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Maternal smoking during pregnancy leaves lasting marks on the child's genetic regulatory machinery contributing to lung disease development later in life

Introduction

Environmental and genetic effects are thought to be the reason for the early origins of asthma and allergy in children. Especially intrauterine exposure to environmental risk factors may have severe and long-lasting effects on health as this is a time of increased biological vulnerability. Prenatal exposure to tobacco smoke has previously been associated with impaired lung development (1) and asthma development (2). Epigenetic mechanisms such as DNA methylation and chromatin modifications were suggested to play a major role in “transmitting” environmental effects into biological function and disease development later in life (3). While the potential of epigenetics as the master regulatory mechanism in determining gene expression and gene function has been recognized, high quality studies investigating such effects have long been missing. Now a hallmark epigenetic study investigating effects of *in utero* tobacco exposure, the best known environmental risk factor for the

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development of asthma in children, on DNA methylation and chromatin modifications has been published.

An international team of scientists led by the Helmholtz Centre for Environmental Research (UFZ) in Leipzig and the German Cancer Research Center (DKFZ)/University of Heidelberg discovered that epigenetic changes associated with maternal smoking during pregnancy lead to extensive genome-wide perturbations in the epigenome of mothers but even more importantly, also in newborns. Published in the open access journal “Molecular Systems Biology” the authors report that epigenetic changes occur preferentially in enhancer regions controlling multiple genes concomitantly and that these changes seem to persist for several years after birth (4). What makes this study so special is the broad approach investigating genetic background, methylation, histone modification, gene expression and *in utero* tobacco exposure in the same individuals over time. Thus, a very detailed picture of the biological mechanisms and consequences of *in utero* tobacco exposure can be drawn for the first time.

Cohort and study design

The study was based on the prospective mother-child cohort entitled LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk), studying the impact of environmental factors on children's health with a particular focus on the prenatal period in 622 mothers and their children since 2006 in Leipzig. Extensive information on environmental exposure during pregnancy and thereafter were collected. For the presented work, whole blood of eight mothers who smoked during pregnancy and eight mothers who were not exposed to tobacco smoke were

analyzed together with cord blood samples from their newborn children. Blood samples from the same individuals were re-analyzed one and four years after birth.

As a critical advantage over previous studies on DNA methylation next generation sequencing instead of methylation chips was employed to achieve comprehensive information on the epigenome at a single-nucleotide resolution. The comprehensive epigenomic data set combined whole genome bisulfite sequencing (WGBS) to analyze methylation, chromatin immuno-precipitation sequencing (ChIPseq) to analyze histone modification of selected chromatin marks, and RNA sequencing (RNAseq) to analyze genome wide gene expression patterns.

Methylation quantitative trait loci distinguish environmental and genomic effects

A large number of significant differences in methylated regions (DMRs) were related to tobacco smoke exposure in mothers (n=1953) and in children (n=1404). Interestingly, the majority of affected regions differed between mothers and children (with only 10% overlap) suggesting that health impact of tobacco smoke on children differs significantly and mechanistically from that observed in actively smoking adults. The next generation sequencing approach in this study allowed to discriminate epigenetic changes correlating with the genotype from those associated with environmental exposure. The researchers found that the individual genomic background determines DNA methylation in a large majority of regions. However, 17-20% of DMRs (1404 in children/1953 in mothers) are associated with tobacco smoking. Notably, the genetic background has been widely ignored in earlier epigenetic studies on the impact of maternal smoking on the epigenome of their children (5).

Enhancer regions of the genome are the prime target of epigenetic dysregulation due to tobacco smoke and persist over years

For the first time the authors show that epigenetic perturbations in DNA methylation by tobacco smoke exposure do not occur randomly in the genome. Instead, differently methylated regions due to tobacco exposure cluster in regulatory elements, predominantly in and around enhancers which regulate gene expression of gene clusters (and not promoters regulating a single gene). In total, tobacco induced epigenetic modification affected more than 400 enhancers that regulate genes known to be involved in diseases such as diabetes, adiposity or even cancer. Overall chromatin of children transformed into a hyper-activated and more accessible chromatin state in response to maternal smoking. Longitudinal analysis showed that not only genetically determined DMRs but also many tobacco smoke induced DMRs were stable for up to four years after birth, suggesting a long-term impact of tobacco related adverse health effects.

