Research paper

Association of maternal prenatal smoking GFI1-locus and cardiometabolic phenotypes in 18,212 adults

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Cigarette smoking, including second-hand exposure, is estimated to account for nearly 6 million deaths annually [1]. First and second-hand exposures are widely recognized as independent risk factors for cardiovascular diseases (CVD), largely determined by dose and duration [1,2]. Proposed direct mechanisms linking cigarette smoking and CVD include increased heart rate and myocardial contractility, inflammation, insulin resistance, and oxidative stress [3,4]. Moreover, the risk may remain even after successful long-term smoking cessation [5]. Similarly, maternal prenatal smoking has implications for birth outcomes, including low birth weight and risk of preterm birth [6], as well as increased risk to the offspring’s later cardio-metabolic health [7,8]. A recent global review reported the highest estimated prevalence of maternal prenatal smoking in Europe, despite the widely known risks [9].

Emerging research suggests that part of the downstream impact of smoking likely persists through altered epigenetic patterns, many of which have been associated with alterations in the gene expression [10]. Of particular importance, altered DNA methylation at AHHR, GFI1, and MYO1G genes is consistently observed among both adult smokers and new-borns exposed to maternal prenatal smoking [10–13]. Evidence on the stability of smoking-related-loci DNA methylation over the lifetime is inconsistent. Many CpGs in former smokers show a reversal of disrupted DNA methylation equivalent to non-smokers within five years of cessation, whereas others show no reversibility even 20–30 years after cessation [10,14]. Similarly, Richmond et al. suggested there were both reversible and permanent changes at smoking-related DNA methylation loci in offspring exposed to maternal smoking during pregnancy [15].

Furthermore, eight GFI1-linked-CpGs with aberrant DNA methylation were reported to partially mediate the association of maternal prenatal smoking with offspring’s adult cardio-metabolic health [8]. We aimed to pursue a life-course approach to...
We hypothesized that DNA methylation changes at GFI1, a candidate gene linking it to cardio-metabolic health, can help bio-marking exposure to past risk. These findings provide a strong foundation for further work to unravel emerging smoking epigenetic markers with downstream detrimental health outcomes and further draws attention to increase awareness on smoking cessation and better prevention strategies.

evaluate the possibility that exposure to maternal smoking in pregnancy influences the health of offspring via epigenetic mechanisms. We hypothesized that DNA methylation changes at GFI1-CpGs, a potential smoking biomarker, persist throughout the life-course and associate with cardio-metabolic phenotypes in adults. We tested this hypothesis in a large meta-analysis involving 22 population-based studies.

2. Material and methods

2.1. Participating studies

We included 22 studies consisting of 18,212 participants, including five pregnancy-birth cohorts, 17 other population-based datasets and their sub-studies: the Avon Longitudinal Study of Parents and Children (ALSPAC) (specifically subset with DNA methylation profiles in the Accessible Resource for Integrated Epigenomic Studies), two studies from the Bogalusa Heart Study (BHS – the European-American and African-American cohorts), the BIOS consortium, the Estonian Genome Centre University of Tartu (EGCUT), the European Prospective Investigation into Cancer and Nutrition (EPIC), the Italian Cardiovascular section (EPICOR), two independent subsets of the ESTHER study, the Cooperative Health Research in the Augsburg Region F4 (KORA F4), the Lifelines Deep (LLD), the London Life Science Population study (LOLPOP), two follow-up datasets from the Northern Finland Birth cohort 1966 (NFBC1966 – 31 years and NFBC1966 – 46 years) and Northern Finland Birth cohort 1986 (NFBC1986), the Western Australian Pregnancy Cohort (RAINE) study, two independent subsets from the Rotterdam Study (RS) –RSIII-1 and RSII-3 II, the Study of Health In Pomerania – Trend (SHIP-Trend), and the Young Finns Study 2011 (YFS). The BIOS consortium represents four studies with coordinated DNA methylation measurements: the Cohort On Diabetes And Atherosclerosis Maastricht (CODAM), the Leiden Longevity Study (LLS), the Netherlands Twin Register Study (NTR) and the prospective Amyotrophic Lateral Sclerosis (ALS) study, the Netherlands (PAN). Among these, five studies (ALSPAC, NFBC1966–31 yr, NFBC1966–46 yr, NFBC1986, and RAINE) participated in the meta-analysis of associations between the eight GFI1-CpGs and maternal prenatal smoking (n = 4230). Detailed data collection and ethical approval of each study are described in supplementary methods in the Appendix A. Subjects with missing information on DNA methylation and multiple births were excluded.

