





Prenatal exposure to tobacco smoke sex dependently influences methylation and mRNA levels of the lgf axis in lungs of mouse offspring

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- 1 Prenatal exposure to tobacco smoke sex-dependently influences methylation and mRNA
- 2 levels of the *Igf* axis in lungs of mouse offspring
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- 16
- 17 Running head: *Igf1r* methylation after prenatal smoke exposure
- 18
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- 26 KFM designed and conducted the experiments, analyzed the data, wrote the manuscript
- 27 WK and MRL prepared lung tissues for further use
- 28 SKE, WT, LK and TP analyzed the data and revised the manuscript
- 29 MNH designed the mouse experiment, analyzed the data and revised the manuscript
- 30

32 ABSTRACT

33	Background: Prenatal smoke exposure is a risk factor for abnormal lung development and
34	increased sex-dependent susceptibility for asthma and COPD. Birth cohort studies show
35	genome-wide DNA methylation changes in children from smoking mothers, but evidence for
36	sex-dependent smoke-induced effects is limited.
37	The insulin-like growth factor (IGF) system plays an important role in lung
38	development. We hypothesized that prenatal exposure to smoke induces lasting changes in
39	promoter methylation patterns of Igf1 and Igf1r, thus influencing transcriptional activity, and
40	contributing to abnormal lung development.
41	Method: We measured and compared mRNA levels along with promoter methylation of <i>Igf1</i>
42	and Igf1r and their protein concentrations in lung tissue of 30-day-old mice which had been
43	prenatally exposed to cigarette smoke (PSE) or filtered air (control). Body weight at 30 days
44	after birth was measured as global indicator of normal development.
45	Results: Female PSE mice showed lower mRNA levels of <i>Igf1</i> and its receptor (<i>Igf1</i> :
46	p = 0.05; <i>Igf1r</i> : $p = 0.03$). Furthermore, CpG-site specific methylation changes were detected
47	in Igf1r in a sex-dependent manner and the body weight of female offspring was reduced after
48	prenatal exposure to smoke, while protein concentrations were unaffected.
49	Conclusion: Prenatal exposure to smoke induces a CpG-site specific loss of <i>Igf1r</i> promoter
50	methylation, which can be associated with body weight. These findings highlight the
51	sex-dependent and potentially detrimental effects of in utero smoke exposure on DNA
52	methylation and Igf1 and Igf1r mRNA levels. The observations support a role for Igf1 and
53	<i>Igf1r</i> in abnormal development.
54 55 56 57	Keywords: "epigenetics" "pyrosequencing" "asthma" "COPD" "fetal programming" "Developmental Origins of Health and Disease"

58 LIST OF ABBREVIATIONS

COPD	Chronic obstructive pulmonary disease		
HPRT	HPRT Hypoxanthine-guanine phosphoribosyltransferase		
IGF	Insulin-like growth factor		
IGF1R	Insulin-like growth factor receptor 1		
IUGR	Intrauterine growth restriction		
PSE	Prenatal smoke exposure		

INTRODUCTION

61	Maternal smoking during pregnancy has detrimental effects for offspring, such as increased
62	risk of pulmonary diseases like as asthma (12, 13, 18) and chronic obstructive pulmonary
63	disease (COPD) (28), but the mechanisms remain largely unknown.
64	Negative gestational outcomes can be caused by epigenetic alterations of which
65	aberrant DNA methylation is commonly analyzed. It primarily refers to 5-methylcytosine
66	which occurs when neighboring a guanidine nucleotide (CpG-site). Often applied to a gene's
67	promoter region, this epigenetic mode can alter a its transcriptional activity.
68	Previous human genome-wide studies have linked prenatal smoke exposure (PSE) to
69	alterations of DNA methylation patterns in blood samples of newborns and children (e.g., 7,
70	14, 15). Such alterations may play a role in abnormal fetal development and increased
71	susceptibility for asthma and COPD. Interestingly, certain DNA methylation marks persist in
72	prenatally exposed children (30) and alterations in DNA methylation due to smoking during
73	pregnancy are still observed later in life. The association of maternal smoking and DNA
74	methylation seems to be more profound in girls than in boys (6), but possible interactions of
75	smoke exposure and the offspring's sex on methylation is rarely investigated.
76	
77	The importance of the insulin-like growth factor (IGF) system to lung development,
78	particularly Igf1 and its receptor Igf1r, is highlighted in Igf1- or Igf1r-depleted mice that show
79	a failure in lung development and diminished growth (10, 20). IGF-1, which exclusively
80	interacts with IGF1R (21), was decreased in female but not male fetuses of asthmatic mothers
81	who smoked during pregnancy (8) and the lower birth weight of female but not male neonates
82	correlated with reduced IGF-1 concentrations (8). For these reasons, we chose <i>Igf1</i> and <i>Igf1r</i> as
83	target genes for our analyses.
84	In lungs of 1-day-old mouse offspring, we previously found reduced mRNA levels of

85 developmentally relevant genes after prenatal smoke exposure (3). Based on these indications,

86 we hypothesized that PSE negatively affects *Igf1* as well as *Igf1r*. To test this postulate, we

87 determined the effect of prenatal smoke exposure on the protein and mRNA levels as well as

88 promoter methylation status of *Igf1* and *Igf1r* in lungs of 30-day-old offspring. These data

were related to each other and the offspring's body weight, a robust indicator of abnormal ornormal development.

91

92 MATERIAL & METHODS

93 Animals & smoke exposure

Female Balb/c mice were obtained from Harlan (Horst, The Netherlands) at 8 to 10 weeks of age. The experimental setup was approved by the local committee on animal experimentation (DEC4575A) and carried out as described previously (4). Offspring (6 male, 5 female) of 11 smoke exposed dams together with 6 male and 8 female offspring from 15 control dams were randomly selected from each nest, weighed, and euthanized 30 days after birth for organ collection. Isolated lungs were immediately frozen in liquid nitrogen and stored at -80 °C until further use.

101

102 **Quantification of IGF1 and IGF1R protein levels in lung homogenates**

103 IGF1 and IGF1R concentrations were measured in homogenized lung tissue (the two smallest

104 right lung lobes, as described in (4)). For quantitative determination of IGF1 concentrations,

105 the Quantikine® ELISA Mouse/Rat IGF-I Immunoassay (R&D Systems Europe, LTD,

106 Abingdon, UK) was used following the manufacturer's instructions. The quantification of

107 IGF1R was performed using the Mouse IGF1R/Igf1 receptor ELISA kit (Sandwich ELISA)

108 (LifeSpan BioSciences, Inc., Seattle, USA) as described by the manufacturer. For IGF1 23 out

109 of 25 samples, and for IGF1R 19 out of 25 samples had sufficient quality for analysis by

110 ELISA.

111

112 DNA & mRNA extraction

- 113 DNA and mRNA were extracted from whole lung tissue using the AllPrep DNA/RNA Mini
- 114 Kit (Qiagen, Venlo, The Netherlands), according to the manufacturer's protocol.
- 115

116 mRNA expression analysis

- 117 qRT-PCR for mRNA expression was performed using qPCR MasterMix Plus (Eurogentec,
- 118 Seraing, Belgium) with commercially available primers for target genes *Igf1* (product
- 119 number: Mm00439560_m1) and *Igf1r* (product number: Mm00802831_m1) (TaqMan® Gene
- 120 Expression Assay, Applied Biosystems, Foster City, CA, USA). Detection of amplification
- 121 reactions was performed using LightCycler® 480 System (Roche Diagnostics GmbH,
- 122 Mannheim, Germany) with cycling conditions as follows: 50°C for 2 min, 90°C for 10 min,
- 123 40 cycles of 95°C for 15 s and 60°C for 1 min. Reactions were performed in triplicate for
- 124 each sample with *Hprt* (Mm03024075_m1) used for normalization. We excluded 1 out of 25
- 125 data points for *Igf1r* and 2 out of 25 for *Igf1* mRNA levels due to large differences between
- 126 housekeeping and target genes.
- 127

128 **Pyrosequencing-based bisulfite PCR analysis**

- 129 In order to assess promoter methylation levels of selected genes, bisulfite sequencing primers
- 130 were designed using PyroMark assay design software (version 2.0, Qiagen). Selection of
- 131 CpG-sites was based on manual identification of CpG dinucleotides, using ENSEMBL
- 132 genome web browser (Ensembl 83: Dec 2015) and transcript location for the identification of
- 133 gene promoter regions. The mouse *Igf1* (ENSMUSG0000020053) has eight transcripts. In
- this study, we focused on transcript *Igf*-005 (ENSMUST00000122386, details in Discussion).
- 135 The analysis of mouse *Igf1r* gene (ENSMUSG0000005533) was done by using transcript

136 ENSMUST0000005671.

137	Extracted genomic DNA from lung (200 ng) was converted with sodium bisulfite (EZ
138	DNA Methylation-Direct TM , Zymo Research, Irvine, CA) following the manufacturer's
139	instructions. In short, the bisulfite conversion was carried out in the dark at 98 °C for 10
140	minutes and 64 °C for 3.5 hours followed by desulphonation of the converted DNA. Gene
141	amplification was done using HotStarTaq® MasterMix Kit (Qiagen, Venlo, The Netherlands).
142	Further specification on amplification conditions and primer sequences are listed in <i>Table 1</i> .
143	Amplification conditions: 95°C for 15 min, 94°C for 30 s, 59°C for 30 s, 72°C for 30 s,
144	40 cycles in a reaction volume of 25 μ L. To assess DNA methylation levels of <i>Igf1</i> and <i>Igf1r</i>
145	promoter methylation, bisulfite sequencing was performed on the PyroMarkQ24 (Qiagen)
146	instrument. Relative levels of methylation at each CpG-site were analyzed with PyroMark
147	Q24 2.0.6 software.

