Exploring epigenetic mechanisms for the long term health impact of the Dutch Famine of 1944-45

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Background

Since the early 1970s, epidemiological studies have reported associations between an adverse prenatal environment and an increased disease risk in later life. Animal studies provided potential mechanisms for some observations, including environmentally induced changes to epigenetic marks during development. Epigenetic marks including DNA methylation (DNAm) can influence the transcription potential of genomic regions and, once changed, can result in long-term biological effects. Animal experiments show that epigenetic changes that are established during early development contribute to phenotypes later in life. In parallel, human studies show changes in DNAm after exposure to a range of adverse prenatal conditions. These DNAm differences may mediate part of the association between adverse prenatal conditions and childhood phenotypes. Systematic epigenome-wide studies investigating the associations among specific adverse conditions, DNAm changes, and later life phenotypes are still largely lacking however. Well-designed epigenome-wide studies are needed to create a catalog of epigenomic regions that are sensitive to the prenatal environment. This information will be important to evaluate the role of very early developmental influences on common human disease.

Developmental origins

The developmental origins hypothesis states that adverse conditions during human development may contribute to adult disease risk. Although for some conditions an association can be demonstrated between early life characteristics and adult health, the mechanisms behind these relationships are largely unclear. The involvement of epigenetic dysregulation is possible however and findings of our group have been a key element in elaborating this hypothesis.

Although most human studies on the developmental origins of health and disease use low birth weight as a proxy for a compromised prenatal development, data from the Dutch famine indicate that such studies can be misleading because the relation between birth weight and nutrition during gestation is variable. We found for instance that during the Dutch famine birth weight was only affected by exposure during late gestation but not early gestation. By contrast, we found that epigenetic differences were seen in adults with normal birth weights who were exposed to famine early in gestation and not in adults who were exposed to famine late in gestation, in spite of their higher risk of low birth weight.

In the setting of the Dutch famine, we have an opportunity to address the impact of a prenatal nutrition shock on adult health and the possible mediation by epigenetic mechanisms in a quasi-experimental setting with a long follow-up time. Prenatal exposure to the Dutch Hunger Winter, a severe war-time famine at the end of World War II, has been associated with an adverse metabolic profile (suboptimal glucose handling, higher body mass index (BMI), elevated total and low-density lipoprotein (LDL) cholesterol) and a higher risk of schizophrenia in later life. The subsequent observation of differential DNA methylation after prenatal famine at promoters and imprinted regions regulating genes involved in metabolism suggests a role for epigenetic mechanisms in these phenotypic associations that may have broader significance for human health. We will here further explore these mechanisms.

The Dutch famine historical setting

The winter of 1944-45 is known as the 'Hunger Winter' in the Netherlands. In 1940, the country was invaded by German forces in WW II. By September 1944, Allied troops had liberated most of the South of the country, but their advance towards the North came to a stop. In support of the Allied war effort, the Dutch government in exile in London called for a national railway strike to hinder German military initiatives. In retaliation, the German authorities blocked all food supplies to the occupied West of the country in October 1944.

Despite the war, nutrition in The Netherlands had been adequate up to October 1944. Thereafter, food supplies became increasingly scarce. Government food rations by April 1945 were as low as 500 kcal per day. Widespread starvation was seen especially in the cities of the western Netherlands. Food supplies were restored immediately after liberation on May 5, 1945. It is therefore possible to define the beginning and the end of the famine period. During the famine there was a sharp drop in fertility, which was larger among manual workers than among those in other occupations and a 300g decline in birth weight among those exposed to maternal undernutrition

during the third trimester. After liberation and the restoration of food supplies, birth weights quickly returned to normal.

Because the Dutch population was typically well fed before and after the Hunger Winter, the circumstances of the famine created what can be regarded as a 'natural experiment' with famine severity estimated from an individual's time and place of birth. In further studies this setting has been used to examine how maternal under-nutrition during specific gestational time windows may affect the subsequent life course of offspring who experienced the famine in-utero.

Dutch famine follow-up studies

Population studies: The famine has been used to examine its impact on health and intelligence at age 18, using records from over 400,000 men examined at conscription for military service. Exposure to the famine was defined by place and date of birth in relation to distributed food rations.

