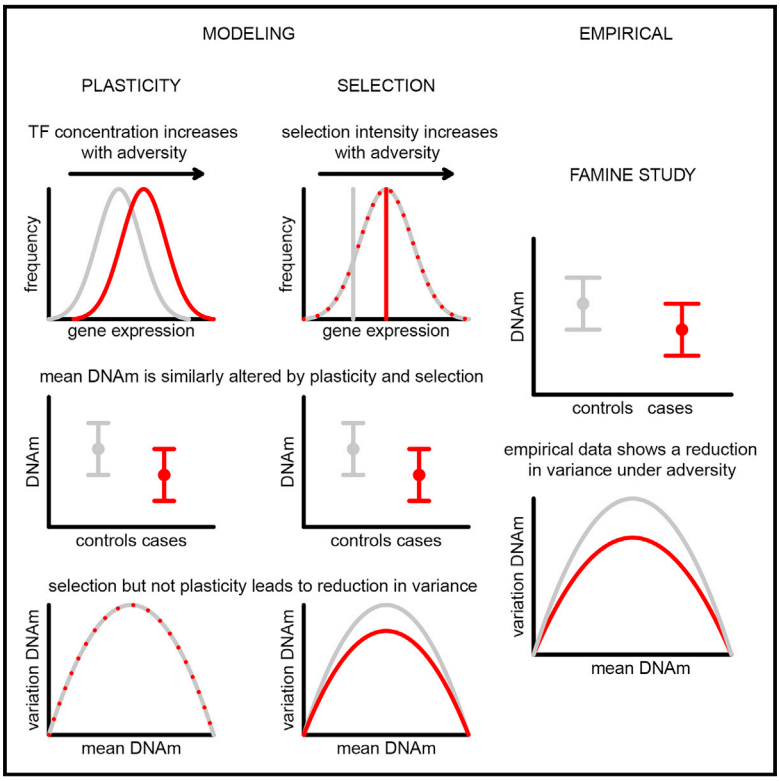
Cell Reports

Selective Survival of Embryos Can Explain DNA Methylation Signatures of Adverse Prenatal Environments

Graphical Abstract



Highlights

- Variation in gene expression and DNA methylation is generated in early development
- Adverse prenatal environments may result in selection on this variation *in utero*
- Selection reduces the variance in DNA methylation at loci that affect survival
- Selection may help explain some of the health effects of prenatal adversity

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In Brief

Tobi et al. hypothesize that prenatal adversity can cause selection on epigenomic profiles *in utero*. Their model predicts that such selection reduces the variance in DNA methylation at genomic regions that contribute to survival, which is testable and detectable in empirical data from the Dutch famine.

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Selective Survival of Embryos Can Explain DNA Methylation Signatures of Adverse Prenatal Environments

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SUMMARY

An adverse intrauterine environment is associated with long-term physiological changes in offspring. These are believed to be mediated by epigenomic marks, including DNA methylation (DNAm). Changes in DNAm are often interpreted as damage or plastic responses of the embryo. Here, we propose that stochastic DNAm variation, generated during remodeling of the epigenome after fertilization, contributes to DNAm signatures of prenatal adversity through differential survival of embryos. Using a mathematical model of re-methylation in the early embryo, we demonstrate that selection, but not plasticity, will generate a characteristic reduction in DNAm variance at loci that contribute to survival. Such a reduction in DNAm variance was apparent in a human cohort prenatally exposed to the Dutch famine, illustrating that it is possible to detect a signature of selection on epigenomic variation. Selection should be considered as a possible mechanism linking prenatal adversity to subsequent health and may have implications when evaluating interventions.

INTRODUCTION

Human and animal studies show intriguing associations between adverse events *in utero* and late-life physiology, behavior, and life history (Gluckman et al., 2007; Brakefield et al., 2005). These associations are widely believed to be mediated by persistent effects of prenatal adversity on epigenomic marks (Gluckman et al., 2007; Heijmans et al., 2009), that is, molecular marks that may bring about mitotically heritable differences in gene expression potential (Jaenisch and Bird, 2003), such as DNA methylation (DNAm). DNAm can control, stabilize, and reflect differences in gene expression potential. DNAm is known to show both positive and negative association with gene expression and is established with DNA methyltransferases and removed in a complex series of reactions mediated by TET enzymes involving hydroxylation of the methyl group. There is substantial evidence for associations between adverse maternal exposures during early gestation and persistent changes in the DNAm profiles of offspring (Sales et al., 2017). However, the mechanism underlying these associations are still poorly understood. Two alternatives are frequently considered. First, adverse conditions *in utero* may compromise the establishment and maintenance of DNAm (Heijmans et al., 2009). Second, DNAm may be actively modified in the embryo to enable a match between its phenotype and the current or future environment (Gluckman et al., 2007). If environments do not match the prediction *in utero*, plasticity can cause maladaptation, and this is commonly invoked to explain the increase in non-communicable disease in many parts of the world.

Another possibility that has been less explored is that embryos with particular epigenomic profiles have different survival probabilities *in utero* (Tobi et al., 2014). Selection on unbiased variation in DNAm, what we refer to as "epigenetic selection," is possible if three key conditions hold: (1) there is stochastic variation in DNAm among embryos, (2) specific DNAm patterns confer differential prospects of embryo survival under adverse conditions, and (3) DNAm patterns arising early in life can be transmitted during cell division and persist during development and, in some cases, even into adulthood. There is increasing evidence in the literature that these conditions are met.

Single-cell transcriptome and methylome studies have revealed stochastic variation in both gene expression and DNAm between genetically identical cells in common environments (Angermueller et al., 2016). Stochastic genome-wide differences in DNAm may arise during early development as the mammalian genome is stripped of its DNAm after fertilization, followed by a period of global re-methylation in the post-implantation embryo

(Messerschmidt et al., 2014). Transient gene expression changes and regulatory circuits controlled by transcription factors play a crucial role in establishing DNAm patterns (Eckersley-Maslin et al., 2016; Greenberg et al., 2017), for example, because the binding of transcription factors to promoter and enhancer regions can locally decrease the probability that cytosines become methylated (Bonder et al., 2017; Maurano et al., 2015).