2.2. Smoking

Maternal prenatal smoking and offspring’s own adult smoking were self-reported. Questions were harmonized to derive a dichotomous variable for maternal smoking as ‘no maternal smoking’ and ‘any maternal smoking’ during pregnancy. Adult own smoking was categorized as current non-smokers and smokers (adult own smoking ≥ 1 cigarette/day).

2.3. DNA methylation measurement and quality control

We used eight GFI1-linked-CpGs: cg04535902, cg09662411, cg09935388, cg10399789, cg12876356, cg18147637, cg14179389, and cg18316974. Each study conducted DNA methylation measurements and quality control. DNA methylation was measured in peripheral whole blood by standard procedures for Illumina HumanMethylation450 or EPIC array. DNA Methylation is described as β-value ranging between 0 (no cytosine methylation) and 1 (complete cytosine methylation). Each study excluded failed samples based on detection P-values, CpG-specific percentage, low DNA concentration, bisulphite conversion efficiency, and other study-specific control metrics (Appendix A) [17].

2.4. Covariates

Covariates were age, sex, and technical covariates for CpGs (batch effects, control probe adjustments, and cell type proportions).
Adjustments for technical variation and cell type proportion in each study are described in the Appendix A.

2.5. Cardio-metabolic phenotypes

We used seven cardio-metabolic phenotypes derived from clinical examinations: body mass index (BMI, weight(kg)/height(m)²), waist circumference (WC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting glucose (FG), systolic blood pressure (SBP), and diastolic blood pressure (DBP). All cardio-metabolic phenotypes were used as continuous variables and standardized (mean = 0, standard deviation = 1). Correction constants were applied to HDL-C, TG, and BP values, if participant reported lipid or blood pressure medication use (Appendix A). According to availability in the participating studies, BMI was available in 18,212, WC in 14,665, HDL-C in 18,212, TG in 18,212, FG in 16,529, DBP in 16,529, and SBP in 16,529 individuals of the total.

2.6. Study-specific statistical analyses

Each study conducted statistical analyses according to the analysis plan. Frequencies and means were computed for descriptive purposes. We used multivariate regression to evaluate three sets of associations: GFI1-CpGs with (i) maternal prenatal smoking (n = 4230), (ii) adult own smoking (n = 13,551), and (iii) cardio-metabolic phenotypes (n = 18,212) (Appendix B Fig. S1). Firstly, analyses in five pregnancy-birth cohort studies were performed using: baseline model, which used any maternal smoking during pregnancy as an exposure plus technical covariates regressed on DNA methylation as an outcome (beta-values), and adjusted model with sex, age (where applicable) and adult smoking as covariates. To assess the impact of adult own smoking on DNA methylation level, we included: baseline model, which used adult own smoking as an exposure plus technical covariates, and DNA methylation as an outcome, and adjusted model including sex and age as covariates. These two analyses were assessed in 20 participating studies. Both maternal and adult smoking showed lower DNA methylation at GFI1-CpGs, and thus we assessed cardio-metabolic phenotypes with respect to risk for lower DNA methylation. In the final analyses, covariance-adjusted models were performed in all participating studies with: baseline model, using DNA methylation as an exposure plus technical covariates, and each cardio-metabolic phenotype as an outcome, and adjusted model, including sex, age and adult smoking as additional covariates.