It was found that famine exposure during gestation was not associated with intelligence as determined by Raven scores. Famine exposure in early and mid-gestation was associated however with an increase in ‘obesity’, defined as a weight/height ratio of 120% or more compared to a reference population.

While benefiting from the large sample provided by a national birth cohort, these studies were limited to men, as women were never conscripted in The Netherlands, and the available data do not include birth records.

Individuals followed from hospital births: We extracted birth records of boys and girls in famine cities in three hospitals in the Netherlands. We selected individuals born before, during and after the famine and traced these to their current addresses through the Dutch population registration system. To avoid potential biases related to the familial clustering of health outcomes across the generations, we also recruited unexposed same-sex siblings as family controls. The survivors in this study were interviewed and examined at the age of 59 years.

We have previously used this information to examine (i) if prenatal famine has an impact on risk factors for metabolic and cardiovascular disease in later life, in particular obesity in relation to Type 2 diabetes mellitus; ii) to identify critical time windows of pregnancy at which fetal programming might occur.

Our studies were based on the follow-up of live-born singleton births at three institutions in famine-exposed cities (the midwifery training schools in Amsterdam and Rotterdam and the university hospital in Leiden). We selected (i) all 2417 births between February 1, 1945 and March 31, 1946 (infants whose mothers were exposed to the famine during or immediately preceding that pregnancy) and (ii) a sample of 890 births from 1943 and 1947 as time controls (infants whose mothers did not experience famine during this pregnancy).

We extracted birth records and submitted the names and addresses at birth to the local population registers for the tracking of current addresses. We then invited by mail all traced members of the

birth cohort to participate in the study. A telephone interview and medical examination were conducted in 2003-05.

We conducted a telephone interview, followed by a clinical examination at the Leiden University Medical Center. All study protocols and materials for data collection were approved by the human subjects committees of all the participating institutions. Participants provided oral consent at the start of the interview and written informed consent at the start of the clinical examination for all study procedures.

For the clinical examinations, participants were asked to fast overnight before a morning appointment. On their arrival at the clinic, we first obtained written informed consents for study examinations and blood collection and storage, including consent for later study of DNA. We then proceeded with medical examinations, interviews, and blood collections, including for further DNA studies.

DNA methylation studies

IGF2 methylation

One of the best-characterized epigenetically regulated loci is insulin-like growth factor (IGF2). IGF2 is a key factor in human growth and development and is maternally imprinted. Imprinting is maintained through the IGF2 differentially methylated region (DMR). When hypomethylated, this leads to expression of IGF2. DMR methylation is a normally distributed quantitative trait that is largely determined by genetic factors in both adolescence and middle age. This mark is stable through middle age. If affected by environmental conditions early in human development, a change in IGF2 DMR methylation could therefore be detected many years later.

We used our study cohort to investigate whether prenatal exposure to famine is associated with persistent differences in methylation of the IGF2 DMR.

We selected the 60 individuals from the Hunger Winter Families Study who were conceived during the famine six decades ago. The exposure period thus included the very early stages of development. These individuals were born after the famine. They were compared with their same-sex siblings to achieve partial genetic matching. We found a significant decrease in IGF2 SMR methylation among exposed individuals compared to their unexposed siblings.

To further investigate the influence of timing, we selected the 62 individuals who were exposed to famine late in gestation. They were born during or shortly after the famine. We found no difference in IGF2 SMR methylation between the exposed individuals and their unexposed siblings.

The mean birth weight of the 62 individuals exposed late in gestation was 296 g lower than the mean (3422g) of 324 reference births in 1943 at the same institution. The lower birth weight underscores the impact of the famine during the Hunger winter notwithstanding the absence of an association with IGF2 DMR methylation. The mean birth weight of the 60 individuals who were

exposed periconceptually was not lower than that of the reference births. IGF2 DMR methylation was not associated with birth weight.

This study provided the first evidence that transient environmental conditions early in human gestation can be recorded as persistent changes in epigenetic information.