Successful embryonic implantation and further development require the expression of specific genes to match the blastocyst physiology with the conditions in the uterus and the endometrium (Altmäe et al., 2012). Such genes are associated with transcription regulation, cell adhesion, and signal transduction in the pre-implantation stage, and with cell growth and signaling pathways. The extent to which these processes are aligned is expected to vary among embryos. This will create variation in the likelihood of successful implantation and development. For instance, the success rate of assisted reproduction is linked to the appropriate degree of global re-methylation of the early blastocyst (Li et al., 2017a). Indeed, in humans more than half of all embryos may fail to implant or abort soon after implantation (Wilcox et al., 1999). This number can be substantially higher under maternal adversity (Bellver et al., 2013; Dechanet et al., 2011; Li et al., 2017b). We therefore expect that maternal adversity could generate intense selection on gene expression and the associated epigenomic profiles, in particular during implantation.

The DNAm maintenance machinery enables transmission of established DNAm profiles during cell division (Jaenisch and Bird, 2003), although DNAm is continuously remodeled during fetal development and cell differentiation (Slieker et al., 2015). In particular, DNAm patterns established early in development may be maintained if these regions do not require extensive remodeling later on (Dominguez-Salas et al., 2014).

Because epigenetic selection removes particular DNAm variants from the population, it may change the distribution of DNAm in survivors. Thus, it should be possible to detect signatures of epigenetic selection *in utero* from DNAm data in human populations (similarly to the rationale for detecting signatures of selection in DNA sequence data). With the aim to derive testable predictions, we implemented a mechanistic model that captures the essentials of the dynamics between transcription factor binding and re-methylation (Chen et al., 2013) and extended this model to encompass multiple cells in a whole embryo. By simulating both the plasticity and epigenetic selection scenarios, we predict that epigenetic selection, but not plasticity, should result in a reduction in DNAm variance at CpG sites that confer a selective advantage. Finally, we illustrate how empirical DNAm data can be used to test for this signature of epigenetic selection and find evidence for reduced variance in a cohort exposed to the Dutch famine, a severe wartime famine at the end of World War II (Lumey et al., 2007).

RESULTS

Modeling Dynamic TFBS Methylation during Epigenomic Remodeling

To generate testable predictions, we modified the mechanistic model of Chen et al. (2013) to model the relationship between transcription factor binding and re-methylation (Figure S1). Although this model excludes significant biological details, these details matter only insofar as they contribute to consistent effects of maternal adversity on transcriptional regulation (i.e., plasticity) or embryo survival (i.e., selection), as these are the processes that generate differences in DNAm among exposed and unexposed groups. We therefore omit several molecular intermediates in the DNAm machinery, intermediates in the process of de- and re-methylation, and other epigenomic marks. Instead, we focus on the variation in DNAm caused by the inherent stochasticity in DNAm and transcription factor binding, transcription factor concentration, and the effects of gene expression on implantation success. Here, we provide only a brief and non-technical summary of the model. The details, including exploration of alternative assumptions and robustness analyses, are provided in the STAR Methods.

Each of our simulations started with 100 embryos, and every embryo consisted of 50 diploid cells containing 75 independent genes. We reduced the complexity of gene regulation to a representation by one transcription factor binding site (TFBS) per gene, assuming ten CpG dinucleotides (CpGs) per TFBS. Because we were interested in the consequences of purely stochastic variation in DNAm, we incorporated no genetic variation between different embryos and no sequence variation between different TFBS.

Our model starts at pre-implantation, when the genome is demethylated and none of the CpGs in the TFBS are methylated. We modeled the dynamics of re-methylation of CpGs within the TFBS as a stochastic process that depends on transcription factor (TF) concentration, later referred to as [TF]. We assumed equal concentrations of a particular TF in all 50 cells constituting an embryo *in silico*. When a TFBS is occupied by a TF, the likelihood that a CpG in a cell will become methylated is decreased (Domcke et al., 2015), as TF binding blocks access for the methylation machinery (Bonder et al., 2017). This process is modeled by simply setting a probability for re-methylation when a TFBS is temporarily vacant. In addition, methylation of a CpG inhibits TF binding (Yin et al., 2017), and hence the likelihood that a neighboring CpG becomes methylated increases. The duration a TFBS is bound by a TF corresponds with the level of gene expression. Because these processes follow simple Michaelis-Menten kinetics, the binding of a TF and the methylation machinery to a TFBS are inherently stochastic. As a result of these dynamic kinetics, both methylation frequencies and gene expression levels are variable among individual cells and embryos. Finally, we assume that DNAm is transmitted during cell division once re-methylation is completed, and hence, any differences in DNAm established early in life will be detectable in post-natal life.

To generate distributions of DNAm in samples of individuals, we first calculated the mean DNAm for each individual CpG within a TFBS over the 50 diploid cells (each of which is 0%, 50%, or 100% methylated) of an individual embryo at the end of each simulation. We then quantified the mean and variance in methylation frequency for each single CpG between embryos. TF binding is positively related to gene expression of the target gene, which is also quantified as the average gene expression of an individual embryo by taking the mean over all cells. The default state is a simulation without maternal adversity. Under