148

149 Calculations and statistical methods

150 Relative gene expression $(2^{-\Delta ct} \text{ method})$ as well as mean % methylation and SEM were

151 calculated in Microsoft® Office Excel 2003. Body weight, mRNA levels, protein

152 concentratios and DNA methylation data were tested for normal distribution of residuals and,

153 if normally distributed, analyzed using multiple linear regression analysis (IBM® SPSS®

version 22 release 22.0.0.1) in order to determine if the effect of prenatal exposure to tobacco

smoke interacts with the effect of sex difference. For statistical post hoc evaluation of the

- 156 subgroups, two-tailed Mann-Whitney U-test was used (GraphPad Prism 5.0 Software,
- 157 SanDiegeo, CA). Correlation analysis of target gene mRNA levels, percent methylation and
- body weight at 30 days after birth was done using linear regression. P-values ≤ 0.05 were

159 considered significant.

161 **RESULTS**

162 Sex-dependent effect of PSE on body weight at 30 days after birth

- 163 In the control group, female offspring had a significantly (p = 0.04) lower body weight when
- 164 compared to male offspring (female: 14.6 g vs. male: 16.5 g, *Figure 1A*). After PSE, female
- 165 offspring showed a significant (p = 0.05) reduction in body weight compared to controls
- 166 (PSE: 13.0 g vs. control: 14.6 g). This decrease was less pronounced in male offspring (PSE:
- 167 14.9 g vs. control: 16.5 g), therefore the sex-specific body weight difference was lost in the
- 168 male PSE group (p < 0.07). An interaction of both parameters, sex and PSE, on the body
- 169 weight was not seen (linear regression).
- 170

171 **Quantification of IGF1 and IGF1R in lung homogenates**

172 *IGF1*

173 Differences of IGF1 concentrations after PSE appeared to be more pronounced in female

- 174 (control: 5965 pg/ml vs. PSE: 4885 pg/ml) than in male offspring (control: 5990 pg/ml vs.
- 175 PSE: 5733 pg/ml), but did not reach statistical significance (data not shown).
- 176 Using linear regression, the variation in IGF1 concentrations contributed to the variation of
- 177 the offspring's body weight by 30% ($R^2 = 0.29$, p = 0.01) (*Figure 1B*). This contribution was
- 178 mostly derived from the prenatally smoke exposed offspring (PSE: r = 0.86, p = 0.002 vs.
- 179 control: r = 0.25, ns). Here, the effect was more pronounced in female (r = 0.98, p = 0.02)
- 180 than in male (r = 0.80, p < 0.06) offspring (*Table 2*). Over all, an association of IGF1
- 181 concentrations in whole lung tissue and the offspring's body weight, independent of the type
- 182 of exposure, was found for female (r = 0.62, p = 0.04), but not for male (r = 0.46, ns)
- 183 30-day-old mice (*Table 2*).

184	IGF1R
185	Similar to the findings for IGF1 protein levels, also the difference in the concentration of
186	IGF1R in lung homogenate did not reach statistical significance when comparing PSE mice to
187	control offspring, but were higher in females (control: 5516 pg/ml vs. PSE: 5807 pg/ml) than
188	in males (control: 4061 pg/ml vs. PSE: 4293 pg/ml; data not shown). Contrasting the
189	observation for IGF1, the variation in IGF1R concentration did not contribute to the variation
190	of the offspring's body weight (linear regression, $R^2 = 0.09$, ns; <i>Figure 1C</i>)
191	
192	Sex-dependent effect of PSE on mRNA concentrations of <i>Igf1</i> and <i>Igf1r</i>
193	Igf1
194	PSE reduced mRNA levels of <i>Igf1</i> in female offspring ($p = 0.05$) (<i>Figure 2A</i>), but not in male
195	offspring. In the control groups, differences of the mRNA levels of male and female offspring
196	were not significant ($p = 0.1$; <i>Figure 2A</i>).
197	Igf1r
198	Figure 2B displays mRNA levels for Igf1r. Again, female mice showed a reduced gene
199	expression after prenatal smoke exposure ($p = 0.03$) (<i>Figure 2B</i>), while no effect was detected
200	in male offspring. Notably, higher base line mRNA levels were seen in female offspring but
201	did not reach statistical significance ($p = 0.07$; <i>Figure 2B</i>).
202	Using linear regression, no interaction of parameters, sex and PSE, was seen for both
203	mRNA levels. However, it revealed a strong positive correlation of mRNA levels between
204	both genes ($R^2 = 0.91$, p < 0.001; <i>Figure 3A</i>).
205	
206	Igf1 gene expression and protein concentrations were only seen to correlate in female PSE,
207	but not in male offspring (linear regression, female: $R^2 = 0.90$, $p = 0.05$ vs. male: $R^2 < 0.01$,

208 ns) wherefore a correlation in all offspring was not seen (*Figure 3B*).

209 Igf1r gene expression, on the other hand, correlated with IGF1R protein concentrations (linear

210 regression; $R^2 = 0.35$, p = 0.02; *Figure 3C*). This effect appears to originate from female

offspring (r = 0.72, p = 0.05; *Table 3*), predominantly from female control animals (r = 0.93,

212 p = 0.02; *Table 3*) while in male offspring no association was seen (r = 0.10; ns).

- 213 Similarly, the variation in gene expression of *Igf1* and *Igf1r* in control animals contributed to
- their variation in body weight by 62% and 69%, respectively (linear regression, *Igf1*:
- 215 p = 0.002; *Igf1r*: p = 0.002; *Tables 2&3*), which was also seen for female but not for male
- offspring (*Igf1*: female: r = -0.76, p = 0.05 vs. male: r = -0.52, ns, *Table 2*; *Igf1r*: female:
- 217 r = -0.86, p = 0.03 vs. male: r = -0.69, ns; *Table 3*).
- 218

219 Effect of PSE on promoter methylation of *Igf1* and *Igf1r*

220 Igf1

221 Figure 4 illustrates the mean percent methylation of each analyzed CpG-site in the promoter

of *Igf1*. The targeted promoter region of *Igf1* did not reveal differences in methylation levels

in any of the analyzed CpG-sites after prenatal smoke exposure. *Figure 5* provides a

sex-specific overview of CpG-site specific data points of *Igf1*, which does not show additionalsignificant findings.

A linear relationship was found between protein concentrations and methylation status of CpG-1509 in all control animals (r = -0.79, p = 0.001). Here, the effect was more

pronounced in female than in male offspring (female: r = -0.93, p = 0.002 vs. male: r = -0.79,

p = 0.06; *Table 2*). This observation is contrasted by a linear relation for all control animals at

230 CpG-1212 (r = 0.64, p = 0.02), which was found in male but not in female offspring (male:

- 231 r = 0.83, p = 0.04 vs. female: r = 0.13, ns; *Table 2*). For that same CpG-site also a linear
- relation was found for *Igf1* mRNA concentrations in all control animals (r = -0.60, p = 0.04).
- 233 This observation was again sex-dependent, as it was only seen in female but not in male

234 control mice (female: r = -0.85, p = 0.02 vs. male: r = -0.46, ns; *Table 2*).

235 Moreover, only for the female PSE group a trend for a linear relationship was found between

- the methylation status at CpG-1180 and protein concentrations (r = 0.93, p = 0.07; *Table 2*) as
- 237 well as body weight (r = 0.86, p = 0.06; *Table 2*).
- 238
- 239 Igflr

240 The mean percent methylation of *Igf1r*'s promoter region is depicted in *Figures 6*, while a

sex-specific overview of CpG-site specific data points is provided in *Figures 7 and 8*. The

- analysis of *Igf1r* promoter allowed three observations:
- Firstly, a sex-independent reduction was found for the %methylation of *Igf1r* CpG-272

(p = 0.04, Figure 6) together with a trend for lower methylation status after prenatal smoke

exposure at CpG-252 (p = 0.08). Within the entire PSE group, significant correlations (linear

- regression) were seen for mRNA concentrations with % methylation at CpG-201 (r = 0.67);
- protein concentrations with CpG-249 (r = 0.78) and CpG-194 (r = 0.92) as well as body

248 weight with CpG-233 (r = 0.62) and CpG-206 (r = -0.66; *Table 3*).

- 249 Secondly, a sex-dependent reduction in methylation levels was found at *Igf1r*
- 250 CpG-233 for male (p = 0.04) and female (p = 0.05) offspring when compared to their control

251 groups (*Figure 7A*). The methylation status of female PSE offspring at this CpG-site was

- significantly lower when compared with male PSE offspring (p = 0.04). Notably, at *Igf1r*
- 253 CpG-206 on the other hand, prenatally smoke exposed female offspring showed higher
- 254 CpG-site specific methylation when compared to male PSE mice (p = 0.02) (*Figure 8*).
- 255 Thirdly, within all analyzed offspring, linear regression revealed a correlation of
- promoter methylation and mRNA concentrations at CpG-201 (r = 0.62) and -17 (r = 0.55).
- 257 This observation was augmented in the male group (r = 0.76 and r = 0.65, respectively;
- 258 *Table 3*). Moreover, in all analyzed offspring, protein concentrations were seen to correlate

259 with methylation status at CpG-201 (r = 0.45), CpG-194 (r = 0.49) and CpG-171 (r = 0.51;

260 **Table 3**) of which the correlation seen for CpG-194 was enhanced in PSE offspring (r = 0.92).

261 Interestingly, linear regression also uncovered that the methylation status at *Igf1r* CpG-233

262 contributed to the variation of body weight at 30 days after birth by 30% (R² = 0.30,

263 p = 0.004; *Figure 7B*). This effect was also seen, sex-independently, for the PSE mice

264 (r = 0.62, p = 0.04; Table 3).

265

266 **DISCUSSION**

According to the "fetal origins of disease" hypothesis (1, 2), an adverse fetal environment has long lasting consequences for the offspring. In this study we investigated the effect of prenatal smoke exposure (PSE) on mRNA and DNA methylation levels of *Igf1* and *Igf1r* as well as

their protein concentrations in lungs of 30-day-old mouse offspring. Our results support the

271 hypothesis that smoking during pregnancy affects mRNA levels of *Igf1* and *Igf1r* in a

sex-dependent way.

