Mendelian randomization study of maternal influences on birthweight and future cardiometabolic risk in the HUNT cohort

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There is a robust observational relationship between lower birthweight and higher risk of cardiometabolic disease in later life. The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that adverse environmental factors in utero increase future risk of cardiometabolic disease. Here, we explore if a genetic risk score (GRS) of maternal SNPs associated with offspring birthweight is also associated with offspring cardiometabolic risk factors, after controlling for offspring GRS, in up to 26,057 mother–offspring pairs (and 19,792 father–offspring pairs) from the Nord-Trøndelag Health (HUNT) Study. We find little evidence for a maternal (or paternal) genetic effect of birthweight associated variants on offspring cardiometabolic risk factors after adjusting for offspring GRS. In contrast, offspring GRS is strongly related to many cardiometabolic risk factors, even after conditioning on maternal GRS. Our results suggest that the maternal intrauterine environment, as proxied by maternal SNPs that influence offspring birthweight, is unlikely to be a major determinants of adverse cardiometabolic outcomes in population based samples of individuals.
There is a robust and well-documented observational relationship between lower birthweight and higher risk of cardiometabolic diseases in later life, including cardiovascular disease (CVD) and type 2 diabetes (T2D). The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that adverse environmental factors in utero or in the early years of life result in increased future risk of cardiometabolic disease1–7. Evidence in favor of DOHaD has primarily come from observational1,2,28 and animal studies9; however, definitive causal evidence from human studies is lacking.

Mendelian randomization (MR) is an epidemiological method used to investigate whether an observational association between an exposure and an outcome represents a causal relationship17. Several studies have recently attempted to use MR to investigate the relationship between lower birthweight and cardiometabolic disease to inform on the validity of DOHaD11–13. However, these MR studies have used sub-optimal methodologies in which only offspring genotypes are considered as genetic instruments to proxy offspring birthweight14. This limitation contrasts strikingly with the argument that many of the DOHaD proponents would make, i.e. that the adverse maternal environment during pregnancy results in low birthweight and increased risk of future cardiometabolic disease1,6. This hypothesis is entirely distinct from postulating that birthweight itself has a direct causal effect on risk of cardiometabolic disease14. Thus, these early MR studies have ignored the potential contribution of the maternal genome (correlated 0.5 with the offspring genome15,16), meaning that any association between offspring SNPs and offspring cardiometabolic risk may in fact be due to maternal genotypes, violating core assumptions underlying MR17, and complicating interpretation of the results. Indeed, Smith and Ebrahim10 in their initial description of the MR methodology, noted that the appropriate way of using MR to investigate the effects of the intrauterine environment on offspring outcomes (in their example maternal folate intake and offspring neural tube defects), was to use maternal genotypes to proxy the intrauterine environment10.

MR principles can be harnessed to test aspects of DOHaD using maternal SNPs that are related to offspring birthweight and/or adverse maternal environmental exposures during pregnancy1,16,18–20. For example, one possibility is to test whether SNPs in the mother that are directly related to offspring birthweight are also associated with offspring cardiometabolic risk factors, after conditioning on offspring genotypes at the same loci. To understand why this analysis would be informative, consider Fig. 1, which illustrates four credible ways in which maternal genotypes related to lower offspring birthweight are also associated with future cardiometabolic risk factors. We investigate whether maternal genotypes associated with lower offspring birthweight are also associated with later life offspring cardiometabolic risk factors. We do not test for the influence of these exposures on offspring cardiometabolic risk in this study, but limit our attention to those that exert an effect on offspring birthweight (a distinction we explore further in the discussion).

We have previously used this paradigm to examine the association between maternal birthweight related SNPs and offspring blood pressure in the UK Biobank study as a preliminary test of the validity of this possible DOHaD mechanism18. Interestingly, this showed that maternal SNPs related to low offspring birthweight were actually associated with lower offspring systolic blood pressure after conditioning on offspring genotype at the same loci (i.e. the opposite of what would be expected if maternal intrauterine effects that reduce fetal growth result in higher later-life cardiometabolic risk). However, the number of mother–offspring pairs used in this previous study was small \((N = 3,886)\) and systolic blood pressure was the only cardiometabolic risk factor investigated. Therefore, the results from this preliminary study need to be replicated and further cardiometabolic risk factors examined. The Norwegian based HUNT Study21, which contains approximately 70,000 genotyped individuals, including 45,849 parent–offspring pairs, is one of the few cohorts where such analyses can be conducted. The average age of the HUNT offspring is approximately 40 years, rendering this cohort not only one of the largest cohorts in the world with genotyped mother–offspring pairs (and father–offspring pairs) with birthweight information, but also one of the few with offspring old enough to have developed adverse cardiometabolic profiles.

In this work, we perform genetic association analyses in up to 26,057 genotyped mother–offspring pairs from the Norwegian HUNT Study in order to investigate whether there is evidence for a causal effect of the intrauterine environment (proxied by maternal SNPs that influence offspring birthweight) on offspring cardiometabolic risk factors. We investigate whether maternal genotypes associated with lower offspring birthweight are also associated with later life offspring cardiometabolic risk factors such as blood pressure, non-fasting glucose levels, body mass index (BMI), and lipid levels, after conditioning on offspring genotype at the same loci. We also perform similar analyses in up to 19,792 father–offspring pairs to investigate whether there is evidence for a postnatal environmental effect (genetic nurture or dynamic effects), rather than an intrauterine environmental effect. In the course of executing these analyses, we implement a computationally efficient genetic linear mixed model that not only enables the investigation of causal questions relevant to the
specific DOHaD mechanism that is the focus of this paper, but also simultaneously accounts for the non-independence between siblings and the considerable cryptic relatedness within the HUNT Study. We show no evidence for a causal effect of the intrauterine environment (as proxied by maternal genetic effects on offspring birthweight) on offspring cardiometabolic risk factors. We do, however, find evidence that offspring SNPs pleiotropically influence both birthweight and future cardiometabolic risk factors, which helps explain the robust observational relationships between the variables.