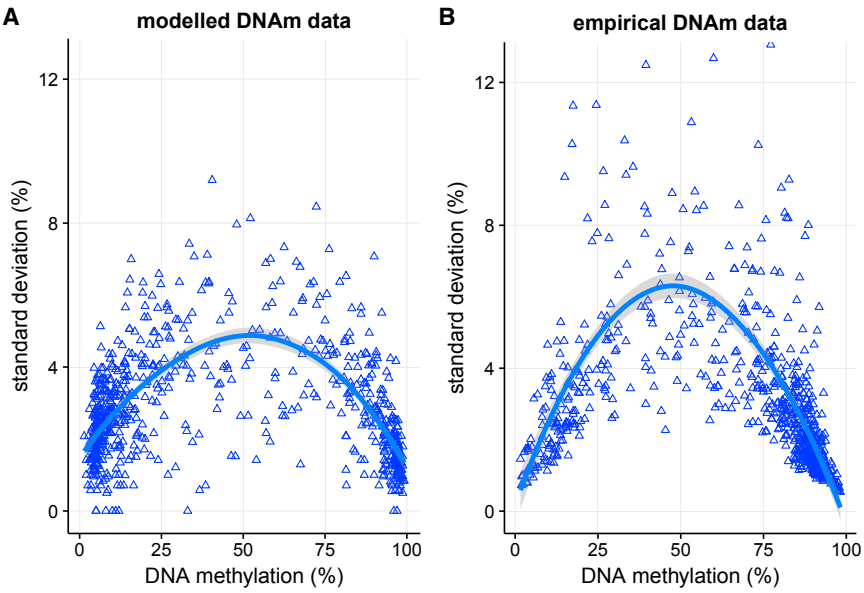

Figure 1. Comparison of Modeled and Empirical DNAm Data

Panels show the trend (locally estimated scatterplot smoothing [LOESS] with span = 1.0 in blue and the 95% confidence interval [CI] in gray, as calculated within the R ggplots2 package) between mean methylation (x axis) and SD (y axis).

(A) DNA methylation (DNAm) for 750 simulated CpGs within 75 TFBS.

(B) DNAm for 750 random CpGs from 463 controls from the Dutch Hunger Winter Families Study.

See also Figure S1.

these assumptions, the modeled distribution of DNAm is characterized by a majority of CpGs with methylation levels close to 0% and 100% and intermediately methylated CpGs with peak variance at 50% DNAm, a mean-variance distribution that also applies to genome-wide DNAm arrays in whole blood from the Dutch Hunger Winter Families Study (Tobi et al., 2015) (Figure 1).

Simulating Plasticity and Epigenetic Selection

We used the above model of re-methylation during development to generate predictions for a series of simulations with the aim of finding a signature of selection in DNAm data in cohorts exposed to prenatal adversity. First, we consider plasticity. Under this scenario, [TF] responds in a coordinated adaptive response to the nutritional conditions *in utero*. We therefore modeled plasticity by increasing or decreasing [TF] upon maternal adversity and studied its effects on the DNAm of the corresponding TFBS. This is contrasted with epigenetic selection. Under this alternative scenario, there are no systematic differences in [TF] *in utero*. Instead, maternal adversity reduces overall embryo survival by favoring embryos with particular gene expression levels (i.e., those favorable for successful implantation and survival). As a consequence, there is selection of a subset of the stochastically arising DNAm profiles.

For each simulated scenario, we modeled 150 genes, 75 "target genes" that either respond plastically (plasticity model) or influence the probability of survival (epigenetic selection model) and 75 "control genes," in a population of embryos with or without maternal adversity. At the end of each simulation, when the *in silico* "genome" had completed its re-methylation (i.e., the DNAm levels remained stable), we characterized the resulting DNAm patterns in each group of embryos. Because the number of embryos is reduced by selection, and this may influence the comparison with the plasticity scenario, we started these simulations with a larger number of embryos and used random subsampling to maintain the same population size across all comparisons.

Model Predictions for Epigenetic Selection

Both plasticity and epigenetic selection caused a shift in mean methylation at the individual CpGs linked to the 75 target genes with a role in plasticity or survival (both increases and decreases in mean DNAm were observed; data not shown). The model simulations showed that under plasticity, the relationship between the mean and variance in methylation was the same in groups of exposed and unexposed individuals (Figure 2A). In contrast, epigenetic selection reduced the variance in DNAm at TFBSs that contribute to survival. This reduction is modest at single CpGs but evident across the entire range of mean DNAm levels (Figure 2B). The relationship between the mean and variance for control genes (i.e., genes whose expression does not contribute to embryo survival) was unaffected by selection (Figure 2C). As expected, the disparity in DNAm profiles between embryos surviving under adverse conditions and controls increased with the intensity of selection, that is, when fewer individual embryos survived (Figure 2D). Hence, the model singles out a modest but consistent reduction in variance in DNAm across its entire range (e.g., 0%–100%) as a key signature of selection.

Robustness Analyses

To study the robustness of the variance reduction under epigenetic selection, we performed simulations under different parameter settings, additional molecular detail, or alternative assumptions. In a large parameter and scenario space, variance was reduced under selection compared with control conditions. The results of the model were robust under conditions causing lower DNAm maintenance and re-methylation, for instance through a shortage of essential (micro-)nutrients for the methylation machinery, and vice versa, also when the likelihood of re-methylation was increased (Figure S2). In the main model, we chose to incorporate only an inverse relationship between transcription and DNAm, but alternative relationships between TF binding and the methylation machinery (Bonder et al., 2017) did not alter the model predictions (Figure S3). Also, the number of CpGs within a TFBS and assuming a gradient of [TF] in the embryo had no considerable effect on variance reduction under selection (Figure S4). Although this shows that the predictions of the model are robust, it is important to note that, inherent to