273 Smoking during pregnancy has a negative effect on the birth weight of a newborn. In 274 our mouse model, we use the body weight at 30 days after birth as a global indicator of 275 abnormal prenatal development. Apart from the *in utero* smoke exposure, housing conditions 276 of all animals were identical. Alterations of the body weight are therefore likely to be caused 277 by the experimental variable, here prenatal smoke exposure. Both male and female offspring 278 showed a reduction of approximately 10% in body weight at 30 days after birth from 279 smoke-exposed mothers compared to their matching control group. Similar findings were 280 described in mice following prenatal smoke exposure by Larcombe et al. (16). 281 In humans, prenatal smoke exposure has previously been linked to intrauterine growth 282 restriction (IUGR) which in turn was shown to increase the risk of developing asthma (35).

283 Moreover, disrupted prenatal lung development was linked to the development of COPD later

284 in life (9). Protein concentrations of IGF1 were reduced in cord plasma of babies born to 285 mothers who had smoked during pregnancy, which may have contributed to fetal IUGR (29). 286 This suggests that prenatal smoke exposure might have some attenuating effects on growth due to 287 reduced IGF1 or a deranged IGF signaling pathway. While no significant effect of prenatal smoke 288 exposure on IGF1 and IGF1R protein levels was detected, we found a strong correlation of IGF1 289 and the offspring's body weight, in particular in the PSE females. Furthermore, protein levels for 290 IGF1R correlated with *Igf1r* gene expression, which again was found for female offspring 291 exclusively. These data are accompanied by the reduced mRNA levels for Igf1 and Igf1r seen in 292 lungs of 30-day-old offspring after prenatal smoke exposure. 293 The simultaneous reduction of mRNA levels for both *Igf1* and *Igf1r* after PSE and 294 hence their strong positive correlation could be explained by a negative feedback loop. This 295 was also proposed by Moreno-Barriuso et al., who suggested that IGF1 could regulate the 296 expression of its own receptor (22).

Given the abrogated lung maturation after Igf1 and/or Igf1r gene deletion (10, 20), the observed reduction of Igf1 and Igf1r gene expression in lungs of 30-day-old mice may reflect abnormal lung development, as at this age the alveolar phase is ending. Although in these mice neither the actual asthma phenotype nor the allergen susceptibility of the 30-day-old offspring was assessed, it is conceivable that a repression of important signaling cascades, such as IGF, during developmental stages of the lung could have long(er) lasting effects later in life.

304

The *Igf1* gene contains 6 exons (23) and so far at least nine different *Igf1* isoforms are known (33). These are generated by utilizing different promoters and splicing variation. Transcripts comprising the first exon are referred to as Class1, those with the second exon as Class2 transcripts (23). Class2 depleted mice did not show an affected viability or phenotypical changes, but a compensatory up-regulation of Class1 transcripts (33). Consequently, we

310	investigated a possible effect of PSE on P1 promoter methylation of the Class1 Igf1 isoform.
311	Our assessment did not reveal any smoke induced effects in 30-day-old mouse offspring but
312	associations of body weight, mRNA levels and protein concentrations with methylation status
313	were found by linear regression. Interestingly, these associations were seen when
314	distinguishing by the offspring's sex and their prenatal exposure. However, the active
315	expression of genes requires an orchestration of many (co-) factors of which DNA
316	methylation can be one out of several epigenetic modes. The lack of a direct correlation was
317	anticipated as the mechanistic link between DNA (de)methylation and gene silencing or
318	activation is complex. Other epigenetic modes (i.e., histone modifications, chromatin
319	remodeling and RNA-based mechanisms (lncRNAs / miRs)) seem to be interlinked, but their
320	chronological order and the exact mechanism(s) that may connect these modes still need to be
321	described to their full extent (reviewed by e.g., 31).
322	

323 Female offspring showed a stronger PSE-induced reduction of *Igf1* as well as *Igf1r* transcripts 324 when compared to male offspring. The implication of a role for sex hormones influencing the 325 expression of Igf1 isoforms is in discussion for a long time and indeed, Class1 transcripts 326 responded to a higher degree to estrogen activation than Class2 transcripts (25). Cord 327 plasma/blood concentrations of IGF1 in female neonates were seen higher than in males (11, 328 36) and a dimorphic expression pattern was suggested. Similarly, we found a trend for higher 329 baseline levels of *Igf1* and *Igf1r* mRNA levels in females when comparing them to male 330 control offspring. Recently, in a COPD mouse model, female mice showed, compared to male 331 mice, an increased morphologic remodeling of the small airways after six months of cigarette 332 smoke exposure (32). These observations suggest either a higher vulnerability of female mice 333 to prenatal insults such as cigarette smoke exposure, or the availability of quicker and/or more 334 efficient compensatory mechanisms to counteract any insult either on a prenatal or early

335 postnatal stage in male mice. This is of interest, as also in humans, the prevalence rate for

336 COPD is higher in women than in men (3.5% vs. 2.9%) (24).

337

338	In contrast to the findings for Igf1, Igf1r promoter methylation was altered after PSE at three
339	CpG-sites. Even though the base line methylation levels at CpG-206 of male and female
340	control groups were similar, after PSE hypomethylation was seen in male and
341	hypermethylation in female offspring, which suggests a possible sex-dependent response to
342	PSE. Similarly, Igf1r mRNA concentrations correlated with methylation status at two CpG-
343	sites (CpG-201 and CpG-17) and in both cases, this association originated from male
344	offspring with an additional contribution of the PSE mice. Moreover, Igf1r CpG-233 was
345	detected to be hypermethylated after PSE and could be linked to the offspring's body weight
346	at 30 days after birth. Interestingly, this can only be seen independently of the offspring's sex,
347	but was also found in the group of prenatally smoke exposed offspring. Lastly, also Igf1r
348	CpG-272 showed sex-dependent hypomethylation. Here, PSE caused a loss of correlation to
349	Igf1r mRNA levels but induced a correlation to the offspring's body weight, predominantly in
350	male mice.
351	Epigenetic marks, such as DNA methylation, are shown to be affected by prenatal

352 smoke exposure and can increase the risk of developing asthma in mice and men (16, 37). The 353 PSE-responsive CpG-sites of the Igf1r promoter region suggest a role for DNA methylation in 354 the expression of Igf1r and its relevance in mediating the IGF system; determining to which

355 extent however, requires further studies.

356 The majority of DNA methylation studies links promoter hypermethylation with gene

357 silencing and the lack of methylation with gene "activation". However, several recent studies

- report upon a positive association of DNA methylation status and gene expression (i.e., 5, 19).
- 359 Other studies find that the link between DNA methylation and gene expression depends on

360	where in the gene sequence the methylation occurs/is detected (gene body vs. flanking
361	regions, transcription start site, 5'-untranslated region etc.). Taking together, these findings
362	suggest that the relation of DNA methylation and gene expression may not be as strict as
363	previously described.
364	In light of these findings and the complex network of several epigenetic modes, we
365	conclude from our observations that differential CpG-site specific methylation after PSE may
366	depend on the offspring's sex.
367	We recognize that we analyzed only one promoter region for the <i>Igf1</i> gene and it could very
368	well be that by doing so, we missed other putative methylation sites. One limitation of our
369	data is the lack of methylation information on other potentially relevant parts of the Igf1r
370	sequence (7 CpG-sites, CpG-146 to -104). Future analysis of this and other potentially
371	regulatory regions is warranted. Nevertheless, methylation changes at CpG-site resolution, as
372	we found for <i>Igfr</i> -233, can be functionally important.
373	Other studies indicated that "CpG-137" and "CpG-611" of the human Igf1 gene may
374	have functional relevance, as they were found to contribute to height and serum IGF1
375	variation in PBMCs of pre-puberty children (26). Additionally, the impact of Igf1 "CpG-137"
376	methylation on serum IGF1 level variation seems to increase in children with idiopathic short
377	stature after treatment with growth hormone (27). Moreover, one CpG-site of the $Igf1r$ gene
378	(cg12562232) was significantly associated with differences in birth weight of monozygotic
379	twins (34).
380	
381	To our knowledge, this is the first study to demonstrate effects of <i>in utero</i> smoke exposure on

382 Igf1r and Igf1 promoter methylation and mRNA levels in mouse lungs. These findings

383 emphasize the sex-dependent effects of PSE and indicate a role of the IGF system,

384 represented here by *Igf1* and *Igf1r*, in (lung) development in mouse offspring. Even though

385 studies could link decreased serum IGF1 levels in the fetal circulation with maternal smoking 386 during pregnancy (e.g., 29), a sex-dependent distinction is rarely done. Notably, maternal 387 smoking was associated with reduced IGF2 methylation in DNA of umbilical cord white 388 blood cells, with a stronger effect in newborn girls than boys (6). Also, Richmond et al. found 389 sex-specific associations for DNA methylation changes in the offspring's cord blood, when 390 compared with non-smokers. Of these associations, one CpG-site at AHRR (cg05575921) was 391 found with a smoke-induced effect larger in girls than in boys whereas another CpG-site at 392 CYP1A1 (cg05549655), the observed effect was larger in boys than in girls (30).

393

In summary, evidence for a sex-specific effect of maternal smoking during pregnancy can be found in human studies, but it is limited. Our data indicate that sex-differences in maternal smoking effects need more attention and may provide important insights into pathogenesis of health effects. Furthermore, the present study provides a sex-specific link between prenatal smoke exposure, epigenetic modifications, body weight, gene expression and protein levels. This information may be used to identify future targets for therapeutic intervention.

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DISCLOSURES

531 FIGURE CAPTIONS

- 532 Figure 1. Body weight [g] comparing control (\circ) with prenatally smoke exposed (\bullet)
- 533 offspring and correlations of body weight with IGF1 and IGF1R concentrations in

534 whole lung tissue.

