Maternal genome-wide association study identifies a fasting glucose variant associated with offspring birth weight

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Abstract

Several common fetal genetic variants have been associated with birth weight, but little is known about how maternal genetic variation influences fetal growth through the intra-uterine environment. To identify maternal genetic variants associated with birth weight, we performed a meta-analysis of 11 genome-wide association studies (GWAS; *n* = 19,626 women of European descent). We selected 18 single nucleotide polymorphisms (SNPs) for replication analysis in up to 13 further studies (*n* = 18,319 women of European descent). One SNP reached genome-wide significance (rs10830963, $P = 2.0 \times 10^{-11}$) in a combined analysis of discovery and replication results. Rs10830963 is intronic in *MTNR1B* and is known from previous GWAS to be associated with fasting glucose levels, type 2 diabetes and gestational diabetes. Each copy of rs10830963-G (the allele associated with higher fasting glucose) corresponded to a 31g [95%CI: 22, 41g] higher offspring birth weight. The association between maternal rs10830963 and birth weight was unaltered by adjustment for any potentially confounding effects of fetal genotype in 8716 maternal-fetal pairs. Although no other SNPs reached genome-wide significance, there was an excess of low *P*-values among SNPs known to be associated with fasting glucose levels. Our study demonstrates that maternal genetic variation at *MTNR1B* influences offspring birth weight and supports a broader role of genetic variation affecting maternal glucose levels in fetal growth. Our study also highlights that the effect sizes of associations between other maternal genetic variants and birth weight are unlikely to exceed 20g per allele, and therefore much larger sample sizes will be required to detect them.

Introduction

The role of common maternal genetic variation in offspring fetal growth is poorly understood. Maternal genotypes may affect the intra-uterine environment by influencing key maternal phenotypes, such as circulating levels of glucose, lipids and other metabolic factors, which may cross the placenta or act upon other maternal attributes such as vascular function. Such maternal environmental effects could in turn influence fetal growth separately from the effects of any growth-related genetic variants that are inherited directly from the mother (**Figure 1**).

Genome-wide association studies (GWAS) have so far identified seven loci in the fetal genome that are robustly associated with birth weight (1, 2). The goal of the current study was to apply the same method to identify *maternal* genetic variants associated with offspring birth weight. As a GWAS is hypothesis-free, it could potentially highlight novel pathways by which the maternal genotype influences offspring birth weight through the intra-uterine environment. We performed a meta-analysis of GWAS of offspring birth weight using maternal genotypes in up to 19,626 women of European descent from 11 studies. We took forward up to 18 potentially important single nucleotide polymorphisms (SNPs) in a total of 18,319 European descent participants from 13 further independent studies. For associated variants, we repeated the analysis adjusting for any potentially confounding effects of fetal genotype using 8716 maternal-offspring pairs.

Results

SNP rs10830963 at the known MTNR1B fasting glucose locus, was associated with birth weight at $P<5x10^{-8}$.

In our Discovery meta-analysis (*N* = 19,626), we observed more associations between maternal SNPs and offspring birth weight than expected under the null hypothesis (**Supplementary Figures 1 and 2**). Of the 18 SNPs taken forward for follow-up in replication studies (including 15 SNPs with $P < 10^{-5}$ in discovery meta-analysis and n=3 SNPs at $P < 10^{-4}$ with robust prior evidence of association with another phenotype potentially relevant to birth weight), only rs10830963 at *MTNR1B,* the known fasting glucose locus, was associated with birth weight at $P < 5 \times 10^{-8}$ in the overall meta-analysis (**Figure 2; Supplementary Figure 3; Supplementary Table 1**). Each additional maternal G-allele, which is associated with higher fasting glucose, was associated with a 31g [95%CI: 22, 41g] higher offspring birth weight ($P = 2.0 \times 10^{-11}$). There was little detectable heterogeneity between studies ($P = 0.36$; **Supplementary Figure 3**).

The association between birth weight and maternal genotype at MTNR1B is not influenced by the fetal genotype.

Using *N* = 8716 mother-child pairs from the ALSPAC, EFSOCH and HAPO studies, we observed that the association between maternal rs10830963 genotype and birth weight did not change on adjustment for fetal genotype. In a meta-analysis of these studies, each additional maternal G-allele was associated with a 21g [95%CI: 3, 38g] higher offspring birth weight in a model adjusted for fetal genotype (compared with 23g [95%CI: 8, 38g] in the same samples before adjustment; **Table 1**).

