Altered DNA methylation in children born to mothers with rheumatoid arthritis during pregnancy

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ABSTRACT

Objectives The main objective of this study was to determine whether the DNA methylation profile of children born to mothers with rheumatoid arthritis (RA) is different from that of children born to mothers from the general population. In addition, we aimed to determine whether any differences in methylation are associated with maternal RA disease activity or medication use during pregnancy.

Methods For this study, genome-wide DNA methylation was measured at cytosine-phosphate-guanine (CpG) sites, using the Infinium Illumina HumanMethylation 450K BeadChip, in 80 blood samples from children (mean age=6.8 years) born to mothers with RA. As controls, blood samples from 354 children (mean age=6.0 years) from the population-based Generation R Study were used. Linear mixed models were performed to investigate differential methylation between the groups, corrected for relevant confounders.

Results A total of 147 CpGs were differentially methylated between blood samples of children born to mothers with RA and the control blood samples. The five most significantly associated CpGs were cg06642177, cg08867893, cg06778273, cg07786668 and cg20116574. The differences in methylation were not associated with maternal RA disease activity or medication use during pregnancy.

Conclusions DNA methylation at 147 CpGs differed between children born to mothers with RA and children born to mothers from the general population. It remains unknown whether the identified associations are causal, and if so whether they are caused by the disease or treatment. More research, including replication of these results, is necessary in order to strengthen the relevance of our findings for the later-life health of children born to mothers with RA.

Key messages

What is already known about this subject?

► Adverse exposures in early life are associated with later-life health.
► Epigenetic changes are thought to be one of the underlying mechanisms.
► There is not much known about the consequences of maternal rheumatoid arthritis (RA) on the offspring’s long-term health.

What does this study add?

► DNA methylation is different in children born to mothers with RA compared with mothers from the general population.

How might this impact on clinical practice or future developments?

► Maternal RA disease during pregnancy might have lifelong consequences for the offspring.
► More research in this particular field must be undertaken.

Adverse exposures in early life are associated with later-life health, which is referred to as the developmental origins of health and disease hypothesis.1–4 Epigenetic processes are thought to be one of the mechanisms underlying the associations of early-life exposures and later-life health outcomes.5–7 DNA methylation is the best studied and understood epigenetic modification.8,9 Factors that have been demonstrated to be associated with fetal DNA methylation include maternal disease,10–15 smoking,14,16 corticosteroids,16 folate depletion17 and cytokines.18 DNA methylation usually occurs at cytosine-phosphate-guanine (CpG) sites.19 The effect of hypermethylation and hypomethylation on gene expression depends on the CpG location.19,20 The most pronounced changes in DNA methylation occur during early pregnancy.7,11

During embryogenesis, there are three germ layers that form in the developing fetus (ectoderm, mesoderm and endoderm). When DNA methylation is altered in early pregnancy, all germ layers are affected.21,22 Rheumatoid arthritis (RA) may be considered as an adverse exposure during pregnancy.23 Therefore, it is plausible that maternal RA may induce changes in fetal DNA methylation, and that it is related with the later-life health of the offspring. Interleukin-6 is known to influence DNA methylation.18 RA treatment during pregnancy includes among others sulfasalazine (SSZ) and corticosteroids such as prednisone. SSZ is a known folate antagonist that crosses the placenta and could influence DNA methylation in this respect.17,24 Furthermore, corticosteroids might influence DNA methylation.25 Especially during early pregnancy, when the placenta is not completely developed, prednisone passively diffuses to the fetus.26–28

In the current study we investigated whether the DNA methylation profile of children born to mothers with RA was different from that of children born to mothers from the general population. Furthermore, we investigated whether any differentially methylated CpGs were associated
with RA disease activity or medication use during pregnancy, or with indicators of future metabolic and cardiovascular diseases. In addition, we examined whether these CpGs were associated with the expression of genes using expression quantitative trait methylation (eQTM) analysis.

