Genetic determinants of glucose levels in pregnancy: genetic risk scores analysis and GWAS in the Norwegian STORK cohort

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Abstract

Objective: Hyperglycaemia during pregnancy increases the risk of adverse health outcomes in mother and child, but the genetic aetiology is scarcely studied. Our aims were to (1) assess the overlapping genetic aetiology between the pregnant and non-pregnant population and (2) assess the importance of genome-wide polygenic contributions to glucose traits during pregnancy, by exploring whether genetic risk scores (GRSs) for fasting glucose (FG), 2-h glucose (2hG), type 2 diabetes (T2D) and BMI in non-pregnant individuals were associated with glucose measures in pregnant women.

Methods: We genotyped 529 Norwegian pregnant women and constructed GRS from known genome-wide significant variants and SNPs weakly associated ($p > 5 \times 10^{-8}$) with FG, 2hG, BMI and T2D from external genome-wide association studies (GWAS) and examined the association between these scores and glucose measures at gestational weeks 14–16 and 30–32. We also performed GWAS of FG, 2hG and shape information from the glucose curve during an oral glucose tolerance test (OGTT).

Results: GRS_{FG} explained similar variance during pregnancy as in the non-pregnant population (~5%). GRS_{BMI} and GRS_{T2D} explained up to 1.3% of the variation in the glucose traits in pregnancy. If we included variants more weakly associated with these traits, GRS_{2hG} and GRS_{T2D} explained up to 2.4% of the variation in the glucose traits in pregnancy, highlighting the importance of polygenic contributions.

Conclusions: Our results suggest overlap in the genetic aetiology of FG in pregnant and non-pregnant individuals. This was less apparent with 2hG, suggesting potential differences in postprandial glucose metabolism inside and outside of pregnancy.

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Introduction

Metabolic adaption during pregnancy is important to ensure growth and development of the foetus and to guarantee that the mothers' elevated energy needs

during the pregnancy are met (1). Glucose is the major energy source for the foetus, which freely crosses the placenta. Two major changes in glucose metabolism

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take place during pregnancy. First, fasting glucose (FG) levels decrease during early pregnancy, mainly due to an increase in plasma volume (2). Secondly, pregnancy has a diabetogenic effect on the mother, with increased insulin resistance usually commencing after the first trimester. Regardless of the changes in maternal glucose metabolism, and the increase in insulin resistance, maternal fasting blood glucose values normally remain relatively stable throughout pregnancy (1). This is largely because of fetal glucose utilization, although postprandial glucose levels tend to increase (3). However, if the maternal beta cells are not able to compensate for insulin resistance by producing more insulin, the mother runs the risk of hyperglycaemia.

The large, multicentre, Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) study showed a clear and graded relationship between plasma glucose values during pregnancy and increased disease burden in the mother and offspring (4, 5). Hence, hyperglycaemia in pregnancy – even without gestational diabetes (GDM) diagnosis – is associated with increased risk of adverse health outcomes in both mother and child (6, 7). Additionally, it has been estimated that around 50% of the women diagnosed with GDM will develop type 2 diabetes (T2D) within 5–10 years, although the exact risk varies with the criteria used and the follow-up time (8, 9). GDM and T2D appear to share both genetic and non-genetic risk factors – such as high BMI – and the overlapping susceptibility may partly explain the increased risk of T2D for women with previous GDM (10, 11).

Glucose levels in pregnancy are usually measured in the fasting state or (one and) two hours after an oral glucose tolerance test (OGTT). Commonly, single measurements – such as fasting glucose levels (FG) and 2h post OGTT glucose levels (2hG) – are used to diagnose GDM. However, different patterns of the glucose levels during an OGTT can yield the same single measurements, and thus, the shape of the glucose curves may provide important additional information. Functional data analysis (FDA) (12) of OGTT glucose curves has previously been used to extract shape information not apparent in commonly used simple measures. Briefly, the most important curve shape characteristic, interpreted as the general glucose level during an OGTT, was positively associated with all OGTT measurements and closely related to the area under the OGTT curve (AUC). The second most important shape characteristic described to what extent the women had a longer than average time to peak and a prolonged time to return to FG levels. The latter curve shape information was found to successfully discriminate between women who did and did not develop GDM later in pregnancy

(13), indicating that the glucose curve shape might hold valuable information of clinical relevance.