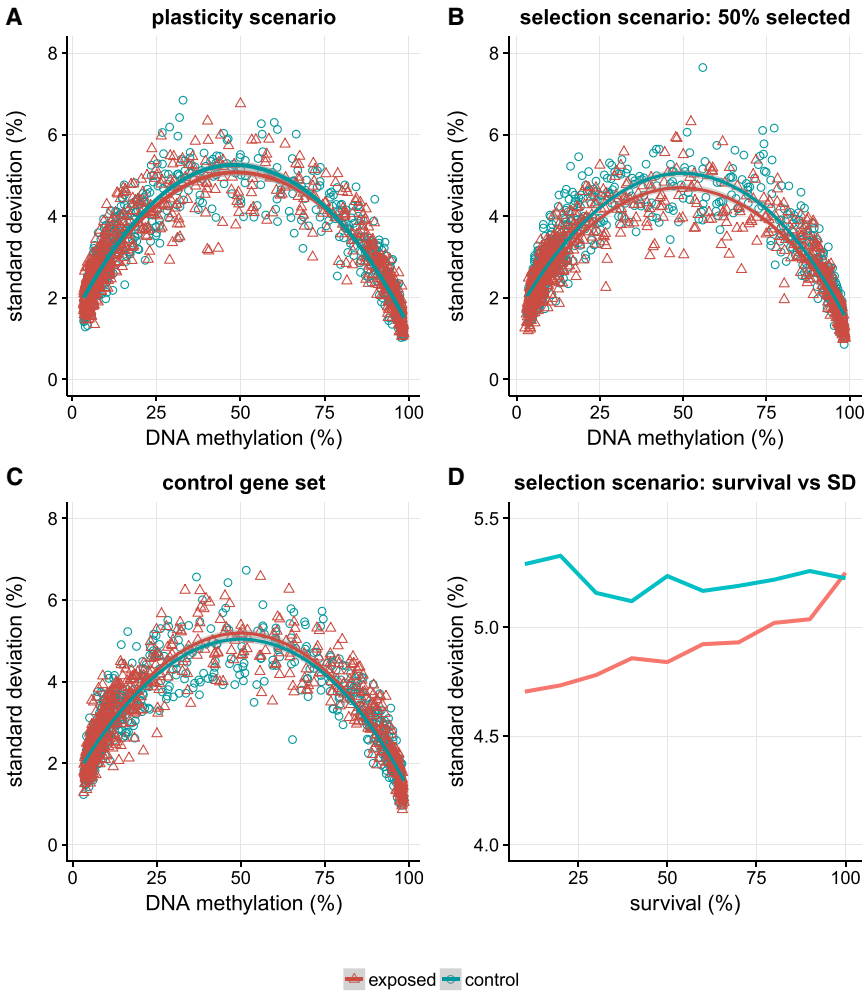

Figure 2. Model Predictions for the Relationship between Mean and SD in DNAm

Blue circles and solid blue trend lines (LOESS with span = 1.0 and 95% CI in gray, as calculated using the R ggplots2 package) denote the observations in the simulated embryos that do not experience prenatal adversity ("controls"). Red triangles and solid red trend lines (identical LOESS settings) are the observations in simulated embryos exposed to maternal adversity ("exposed").

(A) Relation between mean DNAm and SD for 750 CpGs in 75 TFBS linked to genes active in the plastic response.

(B) Relation between mean DNAm and SD for the 750 CpGs in 75 genes linked to survival when 50% of the exposed embryos fail to survive.

(C) The same selection scenario as in (B) but for the 750 CpGs in the 75 TFBS linked to genes that do not contribute to the likelihood of survival (i.e., the control gene set).

(D) SD of DNAm under different cut-offs for survival.

See also Figures S2 and S3 and Table S3.

the stochastic nature of the modeled re-methylation and the selection process itself, the effect sizes were variable across simulations.

Evaluating Model Predictions in Empirical Data

To illustrate how to test for this reduction in variance using empirical DNAm data on populations, we revisited genome-wide DNAm data in whole blood for 422 individuals prenatally exposed to the Dutch famine and 463 unexposed (sibling) controls (Table S1) (Tobi et al., 2015). The Dutch famine is considered a quasi-experimental setting during which the number of births dropped to almost 50% of normal levels (Susser and Stein, 1994) because of a combination of reduced conception, lower implantation success, and an increased rate of fetal deaths in the famine-exposed population (Stein and Susser, 1975).

We compared the variance in DNAm in prenatally exposed individuals and controls across all individual CpGs putatively associated with famine exposure (at $p < 0.001$) during 10 week time frames or during any of these gestational time frames ("any famine exposure"). These loci are enriched for CpGs with intermediate levels of DNAm at (developmental) enhancers and promoters devoid of CpG islands (Tobi et al., 2014). These famine-associated CpGs were contrasted to 1,000 randomly selected control CpGs that had a similar mean and variance as the famine-associated CpGs (in the prenatally unexposed) but that were not associated with prenatal famine exposure ($p > 0.2$). Selecting a set of CpGs with the same mean-variance structure ensures that the comparison is not confounded by differences in the mean and variance of the selected subset itself.

The variance in DNAm at famine-associated CpGs was significantly lower in individuals whose mothers were exposed to the height of the famine in the 10 weeks before conception ($\Delta SD = -0.2\%$, $p_{FDR} = 0.031$; Table 1; Figure 3A). Although similar reductions were suggested for individuals exposed during other exposure periods ($-0.2\% \geq \Delta SD \geq -0.4\%$; Table 1), the reduction was statistically significant for weeks 21–30 of gestation only ($\Delta SD = -0.3\%$, $p_{FDR} = 0.031$; Figure 3B). Both sets of "control" CpGs showed no difference in variance. Similar results were obtained when the exposed individuals were compared with their same-sex, unexposed sibling controls (Table S2).

DISCUSSION

Our main aims in this study were to (1) examine selection on gene expression profiles in early embryos as a possible explanation for the observed associations between prenatal adversity and DNAm in children and adults, (2) use a basic mechanistic model in combination with individual-based simulations to identify predictions for the effect of this selection on DNAm patterns in exposed and control populations, and (3) demonstrate how to test these predictions on empirical DNAm data and provide a

Table 1. Difference in Variance for Differentially Methylated CpGs between Exposed and Control Groups for the Different Periods of Prenatal Exposure to the Dutch Famine

	Famine-Associated CpGs					1,000 Unaffected CpGs	
	N Probes	ΔSD (%)	Corr[a] ΔSD (%)	p Permutations[b]	p FDR	Corr[c] ΔSD (%)	p Permutations[b]
Any famine exposure	543	−0.1	0.0	0.32	0.32	0.01	0.74
Exposure period specific							
−10 to 0	404	−0.2	−0.2	0.010	0.031	0.0	0.67
Weeks 1–10	669	−0.4	−0.2	0.21	0.32	0.1	0.58
Weeks 11–20	520	−0.4	−0.3	0.064	0.13	0.1	0.60
Weeks 21–30	506	−0.3	−0.3	7.3×10^{-3}	0.031	0.0	0.52
Week 31 to delivery	622	−0.2	−0.1	0.29	0.32	0.0	0.47

See also Tables S1 and S2. Corr, correlation; FDR, false discovery rate.

[a]The mean difference in SD between exposed and controls across CpGs associated with prenatal famine exposure; beta values corrected for age, sex, technical variation, and cellular heterogeneity.