**METHODS**

**Study population**

**FEPRA study**

This study is embedded in the Pregnancy-induced Amelioration of Rheumatoid Arthritis (PARA) study, a prospective cohort study on pregnancy and RA. From 2002 to 2008, 369 female patients with RA who had a wish to conceive (or already pregnant) were enrolled. After participation in the PARA study, 196 children and their parents were invited to participate in a follow-up study, the Fetal Programming in Rheumatoid Arthritis (FEPRA) study. For this study, 108 children with a mean age of 6.8 years (SD=1.3) visited Erasmus Medical Centre in Rotterdam, and the parents of 85 children (all of European ancestry) gave informed consent for studies on DNA methylation of their children. Furthermore, the parents of 71 children provided cheek swabs from their children. There were no statistical differences in baseline characteristics between the participating and non-participating group.

**Generation R Study**

The control group consisted of children with a mean age of 6.0 years (SD=0.4), included in the Generation R Study, a population-based prospective cohort study from pregnancy onwards in Rotterdam, the Netherlands. In this study, all pregnant women living in Rotterdam with a delivery date between April 2002 and January 2006 were invited to participate, and 9778 mothers were enrolled. At the age of 6 years, DNA methylation was measured in a subgroup of 493 children of European ancestry.

**Data collection**

**FEPRA study**

In the PARA study, data on mother (eg, disease activity (with the Disease Activity Score in 28 joints using C reactive protein levels, DAS28-CRP³)) and child were collected. For the FEPRA study, data on blood pressure, growth and body composition of the children were measured. Also, blood, which is a mesoderm germ layer derivate, was drawn for DNA methylation analysis. Check swabs were collected for the analysis of DNA methylation in buccal epithelial cells, which is an ectoderm germ layer derivate.

**Generation R Study**

In the Generation R Study, mothers were seen three times during pregnancy. The children were followed from birth until childhood. Data collection in children and their mothers included questionnaires, detailed physical examinations and blood sampling.

**DNA methylation analysis**

Genomic DNA was extracted from whole peripheral blood samples and from the cheek swab samples. Bisulfite conversion of 500 ng of genomic DNA was performed using the Zymo EZ-96 DNA Methylation Kit (Zymo Research, Irvine, California, USA) according to the manufacturer’s protocol.

Genomic methylation profiling was performed using the Infinium Illumina HumanMethylation 450K BeadChip arrays (Illumina, San Diego, USA) according to the manufacturer’s protocol. The Illumina array measures methylation status of 485 512 CpG sites in the gene and non-gene regions across the entire human genome. To prevent possible batch effects, blood and cheek swab samples were measured in the same run.

**Quality control and normalisation**

The data were preprocessed using the minfi package in R V.3.4.1 (www.r-project.org). Samples with incomplete or poor bisulfite conversion, extension, hybridisation or specificity were excluded. In addition, samples with sex mismatch and samples with a call rate <95% were removed. This quality control (QC) was done separately for blood samples and for cheek swab samples. During QC, 5 blood and 14 cheek swab samples from the FEPRA study were excluded, resulting in 80 and 57 samples, respectively. From the Generation R blood samples, 27 were excluded due to corticosteroid use or RA disease in the mother, and 32 were excluded during QC, resulting in 441 blood samples. In addition, 87 cases with missing data from the Generation R Study were excluded, leaving 354 samples to analyse. The intensity values were then quantile normalised using the incorporating Control Probe Adjustment and reduction of global CORrelation (CPACOR) workflow. Methylation at each CpG was calculated as the beta value (beta=intensity of the methylated allele (M)/intensity of the unmethylated allele (U)+M+100)), containing values from 0 to 1. Blood cell composition of the samples was estimated using the Houseman method with the Reinius reference set. The Reinius reference set is not yet validated in children. However, it is the best method available, and it has been used in other epigenetic studies in children. Probes with single nucleotide polymorphisms (SNPs) at single base extension, probes with improper binding, and CpGs on the X and Y chromosome were removed from the analysis.

From the initial 485 512 CpGs, 32 057 were excluded during QC, leaving 453 456 CpGs for analysis.

**Statistical analysis**

For all subjects, descriptive statistics were calculated using Stata V.14.1 (https://www.stata.com/stata14/). Student’s t-tests and χ² tests were used to compare the baseline characteristics. For these analyses, p values <0.05 were considered statistically significant.