Genetic factors are thought to contribute to the increase in blood glucose concentrations during pregnancy. However, whilst more than 100 SNPs have been robustly associated with glycemic traits, hyperglycaemia and T2D in the non-pregnant population (14, 15), there are only a few published studies on such associations in pregnant women (10, 11, 16, 17), and it is still not known how well SNPs robustly associated with glucose levels outside of pregnancy (14) are associated in pregnancy.

In the present study, we genotyped up to 529 pregnant women from the Norwegian STORK cohort with glucose phenotypes measured both in the first and third trimester of pregnancy (18). The placenta is assumed to play an important role in the metabolic changes during pregnancy and the two time points for the OGTTs during pregnancy were chosen to reflect the time before the placenta is well established (around gestational week 20), and the time after the placenta is well established and the foetus is gaining weight, in the third trimester. We constructed genetic risk scores (GRSs) of known SNPs for FG, 2hG, BMI and T2D and examined the association with the glucose variables at the two time points during pregnancy, to study the effect of GRSs on the changes in glucose metabolism during pregnancy. In addition to the longitudinal data, the present study introduces the novel approach of combining GRSs with glucose curve shape information in the analyses. We also present genome-wide association studies (GWAS) for FG, 2hG and shape of the glucose curve during an OGTT. Our aims were to examine the genetic determinants of glucose metabolism during pregnancy, ascertain whether genetic variants robustly associated with glucose metabolism in the non-pregnant population were also associated with the same variables in early and late pregnancy and to see whether known genetic variants associated with T2D and BMI were related to glucose variables during pregnancy.

Subjects and methods

The STORK study

The STORK study is a prospective cohort of 1031 healthy pregnant women of Scandinavian ancestry who registered for obstetric care at the Oslo University Hospital Rikshospitalet from 2001 to 2008 (18). Exclusion criteria were multiple pregnancy, known history of type 1 or type 2 diabetes mellitus, and severe chronic diseases (pulmonary, cardiac, gastrointestinal or renal). Results

of a 75 g OGTT, age, height and weight were recorded at inclusion at gestational weeks (GWs) 14–16. The OGTT was repeated at GW 30–32. This paper includes up to $n=529$ individuals with genotype and repeated clinical and laboratory examinations (Fig. 1). We did not exclude women from the analysis on the basis of GDM diagnosis in pregnancy. The study was approved by the Regional Committee for Medical Research Ethics, Southern Norway, Oslo, Norway (reference number S-2014/224-0119a and S-07392a) and performed according to the Declaration of Helsinki. All participating women provided written informed consent.

Measurements of glucose

All OGTTs were performed after an overnight fast. Blood samples were taken every 30 min for 2 h, for a total of five OGTT measurements per woman. Briefly, venous blood

was drawn in gel tubes, allowed to clot for 30 min, and thereafter centrifuged for 10 min at 1800g. Serum was separated and stored at -80°C . Glucose was measured from frozen serum samples as previously reported (19) from antenatal visits at GW 14–16 and GW 30–32, with the hexokinase method at an accredited clinical chemistry laboratory at Oslo University Hospital, Rikshospitalet (Cobas 6000 from Roche).