**Results**

**Phenotypic correlations.** HUNT offspring with recorded values for birthweight were on average 30.1 years old, with a minimum age of 19, and a maximum age of 85 at the time of measurement used in this study. Descriptive statistics on the mother–offspring and father–offspring pairs are presented in Table 1. It is important to note that only offspring born after 1967 had birthweight recorded and were included in this part of the analysis. Table 2 shows the phenotypic association between own birthweight and SBP, DBP, non-fasting glucose, non-fasting total, LDL and HDL cholesterol, non-fasting triglycerides, and BMI. Consistent with many previous observational epidemiological studies, linear regression yielded negative point estimates of the observational relationship between birthweight and blood pressure, LDL, total cholesterol, and BMI. We also found evidence for positive quadratic terms in the model between birthweight and both BMI and glucose, suggesting U-shaped/J-shaped relationships between these variables. Finally, we found evidence for a positive linear relationship between HDL cholesterol and birthweight with additional evidence for a convex quadratic term indicating small and large babies are likely to have slightly reduced HDL levels in later life.

**Analysis of fetal growth and cardiometabolic risk factors in the HUNT offspring.** We first checked whether the GRs of birthweight associated SNPs from the latest GWAS of birthweight were also related to offspring birthweight in HUNT. The full results are presented in Supplementary Table 1. In short, we found that maternal GRs were strongly associated with increased offspring birthweight after conditioning on offspring GRs in HUNT. Offspring GR was related to offspring birthweight, but this relationship attenuated after controlling for maternal GR. In the case of the GR consisting of SNPs that only had a maternal effect from the Warrington et al. birthweight GWAS, offspring GR was not strongly related to offspring birthweight after controlling for maternal GR. As expected, paternal GR was not associated with offspring birthweight after conditioning on offspring GR. The effect size of the offspring GR was similar in mother–offspring and father–offspring pairs, and did not attenuate after adjusting for paternal GR.

For the primary analyses investigating the effect of GRs on offspring cardiometabolic traits, we had a total of 26,057 mother–offspring pairs and 19,792 father–offspring pairs. HUNT offspring were on average 40 years old, with a minimum age of 19, and a maximum age of 85 at the time of measurement used in this study. Descriptive statistics on all of the outcome variables in the two samples are presented in Table 3. Our asymptotic power...
calculations indicated that we had (≥80%) power to detect a maternal genetic effect that explained as little as 0.04% of the variance in offspring outcome (N = 26,057) (two tailed α = 0.05) and slightly lower power (≥68%) (N = 19,792) to detect a paternal genetic effect responsible for a similar proportion of the offspring phenotypic variance. Due to some missing data in the offspring’s cardiometabolic risk factors, the number of mother–offspring and father–offspring pairs differed slightly across the outcomes (Table 3). Although the sample size for some of the analyses is slightly lower (lowest being 25,461 mother–offspring pairs and 19,339 father–offspring pairs) we retain statistical power to detect an association of maternal GRS with offspring cardiometabolic risk factors (79% and 67%, respectively) using the same parameters as above.

We found little evidence for an association between maternal (or paternal) GRS and any of the offspring cardiometabolic risk factors in later life, after adjusting for offspring GRS (Tables 4, 5; Supplementary Data 1). These tables show the estimated expected change in offspring cardiometabolic outcome per one unit (i.e. allele) increase in maternal/paternal genetic risk score after conditioning on offspring (or maternal/paternal) genetic risk score. These results hold for systolic blood pressure, which had previously been found to associate with maternal GRS in the Warrington et al GWAS of birthweight18. In contrast, there was strong evidence for a relationship between offspring GRS and some of the offspring phenotypes after conditioning on maternal GRS (Table 6). Specifically, there was evidence for a positive association between offspring GRS and both offspring glucose and LDL, and evidence for a negative relationship between offspring GRS and both systolic blood pressure and triglycerides. It is important to note that the blood samples used to measure lipids and glucose were non-fasting samples, which could influence these results.

Cardiometabolic pathology becomes more apparent with increasing age. Indeed, it is possible that younger individuals within the HUNT Study do not show observable compensatory changes in cardiometabolic risk factors, reducing the power of our analyses to detect evidence for the observational associations between birthweight and cardiometabolic risk factors to be causal. We therefore divided our dataset into two strata based on age of the offspring (i.e. offspring under 40 years of age and offspring between 40 and 60 years of age). Our asymptotic power calculations indicated that we had (≥80%) power to detect a maternal genetic effect that explained as little as 0.09% of the variance in offspring SBP (N = 12,037 and N = 11,849) (α = 0.05) and slightly lower power (≥66%) (N = 10,393 and N = 8402) to detect a paternal genetic effect responsible for a similar proportion of the offspring phenotypic variance. Table 7 (and Supplementary Table 2) shows the main results of the stratified analyses compared with those previously reported in the UK BioBank by Warrington et al in their GWAS of birthweight18. Whereas Warrington and colleagues found a significant positive effect of maternal GRS on offspring SBP when adjusting for offspring GRS, we find no effect in the stratified analyses.

**Discussion**

The Developmental Origins of Health and Disease (DOHaD) hypothesis posits that adverse environmental factors in utero or in the early years of life result in increased future risk of cardiometabolic disease1,4,6. In this study, we used an MR paradigm to provide evidence for or against the existence of DOHaD mechanisms that are related to fetal growth and lower birthweight for a range of cardiometabolic risk factors16,18. Specifically, we tested whether a genetic risk score in mothers intended to proxy for maternal intrauterine influences on offspring birthweight was also associated with offspring cardiometabolic risk factors, whilst simultaneously conditioning on offspring GRS constructed from the same birthweight associated loci. There was no strong evidence of association in a sample of over 25,000 mother–offspring pairs from the Norwegian HUNT study, implying that if such an effect on cardiometabolic risk factors exists, it may be small compared to other sources of inter-individual variation, or only affects a few individuals.