Linear mixed models were performed to analyse differential methylation between the groups, using R. The first model was created to compare the blood samples from the FEPRA study with the blood samples of the Generation R Study to determine whether the DNA methylation profile of children born to mothers with RA was different from that of children born to mothers from the general population. This model was corrected for biological covariates (age, body mass index (BMI) SD scores (SDS), adjusted for age and sex according to the Dutch reference values, using the Growth Analyser (V.4.0; Growth Analyser, Rotterdam, the Netherlands, www.growthanalyser.org)) sex, gestational age at delivery, maternal age, folic acid use during pregnancy, socioeconomic status (SES), maternal smoking and white blood cell subtypes), technical covariates (technical batch effects (array identifier (ID) and position on array)) and five hidden confounders. Technical covariates were added as random effects in the models. The hidden confounders were calculated using the CATE package while correcting for the group (RA vs non-RA offsprings), all biological covariates and technical covariates. This resulted in hidden confounders that were independent of all included covariates. The BACON package was used to correct for unobserved covariates in order to reduce test statistic bias and inflation. The genomic inflation factor
eQTM analysis
eQTM is a site at which DNA methylation is known to influence the expression of one or more genes. To analyse whether any of the significant CpGs were linked to the expression of nearby genes, an eQTM analysis was performed. For these analyses, the online BIOS QTL browser (https://biosqtl/molgenis26.target. rug.nl/downloads/biosqtlbrowser/) was used.

RESULTS
Participants
The flow chart of the study population is shown in figure 1. A total of 80 blood and 57 cheek swab samples from the FEPRA study (children born to mothers with RA) and 354 blood samples from the Generation R Study (children born to mothers from the general population) remained for analysis.

Descriptive statistics of the study population are presented in table 1. Overall, children from the FEPRA study were slightly older compared with children in the Generation R Study (p<0.001). In the FEPRA study, 45% of the women did not start using folic acid before or in early pregnancy compared with 92.7% in the Generation R Study (p<0.001). Approximately half of the women (47.5%) from the FEPRA study had a high SES compared with 68.4% of the women from the Generation R Study (p<0.001). One woman (1.3%) from the FEPRA study and 86 women (24.3%) from the Generation R Study smoked periconceptionally or at any time during pregnancy (p<0.001).

DNA methylation analysis
In the first linear mixed model, blood samples from the FEPRA study were compared with blood samples from the Generation R Study, corrected for the covariates mentioned in the Methods section. In total, 147 CpGs were significantly differentially methylated between children in the FEPRA study and children in the Generation R Study (figure 2). The QQ plot is shown in figure 3. The genomic inflation factor (λ) was 1.06.

Table 1  Descriptive statistics of study population

<table>
<thead>
<tr>
<th>Description</th>
<th>FEPRA study (n=80)</th>
<th>Generation R Study (n=354)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age child* (years), mean (SD)</td>
<td>6.8 (1.3)†</td>
<td>6.0 (0.4)†</td>
</tr>
<tr>
<td>BMI SDS child, ** mean (SD)</td>
<td>−0.14 (0.87)†</td>
<td>0.18 (0.74)†</td>
</tr>
<tr>
<td>Sex of the child</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>46 (57.5)</td>
<td>176 (49.7)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>34 (42.5)</td>
<td>178 (50.3)</td>
</tr>
<tr>
<td>Maternal age at delivery (years), mean (SD)</td>
<td>32.9 (3.9)</td>
<td>32.5 (4.0)</td>
</tr>
<tr>
<td>Gestational age (weeks), mean (SD)</td>
<td>39.5 (2.0)†</td>
<td>40.2 (1.5)†</td>
</tr>
<tr>
<td>Gestational age &lt;37 weeks, n (%)</td>
<td>8 (10.0)†</td>
<td>6 (1.7)†</td>
</tr>
<tr>
<td>No use, n (%)</td>
<td>36 (45.0)†</td>
<td>26 (7.3)†</td>
</tr>
<tr>
<td>SES based on educational level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low, n (%)</td>
<td>9 (11.3)†</td>
<td>4 (1.1)†</td>
</tr>
<tr>
<td>Middle, n (%)</td>
<td>33 (41.3)†</td>
<td>108 (30.5)†</td>
</tr>
<tr>
<td>High, n (%)</td>
<td>38 (47.5)†</td>
<td>242 (68.4)†</td>
</tr>
<tr>
<td>Maternal smoking, † n (%)</td>
<td>1 (1.3)†</td>
<td>86 (24.3)†</td>
</tr>
<tr>
<td>DAS28-CRP(3) third trimester, mean (SD)</td>
<td>3.3 (1.1)</td>
<td>–</td>
</tr>
<tr>
<td>Use of medication ≥1 trimester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only prednisone use, n (%)</td>
<td>17 (21.3)</td>
<td>–</td>
</tr>
<tr>
<td>Only sulfasalazine use, n (%)</td>
<td>14 (17.5)</td>
<td>–</td>
</tr>
<tr>
<td>Combination, n (%)</td>
<td>13 (16.3)</td>
<td>–</td>
</tr>
<tr>
<td>Prednisone dose (mg), median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>7.5 (2.5–10.0)</td>
<td>–</td>
</tr>
<tr>
<td>Second trimester</td>
<td>7.5 (5.0–10.0)</td>
<td>–</td>
</tr>
<tr>
<td>Third trimester</td>
<td>6.3 (5.0–10.0)</td>
<td>–</td>
</tr>
<tr>
<td>No medication use, n (%)</td>
<td>36 (45.0)†</td>
<td>–</td>
</tr>
<tr>
<td>Fat percentage SDS, mean (SD)</td>
<td>0.24 (0.97)</td>
<td>–</td>
</tr>
</tbody>
</table>