- 535 A) Prenatally smoke exposed (PSE) and air exposed control mice were euthanized at 30
- 536 days after birth. Prior to euthanasia, the body weight [g] was assessed (individual values
- 537 per group and sex, median depicted as a horizontal line).
- 538 B) The body weight [g] of all offspring correlates with IGF1 concentrations in whole lung
- 539 (linear regression).
- 540 C) A correlation of body weight [g] with IGF1R concentrations in whole lung was not
- 541 found.
- 542 If not stated otherwise, the comparison of displayed groups was not significant.
- 543

544 Figure 2. *Igf1* and *Igf1r* mRNA levels comparing 30d control (0) with prenatally

545 smoke exposed (•) offspring.

- 546 A) *Igf1* and B) *Igf1r* mRNA levels were measured in whole lung tissue of 30-day-old
- 547 prenatally smoke exposed and control mice and corrected for housekeeping gene Hprt. A
- 548 sex-specific reduction of mRNA levels was seen in female mice for both genes, whereas male
- offspring did not show an effect of prenatal smoke exposure on mRNA levels. Data are
- 550 presented per sex and group as individual values with median as a horizontal line. If not stated
- 551 otherwise, the comparison of displayed groups was not significant.

552

553 Figure 3. Correlation of *Igf1* with *Igf1r* mRNA levels in lungs of 30-day-old offspring

and correlations of *Igf1* and *Igf1r* gene expression with protein levels.

555 A) Prenatally smoke exposed (PSE) and air exposed control mice were euthanized at 30 days

556	after birth. Displayed mRNA levels of Igf1 and Igf1R were measured in whole lung tissue and
557	displayed as uncorrected data points. Linear regression revealed a positive relation of mRNA
558	levels between both genes and strong interactions of both mRNA levels were found sex-
559	dependently for all possible groups.
560	B) Based on linear regression, no correlation was found for <i>Igf1</i> gene expression and protein
561	levels in lung homogenates.
562	C) Igflr gene expression correlated with the amount of protein in lung homogenates. This
563	effect was also seen for all female offspring and was most pronounced in the female control
564	group.
565	
566	Figure 4. Methylation of each analyzed CpG-site in the <i>Igf1</i> promoter (mean ± SEM)
567	comparing 30d control (\circ) with prenatally smoke exposed ($ullet$) offspring.
568	DNA from lungs of 30-day-old offspring who were either prenatally smoke exposed ($n = 11$)
569	or in the control group (n = 14) was assessed for $IgfI$ promoter methylation status. No
570	differences were detected. Data are shown as mean \pm SEM. CpG-site annotations are relative
571	to ATG start codon. If not stated otherwise, the comparison of displayed groups was not
572	significant.

574	Figure 5. Methylation	of each analyzed	CpG-site in the <i>Igf1</i>	promoter comparing 30d
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575 control (•) with prenatally smoke exposed (•) offspring.