Our study is, to the best of our knowledge, the largest parent–offspring MR study of DOHaD performed to date. The HUNT Study contains over 25,000 genotyped mother–offspring pairs where the majority of the offspring are middle-aged adults, and are therefore old enough to have begun developing observable signs of cardiometabolic disease. Our asymptotic calculations indicated that we had strong (≥80%) power to detect a maternal genetic effect that explained as little as 0.04% of the variance in offspring outcome (two tailed α = 0.05). In contrast, our previous study in the UK Biobank18 (where we first used this MR paradigm to investigate DOHaD), involved only 3886 mother–offspring pairs, and was likely underpowered. Interestingly, Warrington and colleagues found evidence for a positive relationship between maternal birthweight lowering SNPs and reduced offspring SBP (i.e. the opposite of what DOHaD would predict); however, this result did not replicate in our sample. Possible reasons for the discrepancy include the differences in sample ascertainment across the studies, or that the younger offspring in HUNT did not manifest a large enough effect18.

### Table 1 Descriptive statistics for offspring cardiometabolic risk factors in the phenotypic association analyses.

| Phenotype                  | Mother–offspring pairs | Father–offspring pairs |
|---------------------------|------------------------|------------------------|
|                           | N  | Mean  | SD  | Range   | N  | Mean  | SD  | Range   |
| Birthweight (g)           | 7825 | 3570  | 482 | 1390–5900 | 6875 | 3572  | 480 | 1660–5900 |
| Age                       | 7825 | 30.1  | 6.6 | 19.1–41.4 | 6875 | 30.1  | 6.6 | 19.2–41.4 |
| Sex (% male)              | 7825 | 45.3  | -   | -          | 6875 | 45.7  | -   | -          |
| SBP (mmHg)                | 7792 | 122.9 | 13.4 | 70.0–207.0 | 6846 | 122.8 | 13.4 | 70.0–207.0 |
| DBP (mmHg)                | 7790 | 69.0  | 9.8 | 36.9–117.0 | 6845 | 68.9  | 9.7 | 38.0–120.0 |
| Total cholesterol (mmol/L) | 7684 | 4.95  | 1.16 | 2.29–11.95 | 6727 | 4.95  | 1.17 | 2.29–11.47 |
| LDL cholesterol (mmol/L)  | 7674 | 2.92  | 0.84 | 0.27–6.98  | 6742 | 2.91  | 0.84 | 0.27–7.27  |
| HDL cholesterol (mmol/L)  | 7682 | 1.31  | 0.32 | 0.50–2.80  | 6748 | 1.32  | 0.32 | 0.50–2.80  |
| Triglycerides (mmol/L)     | 7786 | 1.22  | 1.72 | 0.30–11.25 | 6839 | 1.21  | 1.72 | 0.30–11.25 |
| BMI                        | 7803 | 25.79 | 1.19 | 15.64–49.40 | 6853 | 25.28 | 1.17 | 15.96–49.40 |

*SBP systolic blood pressure, DBP diastolic blood pressure, Glucose non-fasting glucose, BMI body mass index, LDL non-fasting low density lipoprotein, HDL non-fasting high density lipoprotein, mmol/L millimol per litre, N number of individuals, SD standard deviation.

*Offspring phenotype first (natural) logarithm transformed in analysis and converted back for overview purposes.
When stratifying our analysis by age, we did find effects in the same direction as our original study for the 40–60 years age group; however, the statistical support for the effect was weak. Taken together, the UK BioBank and HUNT results provide converging evidence that maternal genetic effects that predispose to low offspring birthweight are not associated with increased systolic blood pressure in later life.

In contrast, we did find evidence for association between offspring GRS and a number of offspring cardiometabolic risk factors, even after conditioning on maternal GRS. These results are broadly consistent with the Fetal Insulin hypothesis26–29 and previous studies that have used LD score regression and G-REML approaches to suggest that much of the phenotypic correlation between birthweight and cardiometabolic risk is driven by genetic pleiotropy in the offspring genome rather than DOHaD mechanisms18,30. We note that the direction of the associations involving the offspring GRS and offspring phenotypes are a little difficult to interpret, since the GRS were defined on the basis of maternal genotypic effects on offspring birthweight, whereas these reported associations involve offspring GRS. Offspring genotypes at some of the same loci are known to have quantitatively and qualitatively different effects on offspring birthweight (including the direction of association) compared to the maternal effects. Also important to take into account is the fact that the lipid and glucose measurements were performed in non-fasting samples, which could influence these results, particularly as it is known that mean blood glucose levels and triglycerides are higher in the first three hours after calorie intake31. Nevertheless, our results show clearly that maternal SNPs that influence offspring birthweight have pleiotropic effects on offspring cardiometabolic traits when these same SNPs are transmitted to their offspring.

Another novel facet of our study was the use of the OpenMx software package to model the complicated data structure within the HUNT Study. Using traditional formulations of FIML to model the relatedness structure using a genetic relationship matrix would be computationally prohibitive within the HUNT sample, as maximizing the likelihood would involve an inversion of a matrix of order \( N \). In contrast, our implementation permits complicated tests of association to be performed in the fixed effects part of the model, whilst simultaneously modeling cryptic relatedness in the random effects part of the model in a computationally efficient manner.32. We hope that our implementation will prove useful in complicated genetic analyses of other large scale population-based cohorts where cryptic relatedness/population stratification is likely to be an issue. We have included an example R script in Supplementary Note 1 of the manuscript that can be used as a template by interested researchers. We caution users, however, that specification of the covariance part of the model is more rigid using our speed up in that only two variance components can be fitted simultaneously, one being a residual variance component that is uncorrelated across individuals.