Known maternal fasting glucose-associated variants show more associations with offspring birth weight than expected by chance

Thirteen SNPs known to be associated with fasting glucose in studies of non-pregnant adults (3) (including variants with the largest effects) showed more evidence of association with offspring birth weight in our Discovery meta-analysis than expected under the null hypothesis (**Figure 3; Supplementary Table 2**). There was insufficient evidence to suggest that the associations were more likely to be positive than negative (8 positive associations out of 13; $P = 0.58$), but the maternal fasting glucose-raising alleles of the 5 SNPs showing associations at $P < 0.05$ were all associated with higher offspring birth weight.

Variants previously identified in a fetal GWAS of birth weight showed weaker evidence of association with maternal genotype

Maternal and fetal genotypes are correlated ($r \approx 0.5$), so we would expect to see some evidence of association between maternal genotype and birth weight at SNPs known to influence birth weight through the fetal genotype. Of the seven SNPs known to be robustly associated with birth weight via the fetal genotype (2) none was more strongly associated with birth weight when using the maternal genotype, and most were consistent with the maternal genotype effect size being 50% of that of the fetal genotype (**Supplementary Table 3**).

Discussion

In this study, we have identified a maternal genetic locus that is robustly associated with offspring birth weight. The top SNP, rs10830963, is intronic in *MTNR1B*, which encodes melatonin receptor 1B and is known to be associated with fasting glucose levels (3) and type2 diabetes (4) from previous GWAS of non-pregnant individuals. Previous GWAS of pregnant women have also shown genome-wide significant associations between this locus and both fasting and one-hour plasma glucose levels following oral glucose tolerance test (5), and with gestational diabetes mellitus (GDM) (6). Various other studies have provided further evidence of associations with GDM in Mexican-Americans (7), Finns (8) and British, Chinese, Turkish, Swedish and French (9). As expected, the fasting glucose-raising allele corresponded to higher offspring birth weight in our data.

We also found evidence of a collective effect of known maternal fasting glucose-associated variants on offspring birth weight: more low *P*-values than expected were observed among 13 variants selected, with the glucose-raising allele corresponding to higher offspring birth weight for the 5 most significantly associated SNPs. Among these 5 SNPs, rs4607517 at the *GCK* fasting glucose locus (10) was included in the 18 SNPs taken forward for follow-up analysis. It had a replication *P*-value of 2.7 x 10^{-4}, but did not reach genome-wide significance in the combined analysis (**Supplementary Table 1**). Also among these 5 SNPs was rs7903146 at *TCF7L2* (**Supplementary Table 2**), which has previously shown evidence of association with birth weight in samples that only partially overlap (37%) with our Discovery meta-analysis (11, 12). It is also notable that rs204928, an intronic SNP in *LMO1*, showed some evidence of association with birth weight (P = 4.7 x 10^{-7} in the combined discovery and follow-up meta-analysis). At this locus, rs11041816 (r^2=0.39 with rs204928) has shown prior evidence of association with fasting glucose levels adjusted for BMI at P=2.4 x 10^{-7}, again with the trait-raising allele corresponding to higher birth weight in our data (13). Our study adds to accumulating evidence for a role of maternal *GCK* and *TCF7L2* variation in birth weight (10-12) and demonstrates

the impact of common genetic variations in maternal glucose levels on normal variation in offspring birth weight.

Despite having almost 20,000 samples in the discovery meta-analysis and a combined discovery and follow-up sample of up to 37,747 women, we confirmed association between only one locus and birth weight at a genome-wide level of significance ($P < 5x10^{-8}$). A larger study with greater statistical power would be needed to clarify whether the signals at *GCK* and *LMO1* represent genuine associations with birth weight. The effect size estimates of associations between offspring birth weight and maternal genetic variants known to influence fasting glucose (**Supplementary Table 2**) are modest (<20g per allele) and we estimate that sample sizes of between 50,000 and 270,000 (for SNPs with minor allele frequency between 50% and 5%, respectively) would be needed to provide robust evidence of association at such loci.

We note that variants associated with birth weight from previous fetal GWAS (2) were not among the strongest maternal genotype associations. Even if the maternal genotype is having no effect on birth weight through the intrauterine environment, we would expect to see some evidence of association due to the correlation between maternal and fetal genotypes. However, the maternal genotype effect size estimates were all smaller (**Supplementary Table 3**). This highlights differences between the roles of maternal and fetal genetic variation in fetal growth, and supports that those previously observed associations were not driven by confounding with maternal genotype.