576 DNA of lungs from 30-day-old offspring of PSE and control groups was subjected to bisulfite

- 577 sequencing-based methylation analysis of *Igf1* promoter region. Data of the 8 targeted
- 578 CpG-sites are presented per sex and group as individual values with median as a horizontal
- 579 line. CpG-site annotations are relative to ATG start codon. If not stated otherwise, the
- 580 comparison of displayed groups was not significant.
- 581

```
582 Figure 6. Methylation of each analyzed CpG-site in the Igf1r promoter (mean ± SEM)
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583 comparing **30d** control (\circ) with prenatally smoke exposed (\bullet) offspring.

584 *Igf1r* promoter methylation levels were assessed in lungs of prenatally smoke exposed (PSE)

offspring (n = 11) and compared to control offspring (n = 14). Data are shown as mean \pm

586 SEM. * p < 0.05. CpG-site annotations are relative to ATG start codon. If not stated

587 otherwise, the comparison of displayed groups was not significant.

588

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589 Figure 7. Sex-specific methylation status and correlation of Igf1r CpG-233 comparing
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590 **30d control** (\circ) with prenatally smoke exposed (\bullet) offspring.

- A) Prenatal smoke exposure (PSE) induced reduction of *Igf1r* CpG-233 in male and female
- 592 offspring. Methylation status of *Igf1r* CpG-233 in female PSE offspring is significantly lower
- than in male PSE offspring.
- B) The methylation status of *Igf1r* CpG-233 correlated positively with the offspring's body
- 595 weight [g] at 30 days after birth. Data are shown as individual values. CpG-site annotations
- are relative to ATG start codon. If not stated otherwise, the comparison of displayed groups
- 597 was not significant.

599 Figure 8. Methylation of each analyzed CpG-site in the *Igf1r* promoter comparing 30d

600 control (\circ) with prenatally smoke exposed (\bullet) offspring.

- 601 DNA of lungs from 30-day-old offspring of PSE and control groups was subjected to bisulfite
- 602 sequencing-based methylation analysis of *Igf1r* promoter region. Data of the targeted
- 603 CpG-sites are presented per sex and group as individual values with median as a horizontal
- 604 line. CpG-site annotations are relative to ATG start codon. If not stated otherwise, the
- 605 comparison of displayed groups was not significant.
- 606
- 607

608 Table 1. Bisulfite amplification (F/R) and sequencing (S) primers

Gene	Targeted CpG-site position	Sequences 5' – 3'	
TTTTTTGAGA S2:		R: AAACCAAACTTACCTCAATCTCTTAC S1: AGGTTTTATTTATGGGG S2: GTATTTTAAATTTTTTTGAGA Sequence to analyze: S1: TAGYGTAAAGAGGTAGTGTAGAGTTTTTAATTGGTTTTTGTTTTTATYGATGTGTTAGTATTTTTAAAT TTTTTTGAGA	
	1357-1254 Amplicon length [bp] 212	F: AGAGTAAGAGATTGAGGTAAGTT R: TTACCACAAAAAATAAAATTCTAATCTTC S1: GGGAAAGTATATTTGGAG S2: TTATTGAGAAATAGGTATAAAT Sequence to analyze: S1: AGATATTYGTGGAAAGTATGTAGYGTTTAATTTGGGTTTTTGTAATTTTTTTT	
	1212-1180 Amplicon length [bp] 250	F: TTGGAGAGATATTAGTGGAAAGTATGTAG R: AATTATAATATCATTCAAATCCCTCAACT S: AGAATTTTATTTTTGTGGTAAAG Sequence to analyze: GYGAGTTTATATATATATAAATAGTAGAAGTAGTYGGTTTGAATTATGTTGTTAGTTA	
Gene	Targeted CpG-site position	Sequences 5' – 3'	
Igflr	272-164 Amplicon length [bp] 327	F: GGGGATTTTTTTTAGGAGTTAGATTTA R: ATTTTCCTCCTTCTTCTACATCT S1: TTA TTT GGG ACG AAA TTT S2: GATAAGGAGGGTGG S3: GGAGTYGGGAAGT Sequence to analyze: S1: TTTTTATTTTYGTTTAAAAATAAGAGYGTAGGYGAYGATTTTYGGAAAGYGGYGTGGATAAGGAGG GTGG S2: YGYGGGGYGGTTTTTTAGYGTYGGTAGTAGYGGTTTAYGGGGYGGYGGAGTYGGGAAGT S3: YGGGGYGYGTYGGGGGYGGGTTGTYGGYGTYGTYGGTTTTTATTTGTAAAYGTAGAGATGTAGAAG AAGGAGGAAA	
	17 Amplicon length [bp] 120	F: AGTGAGGATTGAGTTGGAGATTT R: CCTCCCAAACCAAACTTCATTCCTTTAT S: ATTTTTGAGAAAAGGGAATT Sequence to analyze: TYGTTTTAAATAAAAGGAATGAAGTTT	

correlation of / with	IGF1 protein [pg/ml]		all all	all male	all female	all control	all PSE	male control	male PSE	female control	female PSE
		r	0.02	-0.02	0.18	-0.22	0.44	0.16	0.08	-0.26	0.95
Igf1 (2-dCT)		p-value	ns	ns	ns	ns	ns	ns	ns	ns	0.05
		r	0.54	0.46	0.62	0.25	0.86	0.10	0.80	0.33	0.98
weight [g]		p-value	0.01	ns	0.04	ns	0.002	ns	0.06	ns	0.02
_		r	-0.29	-0.28	-0.41	-0.79	0.11	-0.79	0.15	-0.93	0.03
	CpG-1509	p-value	ns	ns	ns	0.001	ns	0.06	ns	0.002	ns
		r	-0.06	-0.19	0.26	-0.27	0.15	-0.50	0.20	0.24	0.28
<u>.</u>	CpG-1465	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
<u></u>	6 6 1420	r	-0.17	-0.33	-0.02	-0.27	-0.09	-0.10	-0.44	-0.60	0.52
- ti	CpG-1430	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>lgH</i> promoter methylation [%]	CpG-1357	r	-0.09	-0.13	-0.12	-0.54	0.29	-0.79	0.38	-0.38	-0.25
netl	CpG-1357	p-value	ns	ns	ns	0.06	ns	0.06	ns	ns	ns
ern	CpG-1341	r	0.28	0.40	0.06	0.51	0.17	0.46	0.50	0.65	-0.37
- not	Cp0-1541	p-value	ns	ns	ns	0.07	ns	ns	ns	ns	ns
101	CpG-1254	r	-0.29	-0.18	-0.45	-0.42	-0.13	-0.17	-0.19	-0.83	0.05
1 12 -	000-1204	p-value	ns	ns	ns	ns	ns	ns	ns	0.02	ns
Ig	CpG-1212	r	0.26	0.49	-0.11	0.64	-0.04	0.83	0.23	0.13	-0.03
-	000-1212	p-value	ns	ns	ns	0.02	ns	0.04	ns	ns	ns
	CpG-1180	r	0.33	0.24	0.58	0.28	0.39	0.42	0.10	0.13	0.93
correlation of /	<i>Igf1</i> (2-dCT)	p-value	ns all all	ns all male	0.08 all female	ns all control	ns all PSE	ns male control	ns male PSE	ns female control	0.07 female PSE
with	1g/1 (2-uC1)	r	-0.17	-0.32	0.01	-0.79	0.00	-0.52	-0.50	-0.76	-0.60
weight [g]											
	CpG-1509	p-value	ns	ns 0.22	ns	0.002	ns	ns	ns 0.51	0.05	ns 0.21
		r	0.08	0.22	0.07	-0.14	0.29	-0.10	0.51	0.51	-0.21
-		p-value	-0.27	-0.33	ns	ns -0.54	ns 0.04	ns 0.61	-0.24	ns -0.71	ns 0.43
_	CpG-1465	r			-0.41	0.07		-0.61		0.07	
~ -		p-value	ns 0.02	ns -0.15	ns 0.22	0.07	ns 0.20	-0.81	ns 0.12	0.50	ns 0.32
<i>lgH</i> promoter methylation [%]	CpG-1430	p-value	ns	-0.15 ns	ns	ns		-0.81 ns		ns	0.32 ns
- ylat		p=varue r	0.13	0.19	0.16	0.15	0.16	0.49	0.01	0.13	0.25
eth	CpG-1357	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
8 - 1		r	-0.03	0.60	-0.21	-0.01	0.29	0.75	0.74	-0.43	-0.05
ote	CpG-1341	p-value	ns	0.05	ns	ns	ns	ns	0.09	ns	ns
шо –		r	0.00	-0.28	0.03	0.20	-0.32	0.03	-0.52	0.15	-0.10
nd z	CpG-1254	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
Igf	G G 1919	r	-0.39	-0.42	-0.53	-0.60	-0.40	-0.46	-0.60	-0.85	-0.25
	CpG-1212	p-value	0.07	ns	0.07	0.04	ns	ns	ns	0.02	ns
-	CpG 1190	r	0.02	-0.26	0.02	0.03	0.18	-0.30	-0.20	-0.31	0.71
	CpG-1180	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
correlation of / with	weight [g]		all all	all male	all female	all control	all PSE	male control	male PSE	female control	female PSE
	C=C 1500	r	-0.16	-0.13	-0.39	0.05	-0.25	0.37	-0.34	-0.44	-0.07
	CpG-1509	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-1465	r	0.09	0.23	0.00	0.02	-0.17	-0.19	0.15	0.04	-0.23
8	сро-1405	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
(g/1 promoter methylation [%]	CpG-1430	r	-0.17	-0.29	-0.32	-0.06	-0.10	0.39	-0.41	-0.59	0.27
	1	p-value	ns -0.02	ns	-0.14	ns 0.06	ns	ns 0.22	ns 0.41	ns 0.11	-0.62
	CpG-1357	r p-value	-0.02 ns	0.05 ns	-0.14 ns	0.06 ns	-0.05 ns	-0.23 ns	0.41	0.11 ns	-0.62 ns
ă -		p-value r	-0.05	-0.28	0.07	0.32	-0.14	-0.07	0.04	0.77	-0.64
otei	CpG-1341	p-value	ns	ns	ns	ns	ns	ns	ns	0.03	ns
ū –	0.01004	r	-0.04	0.11	-0.13	-0.01	0.12	0.85	-0.03	-0.34	0.43
, br	CpG-1254	p-value	ns	ns	ns	ns	ns	0.03	ns	ns	ns
lgf1	C=C 1212	r	0.26	0.51	0.03	0.44	0.06	0.18	0.43	0.45	0.18
ĩ	CpG-1212	p-value	ns	0.09	ns	ns	ns	ns	ns	ns	ns
_											0.07
-	CpG-1180	r p-value	0.19	0.16	0.38	0.15	0.32	0.89 0.02	0.03 ns	-0.07	0.86

Table 2. Correlations between IGF1 protein concentrations, *Igf1* mRNA levels, *Igf1* promoter methylation and the offspring's body weight

correlation of / with	IGF1R protein[pg/ml]	all all	all male	all female	all control	all PSE	male control	male PSE	female control	female PSE
Igflr (2-dCT)		r	0.59	0.10	0.72	0.65	0.66	-0.26	0.55	0.93	1.00
Ig/I/ (2-uC1)		p-value	0.02 -0.30	ns 0.42	0.05 -0.40	0.06 -0.49	ns 0.17	ns 0.44	ns 0.56	0.02 -0.54	Perfect line 0.64
weight [g]		rr p-value	-0.30 ns	ns	-0.40 ns	-0.49 ns		ns	ns	ns	ns
- - - -	CpG-272	r p-value	-0.01 ns	0.26 ns	-0.15 ns	0.02 ns	-0.15 ns	0.59 ns	-0.36 ns	-0.08 ns	-0.09 ns
	CpG-255	r	0.25	0.20	0.29	0.31	0.28	0.24	0.79	0.40	-0.87
	CpG-252	p-value r	ns 0.23	ns 0.27	ns 0.21	ns 0.24	ns 0.71	ns 0.49	ns 0.40	ns 0.29	0.31
	-	p-value r	ns 0.13	ns 0.37	ns -0.03	ns 0.06	0.07 0.78	ns 0.67	ns 0.27	ns -0.03	ns 0.85
	CpG-249	p-value	ns	ns	ns	ns	0.04	ns	ns	ns	ns
	CpG-246	r p-value	0.18 ns	0.21 ns	0.19 ns	0.21 ns	0.21 ns	0.49 ns	-0.29 ns	0.26 ns	-0.21 ns
	CpG-238	r p-value	-0.02 ns	0.36 ns	-0.32 ns	-0.15 ns	0.55 ns	0.72 ns	0.16 ns	-0.30 ns	-0.99 ns
	CpG-233	r	-0.14	0.47	-0.32	-0.19	0.11	0.48	0.82	-0.26	-0.58
-	-	p-value r	-0.25	ns 0.05	ns -0.43	ns -0.26	ns -0.20	ns 0.78	ns -0.81	-0.41	ns -0.71
	CpG-230	p-value r	ns -0.05	ns 0.37	ns -0.18	ns -0.08	ns 0.10	ns 0.86	ns -0.05	ns -0.19	ns 0.27
%] uo .	CpG-228	p-value	ns	ns	ns	ns	ns	0.06	ns	ns	ns
hylati	CpG-223	p-value	-0.02 ns	0.06 ns	-0.02 ns	-0.06 ns	0.17 ns	0.68 ns	-0.93	-0.12 ns	0.83 ns
r metl	CpG-215	r p-value	0.14	0.61	0.02	0.09	0.51	0.39	0.71	0.04	0.64