Our approach has a number of limitations which we discuss in the remaining paragraphs. First, we assume that the maternal SNPs that affect offspring birthweight do so via fetal growth (as reflected in birthweight). This is important, because as many others have noted, it may not be fetal growth/birthweight itself that is relevant for the validity of DOHaD. Rather it could be poor development of different key organs, in key stages of the pregnancy or a particular adverse maternal environment due to famine, disease or a range of other factors. Indeed, it would likely be profitable to use the same framework to investigate the association between offspring cardiometabolic disease and other adverse maternal exposures, such as maternal BMI, maternal alcohol consumption, preeclampsia, and gestational diabetes.
Their effect may be qualitatively and quantitatively different from the maternal effect on birthweight within healthy subjects delivering babies within the normal range. However, even though the mechanisms through which our maternal SNPs influence offspring birthweight are largely unknown (and therefore our genetic risk score is largely unspecific), we know that they play an important part in fetal growth of the offspring. Further MR studies on different maternal exposures are warranted including on those that do not necessarily exert observable effects on offspring birthweight, but proxy other more specific maternal environments. Moreover, we used unweighted GRS of birthweight associated SNPs in our MR framework. Using a weighted maternal GRS and conditioning on a weighted offspring GRS does not completely block the path through the offspring’s genome, increasing type 1 error rate for the maternal effect on offspring cardiometabolic phenotype. To avoid the inflation in type 1 error, we use an unweighted maternal GRS and condition on offspring unweighted GRS, which is sufficient to block this path. However, the main reason for using an unweighted GRS is that weighting SNPs by the strength of association between maternal genotype and offspring birthweight would only be appropriate if the effect of the maternal SNP on the offspring’s cardiometabolic phenotype was mediated through offspring birthweight (i.e. panel B of Fig. 1). However, we believe it is more likely that offspring birthweight is a marker of several latent processes, which may then affect the offspring’s cardiometabolic phenotype (i.e. more akin to panel A of Fig. 1). Using weights derived from a maternal GWAS of birthweight may not accurately reflect SNP associations with these underlying latent processes, particularly if there are many such processes that are relevant for later life disease risk.

Second, our example here, and MR approaches in general, typically test small changes in an exposure. However, it may be that DOHaD mechanisms are important in the genesis of cardiometabolic risk, but only in the case of severe exposures (e.g. famine or obesity) at the extreme ends of the spectrum. These effects may be qualitatively different from small perturbations in the environment that produce relatively subtle variations in the normal healthy population. If DOHaD is only relevant in the case of extreme environmental effects, then MR approaches applied to population data may not be well suited to testing the hypothesis.

Third, although our methods rely on MR principles to inform on the validity of DOHaD (i.e. we use genetic variants to increase our study’s robustness to environmental confounding), we did not perform formal instrumental variables analyses in this manuscript. The reason is that we do not have appropriate estimates of the effect of maternal genotypes on the intrauterine environment. We only have estimates of the relationship between SNPs and offspring birthweight, which is an imperfect proxy of fetal growth restriction. Therefore, it does not make sense to estimate causal effect sizes in our study as in typical MR analyses. However, we note that it may be possible to estimate the effect of a putative latent variable indexing growth restriction using, for example, latent variable models; this is an area of future research for our group.

Fourth, our power calculations show that we were well powered (>80% at $\alpha = 0.05$) to detect an association between maternal genetic risk score and offspring cardiometabolic risk factors responsible for as little as 0.04% of the phenotypic variance. However, whilst our study, to the best of our knowledge, is the largest and most powerful genetic investigation into DOHaD to date, the actual variance in the offspring cardiometabolic risk factor explained by the maternal GRS, depends critically upon the underlying genetic model, and could be even smaller than 0.04%. In an attempt to make this clear, Fig. 2 is a path diagram that illustrates the relationship between maternal GRS, offspring GRS, an intrauterine environment that reduces fetal growth (modeled as a single latent unobserved variable), offspring birthweight and an offspring cardiometabolic risk factor. In this diagram, and consistent with most formulations of DOHaD, we assume that (i) there is no direct causal effect of birthweight on cardiometabolic risk (i.e. no arrow from birthweight to the cardiometabolic risk factor), and (ii) no effect of maternal GRS on the offspring cardiometabolic risk factor that goes through paths other than fetal growth restriction (e.g. no postnatal mechanisms). To make calculations and explication easier, we assume that all variables have been standardized to unit variance. Under this model, the correlation between birthweight and the cardiometabolic risk factor is a function of two processes. One is the effect of the intrauterine environment on birthweight and the cardiometabolic risk factors (i.e. the product of path coefficients $\lambda_1$ and $\lambda_2$). The second is the residual covariance between birthweight and the cardiometabolic risk factors. This latter pathway includes both environmental factors other than fetal growth restriction that affect both phenotypes and the effect of polygenes that are not modeled in the experiment whose joint effects are quantified by the parameter $\Theta$. These correlations could be positive or negative individually, but when combined produce a very small ($|\Theta| < 0.05$) negative phenotypic correlation between birthweight and most of the cardiometabolic risk factors. The point is that, unless the residual covariance between birthweight and the cardiometabolic risk factor is positive, the values for path coefficients $\lambda_1$ and $\lambda_2$ are likely to be very small in order to be consistent with the observed phenotypic correlations.