2.7. Meta-analysis

We used METAL software to conduct inverse variance-weighted fixed effects meta-analysis. We assessed heterogeneity using the P statistic (low-heterogeneity = I² < 50%). Statistical significance was defined by Bonferroni correction for multiple testing at 0.05/4 (P ≤ 0.012), accounting for four clusters of cardio-metabolic phenotypes.

2.8. Supplementary analyses

In the NFBC1966 and 1986, we also examined the correlation between eight GFI1-CpGs. In a conditional analysis, we assessed association between adult own smoking and GFI1-CpGs additionally adjusted for all other GFI1-CpGs. Furthermore, as the full sample is multi-ethnic, the sensitivity analysis was performed to investigate the association between lower DNA methylation at eight GFI1-CpGs and cardio-metabolic phenotypes in a subset of European ancestry. Additionally, we also assessed the association of the eight GFI1-CpGs with former and current adult own smoking in NFBC1966 (Appendix B Tables S2, S5, S7 and S8).

3. Results

3.1. Participant characteristics

Participants were aged 16–81 years at the time of cardio-metabolic phenotype measurements, with the majority between 40 and 60 years. Among these, 17% were current smokers (Table 1). 18% of the participants were exposed to maternal prenatal smoking in the five studies (Appendix B Table S1). All eight GFI1-CpGs had lower mean DNA methylation levels in the group exposed to maternal prenatal smoking compared with unexposed group.

3.2. Correlation structure of the GFI1-CpGs

Fig. 1 displays the correlation matrix between the eight studied GFI1-CpGs in relation to their genomic location. The analysis performed in the NFBC1966 and NFBC1966 described a strong correlation between seven CpGs (cg04535902, cg09662411, cg09935388, cg10399789, cg12876356, cg18146737, and cg18316974). In contrast cg14179389 was weakly correlated with cg09935388 (0.35; P < 0.0001) only (Fig. 1 and Appendix B Table S2).

3.3. GFI1-CpGs DNA methylation and prenatal maternal smoking and offspring’s own smoking exposures

Following meta-analysis from five studies, the prenatal maternal smoking exposure status was associated with lower DNA methylation at cg14179389 (P = 6 × 10⁻³⁹), cg09935388 (P = 9 × 10⁻¹¹), and cg12876356 (P = 0.008) (Fig. 2, Appendix B Table S3). Cg14179389 was found to be the strongest maternal smoking locus and the association was not attenuated when adjusted for age, sex, and adult own smoking (β = 0.03, P = 0.20 × 10⁻²², β = 19.3). Similarly, adult own smoking status was associated with lower DNA methylation at all the studied CpGs. However, cg09935388 was found to be the strongest adult smoking locus (β = 0.07, P = 4.4 × 10⁻⁶⁷); the association being independent of other CpGs in the conditional analysis (Fig. 3; Appendix B Table S4 and S5). In contrast, cg14179389, the strongest above-mentioned prenatal maternal smoking signal did not show association with adult smoking status when conditioned by the DNA methylation at the other seven GFI1-CpGs. In fact, of the eight CpGs studied, only three of them remained associated with adult smoking following conditional analysis including cg09935388, cg18316974, and cg18146737 (P < 0.001) (Appendix B Table S5).

Since smoking exposures were consistently negatively associated with DNA methylation at the GFI1-locus, we assessed the associations of cardio-metabolic phenotypes against lower DNA methylation, to be consistent with the environmental risk itself i.e. increase in smoking.