mote	CpG-209	r	ns 0.36	ns 0.55	0.35	ns 0.31	0.63	ns 0.39	ns 0.72	ns 0.34	ns 0.77
Ig/Ir promoter methylation [%]	-	p-value r	ns 0.16	ns 0.02	ns -0.01	ns 0.02	ns 0.61	ns 0.45	ns -0.78	ns -0.11	ns 0.74
	CpG-206	p-value	ns 0.45	ns -0.02	ns 0.48	ns 0.44	ns 0.66	ns -0.14	ns 0.11	ns 0.50	ns 0.88
	CpG-201	p-value	0.05	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-194	r p-value	0.49	0.29 ns	0.49 ns	0.35 ns	0.92	0.20 ns	0.86 ns	0.42 ns	0.99 ns
	CpG-185	r p-value	0.21	0.41 ns	0.10 ns	0.16 ns	0.65	0.23	0.70 ns	0.11 ns	0.68 ns
	CpG-182	r	ns 0.07	0.27	-0.09	-0.11	ns 0.35	ns 0.42	0.31	-0.57	0.67
		p-value r	ns 0.51	ns -0.17	ns 0.58	ns 0.55	ns 0.50	ns 0.19	ns -0.64	ns 0.62	ns 0.73
	CpG-171	p-value r	0.03	ns 0.30	ns 0.19	0.08	ns 0.41	ns 0.56	ns 0.13	ns 0.08	ns 0.67
	CpG-166	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-164	r p-value	0.36 ns	0.05 ns	0.33 ns	0.25 ns	0.61 ns	0.30 ns	-0.17 ns	0.22 ns	0.88 ns
	CpG-17	r p-value	0.21 ns	-0.29 ns	0.43 ns	0.28 ns	-0.04 ns	-0.66 ns	0.10 ns	0.57 ns	0.30 ns
correlation of / with	Igf1r (2-dCT)	p-value	all all	all male	all female	all control	all PSE	male control	male PSE	female control	female PSI
weight [g]		r	-0.02	-0.10	0.23	-0.83	-0.07	-0.69	-0.51	-0.86	0.80
-		p-value r	ns -0.08	ns -0.13	ns	0.002	ns 0.09	ns -0.76	ns 0.53	0.03	ns -0.12
	CpG-272	p-value	-0.08 ns	-0.13 ns	ns 0.02 ns	0.002 -0.64 0.03	0.09 ns	-0.76 ns	0.53 ns	0.03 -0.36 ns	-0.12 ns
-	CpG-272 CpG-255	r	-0.08	-0.13	ns 0.02	0.002	0.09	-0.76	0.53	0.03 -0.36	-0.12
-	-	r p-value r p-value r	-0.08 ns -0.13 ns -0.17	-0.13 ns -0.11 ns -0.21	ns 0.02 ns -0.03 ns -0.08	0.002 -0.64 0.03 -0.54 0.09 -0.66	0.09 ns 0.22 ns 0.28	-0.76 ns -0.62 ns -0.80	0.53 ns 0.56 ns 0.26	0.03 -0.36 ns -0.15 ns -0.34	-0.12 ns -0.27 ns 0.58
-	CpG-255	r p-value r p-value r p-value r	-0.08 ns -0.13 ns -0.17 ns -0.13	-0.13 ns -0.11 ns -0.21 ns -0.01	ns 0.02 ns -0.03 ns -0.08 ns -0.13	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68	0.09 ns 0.22 ns 0.28 ns 0.43	-0.76 ns -0.62 ns -0.80 ns -0.72	0.53 ns 0.56 ns 0.26 ns 0.41	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54	-0.12 ns -0.27 ns 0.58 ns 0.83
-	CpG-255 CpG-252 CpG-249	r p-value r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns	-0.13 ns -0.11 ns -0.21 ns	ns 0.02 ns -0.03 ns -0.08 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03	0.09 ns 0.22 ns 0.28 ns	-0.76 ns -0.62 ns -0.80 ns	0.53 ns 0.56 ns 0.26 ns	0.03 -0.36 ns -0.15 ns -0.34 ns	-0.12 ns -0.27 ns 0.58 ns
-	CpG-255 CpG-252 CpG-249 CpG-246	r p-value r p-value r p-value r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.12 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns
-	CpG-255 CpG-252 CpG-249	r p-value r p-value r p-value r p-value r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns -0.05 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.12 ns 0.40 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns
	CpG-255 CpG-252 CpG-249 CpG-246	r p-value r p-value r p-value r p-value r p-value r p-value r	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns -0.05 ns -0.13 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.01 ns 0.14 ns 0.04 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns -0.02 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.12 ns 0.40 ns 0.22 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns -0.37 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns 0.11 ns	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.77 0.07	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 0.49 ns 0.65 ns
ution %	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238	r p-value r p-value r p-value r p-value r p-value r p-value r	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns -0.05 ns -0.13	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns 0.04	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns -0.02	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 -0.49 ns -0.64	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.12 ns 0.40 ns 0.22	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.80 ns -0.22 ns -0.37	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns 0.11	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.64 ns -0.77	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns 0.65
ethylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233	r p-value r p-value r p-value r p-value r p-value r p-value r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.13 ns -0.13 ns -0.12	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns 0.04 ns -0.07 ns -0.07	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns -0.02 ns -0.17 ns -0.12	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.50	0.09 ns 0.22 ns 0.43 ns 0.43 ns 0.43 ns 0.40 ns 0.22 ns 0.22 ns 0.24 0.22 0.23 0.23 0.23 0.23 0.23 0.24 0.24 0.25 0.43 0.43 0.40 0.25 0.25 0.25 0.43 0.40 0.25 0.25 0.25 0.25 0.43 0.40 0.5 0.25 0.10 0.25 0.10 0.10 0.10 0.25 0.10 0.10 0.10 0.25 0.25 0.10 0.10 0.10 0.10 0.25 0.10 0.10 0.10 0.10 0.25 0.25 0.10 0.10 0.10 0.25 0.25 0.10 0.10 0.10 0.10 0.10 0.10 0.10 0.25 0.25 0.10 0.10 0.10 0.10 0.25 0.25 0.10 0.10 0.10 0.10 0.25 0.10 0.10 0.10 0.25 0.10 0.10 0.10 0.25 0.10 0.10 0.25 0.10 0.10 0.25 0.10 0.10 0.25 0.10 0.10 0.25 0.25 0.10 0.10 0.25 0.10 0.10 0.25 0.10 0.10 0.25 0.10 0.10 0.55	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns -0.37 ns -0.37 ns -0.42 ns -0.67	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.41 ns 0.41 ns 0.35 ns 0.11 ns 0.35 ns 0.11 ns 0.53	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.64 ns -0.64 ns -0.77 0.07 -0.52 ns -0.47	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns 0.65 ns 0.26 ns 0.29
ter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-228	r p-value r p-value r p-value r p-value r p-value r p-value r p-value r p-value r p-value r	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.13 ns -0.13 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.01 ns 0.14 ns 0.04 ns -0.07 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.12 ns -0.02 ns -0.02 ns -0.07 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.64 0.03 -0.57 0.07	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.12 ns 0.40 ns 0.40 ns 0.20 ns 0.40 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns -0.22 ns -0.37 ns -0.42 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.41 ns 0.41 ns 0.35 ns 0.11 ns 0.35 ns	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.54 ns -0.64 ns -0.64 ns -0.77 0.07 -0.652 ns	-0.12 ns -0.27 ns 0.58 ns -0.22 ns 0.49 ns 0.65 ns 0.26 ns
romoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-228 CpG-223	r p-value p-value r p-value r p-value r p-value r p-value r p-value r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.17 ns -0.18 ns -0.18 ns -0.13 ns -0.13 ns -0.21 ns -0.12 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns -0.04 ns -0.07 ns -0.07 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns -0.02 ns -0.17 ns -0.17 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.50 ns	0.09 ns 0.22 ns 0.23 ns 0.43 ns 0.12 ns 0.40 ns 0.22 ns 0.22 ns 0.22 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns -0.22 ns -0.37 ns -0.42 ns -0.42 ns	0.53 ns 0.56 ns 0.26 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns 0.00 ns 0.000 ns	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.64 ns -0.64 ns -0.77 0.07 -0.52 ns -0.47 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns 0.65 ns 0.26 ns 0.26 ns
<i>ولا</i> ء promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-228	r p-value r p-value	-0.08 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.17 ns -0.17 ns -0.17 ns -0.13 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.13 ns -0.12 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns -0.33 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns -0.17 ns 0.04 ns -0.07 ns -0.07 ns 0.03 ns	ns 0.02 ns -0.03 ns -0.18 ns -0.12 ns -0.12 ns -0.16 ns -0.02 ns -0.17 ns -0.12 ns -0.12 ns -0.12 ns -0.27 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.50 ns -0.50 ns -0.50 ns -0.37 ns -0.51 0.07 -0.50 ns -0.51 0.07 -0.50 ns -0.51 0.07 -0.50 ns -0.51 -0.57 -	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.40 ns 0.40 ns 0.22 ns 0.10 ns 0.22 ns 0.22 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.43 ns 0.40 ns 0.22 ns 0.43 ns 0.40 ns 0.22 ns 0.40 ns 0.22 ns 0.40 ns 0.22 ns 0.40 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.12 ns 0.12 ns 0.22 ns 0.12 ns 0.12 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.22 ns -0.22 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.60 ns -0.80 ns -0.22 ns -0.80 ns -0.22 ns -0.42 ns -0.67 ns -0.62 ns -0.62 ns -0.62 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 -0.80 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.667 ns -0.667 ns -0.667 ns -0.667 ns -0.667 ns -0.667 ns -0.664 ns -0.664 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns 0.11 ns 0.000 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.54 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.41 ns 0.54 ns 0.54 ns 0.54 ns 0.54 ns 0.54 ns 0.54 ns 0.54 ns 0.55 ns 0.54 ns 0.55	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.77 0.07 -0.52 ns -0.47 ns -0.47 ns -0.54 ns -0.54 ns -0.54 ns -0.44 ns -0.54 ns -0.54 ns -0.54 ns -0.44 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.57 ns -0.64 ns -0.77 -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.55 ns -0.55 ns -0.55 ns -0.55 ns -0.55 ns -0.57 -0.55 ns -0.64 -0.77 -0.52 ns -0.52 ns -0.55 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns 0.49 ns 0.65 ns 0.26 ns 0.26 ns 0.26 ns 0.80 ns 0.80 ns