[b]p value obtained by 100,000 permutations for model noted above.

[c]The mean difference in SD between exposed and controls across 1,000 probes not associated with prenatal famine exposure; corrected as noted above.

first assessment of the merits of the hypothesis. The model predicts that selective survival of embryos leads to reduced variance in DNAm at CpGs in groups of individuals exposed to adverse intrauterine exposures as compared with unexposed groups. This indicates that reduced variance in DNAm observed in a population can be a signature of selection during gestation. Our showcase of how to test this hypothesis suggests that selection is a tenable explanation for the DNAm patterns associated with maternal adversity during the earliest stages of development *in utero*.

Epigenetic selection is particularly important to consider when prenatal adversity occurs shortly after conception because mortality during human embryonic development is highest directly before and after implantation (Wilcox et al., 1999). This period is largely captured in the Dutch Hunger Winter Families Study by those pregnancies exposure to famine for at least 10 continues weeks before conception (Lumey et al., 2007). These individuals were conceived at the height of the famine, when rations fell to 500 kcal/day, and a DNAm variance reduction in this exposed group was indeed statistically supported. There was a similar variance reduction for individuals exposed anytime during the first two trimesters, although this was statistically significant only for gestational weeks 21–30, which is the period with the largest number of prenatally exposed individuals and hence the largest study power to detect a statistical difference from controls. Overall, these preliminary results indicate that the DNAm pattern associated with early gestational adversity may have been generated by the selective survival of embryos rather than by embryo plasticity.

However, detecting a signature of selection does not rule out that DNAm in embryos could also respond directly to maternal adversity; both processes may occur simultaneously (Bruckner and Catalano, 2018). Plasticity may be a better explanation for mean changes in DNAm that occur late in gestation, when mortality is lower, and adjusting its physiology will likely aid the fetus to better cope with the adverse intrauterine environment. Furthermore, closer to birth, environmental cues might become relevant for predicting post-natal conditions (Nettle et al., 2013). An interesting example to explore may be the well-established link between growth in late gestation, reduced nephron number, and its associated increased risk for hypertension (He et al., 2010). However, directly testing the adaptive plasticity hypothesis in humans is difficult (Brakefield et al., 2005), because reliable estimates on Darwinian fitness are difficult to obtain in modern societies. Ruling out epigenetic selection may therefore be especially important to studies that invoke adaptive plasticity to explain associations between maternal adversity, DNAm, and phenotypic characters.

Our test for a signature of epigenetic selection in empirical data of the Dutch famine is only a first step to investigate the scope for the hypothesis. More detailed studies are needed. Only small differences in variance were observed in the quasi-experimental setting of the Dutch famine (effect sizes up to 0.1 SDs), despite the severe nutritional deprivation. This may stem partly from our inclusion criterion of nominally significant CpGs that may contain false positives, the fact that we measure the DNAm patterns in blood six decades after the exposure, and the stochastic, and hence noisy, nature of the processes possibly at play. Our simulations suggest that the stochastic nature of re-methylation and selection means that large and multiple datasets are required to robustly identify regions with a lower than expected DNAm variance. The recent formation of large consortia with detailed information on contemporary prenatal conditions and genome-scale DNAm data holds great potential in this respect (Felix et al., 2017). Experimental studies in animal models are another promising route to provide more direct tests for epigenetic selection.

From a theoretical perspective, it will be important to also study how different factors such as age and tissue heterogeneity, DNA sequence variation, and the developmental process itself (e.g., canalization; Pujadas and Feinberg, 2012) can affect the variance of DNAm. In particular, a reduced variance could reflect a more canalized regulation of gene expression. Although the key predictions were unaffected by a number of mechanistic details regarding the process of re-methylation, including that plasticity is caused by shortage of substrate or

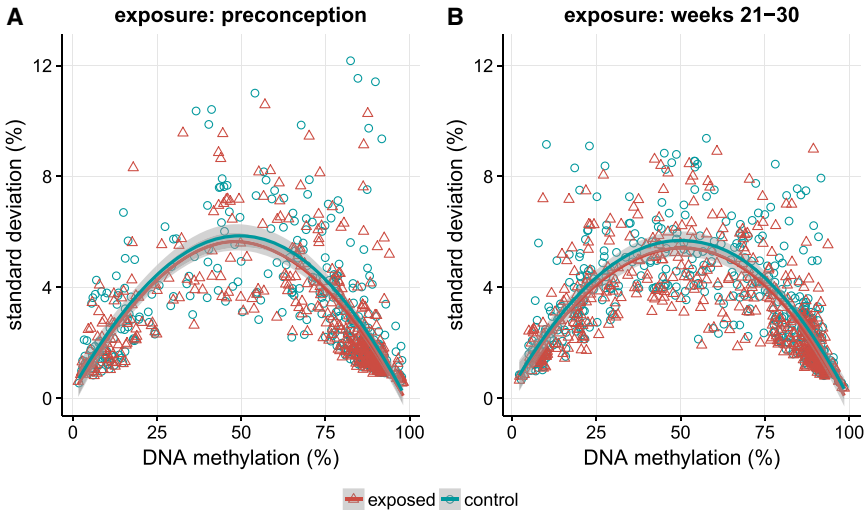

Figure 3. Empirical Data for the Relationship between Mean and SD of DNAm Data in the Dutch Hunger Winter Families Study

Blue circles and solid blue trend lines (LOESS with span = 1.0 and 95% CI in gray, as calculated using the R ggplots2 package) denote embryos that did not experience prenatal exposure to the famine ("controls"). Red triangles and solid red trend lines (identical LOESS settings) are the data for embryos exposed to maternal adversity ("exposed").

(A) Four hundred four CpGs associated with preconception famine exposure ($p<0.001$).

(B) Five hundred six CpGs associated with famine exposure during weeks 21–30 of gestation and controls ($p<0.001$).