Extracting curve shape information from OGTT glucose curves

Extraction of curve shape information from OGTT data at GW 14–16 has previously been described in detail (13). In the present study, OGTT data from GW 14–16 and 30–32 were analysed separately. Seven women included in the study were diagnosed with GDM (WHO99 criteria (20)) after GW 14–16; however, they were only treated with dietary advice and they were therefore included in the analysis at GW 30–32. Briefly, the five OGTT measurements from the 497 women in the study sample with genetic data and complete OGTT data at GW 14–16 were converted into 497 continuous, smooth curves (Supplementary Fig. 1A, see section on supplementary data given at the end of this article). The same procedure was used for the 506 women with genetic data and complete OGTT data at GW 30–32 (Supplementary Fig. 1B). A common smoothing parameter was used in both smoothing procedures (21). These 497 and 506 individually fitted curves formed the basis for two separate functional principal component analyses (FPCAs), in which curve shape information was extracted.

In a FPCA, curve shape information is decomposed into a small set (e.g. two) of common principal component (PC) curves, and a corresponding set of individual PC scores, one score per PC per woman. As shown previously (13), the PC curves can be given physiological interpretations according to the temporal variation (shape) they exhibit. A woman's set of individual PC scores reflects how her individual glucose curve shape corresponds to the general temporal feature expressed by the PC curves. Hence, the PC score values from GW 14–16 and 30–32 were used as outcome variables for the shape information inherent in the OGTT glucose curves. FDA (curve fitting and FPCA) was performed using the FDA package in R (<https://cran.r-project.org/>).

Figure 1

Flowchart for women included in the study. 485 women had 5 OGTT measurements at both GW 14–16 and 30–32. GW, gestational week; OGTT, oral glucose tolerance test.

DNA extraction

At weeks 22–24 and 36–38, peripheral blood mononuclear cells were isolated from venous blood using BD Vacutainer

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CPT Tubes (BD, NJ, USA), centrifuged immediately at 2500rpm for 25 min, buffycoat separated and transferred carefully to nunc-tubes and stored at -80°C until extraction. DNA was extracted using MagNA Pure Isolation Kit and Instrument (Roche Life Science) at weeks 22–24 and with MagMAX Isolation Kit and Instrument (Applied Biosystems) at weeks 36–38, due to changes in instrumentation at the laboratory over the years. To include as many participants as possible, DNA was also extracted from 291 full blood venous samples stored at -70°C containing EDTA from the follow-up study (19), using a salting out procedure (22) (Miller SA, 1215). Where several DNA samples were available only one was used for genotyping.

Genotyping and genome-wide association analysis

DNA samples were genotyped on the Illumina Infinium CoreExome chip using Illumina iScan by the Department of Clinical Sciences, Clinical Research Centre, Lund University, Malmö, Sweden. Of the 1031 individuals who provided blood samples, 455 had low quality or low concentration of DNA or withdrew consent ($n=3$) and could not be analysed further. Of the 573 remaining samples, those with low call rate (i.e. $<95\%$, $n=0$), extreme heterozygosity ($>|\text{mean} \pm (3 \times \text{s.d.})|$, $n=0$), mismatched gender ($n=2$), cryptic relatedness (i.e. one individual (chosen at random) from each related pair, defined as genome-wide IBD >0.185 ($n=6$) was removed from analysis) or of outlying ancestry (defined by ancestry informative PC analysis based on the variance-standardized relationship matrix generated in PLINK 1.9 software package (23) (<https://www.cog-genomics.org/plink/1.9/>), $n=36$) were excluded from analyses (the ancestry of samples is illustrated in Supplementary Fig. 2 which plots principal coordinates obtained via multidimensional scaling). This left 529 participants with useable SNP data. Variants with call rate $<95\%$ (14 730 SNPs), out of Hardy-Weinberg equilibrium (exact $P < 10^{-6}$, 66 SNPs) or with low minor allele frequency (MAF) $<1\%$ (254 802 SNPs) were removed before imputation. Quality control was performed using the PLINK 1.9 software package (23). After quality control, 281 589 variants were left for imputation. The GWAS scaffold was mapped to NCBI build 37 of the human genome, and imputation to the 1000G reference panel (Phase3- <http://www.well.ox.ac.uk/~wrayner/tools/>) for European ancestry was performed using IMPUTEv2.3.2 (24). 84 609 303 SNPs were available after imputation. All SNPs with MAF <0.02 and imputation quality <0.4 were removed from analysis.