Other DNA methylation differences after prenatal famine

Our study of the imprinted IGF2 locus showed a link between prenatal nutrition and DNA methylation. But it remained unclear how common these changes in DNA methylation are and if they could be sex- or timing-specific. To examine this question, we further investigated the methylation of 15 loci implicated in growth and metabolic disease in individuals prenatally exposed to the famine. We found that methylation of INSIGF was lower among individuals who were periconceptionally exposed to the famine (n = 60) compared with their unexposed same-sex siblings and that methylation was higher of IL 10, LEP, ABCA1, GNASAS and MEG3. A significant interaction with sex was observed for INSIGF, LEP and GNASAS. Next, methylation of eight representative loci was compared between 62 individuals exposed late in gestation and their unexposed siblings. Methylation was different for GNASAS and in men for LEP. This shows that persistent changes in DNA methylation can be a common consequence of prenatal famine exposure and could depend on the sex of the exposed individual and the gestational timing of the exposure.

The study shows DNA methylation changes of multiple imprinted and non-imprinted genes with diverse biological functions. This raises the question if associations between early developmental conditions and health outcomes later in life could be mediated by changes in the epigenetic information layer.

Global DNA methylation

Our previous methylation studies show that exposure to a pre-natal famine environment is associated with a persistent decrease in DNA methylation of the IGF2 gene and that study findings on other loci were highly variable. We next studied the relation between pre-natal famine and overall global DNA methylation in adulthood.

For this examination we used the 350 births with pre-natal exposure to the Dutch famine of 1944-45 selected from three birth clinics, 290 time-controls born before or after the famine in the same clinics, and 307 same-sex siblings of either birth group as unexposed family controls.

As measures of genomic DNA methylation, we analysed two repetitive elements, LINE-I (long interspersed nucleotide element 1) and Sat2 (Satellite 2 DNA sequence) by pyrosequencing and MethyLight, respectively, and overall genomic DNA methylation using the Luminometric methylation assay (LUMA).

We found no relation between overall global DNA methylation at age ~58 years and pre-natal famine exposure in any of the three assays. This is in contrast to the previous results on specific loci including IGF2.

DNA methylation signatures

As a next step we concentrated on selected regions in the genome that could be differentially methylated in response to changes in early life exposures and thereby predict adult health outcomes. We defined these as prenatal malnutrition-associated differentially methylated regions (P-DMRs).

The characterization of the genomic regions and biological pathways involved is important to understand environmentally induced plasticity of the epigenome and its potential role in disease. We used reduced representation bisulfite sequencing (RRBS) to generate DNA methylation data in whole blood on 1.2M individual CpG dinucleotides in 24 individuals prenatally exposed to famine and 24 unexposed same-sex sibling as controls. For this analyses, we selected from our data the subset of 24 sibling pairs with a <5-year age difference. We selected an equal number of male and female pairs as well as an equal number of pairs with the control siblings conceived and born before or after the famine period to minimize the potential effects of these possible confounders. We focus on exposure in early gestation since this developmental period represents a window of increased sensitivity and extensive epigenetic reprogramming.

We employed RRBS on DNA from whole blood to obtain single-nucleotide high-resolution DNA methylation data on a genomic scale. Using a step-wise analysis strategy based on extensive genomic annotation followed by technical and biological validation of selected individual regions, we identified specific genomic characteristics of prenatal malnutrition-associated differentially methylated regions (P-DMRs). We also identified pathways accumulating P-DMRs and report individual P-DMRs that show enhancer activity in vitro and were associated with phenotypic outcomes related to early gestational famine exposure.

We demonstrated that P-DMRs preferentially occur at regulatory regions, are characterized by intermediate levels of DNA methylation and map to genes enriched for differential expression during early development. Validation and further exploratory analysis of six P-DMRs highlight the critical role of gestational timing. Differential methylation of the P-DMRs extends along pathways related to growth and metabolism. P-DMRs located in INSR and CPTIA have enhancer activity in vitro and differential methylation is associated with birth weight and serum LDL cholesterol. Our findings suggest that epigenetic modulation of pathways by prenatal malnutrition may promote an adverse metabolic phenotype in later life.

Epigenome wide DNA methylation

To carry out an epigenome-wide association study of DNA methylation to examine the long term impact of a nutrition shock in gestation, we examined the relation of famine exposure during specific 10-week gestation periods, or during any time in gestation, with genome-wide DNA

methylation levels at age 59 years. In addition, we evaluated the impact of exposure during a shorter pre- and post-conception period. DNA methylation was assessed using the Illumina 450k array in whole blood among 422 individuals with prenatal famine exposure and 463 time- or sibling-controls without prenatal famine exposure.