See also Tables S1 and S2.

co-factors (e.g., a lower rate of re-methylation and maintenance), it is important to note that the magnitude of the reduction in variance can be affected. In fact, other forms of selection (e.g., increased survival of both extremes of a distribution) may increase the variance within a population. Another extension of our model is to impose selection within, rather than between, blastocysts. Selection of particular cells within an embryo occurs during normal development (Sancho et al., 2013) and may likewise be hypothesized to play a role in fetal programming. Finally, although we make no assumptions about whether epigenetic selection per se is adaptive to mothers, it may be interesting to explore the conditions under which it evolves under natural selection (van den Heuvel et al., 2016; Nettle et al., 2013).

Understanding the mechanisms of DNAm changes in an adverse prenatal environment has practical and clinical implications. Under epigenetic selection, aberrant DNAm profiles of survivors are not caused by adversity during early life; they arise from the effects of stochastic variation in DNAm on the odds of implantation or post-implantation survival. This hypothesis makes no assumption about specific adaptive or maladaptive consequences of those DNAm profiles that are observed later in life. By contrast, under adaptive plasticity it is posited that an adverse maternal environment induces specific epigenomic profiles and that such adaptations may increase the risk for disease in a mismatched post-natal environment (Gluckman et al., 2007). Hence, although prevention or intervention measures in fetal life could mitigate or neutralize adverse plastic responses, under epigenetic selection, such interventions would merely reduce the selection pressure and not necessarily prevent adverse epigenomic profiles that arise stochastically. Thus, the distinction between induction of DNAm profiles versus selection of pre-existing DNAm profiles may be important for managing the expectations of intervention programs and the ongoing discussion of (epi)genetic determinism and concerns that we are placing blame on past generations for the diseases of our own (Waggoner and Uller, 2015).

In summary, we propose that differential survival of embryos with particular epigenomic profiles ("epigenetic selection") is a tenable explanation for the observed late-life differences in DNAm after early prenatal adversity in humans. By formulating and modeling this alternative, we show that it generates predictions that can be tested using data that are available in human populations. Epigenetic selection needs further consideration as a biological mechanism underlying the association of adversity in early life with health in adulthood.

STAR★METHODS

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- METHOD DETAILS
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- QUANTIFICATION AND STATISTICAL ANALYSIS
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- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and three tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.11.023.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.W.T., J.v.d.H., B.J.Z., B.T.H., and T.U.; Methodology, E.W.T., B.J.Z., and J.v.d.H.; Investigation, E.W.T., J.v.d.H., and L.H.L.; Formal Analysis, E.W.T. and J.v.d.H.; Validation, E.W.T. and J.v.d.H.; Software, J.v.d.H.; Resources, L.H.L.; Data Curation, E.W.T. and L.H.L.; Writing – Original Draft, E.W.T., J.v.d.H., B.T.H., and T.U.; Writing – Review & Editing, E.W.T., J.v.d.H., L.H.L., B.J.Z., B.T.H., and T.U.; Visualization, E.W.T. and J.v.d.H.; Supervision, B.J.Z., B.T.H., and T.U.; Project Administration, L.H.L., B.T.H., and T.U.; Funding Acquisition, L.H.L., B.J.Z., B.T.H., and T.U.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Simulation program. Program coded for C++ that simulates re-methylation dynamics. Program was tested and run under windows (DEV-C++ v5.11)	This paper	GitHub: https://github.com/ecoevomicsjoost/embryo_selection_model
Summarization program. Custom R script that uses input from the simulation program to quantify the relationship between mean and the standard deviation of CpG site methylation frequencies	This paper	GitHub: https://github.com/ecoevomicsjoost/embryo_selection_model
Software and Algorithms		
R package *permute* version 0.9-0	CRAN	https://cran.r-project.org/web/packages/permute/index.html
R version 3.4.1	CRAN	https://cran.r-project.org/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Dr. B. T. Heijmans (B.T.Heijmans@lumc.nl)

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study subjects

The Dutch Hunger Winter Families study is described in detail elsewhere (Lumey et al., 2007). In short, historical birth records were retrieved from three institutions in famine-exposed cities for singleton births between 1 February 1945 and 31 March 1946. Infants were identified whose mothers were exposed to the famine during or immediately preceding that pregnancy and as time-controls infants whose mothers were not exposed to famine during pregnancy from 1943 and 1947. Both groups were invited to participate in a telephone interview and in a clinical examination, together with a same-sex sibling not exposed to the famine (as a family-control). We conducted 1,075 interviews and 971 clinical examinations (of 345 clinic births without a matched sibling and 313 with a matched sibling) between 2003 and 2005.

Ethics approval and consent to participate

The Dutch Hunger Winter Families study was approved by the Institutional Review Board of Columbia University Medical Center and by the Medical Ethical Committee of Leiden University Medical Center and the participants provided verbal consent at the start of the telephone interview and written informed consent at the start of the clinical examination.

Famine exposure

Food rations were distributed centrally and below 900 Kcal/day between November 26, 1944 and May 15, 1945 and the percentage of calories from proteins, fat, and carbohydrates was constant during the famine period (Burger et al., 1948). We defined famine exposure by the number of weeks during which the mother was exposed to < 900 kcal/day after the last menstrual period (LMP) recorded on the birth record (or when missing or implausible [12%], as estimated from the LMP calculated from the birth weights and the date of birth (Lumey et al., 2007). We considered the mother exposed in gestational weeks 1-10, 11-20, 21-30, or 31 to delivery if these gestational time windows were entirely contained within this period and had an average exposure of < 900kcal/day during an entire gestation period of 10 weeks. As the famine lasted 6-months some participants were exposed to famine during two adjacent 10-week periods. Pregnancies with LMP between 26 November 1944 and 4 March 1945 were thus considered exposed in weeks 1-10; between 18 September 1944 and 24 December 1944 in weeks 11-20; between 10 July 1944 and 15 October 1944 in weeks 21-30; and between 2 May 1944 and 24 August 1944 in weeks 31 to delivery. We define individuals exposed to one or at most two of these definitions exposed to 'any' gestational exposure. In addition, individuals with a LMP between 1 February and 12 May 1945 were exposed to an average of < 900kcal/day for an entire 10 weeks before conception (and up to 8 weeks post-conception) and are denoted as the −10 – 0 weeks group (e.g., "preconception").