A total of 8 717 487 SNPs were available for GW 14–16 and 8 709 903 SNPs were available for GW 30–32.

GWAS analysis of the traits FG, 2hG and curve shape variables (PC1 and PC2) was performed on the expected allele counts using linear regression assuming an additive genetic model in the software package SNPTEST (25). The QQman R package (26) was used for visualization. Test statistics with p values $\leq 5 \times 10^{-8}$ were considered genome-wide significant.

Generating GRSs

In order to construct GRSs, we used publicly available GWAS data for FG (14) ($n=46\,186$), 2hG (14) ($n=42\,854$), BMI (27) ($n=339\,224$) and T2D (28) ($n=26\,676$ T2D cases and 132 532 controls). We constructed weighted GRS based on the published regression coefficients from the discovery meta-analysis of these papers using the PLINK 1.9 software package (23). We constructed scores using known variants only (i.e. genome-wide significant SNPs from the above papers) and using nominally associated variants selected from across the genome meeting a particular p -value threshold (i.e. $p < 5 \times 10^{-7}$; $p < 5 \times 10^{-6}$; $p < 5 \times 10^{-5}$; $p < 5 \times 10^{-4}$; $p < 0.005$; $p < 0.05$; $p < 0.1$; $p < 0.2$; $p < 0.3$; $p < 0.4$; $p < 0.5$; $p < 0.6$; $p < 0.7$; $p < 0.8$; $p < 0.9$ and all p values) in order to capture polygenicity in these traits (29, 30). For the known risk scores, if the papers reported several SNPs at one locus, LD was checked and a set of independent (pairwise $r^2 < 0.2$) SNPs with the lowest p values were selected to contribute to the scores. Details of the genome-wide significant variants used in construction of the known GRS can be found in Supplementary Tables 1, 2, 3 and 4.

To evaluate the ability of GRS of known SNPs to explain variation in glycemic related traits in pregnancy, we fitted a multiple regression model that contained a term capturing the effect of the known variants. We also investigated whether polygenicity might help explain variation in our traits of interest. This was done by including an extra term that captured the effect of the GRS of nominally associated variants at the different p value thresholds. In order to guard against the possibility of residual population stratification contaminating our results, we also included covariate terms for the first two ancestry informative PCs in the model. The variance explained by the GRS was quantified by reporting the difference in r -squared between the full regression model containing all effects and a nested sub-model containing only the effects of the ancestry informative PCs. Scores yielding p values ≤ 0.05 in this comparison model were

considered significant. To account for the 32 statistical tests (4 GRS $\times$ 8 outcomes), we also evaluated our results against a Bonferroni corrected α level of 0.0016. All analyses were performed using RStudio, version 1.0.153.

Power calculations

To estimate the power in our study, we used the genetic power calculator (31). We calculated the power for a GWAS assuming a two tailed type 1 error rate and an $\alpha=5\times 10^{-8}$. We examined power to detect association at variants known to affect FG measures and power to detect association using GRS. Here we assumed a one tailed type 1 error rate of $\alpha=0.05$ given that we were making a specific directional hypothesis with regards to these known variants. Finally, we estimated the power to detect variation explained by the known GRS assuming a one degree of freedom test with two tails with $\alpha=0.05$.

Results

Sample characteristics and glucose variables

Characteristics of the study sample, and glucose measurements at GW 14–16 and 30–32 are shown in

Table 1. Except for a small difference in age ($p=0.02$), (i.e. those with available genetic data were slightly older than those without), the clinical characteristics of women with genetic data available were not significantly different from those without genetic data ($0.14\leq p\leq 0.93$). The individually fitted OGTT glucose curves at GW 14–16 and 30–32 are shown in Supplementary Fig. 1A and B.