### Table 3 Descriptive statistics for offspring cardiometabolic risk factors in the primary analyses.

| Phenotype                  | Mother-offspring pairs | Father-offspring pairs |
|----------------------------|------------------------|------------------------|
|                            | N          | Mean  | SD   | Range  | N          | Mean  | SD   | Range  |
| Age (% male)               | 26,057    | 41.4  | 12.7 | 19.1-83.2 | 19,792    | 39.3  | 12   | 19.1-84.8 |
| SBP (mmHg)                 | 25,946    | 128.3 | 17.3 | 70.0-218.0 | 19,711    | 126.9 | 16.4 | 70.0-218.0 |
| DBP (mmHg)                 | 25,940    | 73.9  | 11.9 | 36.0-134.0 | 19,711    | 72.8  | 11.5 | 38.0-126.0 |
| Glucose (mmol/L)*          | 25,461    | 5.16  | 1.20 | 2.29-12.81 | 19,339    | 5.16  | 1.19 | 2.29-12.81 |
| Total cholesterol (mmol/L) | 25,589    | 5.31  | 1.08 | 2.00-10.90 | 19,423    | 5.22  | 1.07 | 2.10-10.80 |
| LDL cholesterol (mmol/L)   | 25,533    | 3.26  | 0.95 | 0.14-8.60  | 19,392    | 3.19  | 0.93 | 0.27-8.60  |
| HDL cholesterol (mmol/L)   | 25,560    | 1.33  | 0.33 | 0.50-2.90  | 19,412    | 1.33  | 0.33 | 0.50-2.80  |
| Triglycerides (mmol/L)*    | 25,916    | 1.35  | 1.73 | 0.18-11.70 | 19,680    | 1.32  | 1.73 | 0.49-11.70 |
| BMI*                      | 25,946    | 26.31 | 1.17 | 15.03-50.40 | 19,715    | 26.31 | 1.17 | 15.80-50.40 |

*SBP systolic blood pressure, DBP diastolic blood pressure, Glucose non-fasting glucose, BMI body mass index, LDL cholesterol non-fasting low density lipoprotein, HDL cholesterol non-fasting high density lipoprotein, mmol/L millimol per litre, N number of individuals, SD standard deviation.

*Offspring phenotype first (natural) logarithm transformed in analysis and converted back for overview purposes.
### Table 4 Results of regressing offspring cardiometabolic risk factors on maternal GRS\(^a\) after conditioning on offspring GRS\(^a\) in mother–offspring pairs.

| Outcome                  | Autosomal SNPs (N = 204) | Autosomal SNPs with maternal effect (N = 71) | Autosomal SNPs with maternal effect only (N = 31) |
|--------------------------|--------------------------|---------------------------------------------|-----------------------------------------------|
|                          | Effect estimate | SE | p-value | Effect estimate | SE | p-value | Effect estimate | SE | p-value |
| SBP (mmHg)               | −0.0090        | 0.0066 | 0.1772  | 0.0008        | 0.0066 | 0.8972  | −0.0056        | 0.0066 | 0.3968  |
| DBP (mmHg)               | −0.0089        | 0.0065 | 0.1713  | −0.0049       | 0.0065 | 0.4512  | −0.0071        | 0.0064 | 0.2676  |
| Glucose (mmol/L)\(^b\)  | −0.0006        | 0.0071 | 0.7985  | −0.0021       | 0.0061 | 0.7854  | −0.0018        | 0.0070 | 0.8014  |
| Total cholesterol (mmol/L) | −0.0028        | 0.0069 | 0.7123  | 0.0040        | 0.0068 | 0.5344  | −0.0034        | 0.0068 | 0.6185  |
| LDL cholesterol (mmol/L) | −0.0042        | 0.0066 | 0.5295  | 0.0016        | 0.0066 | 0.8060  | −0.0016        | 0.0066 | 0.8091  |
| HDL cholesterol (mmol/L) | 0.0071         | 0.0068 | 0.2892  | 0.0049        | 0.0065 | 0.4570  | 0.0095         | 0.0066 | 0.1527  |
| Triglycerides (mmol/L)\(^b\) | −0.0037        | 0.0070 | 0.6577  | 0.0030        | 0.0069 | 0.6518  | −0.0097        | 0.0069 | 0.1628  |
| BMI\(^b\)               | −0.0111        | 0.0071 | 0.1217  | 0.0002        | 0.0068 | 0.8502  | −0.0118        | 0.0071 | 0.0910  |

The regression coefficients give the estimated expected change in offspring cardiometabolic outcome (in the units listed in column 1) per one unit increase in maternal genetic risk score after conditioning on offspring genetic risk score. Effect estimates and standard errors are standardized. \(p\)-values reflect minus two log-likelihood ch-square tests between the full model and a sub-model where the relevant parameter is fixed to zero. All \(p\)-values are two sided uncorrected for multiple testing. All analyses are adjusted for age, sex, measurement occasion, and GRS of offspring. GRS genetic risk score, SBP systolic blood pressure, DBP diastolic blood pressure, Glucose non-fasting glucose, BMI body mass index, LDL non-fasting low density lipoprotein, HDL non-fasting high density lipoprotein, mmol/L millimol per litre, SNP single nucleotide polymorphism, N number of individuals, SE standard error. \(^a\)Maternal and offspring GRS were coded so that increasing dosages reflected maternal alleles associated with increased offspring birthweight based on conditional GWAS results previously published. \(^b\)Offspring phenotype first (natural) logarithm transformed.

### Table 5 Results of regressing offspring cardiometabolic risk factors on paternal GRS\(^a\) after conditioning on offspring GRS\(^a\) in father–offspring pairs.