METHOD DETAILS

Mathematical model

We modeled CpGs (a vector, *M*), which can be in a methylated or un-methylated state. Generally, TFs and proteins that methylate CpGs compete for binding to the DNA (Domcke et al., 2015) (see Figure S1 for a schematic overview). Demethylation of target sites leads to an increase in TF binding (Maurano et al., 2015), while the binding of TFs results in resistance to *de novo* DNAm by DNMTs (Brandeis et al., 1994; Maurano et al., 2015). To encapsulate these dynamic behaviors we follow the logic of the modeling approach by Chen et al. (Chen et al., 2013). The probability of binding of TF and DNAm is negatively related to the presence of the other. A higher activity of TFs will therefore reduce the probability that DNA will become methylated, while more methylation in a TF binding site (TFBS) decreases the probability of TF binding. DNAm therefore causes a lower gene expression for the target gene. For each TFBS we modeled 10 CpGs and each TFBS was replicated in 50 cells (the approximate maximum number of cells in a human pre-implantation blastocyst (Herbert et al., 1995)). Molecular stochasticity gives rise to variation in DNAm between cells and between individuals. We model no differences in DNA sequence between embryos or TFBS within a cell of one embryo. We did therefore not explicitly model CpG islands, which are hypo or hyper-methylated and generally lack DNAm variation.

The dynamics of gene expression and DNAm depend on the concentration of transcription factors, which reduces the ability of DNAm transferases (DNMT) to methylate CpGs (i.e., increased availability of TFs reduces methylation on average). We modeled gene expression (G) as,

$$G = \frac{[TF]^p}{[TF]^p + (H\omega)^p}, \tag{1}$$

where [TF] is the concentration of active TF, the parameter H is the half-saturation value, p determines the rate at which gene expression increases with [TF], and the parameter ω modulates this relationship and is itself a function of methylation state. Following Chen et al. (Chen et al., 2013) for a given locus *i* in cell *j*, ω_{ij} is

$$\omega_{ij} = \prod_{k=1}^{l} q^{M_{ijk}}, \tag{2}$$

where *l* is the number of modifiable loci (CpGs) in the TFBS, and *q* is the effect of methylation of a single locus on ω_{ij}. This is 1 if methylation has no effect or > 1 for a negative effect of methylation on transcription. Parameter M_{ijk} thus describes the methylation status for each TFBS at the *k*th locus, in the *j*th cell for the *i*th individual. In all simulations that follow we set $q = 1.2$.

The dynamics of re-methylation over time is defined by the probability of an un-methylated site becoming methylated and vice versa. The probability of methylation of an un-methylated site is inversely related to the binding of a TF, i.e.,

$$\Pr\{M_{ijk,t} = 0,\ M_{ijk,t+1} = 1\} = 1 - G, \tag{3}$$

where $M_{ijk,t}$ and $M_{ijk,t+1}$ are the states of methylation of the *k*th locus (individual *i* and cell *j*) in a TFBS at times *t* and *t+1* respectively, and *G* is the gene expression (Equation [1]). Therefore, the probability of a locus to remain un-methylated is equal to the concentration of a TF. We assume that the latter is independent of transcription, i.e.,

$$\Pr\{M_{ijk,t} = 1, M_{ijk,t+1} = 1\} = m, \tag{4}$$

where *m* is a constant. The probability that a CpG site loses its methylation is therefore (1-*m*).

We initiate every simulation with a [TF] and all loci begin as un-methylated, i.e., all values of *M* are set to zero. We simulated 'genes' with different levels of expression by stepwise increasing [TF] (details in Table S3). Gene expression is calculated per cell and, within an embryo, [TF] is equal for all cells. Because methylation status as determined by Equation [2] is zero for all potential methylation sites, the [TF] determines the probability of CpG dinucleotides being methylated, following Equation [3]. Then for every given site we compare a randomly drawn number from a uniform distribution with the outcome of Equation [3]. A CpG dinucleotide is methylated when this random number is lower than the probability calculated under Equation [3]. In the next time step, some sites will be methylated, while others remain un-methylated. Again by Equation [2] we determine the methylation status of every site and determine the probability of methylation for sites that are not methylated. However, for sites that are methylated we determine the probability that this methylation is maintained, according to Equation [4]. Therefore, for every time step we iterate over all individuals, cells and TFBS and CpGs within TFBS and determine states and state changes. For every time step we can calculate the gene expression of an individual by taking the mean over all cells. This process is repeated for every CpG site. Note that for a specific gene we therefore generate one gene expression level, while multiple mean levels of methylation exist.

Simulations

For the epigenetic selection hypothesis, the likelihood of implantation of an embryo depends on its gene expression level, which itself is not caused by maternal adversity but varies as a result of the stochastic nature of TF and DNAm binding (as outlined above). The resulting gene expression levels, as well as the values of methylation frequencies (mean methylation over all cells) are saved before (as control) and after selection (as cases). The intensity of selection was varied through the proportion of embryos that would

successfully implant. Because the number of individuals after selection is lower, and this could potentially influence the variation, we subsample the controls to the same N as the cases. To induce variation in the mean methylation between different genes we altered the [TF]. Thus, the effects of selection are compared between controls and selected embryos for a distribution of gene expression varying on a continuous scale between 0 and 1 in mean methylation frequency (see Figure 2).

For the adaptive plasticity hypothesis, cases (those under adversity) had a simulated concerted change in [TF] for a gene responding to maternal adversity (as a simulation of adaptive plasticity). On the contrary, to simulate control individuals, [TF] did not change (in effect creating a mean difference in gene expression between cases and controls). We assumed similar [TF] for all cells within individual embryo's and furthermore assumed no differences in [TF] between individuals at the start of the simulation. As above, the mean and standard deviation in methylation frequencies were computed for individuals CpG dinucleotides and mean and standard deviation were compared between controls and cases (see Figure 2). All values of constants used can be found in Table S3. Means and standard deviations were calculated in the in the R programming environment (R Development Core Team, 2009), while all simulations were performed in C++.