At GW 14–16, results from the FPCA showed that the first two components describing the shape of the glucose curve (PC1 and PC2, Supplementary Fig. 1C and D) explained 89 and 8% of the temporal variation between the fitted curves, respectively. Similarly, at GW 30–32, results from the FPCA showed that the first two PCs (PC1 and PC2, Supplementary Fig. 1E and F) explained 86 and 10% of the temporal variation between the fitted curves, respectively. Since PC1 scores were highly correlated with AUC ($r=0.999$ (GW 14–16) and $r=0.997$ (GW 30–32)) the label ‘General glucose level’ was chosen for this variable. PC2 scores, on the other hand, corresponded to time of the highest glucose level and the label ‘Timing of glucose curve peak’ was therefore chosen for this variable. The PCs were labelled according to the physiological information they comprised, based on plots of how an individual curve differed from the mean curve if the corresponding PC scores are low or high (Supplementary Fig. 1C and D) (12, 13). Descriptive statistics for the corresponding

Table 1 Descriptive statistics for the study sample and the women in the cohort for whom genetic data were not available. The values presented are mean (s.d.) or frequency (%) unless otherwise stated.

Sample characteristic	Study sample ($n=529^*$)	Not in study sample ($n=502^{**}$)
Age of mother (years)	31.6 (3.8)	31.0 (4.0)
First time mothers	270 (52%)	275 (55%)
University education	458 (87%)	427 (85%)
Daily smokers	12 (2%)	10 (2%)
Pre-gestational BMI (kg/m ²) (self-reported)	23.8 (3.9)	23.5 (3.6)
OGTT at gestational weeks 14–16		
Fasting glucose (mmol/L; $n=517$)	4.6 (0.4)	4.6 (0.4)
2h glucose (mmol/L; $n=506$)	4.8 (1.2)	4.8 (1.2)
PC1, ‘General glucose level’ $^{\circ}$ ($n=497$)	0.0 (13.0)	–
PC2, ‘Timing of glucose curve peak’ $^{\circ\circ}$ ($n=497$)	0.0 (3.9)	–
OGTT at gestational weeks 30–32		
Fasting glucose (mmol/L; $n=521$)	4.6 (0.4)	4.6 (0.5)
2h glucose (mmol/L; $n=512$)	6.2 (1.5)	6.2 (1.4)
PC1 scores, ‘General glucose level’ $^{\circ}$ ($n=506$)	0.0 (14.7)	–
PC2 scores, ‘Timing of glucose curve peak’ $^{\circ\circ}$ ($n=506$)	0.0 (4.9)	–
Weight gain from gestational weeks 14–16 to 30–32	7.8 (2.7)	7.5 (2.5)
Gestational age at birth (weeks)	40.1 (1.6)	39.8 (2.0)
Birth weight (g)	3615 (557)	3560 (591)

*Exact numbers are given for all outcome variables. Other numbers may vary due to missing values; **numbers may vary due to missing values; $^{\circ}$ explains 89 and 86% of the temporal variation in the 497 and 506 OGTT glucose curves from gestational weeks 14–16 and 30–32 respectively. The PC curves at gestational weeks 14–16 and 30–32 were not identical, but both was given the same label ‘General glucose level’, and values of PC1 scores are correlates strongly to area under the curve ($r=0.999$ and $r=0.997$ at GW 14–16 and 30–32 respectively); $^{\circ\circ}$ explains 8 and 10% of the temporal variation in the total sample of OGTT glucose curves. High values of PC2 scores correspond to late glucose peaks.

BMI, body mass index; OGTT, oral glucose tolerance test; PC, principal component.