Epigenetic dysregulation affects JNK2, a member of the WNT pathway, potentially involved in lung disease

The authors selected a methylation change in an enhancer region interacting with the promoter of the c-Jun N-terminal kinase 2 (JNK2) gene, also known as MAPK9, for analysis in the whole LINA cohort as this gene is a member of the WNT signaling cascade, which has been implicated in a variety of tobacco smoke-induced lung diseases such as COPD (6). In the entire LINA cohort and a further independent birth cohort (LISA, n=2443) the authors validated the observed methylation change and associated hypomethylation of this enhancer, in part due to tobacco smoke exposure and in part to genetic background, with JNK2 gene expression. JNK2

hypomethylation was also associated with an 40% increased risk for late onset wheezing in both cohorts. Furthermore, JNK2 knock-out mice showed markedly decreased airway inflammation in an ovalbumin asthma model.

Conclusion

This is by far the most advanced study on environmental effects in epigenetic regulation of complex diseases published to date. It fulfils and even exceeds all necessary criteria for reliable results in epigenetics recently suggested (7). It sets technology standards for epigenetic analysis and provides new insight on how tobacco smoke exerts its biological activity especially in children and on how asthma may be triggered. The applied approach was truly genome wide and unbiased. Although the initial hypothesis was based on only 16 mother-child-pairs sequenced at the time of birth and at two further time points this in depth, multiple layer analysis was able to produce results by longitudinal targeted methylation and transcriptional analysis validated in several hundred children of the LINA and LISA cohorts.

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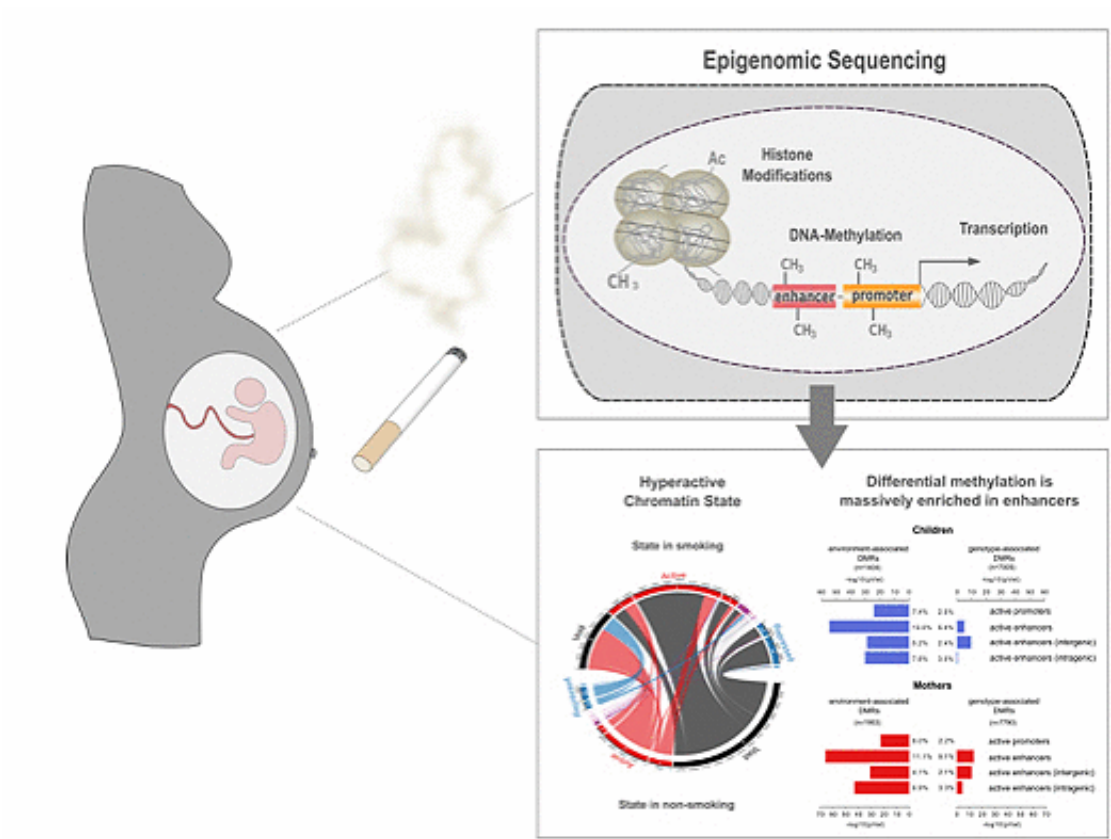
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Figure 1

Genome-wide epigenomic and transcriptomic data reveal that maternal smoking induces important changes in the methylome of mothers and their children that affect in particular enhancer regions. These changes are stably maintained for years in the epigenome of the child.

(from reference 4 with permission)



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