<i>IgHr</i> promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-228 CpG-223	r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.17 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.18 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns -0.10 ns	-0.13 ns -0.11 ns -0.21 ns -0.01 ns 0.14 ns 0.14 ns -0.07 ns -0.07 ns -0.07 ns -0.03 ns 0.37 ns 0.45 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.02 ns -0.12 ns -0.12 ns -0.12 ns -0.02 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.02 ns -0.12 ns -0.12 ns -0.02 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.50 ns -0.37 ns 0.14 ns 0.07 ns	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.40 ns 0.40 ns 0.20 ns 0.21 ns 0.22 ns 0.23 ns 0.23 ns 0.23 ns 0.24 ns 0.24 ns 0.25 ns 0.25 ns 0.25 ns 0.26 ns 0.27 ns 0.28 ns 0.28 ns 0.43 ns 0.40 ns 0.40 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.12 ns 0.12 ns 0.10 ns 0.12 ns 0.12 ns 0.10 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.26 ns 0.12 ns 0.26 ns 0.12 ns 0.26 ns 0.12 ns 0.26 ns 0.12 ns 0.26 ns 0.26 ns 0.12 ns 0.26 ns 0.26 ns 0.12 ns 0.26 ns 0.26 ns 0.21 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.80 ns -0.22 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.67 ns -0.61 ns -0.61 ns -0.62 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.62 ns -0.62 ns -0.62 ns -0.62 ns -0.62 ns -0.62 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.667 ns -0.09 ns -0.667 ns -0.664 ns -0.16 ns -0.165 ns -0.16 ns -0.16 ns -0.16 ns -0.16 ns -0.16 ns -0.16 ns -0.16 ns -0.16	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.00 ns 0.00 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.20 ns 0.35 ns 0.35 ns 0.20 ns 0.35 ns 0.35 ns 0.20 ns 0.35 ns 0.20 ns 0.35 ns 0.20 ns 0.35 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.20 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.29 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.29 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 0.29 ns 0.28 0.29 0.59 0.50 0.55	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.64 ns -0.77 0.07 -0.77 0.07 -0.52 ns -0.47 ns -0.47 ns -0.51 ns -0.54 -0.57 -0.54 -0.54 -0.57 -0.54 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.54 -0.57 -0.57 -0.52 -0.55 -0.52 -0.55 -0.55 -0.55 -0.55 -0.57 -0.55 -0.	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns 0.49 ns 0.65 ns 0.26 ns 0.26 ns 0.29 ns 0.80 ns 0.09 ns
<i>IgHr</i> promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-230 CpG-228 CpG-223 CpG-223	r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns -0.05 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.18 -0.19 -0.18 -0.18 -0.19 -0.18 -0.19 -0.18 -0.18 -0.18 -0.19 -0.18 -0.19 -0.18 -0.18 -0.19 -0.18 -0.19 -	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns -0.07 ns -0.07 ns -0.07 ns -0.03 ns -0.03 ns -0.23 -0.01 -0.17 -0.14 -0.17 -0.07 -0.5 -0.07 -0.5 -0.03 -0.5 -0.13 -0.03 -0.5	ns 0.02 ns -0.03 ns -0.13 ns -0.12 ns -0.16 ns -0.17 ns -0.17 ns -0.17 ns -0.27 ns -0.02 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.50 ns -0.37 ns 0.14 ns 0.07	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.40 ns 0.40 ns 0.40 ns 0.22 ns 0.41 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.28 ns 0.43 ns 0.40 ns 0.40 ns 0.20 ns 0.40 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.10 ns 0.12 ns 0.12 ns 0.10 ns 0.10 ns 0.12 ns 0.12 ns 0.10 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.10 ns 0.21 ns 0.00	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.72 ns -0.22 ns -0.22 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.67 ns -0.69 ns -0.60 -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.22 ns -0.22 ns -0.67 -0.80 ns -0.22 ns -0.67 -0.80 ns -0.22 ns -0.67 -0.80 -0.67 -0.67 -0.67 -0.67 -0.67 -0.80 -0.80 -0.67 -0.67 -0.80 -0.67 -0.67 -0.80 -0.67 -0.80 -0.67 -0.67 -0.64	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.35 ns 0.35 ns 0.35 ns 0.35 ns 0.00 ns 0.00 ns 0.28 ns 0.29 ns 0.26 0.35 0.35 0.35 0.35 0.35 0.5 0.28 0.35 0.35 0.5 0.28 0.35 0.5 0.26 0.35 0.5 0.26 0.35 0.5 0.26 0.26 0.35 0.5 0.26 0.35 0.5 0.5 0.26 0.26 0.35 0.5 0.5 0.6 0.26 0.35 0.5 0.5 0.6 0.7 0.5 0.6 0.7 0.7 0.7 0.7 0.7 0.7 0.7 0.7	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.77 0.07 -0.52 ns -0.77 0.07 -0.52 ns -0.47 ns -0.54 ns -0.54 ns -0.47 ns -0.54 ns -0.47 ns -0.54 ns -0.47 ns -0.64 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.64 -0.77 -0.52 ns -0.52 ns -0.52 ns -0.77 -0.52 ns -0.64 -0.77 -0.52 ns -0.67 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.52 -0.53 -0.52 -0.55 -0.55 -0.55 -0.55 -0.55 -0.55 -0.55 -0.55 -0.77 -0.55 -0	-0.12 ns -0.27 ns 0.58 ns 0.83 ns -0.22 ns 0.49 ns 0.26 ns 0.26 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.26 ns 0.26 ns 0.22 ns 0.22 ns 0.22 ns 0.49 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.80 ns 0.26 ns 0.26 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.80 ns 0.26 ns 0.80 ns 0.26 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
<i>I</i> g/ <i>I</i> r promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-233 CpG-233 CpG-230 CpG-228 CpG-223 CpG-223 CpG-215 CpG-209	r p-value r r p-value r r p-value r r p-value r r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.18 ns -0.05 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.10 ns -0.17 -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.16 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.05 ns -0.21 ns -0.05 ns -0.21 ns -0.21 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.08 ns -0.08 ns -0.08 ns -0.09 ns -0.09 ns -0.08 ns -0.09 ns -0.062 -0.09 ns -0.09 ns -0.062 -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 ns -0.09 -0.09 ns -0.09 -0.	-0.13 ns -0.11 ns -0.21 ns -0.01 ns 0.14 ns -0.07 ns -0.07 ns -0.07 ns -0.07 ns -0.03 ns -0.03 ns -0.25 ns 0.25 0.45 0.45 0.45 0.56 0.45 0.45 0.45 0.56 0.46 0.45 0.45 0.56 0.45 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<i>IgItr</i> promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-233 CpG-233 CpG-230 CpG-230 CpG-228 CpG-223 CpG-215 CpG-209 CpG-206 CpG-201	r p-value r r p-value r r p-value	-0.08 ns -0.13 ns -0.17 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.10 ns -0.21 ns -0.21 ns -0.12 ns -0.12 ns -0.10 ns -0.12 ns -0.12 ns -0.12 ns -0.13 ns -0.13 ns -0.16 ns -0.17 ns -0.17 ns -0.17 ns -0.17 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.18 ns -0.19 ns -0.19 ns -0.19 ns -0.19 ns -0.19 ns -0.19 ns -0.19 ns -0.19 ns -0.110 ns -0.110 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.112 ns -0.13 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.33 ns -0.10 ns -0.33 ns -0.10 ns -0.33 ns -0.10 ns -0.10 ns -0.33 ns -0.10 ns -0.20 ns -0.33 ns -0.20 n -0.20 n -0.20 n -0.20 n -0.20 n -0.20 n -0.20 n -0.20 n -0.20 n -0.20 -0.20 n -0.20 n -0.20 n -0.20 n -0.20 -0.0	-0.13 ns -0.11 ns -0.21 ns -0.01 ns 0.14 ns 0.14 ns -0.07 ns -0.07 ns -0.07 ns 0.03 ns 0.37 ns 0.37 ns 0.25 ns 0.25 ns	ns 0.02 ns -0.03 ns -0.08 ns -0.13 ns -0.12 ns -0.16 ns -0.02 ns -0.12 ns -0.17 ns -0.02 ns -0.12 ns -0.02 ns -0.02 ns -0.02 ns -0.02 ns -0.02 ns -0.027 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 -0.57 0.07 -0.50 ns -0.37 ns -0.33 -0.37 ns -0.33 -0.37 ns -0.33 -0.37 ns -0.03 -0.53 -0.37 ns -0.03 -0.53 -0.53 -0.57 -0.50 ns -0.53 -0.57 -0.50 ns -0.50 ns -0.53 -0.57 -0.50 ns -0.53 -0.57 -0.53 -0.57 -0.50 -0.50 -0.57 -0.50 -0.	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.12 ns 0.40 ns 0.40 ns 0.22 ns 0.10 ns 0.22 ns 0.22 ns 0.22 ns 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.21 ns 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.28 0.12 ns 0.40 ns 0.22 ns 0.10 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.22 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.22 ns 0.12 ns 0.22 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.12 ns 0.21 ns 0.21 ns 0.26 ns 0.21 ns 0.26 ns 0.21 ns 0.26 ns 0.26 ns 0.21 ns 0.26 ns 0.57	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.72 ns -0.80 ns -0.22 ns -0.22 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.60 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.80 ns -0.22 ns -0.22 ns -0.42 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.667 ns -0.09 ns -0.667 ns -0.09 ns -0.664 ns -0.664 ns -0.16 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.664 ns -0.066 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.54 ns 0.35 ns 0.11 ns 0.00 ns 0.11 ns 0.00 ns 0.28 ns 0.24 ns 0.24 ns 0.25 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.54 ns 0.54 ns 0.55 0.25 ns 0.54 ns 0.55 0.54 ns 0.55 0.55 ns 0.54 ns 0.55 ns 0.26 ns 0.55 ns 0.28 ns 0.29 ns 0	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.47 ns -0.64 ns -0.64 ns -0.77 0.07 -0.52 ns -0.47 ns -0.47 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.64 ns -0.64 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.55 ns -0.64 ns -0.64 ns -0.77 -0.52 ns -0.47 ns -0.52 ns -0.52 ns -0.47 ns -0.52 ns -0.52 ns -0.47 ns -0.52 ns -0.52 ns -0.47 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.52 ns -0.55 ns -0.52 ns -0.52 ns -0.55 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns 0.62 ns 0.49 ns 0.65 ns 0.26 ns 0.26 ns 0.20 ns 0.26 ns 0.80 ns 0.20 ns 0.26 ns 0.26 ns 0.22 ns 0.26 ns 0.26 ns 0.26 ns 0.26 ns 0.22 ns 0.26 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.56 ns 0.57 ns 0.58