To conclude, we have identified a robust association between maternal genetic variation at the *MTNR1B* fasting glucose locus and offspring birth weight. Our study (i) supports a broader role in fetal growth of genetic variation affecting levels of maternal glucose, and (ii) highlights that individual common maternal genetic variants, well captured by our GWAS approach, are unlikely to influence offspring birth weight by more than about 20g per allele. Detection of associations

between maternal genotype and offspring outcomes will necessitate larger sample sizes than typical genotype-phenotype association studies.

Materials and Methods

Discovery studies, genotyping and imputation

We studied 19,626 unrelated women of European ancestry from eleven studies with maternal genome-wide genotypes and offspring birth weight available. These included two sub-samples from the 1958 British birth cohort (1958BC-WTCCC2, n = 836; 1958BC-T1DGC, n = 858); the Avon Longitudinal Study of Parents and Children (ALSPAC, n = 7,304); a sub-sample of the Danish National Birth Cohort from the Genetics of Extreme Overweight in Young Adults study (DNBC-GOYA, n = 1,805); population-based controls from a case-control study of pre-term birth in the DNBC (DNBC-PTBCTRLS, n = 1,656); the Hyperglycemia and Adverse Pregnancy Outcome study (HAPO, n = 1,280); the Norwegian Mother and Child cohort study (MoBa, n = 650); the Northern Finland 1966 Birth Cohort study (NFBC1966, n = 2,035); the Netherlands Twin Register (NTR, n = 707); the Queensland Institute of Medical Research study of adult twins (QIMR, n = 892); the Twins UK study (TwinsUK, n = 1,603).

Genotypes in each study were obtained through high-density SNP arrays and up to ~2.5million autosomal SNPs were imputed to HapMap Phase II. Study protocol was approved at each study centre by the local ethics committee and written informed content had been obtained from all participants and/or their parent(s) or legal guardians. Study descriptions and basic characteristics of samples in the discovery phase are presented in **Supplementary Table 4**.

Genome-wide association analysis within discovery studies

We converted offspring birth weight (BW, grams) to a z-score ((BW value - mean(BW))/ standard deviation(BW)) to allow comparison of data across studies. We excluded multiple births, stillbirths, congenital anomalies (where known), and births before 37 weeks of gestation (where known). We assessed the association between each SNP and offspring birth weight using linear regression of the birthweight z-score against maternal genotype (additive genetic model), with sex and gestational

age as covariables. Ancestry principal components were included as covariables where necessary in the individual studies. Genome-wide association analyses were conducted using PLINK (14), SNPTEST (15), Mach2qtl (16) or Beagle (17) (see **Supplementary Table 4**).

Genome-wide meta-analysis of discovery studies

Prior to meta-analysis, SNPs with a minor allele frequency (MAF) <0.01 and poorly imputed SNPs (info<0.8 (PLINK), r2hat <0.3 (MACH or Beagle) or proper_info <0.4 (SNPTEST)) were filtered out. To adjust for inflation in test statistics generated in each cohort, genomic control (18) was applied once to each individual study (see **Supplementary Table 4** for λ values in each study). Data annotation, exchange and storage were facilitated by the SIMBioMS platform (19). Quality control of individual study results and fixed-effects inverse variance meta-analyses were undertaken by two meta-analysts in parallel at different study centres using the software package METAL (2009-10-10 release) (20). We obtained association statistics for a total of 2,422,657 SNPs in the meta-analysis for which at least 7 of the 11 studies were included. The genomic inflation factor, λ, in the overall meta-analysis was 1.007. Effect sizes from combined meta-analysis are reported both as a z-score and in grams: to obtain the approximate effect size in grams, we multiplied by 484g, the median standard deviation of birth weight in a representative sample of European studies (1).