*At time of the blood sampling.
**P<0.001.
†During pregnancy.

BMI, body mass index; DAS28-CRP(3), Disease Activity Score in 28 joints using C reactive protein levels; FEPRA, Fetal Programming in Rheumatoid Arthritis; SDS, SD score; SES, socioeconomic status.
mothers with RA than in those from the general population at all five CpGs with the lowest p values. Methylation at the five CpGs with the largest effect sizes was lower in children born to mothers with RA, with the exception of cg06656994.

Subsequent analysis of DNA methylation
The mean DAS28-CRP(3) in the first, second and third trimesters (3.6, 3.5 and 3.3, respectively) were highly correlated (>0.6). The DAS28-CRP(3) in the third trimester was available in all patients. Therefore, this timepoint was chosen for the analysis. None of the 147 CpGs were significantly associated with maternal RA disease activity (DAS28-CRP(3)) in the third trimester or medication (prednisone or SSZ) use. In addition, none of the CpGs were associated with BMI SDS or fat percentage SDS in the children.

Analysis in buccal epithelial cells
A total of 10 out of the 147 CpGs significantly associated with maternal RA in blood were also associated in buccal epithelial cells. From these, four were in the same direction as in blood (table 3). CpG cg11336323 was located in a promoter region.

eQTM analysis
Two CpGs, cg21384971 and cg11220663, were associated with expression of the COPZ2 and ADD2 genes, respectively (table 4). These two genes were also the nearest genes to those CpGs.45 Both CpGs were hypermethylated in the children born to mothers with RA and were associated with decreased expression of COPZ2 and ADD2 in the BIOS eQTM lookup browser.

DISCUSSION
This is the first study investigating the differences in DNA methylation of children born to mothers with RA compared with children born to mothers from the general population. In this unique study, all participants were followed prospectively from pregnancy onwards. Our study showed differential DNA methylation between the two groups. The differentially methylated CpG sites were not associated with disease activity and/or medication use, nor to BMI SDS and fat percentage SDS.

In total, 147 CpGs were significantly associated with maternal RA after adjustment for multiple biological and technical covariates and hidden confounders. Of the five most significant CpGs, interestingly, two (cg06642177 and cg07786668) have been associated with myocardial infarction.47 The most significant CpG, cg06642177, is located on chromosome 6 near the SLC2A12 gene, 48 associated with insulin sensitivity,48 49 heart failure and diabetes,50 in animal models, cg07786668, located on chromosome 16, is located in the ZFHX3 gene. ZFHX3 has been associated in multiple human studies with atrial fibrillation,51–55 coronary heart disease56 and obesity in a Korean population.57 cg20116574 was annotated to the NCOA5, a protein coding gene, which has been associated with diabetes mellitus type 2 in animal models.58 59

From the remaining 142 CpGs, 1 (cg17218495), annotated to the SMARCA4 gene, has independently and significantly been associated with myocardial infarction.60 The other significant CpGs from our study have not been associated with disease phenotypes.