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PC scores are given in Table 1. It was noted that neither the mean curves, nor the PC curves at GW 14–16 and 30–32 were identical (Supplementary Fig. 1C, E and D, F, respectively), and the PC scores from the two time points should therefore not be directly compared.

Genetic risk scores

We found that the GRS for FG (GRS_{FG}) explained a similar amount of variance in FG in pregnancy at both measurement occasions as in the MAGIC consortium data (Table 2 and Supplementary Fig. 7). GRS_{2hG} consisting of known variants did not explain 2hG variation during pregnancy well (Table 2). However, when we included nominally associated variants in construction of the GRS by relaxing the p -value threshold for the inclusion of SNPs in the risk score, we explained far larger proportions of the variance in 2hG during pregnancy, highlighting the importance of polygenic contributions to the trait (Supplementary Fig. 8). A similar trend was seen for GRS_{T2D} (Table 2), where known variants explained a small significant proportion of the variance in 2hG and PC1. This proportion increased with the inclusion of nominally associated SNPs from the T2D GWAS (Supplementary Fig. 9). GRS_{BMI} explained some of the variance in FG, 2hG and PC1 (Table 2), and an increase in variance explained was also observed when the p value threshold was lowered (Supplementary Fig. 10). PC2 was not significantly associated with any of the risk scores.

We had between 85 and >99% power to detect association (assuming a two tailed $\alpha=0.05$) for a GRS that explained the smallest and largest expected proportions

of the trait variance (i.e. the GRS of known variants is expected to explain 1.7% of the variation in 2hG and 4.8% of the variance in FG according to the literature (14)). Thus, we are well powered to detect association between our GRS and glucose related traits in pregnancy (should the underlying genetic aetiology of the traits be similar).

Genome-wide association study

The results from the GWAS analysis can be found in the Supplementary data and in Supplementary Figs 3 and 4. Our main findings are shown in Supplementary Table 5. The results from the look-up of known SNPs are shown in Supplementary Tables 1, 2, 3 and 4. However, our study had low power (<40%) to detect single loci that explained 5% of the phenotypic trait variance at genome-wide levels of significance ($\alpha=5 \times 10^{-8}$) (power=1% for a variant explaining 2% of the variation). Since it would be very unusual for quantitative trait loci underlying complex traits to explain such large proportions of the phenotypic variance, it follows that we must be cautious in interpreting the results. In the case of our look-up of known glucose variants, we had 49% power to detect SNPs explaining 0.5% of the phenotypic variance, and 81% power to detect variants explaining 1.2% of the variation in the trait. Previously published GWAS indicate that most of the known loci contributing to glucose-related traits do not explain such large proportions of the phenotypic variance in individuals in the non-pregnant population. Assuming the same in our sample of pregnant individuals suggests that we are also underpowered to detect specific known loci.

Table 2 Phenotypic variance explained (%) by GRS of known SNPs on outcome variables in GW 14–16 and 30–32. Results are shown as difference in R^2 between the full regression model containing all effects and a nested sub-model containing only the effect of the ancestry informative principal components.

	GRS_{FG}		GRS_{2hG}		GRS_{T2D}		GRS_{BMI}	
	% VE	p value	% VE	p value	% VE	p value	% VE	p value
FG GW 14–16	4.95*	5.55×10^{-7}	0.02	0.779	0.05	0.636	0.36	0.181
FG GW 30–32	4.01*	5.09×10^{-6}	0.42	0.142	0.07	0.562	1.04	0.021
2hG GW 14–16	2.37*	0.001	0.24	0.277	0.69	0.065	0.78	0.048
2hG GW 30–32	0.90	0.033	0.46	0.128	1.31	0.010	0.37	0.171
PC1 GW 14–16	2.27*	0.001	0.25	0.263	1.25	0.013	0.79	0.048
PC1 GW 30–32	1.43	0.007	0.46	0.127	0.66	0.067	1.07	0.020
PC2 GW 14–16	0.00	0.917	0.01	0.853	0.00	0.904	0.08	0.531
PC2 GW 30–32	0.00	0.910	0.02	0.750	0.16	0.367	0.05	0.599

Significant results ($p \leq 0.05$) are shown in bold, and results significant with a Bonferroni correction ($p \leq 0.0016$) are marked with *. Known SNPs are defined from previously published GWAS (p values are available in Supplementary Tables 1, 2, 3 and 4). Percentages correspond to the left point in Supplementary Fig. 7, 8, 9 and 10 labelled known.