| Outcome                  | Autosomal SNPs (N = 204) | Autosomal SNPs with maternal effect (N = 71) | Autosomal SNPs with maternal effect only (N = 31) |
|--------------------------|--------------------------|---------------------------------------------|-----------------------------------------------|
|                          | Effect estimate | SE | p-value | Effect estimate | SE | p-value | Effect estimate | SE | p-value |
| SBP (mmHg)               | −0.0008        | 0.0076 | 0.9143  | −0.0008        | 0.0076 | 0.9143  | −0.0008        | 0.0076 | 0.9143  |
| DBP (mmHg)               | −0.0048        | 0.0075 | 0.5238  | 0.0061        | 0.0074 | 0.4114  | 0.0025        | 0.0075 | 0.7412  |
| Glucose (mmol/L)\(^b\)  | 0.0031         | 0.0081 | 0.6102  | 0.0086        | 0.0080 | 0.2786  | 0.0031        | 0.0075 | 0.8362  |
| Total cholesterol (mmol/L) | −0.0027        | 0.0079 | 0.7318  | −0.0085       | 0.0078 | 0.2798  | −0.0102        | 0.0079 | 0.1951  |
| LDL cholesterol (mmol/L) | 0.0009         | 0.0075 | 0.9445  | 0.0019        | 0.0074 | 0.8027  | −0.0033        | 0.0075 | 0.6637  |
| HDL cholesterol (mmol/L) | −0.0011        | 0.0079 | 0.8912  | −0.0046       | 0.0078 | 0.5494  | −0.0131        | 0.0077 | 0.0993  |
| Triglycerides (mmol/L)\(^b\) | −1.2 × 10^{-03} | 0.0080 | 0.8851  | −0.0039       | 0.0079 | 0.6087  | −0.0039        | 0.0079 | 0.6238  |
| BMI\(^b\)               | −0.0016        | 0.0082 | 0.6710  | −0.0001       | 0.0068 | 1       | 0.0119         | 0.0080 | 0.1414  |

The regression coefficients give the estimated expected change in offspring cardiometabolic outcome (in the units listed in column 1) per one unit increase in paternal genetic risk score after conditioning on offspring genetic risk score. Effect estimates and standard errors are standardized. \(p\)-values reflect minus two log-likelihood ch-square tests between the full model and a sub-model where the relevant parameter is fixed to zero. All \(p\)-values are two sided uncorrected for multiple testing. All analyses are adjusted for age, sex, measurement occasion and GRS of offspring. GRS genetic risk score, SBP systolic blood pressure, DBP diastolic blood pressure, Glucose non-fasting glucose, BMI body mass index, LDL non-fasting low density lipoprotein, HDL non-fasting high density lipoprotein, mmol/L millimol per litre, SNP single nucleotide polymorphism, N number of individuals, SE standard error. \(^a\)Paternal and offspring GRS were coded so that increasing dosages reflected maternal alleles associated with increased offspring birthweight based on conditional GWAS results previously published. \(^b\)Offspring phenotype first (natural) logarithm transformed.
The variance in birthweight explained by the maternal GRS is a function of the direct association between the SNPs and the intrauterine environment (the path coefficient $\gamma$), and the effect of the intrauterine environment on birthweight (the path coefficient $\lambda_1$—the precise formula being $\gamma^2\lambda_1^2$). The variance explained in the cardiometabolic risk factor by the maternal GRS is equal to the product of the SNPs’ direct effect on the intrauterine environment (path coefficient $\gamma$ in Fig. 2), multiplied by the effect of the intrauterine environment on the cardiometabolic risk factor (path coefficient $\lambda_2$ in Fig. 2) all squared. There are an infinite number of ways these parameters can vary to make the underlying model consistent with the pattern of observed correlations and the proportion of variance explained in birthweight by the maternal GRS. To give the reader an idea of the potentially small numbers involved, we assume that the correlation between birthweight and the cardiometabolic risk factor is completely explained by the intrauterine environment and $\lambda_1 = -0.5$ and $\lambda_2 = 0.1$ (so that the observed correlation $r = \lambda_1 \lambda_2 = -0.05$).

In order for the underlying model to also be consistent with the maternal GRS explaining a small percentage of the variance in birthweight (say 0.5% of the variance), then the path coefficient between the maternal GRS and the latent intrauterine variable $\gamma$ would equal $\sqrt{0.005/0.1} = 0.1414$. These values in turn would imply that the variance explained in the cardiometabolic risk factor by the maternal genetic risk score would be $0.1414^2 \times 0.1^2 = 0.0237$, which is a small proportion of the variance, and one that we are only moderately well powered to detect (>50%) in our study. Our point, however, is that the proportion of variance in the outcome explained by the maternal GRS may be very small, and so power may only be moderate despite the very large sample size of HUNT. The corollary to this is that we are very well powered to detect larger effects of the intrauterine environment influencing offspring birthweight on cardiometabolic risk factors, and the fact that we do not detect these suggests that if such an effect is present, it is likely to be small.

Finally, we recognize that our act of conditioning on offspring GRS, may have induced a (spurious) correlation between maternal GRS and paternal GRS due to conditioning on a collider variable, potentially biasing the results of our maternal GRS analyses. However, any such bias is likely to be small in magnitude as it relies on the existence of (and is proportional to the size of) direct paternal genetic effects from the same SNPs on the offspring phenotype. As sizeable paternal genetic effects on offspring cardiometabolic risk are unlikely at these loci, we doubt that collider bias is a serious impediment to the validity of our study.

In conclusion, we did not find evidence for a causal effect of the intrauterine environment (as proxied by maternal genetic effects on offspring birthweight) on offspring cardiometabolic risk factors in a population-based sample of individuals. We did, however, find evidence of genetic pleiotropy between offspring birthweight and offspring cardiometabolic risk factors which helps explain the robust observational relationships between the variables.

### Methods

**HUNT study.** The Nord-Trøndelag Health Study (HUNT) is a large population-based health study of the inhabitants of Nord-Trøndelag County in central Norway that commenced in 1984. A comprehensive description of the study population has been previously reported. Approximately every 10 years the entire adult population of Nord-Trøndelag (~90,000 adults in 1995) is invited to attend a health survey which includes comprehensive questionnaires, an interview, clinical examination, and detailed phenotypic measurements (HUNT1 1984 to 1986; HUNT2 1995 to 1997; HUNT3 2006 to 2008 and HUNT4 2017 to 2019). These surveys have high participation, with 89%, 69%, 54%, and 54% of invited adults participating in HUNT1, 2, 3, and 4, respectively. Additional phenotypic information is collected by integrating national registers. Approximately 90% of...
participants from HUNT2 and HUNT3 were genotyped in 201535, and the gen-
type and phenotype data used in the subsequent analysis are exclusively from these
two surveys.