Out of the initial 147 significant CpGs, 10 were also significantly differentially methylated in buccal epithelial cells obtained by cheek swabs. From these, four were in the same direction as in blood. When DNA methylation is altered in more than one germ layer derivative, it is likely that these alterations occurred in early development.21 22 These four CpGs have not been associated with disease phenotypes in humans. Unfortunately, in the Generation R Study, DNA methylation in buccal epithelial cells was not available. There were no publicly available epigenetic data sets on buccal epithelial cells in healthy children with a similar age and background.

As mentioned before, CpGs that are associated with eQTM can influence the expression levels of genes.60 In our study, from the 147 significant CpGs, 2 were present in the BIOS QTL browser. cg21384971 is associated with less expression of the...
COPZ2 gene, which has been studied as a therapeutic opportunity for proliferation-independent selective killing of tumour cells.\textsuperscript{60} cg11220663 is associated with less expression of the ADD2 gene, also known as beta-adducin. ADD2 is involved in multiple pathogenic processes with a wide range of diseases.\textsuperscript{61} ADD2 gene variants are associated with hypertension,\textsuperscript{62} cancer\textsuperscript{63} and systemic lupus erythematosus.\textsuperscript{63}

Pathway analysis of the genes annotated to the 147 significant CpGs, using the WebGestalt (WEB-based Gene Set Analysis Toolkit),\textsuperscript{64} did not result in significant pathways (data not shown).

Thus, some of the associated CpGs (cg06642177, cg07786668 and cg17218495) have been associated with cardiovascular disease in previous studies, while others are located in or near genes that are associated with cardiovascular or metabolic disease. Maternal RA during pregnancy, a chronic inflammatory disease, might be associated with later-life health and disease risk in the offspring.

None of the significant CpGs were associated with RA disease activity or medication use during pregnancy. However, this may have been due to a lack of power, since these analyses were performed in the 80 samples of the children born to women with RA. The same power problem also applied to the analysis of the CpGs with indicators for future metabolic and cardiovascular disease (BMI SDS and fat percentage).

Remarkably, a large percentage of women with RA did not use folic acid before or during pregnancy. Even though this is outside the scope of our study, this requires additional attention during preconceptional counselling.

Our study has some limitations. First, although in its kind it is a large study, a study on DNA methylation including 80 subjects and 354 controls is still relatively small. Despite this, a large number of CpGs reached significance. Correcting for biological and technical covariates, as well as hidden confounders, and using BACON resulted in a λ near 1, reflecting that there was no inflation. Second, we were not able to collect a new independent cohort of children born to mothers with RA to replicate the results. At the time our study was performed, there were no other comparable prospective studies available. Currently, European research groups are conducting new prospective cohort studies on the impact of RA on pregnancy and offspring. We encourage these research groups, possibly with international collaborations, to replicate our study.

Thus, the results of this study may support follow-up research of children born to mothers with RA. Based on our data, we recommend that at least indicators for future cardiovascular and metabolic disease should be considered. The effects of RA disease activity and medication use on DNA methylation should be investigated in studies with larger sample sizes. Furthermore, in the last years the use of tumour necrosis factor (TNF) inhibitors during pregnancy in patients with RA has increased. This often results in lower RA disease activity during pregnancy. Future research should also cover the effects of the use of TNF inhibitors on the differentially methylated CpGs in children born to mothers with RA.

In addition, it would be interesting for a future study to measure DNA methylation in the mothers of the children from our study and compare that with the methylation of their offspring. Since