3.4. Meta-analysis: eight GFI1-CpGs with lower DNA methylation and cardio-metabolic phenotypes

The associations between GFI1-CpGs and cardio-metabolic phenotypes from the meta-analysis are presented in Fig. 4 and Appendix B Table S6. Lower DNA methylation at cg14179389 was associated with increased WC, TG, and BP after a Bonferroni-correction set at P ≤ 0.012, with associations being enhanced with WC and BP when adjusted for sex, age, and adult own smoking (WC β = 0.04; BP β = 0.04; 0.0002 ≤ P ≤ 0.001). Cg14179389 consistently showed the lowest heterogeneity of the eight CpGs (P = 0.05). In contrast, lower DNA methylation at cg09935388 was associated with decreased BMI, WC, and BP, although similarly to cg14179389 showed association with increased TG. After adjustments, the associations remained showing moderate attenuation with TG (BMI β = 0.06, WC β = 0.05; BP β = 0.03, TG β = 0.01; 1 × 10⁻³ ≤ P ≤ 0.01). Lower DNA methylation at cg12876356, cg18316974, and cg09662411 was associated with decreased BMI, WC, BP and increased TG and after adjustments,
associations with decreased BMI and WC survived Bonferroni-correction ($5 \times 10^{-8} \leq P < 9 \times 10^{-5}$). Similarly, lower DNA methylation at cg18146737 was associated with decreased BMI and WC at cg04535902, with decreased BMI when adjusted ($1 \times 10^{-7} \leq P \leq 0.001$). Lower DNA methylation at cg10399789 showed no associations following adjustments ($P > 0.04$). Lower heterogeneity was observed in only European ancestry individuals, rather than the full sample, for the association between $GFI1$-CpGs with BMI and WC ($0 \leq I^2 < 40$).

**Table 1**

Characteristics of the participants of studies in the meta-analysis.

<table>
<thead>
<tr>
<th>Study Acronym</th>
<th>Sample size</th>
<th>Males, N (%)</th>
<th>Age, mean (SD), years</th>
<th>Current smokers, N (%),</th>
<th>BMI, mean (SD), kg/m²</th>
<th>WC, mean (SD), cm</th>
<th>TG, mean (SD), mmol/l</th>
<th>HDL-C, mean (SD), mmol/l</th>
<th>FG, mean (SD), mmol/l</th>
<th>DBP, mean (SD), mmHg</th>
<th>SBP, mean (SD), mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS PAC</td>
<td>1530</td>
<td>563 (37)</td>
<td>49-1 (5-8)</td>
<td>172 (11)</td>
<td>26-8 (4-8)</td>
<td>89-2 (13-2)</td>
<td>1-2 (0-7)</td>
<td>1-4 (0-4)</td>
<td>5-4 (1-1)</td>
<td>74 (10)</td>
<td>123 (14)</td>
</tr>
<tr>
<td>BHS_EA</td>
<td>680</td>
<td>208 (31)</td>
<td>42-2 (4-5)</td>
<td>168 (25)</td>
<td>30-6 (9)</td>
<td>98-6 (16-4)</td>
<td>1-7 (1-2)</td>
<td>1-2 (0-3)</td>
<td>4-7 (1-2)</td>
<td>81 (9)</td>
<td>117 (14)</td>
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<tr>
<td>BHS_AA</td>
<td>288</td>
<td>113 (39)</td>
<td>43-2 (4-5)</td>
<td>96 (33)</td>
<td>32-5 (8-6)</td>
<td>100-9 (17-7)</td>
<td>1-3 (1-0)</td>
<td>1-3 (0-4)</td>
<td>5-0 (2-0)</td>
<td>89 (14)</td>
<td>131 (22)</td>
</tr>
<tr>
<td>CODAM</td>
<td>160</td>
<td>86 (54)</td>
<td>65-6 (8-6)</td>
<td>25 (16)</td>
<td>28-4 (9-3)</td>
<td>NA</td>
<td>1-5 (0-7)</td>
<td>1-4 (0-3)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>EGCUT</td>
<td>312</td>
<td>156 (50)</td>
<td>50-2 (17)</td>
<td>56 (18)</td>
<td>27-4 (5-6)</td>
<td>91-6 (14-9)</td>
<td>1-3 (0-8)</td>
<td>1-6 (0-5)</td>
<td>5-1 (0-7)</td>
<td>80 (10)</td>
<td>128 (18)</td>
</tr>
<tr>
<td>EPICOR</td>
<td>584</td>
<td>376 (64)</td>
<td>63 (17)</td>
<td>194 (33)</td>
<td>26-6 (3-8)</td>
<td>90-6 (11-7)</td>
<td>1-5 (0-9)</td>
<td>1-7 (0-5)</td>
<td>6-1 (1-6)</td>
<td>87 (11)</td>
<td>140 (21)</td>
</tr>
<tr>
<td>ESTHERa</td>
<td>1000</td>
<td>500 (50)</td>
<td>62-1 (6-5)</td>
<td>186 (19)</td>
<td>27-8 (4-3)</td>
<td>NA</td>
<td>1-3 (0-9)</td>
<td>1-3 (0-4)</td>
<td>5-6 (1-2)</td>
<td>87 (12)</td>
<td>146 (22)</td>
</tr>
<tr>
<td>ESTHERb</td>
<td>864</td>
<td>390 (45)</td>
<td>62-1 (6-5)</td>
<td>174 (21)</td>
<td>27-7 (4-8)</td>
<td>NA</td>
<td>1-5 (0-9)</td>
<td>1-3 (0-4)</td>
<td>5-7 (2-0)</td>
<td>89 (12)</td>
<td>148 (22)</td>
</tr>
<tr>
<td>KORAF4</td>
<td>1701</td>
<td>831 (49)</td>
<td>60-9 (8-9)</td>
<td>243 (14)</td>
<td>28-1 (4-6)</td>
<td>95-4 (13-9)</td>
<td>1-6 (1-1)</td>
<td>1-5 (0-4)</td>
<td>5-0 (0-9)</td>
<td>79 (11)</td>
<td>130 (21)</td>
</tr>
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<td>LLD</td>
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<td>446 (42)</td>
<td>45-2 (13-5)</td>
<td>439 (42)</td>
<td>25-3 (4-1)</td>
<td>88-2 (12-5)</td>
<td>1-4 (0-2)</td>
<td>1-6 (0-4)</td>
<td>5-2 (0-1)</td>
<td>70 (9)</td>
<td>119 (13)</td>
</tr>
<tr>
<td>LLSD</td>
<td>631</td>
<td>301 (48)</td>
<td>58-9 (6-6)</td>
<td>85 (13)</td>
<td>25-4 (3-5)</td>
<td>NA</td>
<td>1-9 (1-2)</td>
<td>1-4 (0-4)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>LOLLIPOP</td>
<td>3842</td>
<td>2386</td>
<td>50 (10-3)</td>
<td>304 (8)</td>
<td>27-5 (4-4)</td>
<td>96-9 (11-2)</td>
<td>1-7 (1-1)</td>
<td>1-3 (0-3)</td>
<td>5-4 (1-1)</td>
<td>81 (11)</td>
<td>139 (11)</td>
</tr>
</tbody>
</table>