$I_{\mathcal{K}} f_{\mathcal{T}}$ promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-238 CpG-233 CpG-230 CpG-230 CpG-223 CpG-223 CpG-215 CpG-209 CpG-200 CpG-201 CpG-201	r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.05 ns -0.021 ns -0.05 ns -0.021 ns -0.08 ns -0.08 ns -0.08 ns -0.08 ns -0.09 ns -0.09 ns -0.00 ns -0.00 ns -0.08 ns -0.08 ns -0.09 ns -0.09 ns -0.09 ns -0.00 ns -0.00 ns -0.00 ns -0.08 ns -0.00 -0.00 -0	-0.13 ns -0.11 ns -0.21 ns -0.01 ns -0.17 ns 0.14 ns -0.07 ns -0.07 ns -0.07 ns -0.07 ns -0.03 ns -0.37 ns 0.45 ns 0.25 ns 0.45 ns 0.25 ns -0.17 ns -0.01 ns -0.17 ns -0.14 -0.07 ns -0.37 ns -0.25 ns -0.25 ns -0.25 ns -0.25 ns -0.25 ns -0.25 ns -0.25 -0.37 ns -0.25 -0.25 -0.37 -0.57 -	ns 0.02 ns -0.03 ns -0.08 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.22 ns -0.17 ns -0.27 ns -0.17 ns -0.23 ns	0.002 -0.64 0.03 -0.54 0.09 -0.66 0.03 -0.68 0.02 -0.71 0.01 -0.49 ns -0.64 0.03 -0.57 0.07 ns 0.14 ns 0.07 ns -0.03 ns -0.37 ns 0.14 ns 0.07 ns -0.03 ns -0.03 ns -0.57 0.07 0.07 -0.03 ns -0.03 -0.57 0.07 -0.03 -0.03 -0.57 0.07 -0.03 -0.03 -0.57 -0.03 -0.03 -0.57 -0.03 -0.03 -0.57 -0.03 -0.03 -0.57 -0.03 -0.03 -0.57 -0.03 -0.57 -0.03 -0.03 -0.57 -0.03 -0.57 -0.03 -0.57 -0.03 -0.57 -0.03 -0.57 -0.50 -0.57 -0.50 -0.57 -0.57 -0.50 -0.57 -0.50 -0.57 -0.57 -0.50 -0.57 -	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.40 ns 0.40 ns 0.40 ns 0.10 ns 0.10 ns 0.12 ns 0.12 ns 0.26 0.67 0.00 0.67 0.00 0.67 0.00	-0.76 ns -0.62 ns -0.80 ns -0.72 ns -0.80 ns -0.80 ns -0.22 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.69 ns -0.62 ns -0.37 ns -0.42 ns -0.62 ns -0.37 ns -0.42 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.72 ns -0.72 ns -0.72 ns -0.80 ns -0.22 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.67 ns -0.66 ns -0.66 ns -0.66 ns -0.664 ns -0.664 ns -0.664 ns -0.72 ns -0.664 ns -0.72 ns -0.72 ns -0.664 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.67 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.77 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns -0.72 ns	0.53 ns 0.56 ns 0.26 ns 0.41 ns 0.35 ns 0.35 ns 0.35 ns 0.00 ns 0.00 ns -0.28 ns 0.28 ns 0.20 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.20 0.35 0.35 0.20 0.35 0.20 0.35 0.35 0.20 0.35 0.35 0.20 0.35 0.00 0.35 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.	0.03 -0.36 ns -0.15 ns -0.34 ns -0.54 ns -0.54 ns -0.47 ns -0.64 ns -0.77 0.07 -0.64 ns -0.77 0.07 -0.52 ns -0.47 ns -0.47 ns -0.51 ns -0.54 ns -0.47 ns -0.54 ns -0.54 ns -0.47 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.54 ns -0.47 ns -0.55 ns -0.47 ns -0.55 ns -0.47 ns -0.55 ns -0.47 ns -0.55 ns -0.55 ns -0.47 ns -0.55 ns -0.77 0.07 -0.55 ns -0.47 ns -0.55 ns -0.77 0.07 -0.55 ns -0.47 ns -0.55 ns -0.47 ns -0.52 ns -0.47 ns -0.55 ns -0.47 ns -0.55 ns -0.49 ns -0.56 ns -0.55 ns -0.55 ns -0.56 ns -0.55 ns -0.55 ns -0.59 ns -0.59 ns -0.19 ns -0.49 ns	-0.12 ns -0.27 ns 0.58 ns 0.83 ns 0.49 ns 0.49 ns 0.49 ns 0.26 ns 0.26 ns 0.20 ns 0.22 ns 0.23 ns 0.58 ns 0.49 ns 0.26 ns 0.26 ns 0.26 ns 0.27 ns 0.21 ns 0.21 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.22 ns 0.26 ns 0.30 ns 0.26 ns
$I_{\mathbf{g}} I_{\mathbf{r}}$ promoter methylation [%]	CpG-255 CpG-252 CpG-249 CpG-246 CpG-233 CpG-233 CpG-230 CpG-230 CpG-228 CpG-223 CpG-215 CpG-209 CpG-206 CpG-201	r p-value r p-value	-0.08 ns -0.13 ns -0.17 ns -0.13 ns -0.13 ns -0.13 ns -0.05 ns -0.05 ns -0.05 ns -0.21 ns -0.21 ns -0.21 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.12 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.13 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.15 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.21 ns -0.08 ns -0.08 ns -0.09 ns -0.10 ns -0.12 ns -0.10 ns -0.20 ns -0.20 ns -0.21 ns -0.08 ns -0.20 ns -0.20 ns -0.20 ns -0.21 ns -0.20 ns -0.21 ns -0.20 ns -0.20 ns -0.22 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.20 ns -0.22 -0.02 -0.22 -0.22 -0.22 -0.22 -0.02 -0.02 -0.22 -0.02 -0.02 -0.02 -0.22 -0.02 -	-0.13 ns -0.11 ns -0.21 ns -0.01 ns 0.14 ns 0.04 ns -0.07 ns -0.07 ns -0.07 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-0.49 ns -0.64 0.03 -0.57 0.07 -0.50 ns -0.57 0.07 -0.50 ns -0.37 ns 0.14 ns 0.02 -0.37 ns 0.14 ns 0.07 -0.68 0.03 -0.57 0.07 -0.50 ns -0.68 0.03 -0.57 0.07 -0.50 ns -0.68 0.03 -0.57 0.07 -0.50 ns -0.68 0.03 -0.57 0.07 -0.50 ns -0.57 0.07 -0.50 ns -0.57 0.07 -0.50 ns -0.37 ns 0.02 -0.57 0.07 -0.50 ns -0.64 0.03 -0.57 0.07 -0.50 ns -0.64 0.07 -0.50 ns -0.57 0.07 -0.50 ns -0.68 0.07 -0.50 ns -0.68 0.07 -0.50 ns -0.57 ns -0.03 ns -0.57 ns -0.03 ns -0.03 ns -0.03 ns -0.03 ns -0.03 ns -0.03 ns -0.03 -0.57 0.07 -0.50 ns -0.03 -0.57 -0.02 -0.03 -0.57 -0.03 -0.57 -0.03 -0.57 -0.03 -0.57 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.03 -0.57 -0.02 -0.02 -0.03 -0.57 -0.02 -0.02 -0.02 -0.02 -0.03 -0.52 -0.03 -0.57 -0.02 -0.02 -0.03 -0.24 -0.52 -0.54 -0.54 -0.55 -0.57 -0.02 -0.58 -0.57 -0.02 -0.02 -0.02 -0.54 -0.54 -0.54 -0.57 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.02 -0.54 -0.5	0.09 ns 0.22 ns 0.28 ns 0.43 ns 0.43 ns 0.40 ns 0.40 ns 0.20 ns 0.40 ns 0.21 ns 0.28 ns 0.43 ns 0.43 ns 0.43 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Table 3. Correlations between IGF1R protein concentrations, *Igf1r* mRNA levels, *Igf1r* promoter methylation and the offspring's body weight

	CpG-166	r	0.01	0.06	0.08	0.04	-0.39	0.46	-0.40	-0.26	-0.90
		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
		p-value r	0.27	0.07	0.22	-0.06	0.08	-0.34	-0.03	-0.23	0.22
	CpG-164	p-value	ns	ns		ns		ns	ns	ns	ns
				0.65	ns 0.59	0.53	-0.22	0.75	-0.04	0.50	-0.64
	CpG-17	p-value	0.55	0.65	0.39	0.09		0.75 ns	-0.04 ns	0.30	-0.64 ns
correlation of / with	weight [g]	p-value	all all	all male	all female	all control	all PSE	male control	male PSE	female control	female PSE
		r	0.20	0.22	0.17	0.27	-0.57	-0.07	-0.77	0.38	-0.63
	CpG-272	p-value	ns	ns	ns	ns	0.07	ns	0.07	ns	ns
		r	0.05	0.17	-0.06	-0.09	-0.58	-0.22	-0.42	-0.19	-0.79
	CpG-255	p-value	ns	ns	ns	ns	0.06	ns	ns	ns	ns
		r	0.14	0.26	0.06	0.04	-0.45	0.11	-0.44	-0.14	-0.03
	CpG-252	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
		p vulue r	0.18	0.03	0.32	0.19	-0.28	-0.11	-0.54	0.21	0.43
	CpG-249	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
		p-value r	0.22	0.30	0.06	0.25	-0.57	0.03	-0.65	0.11	-0.52
	CpG-246	p-value	ns	ns	ns	ns	0.07	ns	ns	ns	ns
		p-value r	0.23	0.04	0.47	0.21	-0.19	-0.39	-0.47	0.46	0.38
	CpG-238	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
		p-value r	0.55	0.47	0.47	0.33	0.62	-0.03	0.42	0.12	0.64
_	CpG-233	p-value	0.004	ns	ns	ns	0.02	-0.05 ns	ns		ns
	CpG-230	p-value r	0.22	0.04	0.26	0.24	-0.17	-0.19	-0.68	0.18	0.21
		p-value									
		<u> </u>	ns 0.03	-0.18	ns 0.14	ns 0.43	ns -0.53	ns 0.51	-0.66	ns 0.49	-0.64
<u>`</u>	CpG-228	r					0.09				
ie —		p-value	ns 0.36	ns 0.06	ns 0.52	ns 0.54	0.09	ns 0.21	ns -0.59	ns 0.66	ns 0.48
ylai	CpG-223 CpG-215 CpG-209 CpG-206 CpG-201 CpG-194	r	0.36		0.32					0.00	
— ţ		p-value	0.08	ns 0.11		0.05	ns 0.29	ns 0.20	ns 0.29		ns 0.02
Ĕ		r		0.11	0.26	0.00		-0.29		0.20	
— ter		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
e e		r	-0.29	-0.01	-0.49	-0.26	-0.39	-0.45	-0.05	-0.40	-0.55
ord —		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>lg/1r</i> promoter methylation [%]		r	-0.30	-0.22	-0.11	-0.11	-0.66	-0.52	-0.89	0.20	-0.40
Ig		p-value	ns	ns	ns	ns	0.03	ns	0.02	ns	ns
		r	-0.20	-0.37	0.04	-0.38	-0.33	-0.73	-0.73	-0.19	0.61
_		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
		r	-0.28	0.06	-0.39	-0.02	-0.44	0.59	-0.27	-0.19	-0.25
		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-185	r	-0.32	-0.14	-0.34	-0.38	-0.45	-0.40	-0.23	-0.35	-0.55
		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-182	r	0.03	0.03	0.19	-0.01	0.14	0.21	0.35	0.47	0.08
	CPG-102	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-171 CpG-166	r	-0.39	-0.42	-0.35	-0.39	-0.42	-0.79	-0.76	-0.44	-0.01
		p-value	0.06	ns	ns	ns	ns	0.06	0.08	ns	ns
		r	0.06	-0.02	0.13	-0.03	0.22	-0.53	0.32	0.27	0.03
		p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-164	r	-0.01	0.22	0.14	-0.04	-0.13	0.01	-0.12	0.19	0.20
	Сро-104	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns
	CpG-17	r	0.07	-0.10	0.25	-0.19	0.05	-0.68	-0.14	0.03	0.02
	сро-17	p-value	ns	ns	ns	ns	ns	ns	ns	ns	ns

