### Table 2

<table>
<thead>
<tr>
<th>CpG</th>
<th>Beta*</th>
<th>SE</th>
<th>P value</th>
<th>Nearest gene (±bp)</th>
<th>Chr</th>
<th>bp</th>
<th>Location†</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg06642177</td>
<td>0.028</td>
<td>0.002</td>
<td>1.32×10⁻²⁴</td>
<td>SL2CA12 (+122 529)</td>
<td>6</td>
<td>134 496 341</td>
<td>–</td>
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<tr>
<td>cg08867893</td>
<td>0.018</td>
<td>0.002</td>
<td>7.66×10⁻²⁴</td>
<td>ZNF365 (+221)</td>
<td>10</td>
<td>64 134 160</td>
<td>–</td>
</tr>
<tr>
<td>cg06778273</td>
<td>0.024</td>
<td>0.002</td>
<td>3.77×10⁻¹³</td>
<td>TNPFSF18 (+4995)</td>
<td>1</td>
<td>1 137 117</td>
<td>–</td>
</tr>
<tr>
<td>cg07786668</td>
<td>0.026</td>
<td>0.002</td>
<td>7.11×10⁻¹³</td>
<td>ZFHX3 (-10 142)</td>
<td>16</td>
<td>73 092 391</td>
<td>–</td>
</tr>
<tr>
<td>cg20116574</td>
<td>0.019</td>
<td>0.002</td>
<td>5.91×10⁻¹⁹</td>
<td>NCOA5 (+435)</td>
<td>20</td>
<td>44 718 168</td>
<td>–</td>
</tr>
<tr>
<td>cg16930947</td>
<td>-0.050</td>
<td>0.008</td>
<td>3.22×10⁻¹¹</td>
<td>–</td>
<td>8</td>
<td>88 984 447</td>
<td>–</td>
</tr>
<tr>
<td>cg14856454</td>
<td>-0.044</td>
<td>0.006</td>
<td>1.64×10⁻¹³</td>
<td>MIL16 (+303)</td>
<td>17</td>
<td>36 862 199</td>
<td>–</td>
</tr>
<tr>
<td>cg12360123</td>
<td>-0.043</td>
<td>0.008</td>
<td>1.61×10⁻⁰⁸</td>
<td>–</td>
<td>10</td>
<td>79 984 352</td>
<td>–</td>
</tr>
<tr>
<td>cg06556994</td>
<td>0.038</td>
<td>0.005</td>
<td>9.67×10⁻¹³</td>
<td>FAM163A (+903)</td>
<td>1</td>
<td>179 713 176</td>
<td>–</td>
</tr>
<tr>
<td>cg17483482</td>
<td>-0.037</td>
<td>0.006</td>
<td>4.62×10⁻¹⁰</td>
<td>–</td>
<td>1</td>
<td>117 152 162</td>
<td>–</td>
</tr>
</tbody>
</table>

†Location in promoter, enhancer or unknown (–).

*Beta represents the difference in DNA methylation at the given CpG site in children born to mothers with RA (FEPRA study) as compared with children born to mothers from the general population (Generation R Study).

### Table 3

<table>
<thead>
<tr>
<th>CpG</th>
<th>Beta*</th>
<th>SE</th>
<th>P value</th>
<th>Nearest gene (±bp)</th>
<th>Chr</th>
<th>bp</th>
<th>Location†</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg12998206</td>
<td>0.1029</td>
<td>0.022</td>
<td>4.00×10⁻⁰⁶</td>
<td>–</td>
<td>12</td>
<td>49 239 429</td>
<td>–</td>
</tr>
<tr>
<td>cg03654106</td>
<td>0.0727</td>
<td>0.016</td>
<td>9.50×10⁻⁰⁶</td>
<td>–</td>
<td>19</td>
<td>49 539 527</td>
<td>–</td>
</tr>
<tr>
<td>cg02613964</td>
<td>0.058</td>
<td>0.014</td>
<td>7.57×10⁻⁰⁶</td>
<td>–</td>
<td>3</td>
<td>44 690 321</td>
<td>–</td>
</tr>
<tr>
<td>cg11336323</td>
<td>-0.092</td>
<td>0.022</td>
<td>1.63×10⁻⁰⁴</td>
<td>–</td>
<td>19</td>
<td>41 946 040</td>
<td>–</td>
</tr>
</tbody>
</table>

†Location in promoter, enhancer or unknown (–).

*Beta represents the difference in DNA methylation at the given CpG site in buccal epithelial cells from children born to mothers with RA (FEPRA study) as compared with blood samples from children born to mothers from the general population (Generation R Study).

Table 2: The five most significant CpGs (white rows) and the five CpGs with the largest effect size (grey rows) from the linear mixed model: DNA methylation in blood samples from children born to mothers with RA (FEPRA study) compared with children born to mothers from the general population (Generation R Study).

Table 3: CpGs that were differentially methylated in the same direction in both blood and in buccal epithelial cells.
In this research, maternal RA during pregnancy is associated with differential DNA methylation in offspring. It remains unknown whether the identified associations are causal, and if so whether they are caused by the disease or treatment. Some of the differentially methylated CpGs or their nearby genes were associated with cardiovascular or metabolic disease. Maternal RA disease might have lifelong consequences for the offspring. However, more research in this particular field must be undertaken in order to strengthen the relevance of our findings.

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Rheumatoid arthritis


