**Specific aim 1.** Investigate whether the methylation differences related to maternal cotinine that we detected at birth persist to later childhood (ages 8-11 years).

**Specific Aim 2.** Examine the association between maternal smoking during pregnancy, assessed by plasma cotinine, and the development of asthma in the child ascertained at follow-up at age seven years.

**Specific Aim 3.** Investigate whether methylation differences at birth related to maternal cotinine may be a biomarker of the risk of developing asthma from exposure to maternal smoking during pregnancy.

This proposal addresses FDA research area #4 and specific questions 5, 25, 28, 29, 30.

**Methods:** MoBa is a population-based cohort study that includes over 100,000 pregnancies between 1999 and 2008. Blood samples were collected on mothers twice in pregnancy and cord blood was collected from newborns. The cohort is followed with questionnaires completed by the mother at ages 6, 18 and 36 months, 5, 7, and 8 years and linkage to Norwegian health registries. We now propose to collect a blood sample on 824 children at ages 8-11 years to assess methylation using the same platform as in our cord blood analysis, the Illumina450K, in the same laboratory.

**Significance:** This proposal will investigate whether the epigenetic changes that we have identified at birth in relation to maternal smoking during pregnancy persist into later childhood and thus can be biomarkers for the development of asthma due to this important *in utero* exposure. Because the fetus is exposed via the bloodstream rather than via inhalation, epigenetic biomarkers of fetal exposure to a smoking mother may be
especially relevant to novel and emerging tobacco products, some of which are not inhaled.

This experienced, multidisciplinary team includes researchers at NIEHS, the Norwegian Institute of Public Health, the University of Bergen, Duke University, and the University of Bristol who have a strong history of collaboration.