Follow-up of 18 signals in additional studies

We selected 15 SNPs that surpassed a *P*-value threshold of $P < 1 \times 10^{-5}$ for follow-up in additional, independent studies. Of these, one SNP (rs11020124) was in linkage disequilibrium (LD; $r^2 = 0.63$, 1000 Genomes Pilot 1 data) with SNP rs10830963 at the *MTNR1B* locus known to be associated with fasting glucose and Type 2 diabetes (4). We assumed that these represented the same association signal. Given its robust association with maternal glycemic traits likely to impact on offspring birth weight, we took only rs10830962 forward for follow-up at this locus. We then used the National Human Genome Research Institute (NHGRI) catalog of published GWAS

(http://www.genome.gov/gwastudies, accessed September 2012) to query the SNPs associated with birth weight between $P = 10^{-4}$ and $P = 10^{-5}$. We identified three further SNPs at loci with robust evidence ($P < 5 \times 10^{-8}$) of association with other phenotypes, and therefore higher prior odds of association with birth weight: rs2971669 near *GCK* ($r^2 = 0.73$ with rs4607517 associated with fasting glucose)(10); rs204928 in *LMO1* ($r^2 = 0.90$ with rs110419 associated with neuroblastoma)(21) and rs7972086 in *RAD51AP1* ($r^2 = 0.27$ with rs2970818 associated with serum phosphorus concentration)(22). We took forward SNPs rs4607517, rs204928 and rs7972086 for follow-up at these loci, giving a total of 18 SNPs to be examined in additional studies.

The descriptions, genotyping details and basic phenotypic characteristics of the follow-up studies are presented in **Supplementary Table 5**. Of a total of thirteen follow up studies (n = 18,319 individuals), 9 studies (n = 15,288) provided custom genotyping of between 4 and 18 SNPs, while 4 studies (n = 3,031 individuals) had *in silico* genome-wide or exome-wide SNP genotypes available. Where SNPs were imputed, we included only those with quality scores (r2hat or proper_info) >0.8. We excluded directly genotyped SNPs showing evidence of deviation from Hardy-Weinberg Equilibrium at *P* <0.0028 (Bonferroni corrected for 18 tests). Where genotypes were unavailable for the index SNP, we used the SNP Annotation and Proxy (SNAP) Search Tool to find proxy SNPs based on LD in the 1000 Genomes Pilot 1 dataset ($r^2 > 0.8$; https://www.broadinstitute.org/mpg/snap/ldsearch.php; accessed September 2012; see **Supplementary Table 6**).

Overall meta-analysis of discovery and follow-up samples

We performed inverse variance, fixed-effects meta-analysis of the association between each SNP and birth weight z-score in up to 24 discovery and follow-up studies combined (maximum total n = 37,747) using the user-written Stata command, metan (23). We estimated the percentage of total variation among study estimates that was due to between-study heterogeneity using Cochran's Q

test. To report the final results from the combined meta-analysis, we additionally excluded SNPs from the discovery studies that had imputation quality scores (r2hat or proper_info) <0.8.

Analyses of the MTNR1B variant in mother-child pairs

To check that the association between maternal rs10830963 genotype and offspring birth weight was not influenced by fetal rs10830963 genotype, we analysed n=8716 mother-child pairs from 3 studies with both maternal and fetal direct genotypes available (ALSPAC, EFSOCH, HAPO (non-GWAS)). In the ALSPAC study, direct genotypes were used due to the poor imputation quality (r^2<0.8) of rs10830963 in the GWAS dataset (genotyping performed at LGC Genomics, Hoddesdon, UK (www.lgcgroup.com) using KASP^{TM} genotyping chemistry: call rate >95%; HWE P > 0.05; concordance between duplicate genotyped samples >99%). Although mother-child pairs were also available in the HAPO (GWAS), DNBC-PTBCTRLS and Generation-R studies, the imputation quality of either maternal or fetal rs10830963 or both was r^2<0.8 so we did not include those studies. We used linear regression to test the association between maternal genotype and birth weight z-score, while adjusting for fetal genotype. We compared this with the model unadjusted for fetal genotype in the same sample. Genotypes were coded additively for the number of glucose-raising alleles and models were adjusted for sex and gestational age. We combined the results from the individual studies using inverse variance meta-analysis with fixed effects.

Testing associations with birth weight of known glucose-associated variants

We selected the first 16 SNPs that were associated with fasting glucose at $P < 5 \times 10^{-8}$ in studies of non-pregnant individuals, including those with the largest effect sizes (3). We hypothesised that these SNPs in the pregnant mothers would show more associations with offspring birth weight than expected by chance, and that the associations would be between the maternal glucose-raising allele and higher offspring birth weight. We excluded SNPs rs780094 and rs174550 at *GCKR* and *FADS1* due to known effects on other metabolic traits (3, 24-26). We additionally excluded rs11708067 at

ADCY5 as the fetal glucose-raising allele is associated with lower birth weight (1). We used the results from the discovery meta-analysis to create a QQ plot of these 13 associations. We additionally used the binomial probability (sign) test to assess whether there was more evidence of positive or negative association with birth weight than the 50% expected under the null distribution.