The HUNT Study was approved by the Regional Committee for Medical and
Health Research Ethics, Norway and all participants gave informed written consent
(REK Central application number 2018/2488).

Genotyping, quality control, and imputation. DNA from 71,860 HUNT samples
was genotyped using one of three different Illumina HumanCoreExome arrays
(HumanCoreExome12 v1.0, HumanCoreExome12 v1.1, and UM HUNT Biobank
v1.0)35. Genomic position, strand orientation, and the reference allele of genotyped
variants were determined by aligning their probe sequences against the human
genome (Genome Reference Consortium Human genome build 37 and revised
Cambridge Reference Sequence of the human mitochondrial DNA; http://genome.
ucsc.edu) using BLAT36. Ancestry of all samples was inferred by projecting all
samples into the space of the principal components of the Human
Genome Diversity Project (HGDP) reference panel (938 unrelated individuals;
human core exome18 were extracted from the HUNT imputed genotype data in dosage
format using plink239. Dosages were coded so that increasing dosages re-

Identifying genotyped parent–offspring pairs. Before the kinship analysis, the
plink files with genotyped SNPs underwent a second stage of cleaning. Any indi-
viduals whose inferred sex contradicted their reported gender (N = 348) as well as
individuals showing high or low heterozygosity (±5 SD from the mean) (N = 412)
were removed (760 individuals in total). In addition, variants with minor allele
frequency <0.005 or more than 5% missing rate were removed. Parent–offspring
pairs were identified by kinship analysis using the KING software version 2.2.414.1
Only genotyped SNPs shared across the arrays on autosomal chromosomes were
used for the analysis – a total of 257,488 SNPs.

From the analysis, 46,428 parent–offspring relationships were identified, in
addition to 35,373 full sibings, 128,334 second degree relationships and 386,619
third degree relationships based on the kinship analysis performed using the KING
software and recommended thresholds for relatedness implemented as part of this
package41. Any parent–offspring pair with 15 years or fewer difference in birth year
was removed from further analyses. After removing these pairs, a total of 26,057
mother–offspring pairs and 19,792 father–offspring pairs of European ancestry
with genotype information passing QC were identified. Each parent had between
one and eight offspring available for analysis. Supplementary Table 3 shows the
number of offspring per mother/father available for analysis.

Genetic risk scores. SNPs previously associated with own or offspring birthweight
at genome-wide levels of significance in the Early Growth Genetics (EGG) Con-
sortium paper18 were extracted from the HUNT imputed genotype data in dosage
format using plink239. Dosages were coded so that increasing dosages reflected
maternal alleles associated with increased offspring birthweight based on condi-
tional genome-wide association study (GWAS) results previously published18.

Unweighted genetic risk scores (GRS) were constructed by simply adding the
expected number of increasing birthweight alleles together for each individual. We
used unweighted scores because we do not know the extent to which each allele
influences growth restriction, and so weighting the scores by e.g. their observed
effect on birthweight would be less appropriate. Three GRS were constructed—one
using all autosomal SNPs shown to have an effect on birthweight (N = 204) from the
recent EGG Consortium GWAS paper of birthweight18 (that found 205 auto-

Outcome variables. During the health surveys (HUNT1–4)21 clinical examination,
and detailed phenotypic measurements were performed on all participants, data
from HUNT3 or HUNT2 were used in the subsequent work. For all cardiomet-
abnol risk factors in the offspring (BMI, systolic blood pressure (SBP), diastolic
blood pressure (DBP), non-fasting glucose (Glucose), total cholesterol, high density
lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and
triglycerides), the most recent value (e.g. values measured in HUNT3) was used if
available. If the individuals were not a part of HUNT3, measurements from HUNT2
were used. Age at participation was calculated to correspond with the
health survey chosen. Blood pressure was taken three times during the clinical
examination, and SBP and DBP measurements were calculated as the average of
the second and third measurement. For individuals who only had two blood
pressure measurements taken (12% of offspring in the mother

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Fig. 2 Path diagram of the relationship between maternal Genetic Risk Score (GRS), offspring GRS, the intrauterine environment, offspring birthweight and an offspring cardiometabolic risk factor. Variables within square boxes represent observed variables, whereas variables in circles represent latent unobserved variables. Unidirectional arrows represent causal relationships from tail to head, whilst two headed arrows represent correlational relationships. Greek letters on one headed arrows represent path coefficients which quantify the expected causal effect of one variable on the other. Greek letters on two headed arrows represent covariances between variables. The two epsilon variables represent residual latent factors (both environmental and genetic) that are not modeled in the study. The coefficient $\Theta$ represents the covariance between the residual terms.

We assume that all variables are standardized to have unit variance. Consequently, the residual variance of the offspring GRS is set to 0.75 since $\frac{1}{4}$ of the variance comes from the maternal genotype. For the purposes of the power calculation described in the discussion, we assume that maternal single nucleotide polymorphism (SNP)s that affect offspring birthweight do so through a single latent intrauterine factor, and that this factor also exerts long term effects on the offspring cardiometabolic risk factor of interest.

Phenotypic relationship between birthweight and cardiometabolic risk factors. Own birthweight was available for individuals in HUNT after linking with the Medical Birth Registry of Norway (MBRN) using the unique 11-digit identification numbers assigned to all Norwegian residents. This was performed by a third party and the researchers only had access to de-identified data. The registry commenced in 1967, when health authorities began reporting pregnancy-related data; therefore, birthweight measurements were only available for HUNT participants born in 1967 or later. The validity of information on birthweight in the MBRN has previously been reported as very good. Individuals in HUNT with pants born in 1967 or later. The validity of information on birthweight in the squared was included as a between birthweight and cardiometabolic risk factors, as has been observed in interest.