Data shown as N (%) or mean (SD). According to availability in the participating studies, BMI was available in 18212, WC in 14665, HDL-C in 18212, TG in 18212, FG in 16529, DBP in 16529, SBP in 16529 individuals of the total.

Abbreviations: BMI – Body Mass Index; WC – Waist Circumference; TG – Triglycerides; HDL-C – High Density Lipoprotein Cholesterol; FG – Fasting Glucose; DBP – Diastolic Blood Pressure; SBP – Systolic Blood Pressure, NA - not available.

a Study names: The Avon Longitudinal Study of Parents and Children (ALSPAC) (specifically subset with DNA methylation profiles in the Accessible Resource for Integrated Epigenomic Studies, ARIES), the two studies from Bogalusa Heart Study (BHS – European American (EA) and African American (AA)), the Cohort On Diabetes And Atherosclerosis Maastricht (CODAM), the Estonian Genome Centre, University of Tartu (EGCUT), the Italian cardiovascular section of EPIC (EPICOR), the Cooperative Gesundheitsforschung in der Region Augsburg (COGAT, Collaborative Health Research in the Augsburg Region) F4 (KORAF4), the two independent subsets of the Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der Altersbevölkerung (ESTHERa and ESTHERb), the Lifelines Deep (LLD), the London Life Science Population study (LOLLIPOP), the two follow-up datasets from Northern Finland Birth cohort 1966 (NFBC1966–31 years and NFBC1966–46 years), Northern Finland Birth cohort 1986 (NFBC1986), the Netherlands Twin Register study (NTR), the Prospective Amyotrophic Lateral Sclerosis study Netherlands (PAN), The Western Australian Pregnancy cohort study (RAINE), the two independent studies from Rotterdam Study (RS) – RSII-1 and RSII-3, III-2, the Study of Health in Pomerania – Trend (SHIP-Trend), and the Young Finns Study 2011 (YFS). The CODAM, LLS, NTR, and PAN belong to the BIBS consortium with coordinated DNA methylation measurements.