Robustness to parameter values and mechanistic assumptions

A robustness analysis was performed by altering values of the parameters used in each simulation (Table S3), as well as running simulations with alternative mechanistic assumptions. Throughout the robustness analyses, we calculated the ratio of standard deviation of methylation at a mean of 0.5 methylation frequency between selected and control samples. Selection intensity was taken to be 50% throughout the analysis, which corresponds to that of the main paper. For every parameter combination we estimated this ratio in 50 replicate populations and calculated the mean, standard deviation, minimum, and maximum of these ratio values.

Half saturation and methylation maintenance rate

The half saturation parameter (H) determines the concentration of TF and methylation status of the TF binding site (TFBS) at which gene expression is initiated (i.e., binding of the TF). A higher H results in higher gene expression for a certain methylation level and [TF]. The methylation maintenance parameter m is the probability that a site that is methylation will remain methylated from the first to a second-time point. Together, these parameters can influence the relationship between [TF], methylation, and gene expression and therefore potentially the strength of the effect of epigenetic selection on the variance in DNAm.

In the main paper, a decrease in variance is described for selected embryos compared to controls. This mean relative decrease in variance was evident for different levels of H and m (Figure S2). However, with high levels of H ($H = 20$) and high levels of methylation maintenance ($m > 0.96$), the distribution of the mean ± SD of the estimated ratios overlapped with 1, indicating no reduction in variance. Furthermore, in some individual simulations the ratio was higher than 1, which implies that the pattern will not always be detected even with reasonably strong selection, or only be evident for a subset of the genes that influence embryo survival.

Half saturation and recruitment of DNMTs by TFs

In the main paper, we report the results from simulations in which the binding of TFs restricted access for DNMTs, and therefore *de novo* methylation. However, within the genome, there are loci for which gene expression actually recruits DNMTs. Therefore, simulations were performed to study the effect of a positive relationship between gene expression and DNAm (Figure S3, upper row). Because the half saturation parameter, H, might interact with this effect, we ran simulations for a range of values of H. Under all scenarios, the mean ratio of variance between cases and controls was lower than unity, indicating a variance reduction similar to that described for the main model set-up. As expected, with low values of H (i.e., where methylation has little effect on gene expression), the effect of epigenetic selection is smaller as well. However, the fraction of TFBSs that positively or negatively affect methylation rate, by recruitment or competition with DMNTs, had no considerable effect on the reduction in DNAm variance upon epigenetic selection.

Number of loci per TFBS, methylation effect and gradient [TF]

While in the main paper 10 CpG sites per TFBS were modeled, the genome varies in the number of epigenetic loci that can affect the methylation dynamics. Furthermore, not all cells in an embryo will have the same [TF]. The interactive effect of these parameters was also studied (Figure S4).

Allowing for a gradient did not affect the ratio of variance between cases and controls in any of the settings for number of loci and effect of methylation per CpG site. However, if methylation has a relatively small effect on gene expression, the reduction in variance is larger when the number of loci is small. With an increasing effect of methylation on gene expression, the effect of selection on variance reduction increased for those TFBSs that have a larger number of CpG sites, while it remained relatively unaltered for those that have a small number of CpG sites. It is therefore expected that the number of CpG sites in a TFBS can affect the magnitude of variance reduction under epigenetic selection.

QUANTIFICATION AND STATISTICAL ANALYSIS

DNAm data

DNAm was measured using the Illumina Infinium Human Methylation 450K BeadChip (450k array) and described in detail elsewhere (Tobi et al., 2015). Briefly, samples were randomly distributed per 96-well plate and 450K array. Sample dependent and sample

independent quality metrics were assessed (van Iterson et al., 2014) and bisulfite conversion efficiency was assessed using dedicated probes and sequencing the IGF2 DMR0. Sample swaps were excluded by re-measuring genotypes measured on the 450K array and gender assessment using X chromosome CpG dinucleotides. We used *noob* and *Functional Normalization* from the *minfi* package to normalize data. Individual measurements with a detection p value > 0.01 or zero intensity value in the used color-channel were set as missing. The measurement success rate per sample was > 99%. Next, we removed a-specific/polymorphic, non-autosomal, < 95% success rate, and completely methylated or unmethylated (in bisulfite sequencing datasets) probes.

Statistics

We previously used generalized estimation equations (GEE) with a Gaussian link function to evaluate the association between DNAm percentage (the 450k array β-value x 100) and famine exposure (Tobi et al., 2015). In short, per 10-week exposure period and for 'any' gestational exposure we compared individuals that meet an exposure definition to all 463 prenatally unexposed time- and family-controls, controlling for correlation within sib-ships and adjusting for age, gender, row on the 450k array and bisulfite conversion plate, scan batch, and cellular heterogeneity. We took CpG dinucleotides further for an assessment of the variance in DNAm when the analysis for a difference in DNAm between famine exposed and controls showed a p value of < 0.001. As control set we took 1000 CpG dinucleotides not associated with famine exposure (p > 0.2) drawn by using de D-optimum criterion, which ensures that the 1000 reflect the distribution of both the mean and variance of the methylation of the famine associated CpG dinucleotides.

For each single exposure period we calculated the difference in variance with and without correcting the Beta values for the above-mentioned covariates using a simple linear regression. We calculated that standard deviation (SD) (as measure of the variation) of DNAm (%) per CpG for the exposed and controls separately and finally calculated the mean difference between the famine exposed and (sibling) controls over all selected CpGs. The significance of this difference was established by 100,000 permutations as the correlation between CpGs and within sibling pairs and the parabolic relation between SD and mean methylation violates assumptions required for (non)-parametric tests. We performed restricted permutations using the R package permute (version 0.9-0) as to assess how remarkable our observation is given our same-sex sibling design. We randomly assigned the 0-1 or 0-0 status for famine exposure status between sibling pairs (we do not have 1-1 pairs) and then randomly flipped exposure status within a pair. In conjuncture, we randomly assigned famine exposure status to individuals without a sibling in the study. Each permutation had exactly the same number of exposed individuals in the sibling pairs and unrelated individuals as in the actual cohort.

DATA AND SOFTWARE AVAILABILITY

Empirical DNAm data generated or analyzed during this study have been published (Tobi et al., 2015), these data are available on reasonable request. Simulation program (C++) and custom R script to analyze these data are deposited at GitHub at https://github.com/ecoevomicsjoost/embryo_selection_model.