We found that famine exposure during gestation weeks 1-10, but not weeks 11-20, 21-30 or 31-delivery, was associated with an increase in DNA methylation of CpG dinucleotides cg20823026 (FAM150B), cg10354880 (SLC38A2) and cg27370573 (PPAP2C) and a decrease of cg11496778 (OSBPL5/MRGPRG). These changes represent a shift of 0.3-0.6 standard deviations and are linked to genes involved in growth, development and metabolism.

These findings provide additional evidence that the early gestation period is a critical time-window during which the prenatal environment may affect the human blood methylome.

DNA methylation as link between prenatal adversity and adult health

Our previous DNAm studies on prenatal famine suggested that epigenetic mechanisms, including changes in DNA methylation (DNAm), may underlie the relationship between adverse intrauterine conditions and adult metabolic health.

We therefore evaluated the possible role of DNAm in whole blood as a mediator of the association between prenatal famine exposure and metabolic health in 422 individuals exposed to famine in utero and 463 (sibling) controls. We implemented a two-step analysis. We first carried out a genome-wide exploration across 342,596 cytosine-phosphate-guanine dinudeotides (CpGs) for potential mediators of the association between prenatal famine exposure and adult body mass index (BMI), serum triglycerides (TG), or glucose concentrations. We then carried out a formal mediation analysis.

To establish mediation, we first re-examined the relation between famine exposure any time during gestation ("famine exposure") and adult BMI, glucose, TG, and LDL-C outcomes in individuals with genome-wide DNAm data. Next, we examined genome-wide if DNAm at specific cytosine-phosphate-guanine (CpG) dinucleotides was associated with both famine exposure and the outcome of interest and subjected the identified candidate CpGs to a formal mediation test to determine the extent to which DNAm mediated the association between prenatal famine and adult outcomes. These analyses were repeated for exposure during early gestation as an especially sensitive period of gestation.

We found that DNAm mediated the association of prenatal famine exposure with adult BMI and TG but not with glucose. DNAm at PIM3 (cg09349128), a gene involved in energy metabolism, mediated 13.4% of the association between famine exposure and BMI. DNAm at six CpGs, including TXNIP (cg19693031), influencing (t cell function, and ABCGI (cg07397296), affecting lipid metabolism, together mediated 80% of the association between famine exposure and TG.

Analyses restricted to those exposed to famine during early gestation identified additional CpGs mediating the relationship with TG near PFKFB3 (glycolysis) and METTL8 (adipogenesis).

Using a systematic genome-wide approach, we have therefore demonstrated that DNAm at specific CpGs mediates a considerable proportion of the associations between prenatal famine exposure and later-life adiposity and serum TG levels. Our data are consistent with the hypothesis that the associations between exposure to an adverse environment during early development and health outcomes in adulthood are mediated by epigenetic factors. The specific causal mechanism awaits elucidation, but could be driven by the selective survival of embryos under adverse conditions.

Prospects

In the above studies we demonstrated that the circumstances of the Dutch famine provide a powerful, quasi-experimental setting in humans for the discovery of epigenetic marks that are sensitive to the prenatal nutrition environment. They also provide proof of concept of the potentially long-term health impact of shocks in early life.

Studies that examine other prenatal shocks could also add to the catalog of labile epigenetic marks that are vulnerable to disturbances of the prenatal environment. The catalog could be used as a monitoring tool for early detection of adverse prenatal conditions. Moreover, the catalog may be applied to epidemiologic studies of being overweight or obese and related health conditions diabetes and cardiovascular disease when data on development are lacking. The insights could be applied to biobank studies that are suitable for epigenomics research, preferably incorporating various tissues or separated cell types, extensive longitudinal samplings, and adequate resources to study the transcriptome.

Despite the challenges that lie ahead, the study of early development in humans is likely to be a promising route for the further identification of epigenomic risk factors for human adult disease. In particular, studies that aim at the integration of epigenomic and genetic information may eventually reveal genomic risk factors that are more powerful than the current ones solely based on DNA sequence variation.

Understanding how epigenetic control is shaped by early life challenges may increasingly clarify its role in promoting health over the lifespan and ultimately suggest new ways to prevent human disease.

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