b Sample size of the studies with DNA methylation data.

c Current smoking was defined as smoking ≥1 cigarette per day.

**Fig. 1.** Map and correlation clustering of DNA methylation at eight $GFI1$-CpGs on human chromosome 1 (HapMap build 37).
The independent results of all the associations from each of the 22 studies are present in the Appendix B Tables S9, S10, S11 and S12.

4. Discussion

The present meta-analysis has corroborated the association of lower DNA methylation at the eight GFI1-CpGs with maternal prenatal and adult own smoking exposure, as well as uniquely identifying lower DNA methylation at cg14179389, a prenatal maternal smoking-related locus, as a risk factor for adult adiposity and blood pressure levels. Importantly, lower DNA methylation at all the CpGs indicates risk for higher triglyceride levels.

Recently, studies have shown GFI1-CpGs to mediate low birth weight due to prenatal maternal smoking exposure [16], and to associate with sudden infant death syndrome (SIDS) [18]. One striking finding...
from our study was the long lasting association between exposure to maternal prenatal smoking and lower DNA methylation at cg14179389 until adulthood. Moreover, lower DNA methylation at cg14179389 was also associated with increased adult WC, SBP, and DBP, suggesting a risk for adiposity and hypertension. The meta-analysis revealed a consistent effect size and direction of association in all studies, highlighting the reproducibility of findings. Furthermore, the associations persisted and were reinforced after adjusting for adult own smoking, supporting robustness and postnatal stability of maternal smoking-related DNA methylation locus. Previous studies have observed cg14179389 as the most consistent and strongest signal associated with maternal prenatal smoking among GFI1-CpGs [13,16]. These findings also include an appreciable overlap with previously identified evidence for influence of maternal smoking on the offspring’s risk for obesity, hypertension, hyperlipidaemia and cardiovascular disease [19,20]. As hypothesized, similarity in influences of maternal smoking and cg14179389 on cardio-metabolic health identifies consequences for childhood development, and suggests there may be an underlying regulatory role for epigenetic changes in relation to detrimental cardio-metabolic health outcomes. We speculate that functionally important DNA methylation changes at cg14179389 in adults are present from birth due to smoking exposure in-utero.