Associations between birth weight and maternal genotype at SNPs previously identified in a fetal GWAS of birth weight

Maternal and fetal genotypes are correlated ($r \approx 0.5$), so we would expect to see some evidence of association between maternal genotype and birth weight at SNPs known to influence birth weight through the fetal genotype. To check that previously reported fetal genotype associations were not driven by maternal genotype effects, we queried the results of our Discovery meta-analysis for 7 SNPs known to be robustly associated with birth weight via the fetal genotype ($P < 5 \times 10^{-8}$)(2).

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Statistical Analysis: J.T., R.M.F., R.C.R., D.A.L., G.M., D.M.E., E.K-M., L.P., B.F., F.G., C.A., J.F.F., J-J.H., M.G.H., D.M.S., M.N., K.L.L., M.H., C.L.R., C.P., A.E., S.D., V.H., J.N.P., S.E.M., P.A.L., A.C., D.J.B., R.M., V.S., J.A.M., W.A., S.M., S.J.B.

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Figure legends

Figure 1. A schematic diagram illustrating that maternal genetic factors may influence fetal growth indirectly through the intra-uterine environment, or directly through inheritance by the fetus.

Figure 2. Regional plot of the association between maternal genotype and offspring birth weight at the *MTNR1B* locus. SNP position (NCBI build 36 coordinates; x-axis) and trait association ($-\log_{10}$ *P*-value; left y-axis) are shown, and the colours reflect linkage disequilibrium of each SNP with rs10830963 (based on pairwise r^2 values from HapMap). Recombination rates are from HapMap (right y-axis). The *P*-value for rs10830963 in the combined analysis is represented by a purple diamond, and that from the discovery stage analysis by a purple circle. Removal of discovery studies in which rs10830963 was imputed with quality score (r^2hat or proper_info) <0.8 resulted in a *P*-value in the combined analysis of 2.0×10^{-11} (see **Table 1**).

Figure 3. Quantile-quantile plot of associations (from the meta-analysis of 19,626 discovery samples) between birth weight and 13 maternal SNPs known to be associated with fasting glucose. Observed versus expected $-\log_{10}$ *P*-values are plotted for all SNPs and the black line represents expected $-\log_{10}$ *P*-values under the null distribution. The grey area defines the 95% concentration bands, which are an approximation to the 95% confidence intervals around the expected line. Triangles indicate associations between the glucose-raising allele and higher birth weight; circles indicate associations between the glucose-raising allele and lower birth weight.