Single nucleotide polymorphism (SNP)s that affect offspring birthweight ¼ of the variance comes from the maternal genotype. For the purposes of 10 Medical Birth Registry of Norway (MBRN) using the unique 11-digit identifiers. Phenotypic relationship between birthweight and cardiometabolic risk factors (both environmental and genetic) that are not modeled in the study. The problem then reduces from a dependence between outcome trait values, leaving the random effects uncorrelated.

\[
\Sigma = \mathbf{A}_G + \mathbf{I}_u,
\]

where $\Sigma$ is the expected $N \times N$ phenotypic covariance matrix, $\mathbf{A}$ is an $N \times N$ genetic relationship matrix calculated using the GCTA software version 1.9352, $\mathbf{I}_u$ is an $N \times N$ identity matrix, $\mathbf{A}_G$ and $\mathbf{I}_u$ are variance components due to additive genetic and residual sources of variation respectively, and $N$ is the number of individuals in the analysis. When creating the GRM it was important to exclude the SNPs (and the SNPs in linkage disequilibrium around them) used in the genetic risk scores. Otherwise we would risk some of our association signal in the fixed effects part of the model betty attenuated because we also also would also the random effects part of the model. The GRM was therefore calculated after excluding the known birthweight SNPs and any SNPs 1 Mb away from them. Estimating these parameters using FIML in OpenMx is computationally intensive in that it involves inverting a matrix of order $N$. Indeed, our initial attempts to do this suggested that fitting the model to the HUNT data this way may not be possible given the limitations of our computing hardware. We therefore reimplemented the statistical model using a factor rotation which converted the problem from one involving an $N \times N$ matrix, to one involving a $1 \times 1$ matrix (see Supplementary Note 2 for details). Our implementation involved first performing a singular value decomposition of the genetic relationship matrix (A), and then gene multiplying the matrices of outcomes and fixed effects respectively by the matrix of eigenvectors. This pre-multiplication has the effect of ‘rotating away’ the dependence between outcome trait values, leaving the random effects uncorrelated. The problem then reduces from $N$ correlated observations (modeled by an $N \times N$ matrix), to $N$ independent observations, greatly facilitating computation. An R script with code illustrating our method is included in the Supplementary Note 1.

We first tested the relationship between maternal GRS and offspring birthweight in $N = 7,825$ mother–offspring pairs and $N = 6,875$ father–offspring pairs to confirm that our GRS explained some of the variance in offspring birthweight. We then performed our primary analyses testing the relationship between maternal GRS and each of the offspring cardiovascular risk factors, whilst conditioning on offspring GRS. We performed the same analyses in father–offspring pairs to assess whether there was evidence for a postnatal effect from either parent (Fig. 1d). In addition to analyzing all of the offspring together, we stratified the data into two groups; one group with offspring under age 40 at the time of measurement and one for offspring between 40 and 60 years of age. This was done to obtain a sample that would be easier to compare with the previous analysis of GBP and in the UK Biobank Study by Warrington and colleagues. Age strata for individuals over 60 is not presented due to the low number of individuals. A flowchart of the sample selection is presented in Fig. 3.

Power calculations. We were interested in the statistical power of our approach to detect maternal genetic effects on offspring cardiometabolic risk factors. We therefore used the Maternal and Offspring Genetic Effects Power Calculator to calculate power to detect association. We assumed $N = 26,057$ complete mother–offspring pairs, the absence of offspring genetic effects, a Type I error rate of $\alpha = 0.05$ (the presence/absence of offspring genetic effects has little influence on power to detect maternal genetic effects so long as the proportion of variance explained is small).
Fig. 3 Flowchart showing the number of individuals (N) participating in the analysis and the exclusion criteria for each analysis. A total of 69,716 genotyped HUNT participants were recruited from either HUNT2 or HUNT 3. Of these 46,428 parent–offspring relationships were identified using the King software. Parent–offspring pairs with ≤15 years difference in birth year were removed leaving 26,058 mother–offspring pairs and 19,792 father–offspring pairs for the main analysis. Birthweight was only available for offspring born after 1967. Additionally, for analyses involving birthweight as an outcome, offspring were excluded if they were part of a multiple birth, had congenital malformation, were born where the birth was induced or performed with C-section, their birthweight ≤1000 g, or were born before 258 days of gestation.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The empirical datasets used with the HUNT study will be archived with the study and will be made available to individuals who obtain the necessary permissions from the study’s Data Access Committee. Due to privacy issues, access to individual level data requires permission from the HUNT Study, the Medical Birth Registry of Norway and the regional committee for medical research ethics. Requirements for access to data from the HUNT Study are described at [www.ntnu.edu/hunt](http://www.ntnu.edu/hunt).

**Code availability**
Example code is provided in Supplementary Note 1.

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Author contributions

G.H.M. contributed to the design of the work, the data analysis, interpretation of the data and the draft of this manuscript. B.B. contributed to the acquisition and interpretation of the data and substantively revised the manuscript. C.W. contributed to the acquisition and analysis of the data. B.O.A. contributed to the acquisition and interpretation of the data and substantively revised the manuscript. K.I.B. contributed to the interpretation of the data and substantively revised the manuscript. G.W. contributed to the design of the work and interpretation of the data. M.C.N. contributed to the analysis and substantively revised the manuscript. R.M.F. contributed to the interpretation of data and substantively revised the manuscript. G.D.S. contributed to the interpretation of data and substantively revised the manuscript. D.A.L. contributed to the interpretation of data and substantively revised the manuscript. R.M.K. contributed to the analysis and substantively revised the manuscript. N.M.W. contributed to conception, design of the work, interpretation of the data and substantively revised the manuscript. D.M.E. contributed to conception, design of the work, interpretation of the data and the draft of this manuscript. All authors approved the submitted version.

Competing interests

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Additional information

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