Fig. 4. Forest plot showing meta-analysis effect sizes of cardio-metabolic phenotypes in SD change by one SD lower DNA methylation at eight GFI1-CpGs across all the participating studies (n = 18,212). Model 1: Cardio-metabolic Phenotype = CpG + technical covariates; Model 2: Cardio-metabolic Phenotype = CpG + technical covariates + sex + adult smoking + age. Bonferroni corrected P-value threshold of P < 0.012 has been used for this analysis. Open and closed symbol indicate p < 0.012 and p < 0.012, respectively. Standardized values with mean = 0 and standard deviation = 1 were used for cardio-metabolic phenotypes and CpG methylation across all the studies. β can be interpreted as SD change in cardio-metabolic phenotype per 1-SD decrease in methylation. According to availability in the participating studies, we had BMI for 18212, WC for 14665, HDL-C for 18212, TG for 18212, FG for 16529, DBP for 16529, and SBP for 16529 of the total 18212 individuals. Abbreviations: BMI – Body Mass Index, BP – Blood Pressure, CI - Confidence Interval, HDL-C – High Density Lipoprotein Cholesterol. Measurement units for each cardio-metabolic phenotype are given in Table 1.
In contrast, lower DNA methylation at the other six GFI1-CpGs (cg09935388, cg12876356, cg18316974, cg09662411, cg18146737, cg04535902) was associated with decreased BMI, WC, and BP. Of these all BMI and WC and the most of BP associations survived Bonferroni correction. The associations were of similar magnitude, although directionally opposite to cg14179389. Furthermore, adult own smoking showed a confounding effect in attenuating the associations. These findings are in agreement with the observational studies that show highly complex and non-linear associations between smoking and cardio-metabolic health [2,5,21]. Snee et al. observed a U-shaped relationship between the number of cigarettes/day and BMI, with lowest BMI in those smoking 6–10 cigarettes/day; smoking cessation was associated with an initial increase in weight compared to those who continued smoking [22]. Increased risk of obesity among smokers is observed in a dose dependent manner, where former heavy smokers are more likely to be obese than former light smokers and have greater risk for CVD events [5,21]. Higher BMI in heavy smokers likely reflects clustering of risky behaviours that is conducive to weight gain. Paradoxically, whilst smoking acutely increases BP, smokers are observed to have slightly lower BP levels than non-smokers, especially in young adulthood, in larger epidemiological studies [23]. The comparable observations between six GFI1-CpGs and smoking with cardio-metabolic phenotypes raises the intriguing possibility that cigarette smoking induces epigenetic modifications at these CpGs, which, at least in part, may reflect the detrimental impact of smoking on cardio-metabolic health. Significant associations in our study between adult own smoking and lower DNA methylation at GFI1-CpGs across the participating studies support this hypothesis (Fig. 1, Appendix B Table S5). The observed epigenetic alterations may also partly indicate potential pathways for complex associations between smoking and BP.

Interestingly, lower DNA methylation at all eight CpGs showed association with higher TG in technically corrected models, but adjustment for adult smoking attenuated the associations, indicating a strong confounding or mediation effect. Consistency in direction of effect across all CpGs implies a concordant influence of both maternal and adult smoking induced epigenetic alterations at the GFI1-CpGs on TG. Previous evidence shows that maternal prenatal smoking exposure is associated with hyperlipidaemia in offspring [19]. Similarly, adult smokers have hyperlipidaemia and the influence of smoking cessation on lipid profiles seems to be quite modest and higher triglyceride levels pose significant risk to CVDs [24,25].

Lower heterogeneity was observed only in the European ancestry individuals, rather than the full sample, for the association between GFI1-CpGs with BMI and WC, indicating population-specific influence pertaining to adiposity (Appendix B Table S7). The studies excluded here were of the African American and South Asian ancestry. There is strong evidence that at any given BMI, the health risks are markedly higher in some ethnic groups than others. Asians have higher weight-related disease risks at a lower BMI and South Asians, in particular, have especially high levels of body fat and are more prone to developing abdominal obesity than Caucasians [26].

We observed contrasting associations of the different GFI1-CpGs (cg14179389 vs six other CpGs) with cardio-metabolic phenotypes. In the NFBC1966 and NFBC1986, we observed that cg14179389 differed and did not correlate with the other CpGs, while the other seven CpGs were highly correlated with each other (Fig. 1, Appendix B Table S2). This is supported by a recent population-level study that identified differential DNA methylation quantitative trait loci (meQTL) at these eight GFI1-CpGs, where all but one of the CpG sites (cg14179389) were highly correlated with the others, and formed contiguous clusters under the control of one meQTL [27]. Furthermore, cg14179389 association with adult smoking disappeared when adjusted for other CpGs whilst cg09935388, cg18316974, and cg18146737 showed independent associations (Appendix B Table S5). This explains the differences in their independent biological functions. Although some CpGs were associated with both maternal and adult smoking, perhaps due to the reversible nature of DNA methylation, their association with cardio-metabolic phenotypes was similar to functional consequence of own smoking in later life. Longitudinal analysis has provided evidence of rapid reversibility of DNA methylation in general during early development, particularly during the immediate postnatal years, with stabilization beyond age 7, suggesting a ‘catch up’ mechanism in early life [15,28]. In contrast, the unperturbed effect of cg14179389 due to maternal smoking in our study indicates persistent disruption of DNA methylation due to in-utero smoking exposure at this particular site.