Figure 1

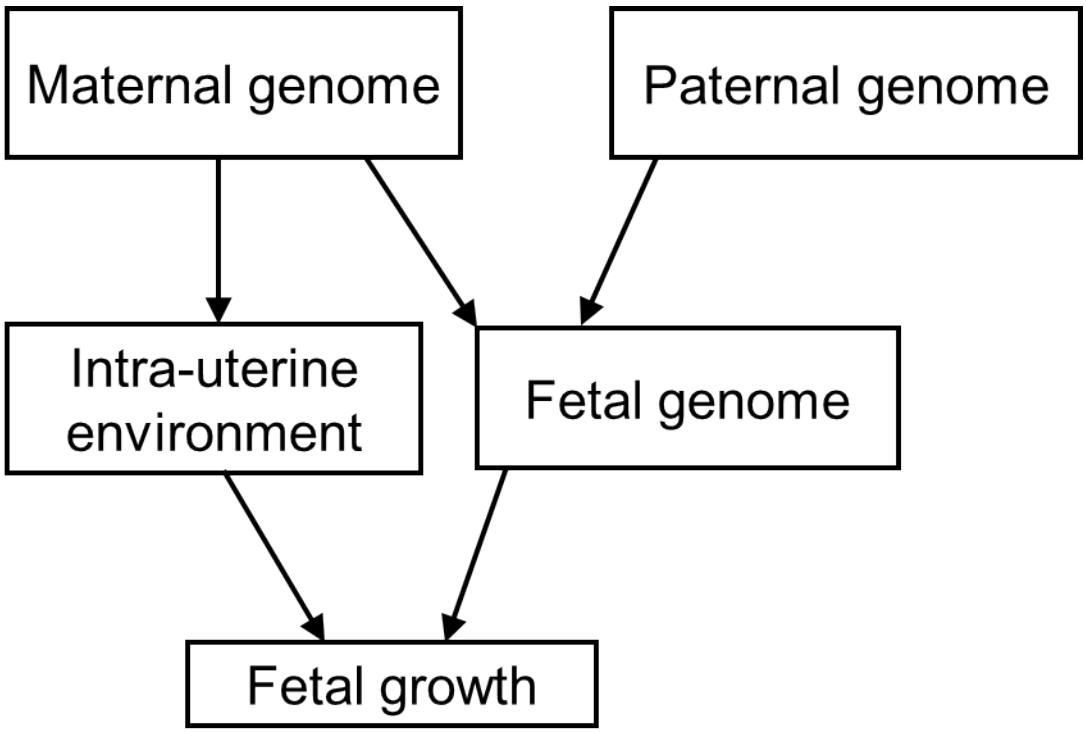

Figure 2

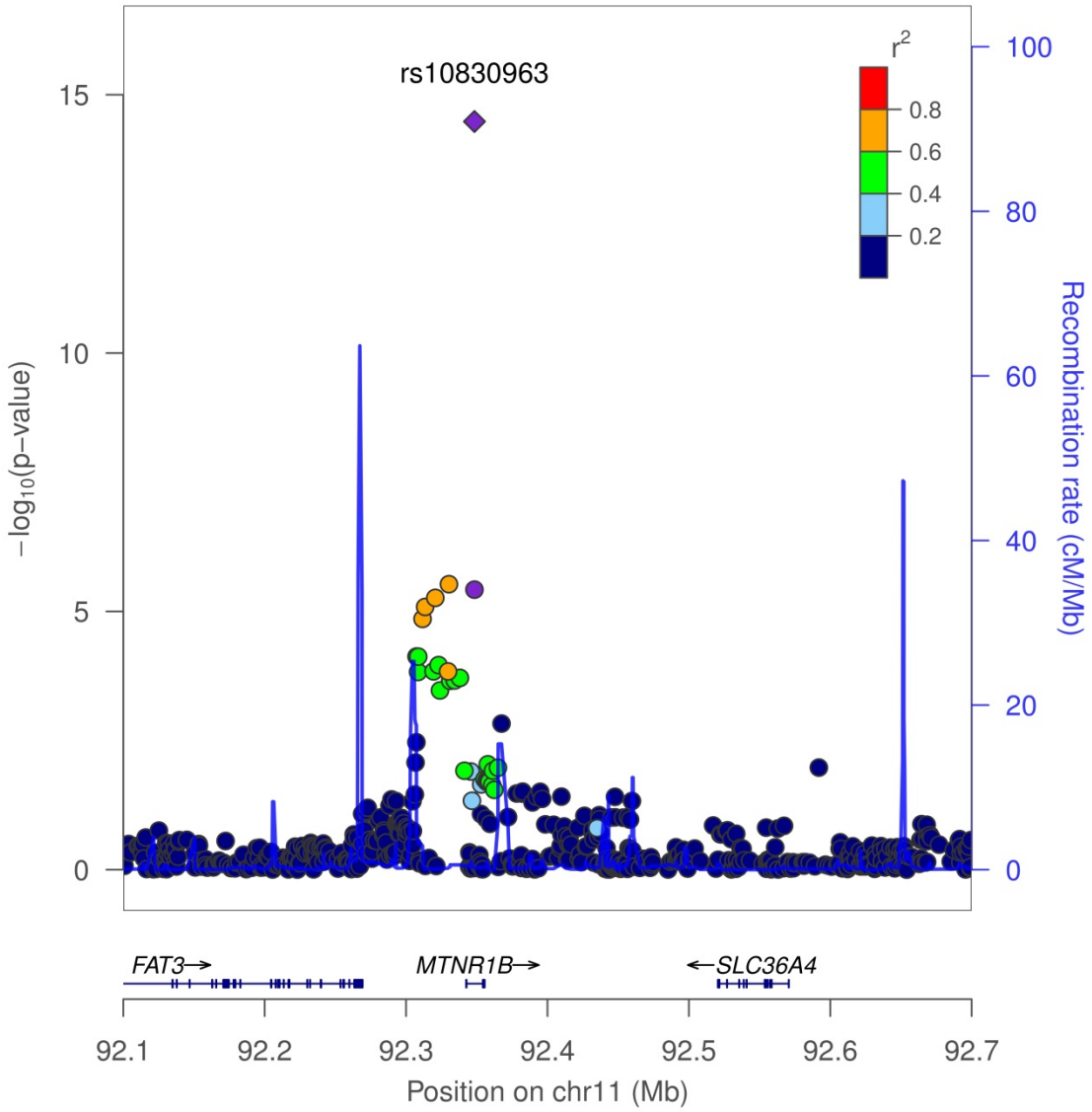

Figure 3

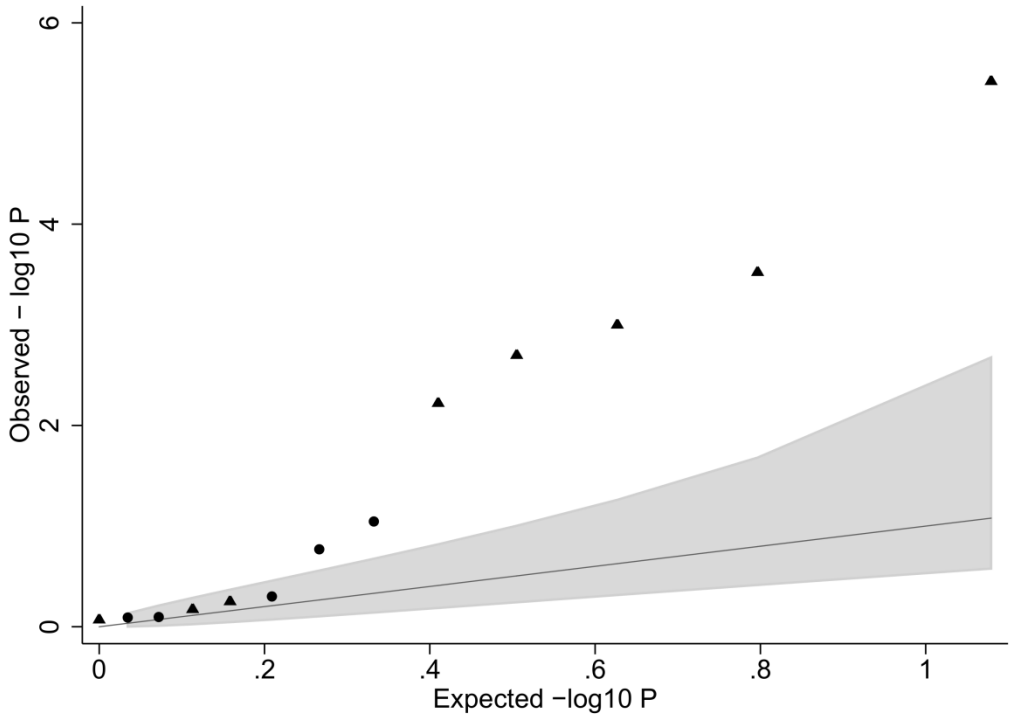

Table 1. Association between maternal *MTNR1B* SNP rs10830963 and offspring birth weight z-score unadjusted and adjusted for fetal genotype in mother-child pairs

Study	N mother-child pairs	Difference in offspring birth weight z-score per maternal rs10830963 G-allele, UNADJUSTED for fetal genotype (95% CI)	P-value (P-het)	Difference in offspring birth weight z-score per maternal rs10830963 G-allele, ADJUSTED for fetal genotype (95% CI)	P-value (P-het)
ALSPAC (genotyped SNP)	4608	0.031 (-0.014, 0.076)	0.180	0.025 (-0.028, 0.077)	0.357
EFSOCH	666	0.162 (0.053, 0.272)	0.004	0.169 (0.040, 0.298)	0.010
HAPO (non-GWAS)	2471	0.056 (0.001, 0.112)	0.046	0.060 (-0.002, 0.123)	0.060
Meta-analysis	8716	0.047 (0.016, 0.078) In grams: 23 (8, 38)	0.003 (0.13)	0.043 (0.007, 0.079) In grams: 21 (3, 38)	0.019 (0.13)

All association results are adjusted for sex and gestational age.