Collectively, these observations suggest evidence of two concepts (Fig. 5). First, maternal prenatal smoking induces a foetal response modulated through persistent epigenetic disruption. Second, adult own smoking, that may be potentially influenced by maternal smoking, induces similar epigenetic changes, which may play a role in the underlying pathways towards adverse consequences of smoking on cardiovascular risk. It is important to consider that many other environmental factors contribute to the cardiovascular risk (e.g. physical activity, stress, sedentary lifestyle, diet, alcohol consumption), and associated DNA methylation disruption following exposures to the maternal prenatal and own smoking could only explain a partial mediating role.

This study represents a major effort to perform a large-scale meta-analysis of maternal prenatal smoking, DNA methylation at GFI1-locus and cardio-metabolic phenotypes. A wide range of phenotypic data was available, facilitating assessment of the functional consequences of DNA methylation changes over a varied age range. The study replicates and confirms previously reported associations of lower DNA methylation at the GFI1-CpGs with exposure to own and maternal prenatal smoking.

We acknowledge that large collaborations utilizing summary level data, although useful in enhancing power to detect associations, may limit the ability to undertake multiple sensitivity analyses. We were unable to fully analyse DNA methylation changes over the life-course and disentangle the interaction of age with DNA methylation, to support emerging evidence that shows reversibility of DNA methylation patterns [29]. Another limitation was the use of leucocytes, which were the source of DNA used. They are composed of several cell types each with cell-type specific DNA methylation patterns and thus differences in these cell types could potentially confound the observed associations. Adjustment for derived cell type proportions was included in the analysis to overcome this eventuality. Our study included eight GFI1-sites associated with maternal prenatal smoking. We recognize that further work exploring the associations between DNA methylation of other adult and maternal prenatal smoking related loci and cardio-metabolic phenotypes could yield additional insights into the role of epigenetic markers that may jointly affect cardio-metabolic health. In addition, lack of gene expression data across studies limited insight into the molecular mechanisms. Additional evidence is needed to support GFI1 as the causal gene responsible for the observed findings. However, in a recent animal study, GFI1 did affect the systemic inflammation through the NE-dependent-C/EBPa-GFI1 pathway that predisposes to metabolic dysfunction and obesity [30]. Translating these findings to human data would be clinically relevant in light of our findings.

5. Conclusion

Our findings support evidence that epigenetic factors at the GFI1-locus, that are associated with exposures to smoking in-utero or adulthood are also linked to cardio-metabolic risk factors, specifically suggesting a role in hypertriglyceridaemia. The findings support an underlying epigenetic component of the epidemiologically observed cardio-metabolic risk by maternal prenatal and adult smoking. The fact that these epigenetic factors associate with cardio-metabolic risk in later life even among non-smokers exposed to in-utero smoking may have important clinical implications. Such epigenetic loci might serve as objective biomarkers of past environmental exposures that
could be used for preventive health measures. Our findings provide a strong foundation for further work to unravel emerging epigenetic markers with downstream detrimental health outcomes, and deliver strong evidence to support the early origin of adult health. It draws attention to increase awareness on smoking cessation and better prevention strategies.

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**Fig. 5. Model representing the potential mechanistic pathways in the study.**
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Conflicts of interest

Niek Verweij is employee at Genomic Plc. Other authors have nothing to disclose.

Contributors

SS, MRJ, and PP conceptualised and designed the study. PP wrote the first draft and analysed the data. PP, MRJ, and SS had full access to the data. All authors acquired and interpreted the data, critically reviewed the manuscript, provided technical or material support and approved the final version to be published. SS and MRJ supervised the study.

Appendix A. Supplementary data

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