%VE, % variance explained; 2hG, 2-h post OGTT; BMI, body mass index; FG, fasting glucose; GRS, genetic risk score; GW, gestational week; PC, principal component; T2D, type 2 diabetes.

Discussion

By analysing GRS of known and nominally associated variants, we found that SNPs robustly associated with glucose measurements in the non-pregnant population also explain significant proportions of the variance in the glucose measurements in a cohort of pregnant Norwegian women. Furthermore, we found that some single genetic variants associated with glucose metabolism in the non-pregnant population are also associated with the same measures during pregnancy – although the statistical power for these confirmatory analyses was low.

GRS_{FG} explained a similar percentage of variance in FG values during pregnancy as in non-pregnant individuals (14). These finding was consistent in early and late pregnancy and the associations were still significant after Bonferroni correction. GRS_{FG} also explained a small, but significant percentage of the variation in 2hG and the general OGTT glucose level (PC1). Due to the strong association between GRS_{FG} and both FG and 2hG, and our previous findings of high correlations between PC1 scores and all OGTT glucose measurements (13), the observed effect on PC1 was expected.

Surprisingly, no effects on the glucose phenotypes were found for GRS_{2hG}. However, SNPs just beneath the genome-wide significance level (e.g. p -value threshold of $<5 \times 10^{-7}$) explained more of the variance in this trait. This might suggest that SNPs weakly associated with 2hG in the non-pregnant population may be more important during pregnancy. Our findings highlight the importance of the polygenic contributions to the trait.

The fact that GRS_{FG} explained a similar percentage of variation in the trait both early and late in pregnancy compared to a non-pregnant population, whereas GRS_{2hG} explained a negligible (and not significant) proportion of the variation in 2hG and PC1 during pregnancy is interesting as we know that FG values normally remain relatively stable throughout pregnancy (1) but that the postprandial glucose levels tend to increase (3). This leads us to speculate that the underlying genetic mechanisms for FG are similar in both pregnant and non-pregnant individuals, but that the mechanisms differ for postprandial glucose levels. More studies to understand the mechanisms for the increase in postprandial glucose levels during pregnancy are therefore important. It is, however, important to note that there are fewer known SNPs associated with 2hG than FG – explaining less of the variance in the trait – and thus we have less power to detect a relationship that might be there. Larger GWAS on 2hG could reveal more

associated SNPs – generating a better GRS – and larger GWAS on pregnant women with OGTT could assist in demonstrating a significant genetic overlap.

GRS_{BMI} explained some of the variation in the glucose measurements during pregnancy, suggesting that genetically determined BMI has a significant effect on glucose related variables during pregnancy. This is consistent with the fact that BMI is a shared risk factor for both GDM and T2D (32, 33). GRS_{T2D} explained a significant proportion of the variation in 2hG and PC1 scores. It should be noted that the associations involving GRS_{BMI} and GRS_{T2D} were not significant after Bonferroni correction. Just as for GRS_{2hG}, the polygenic GRS based on SNPs with p value thresholds below genome-wide significance explained more variance than the GRS of just the known SNPs alone. As it is the 2hG value, and not FG, that is being explained by this risk score, this GRS might reflect beta cell function and/or insulin resistance during the OGTT. The involvement of GRS_{T2D} suggests some genetic overlap between GDM and T2D, which is in accordance with previous literature (10, 11). One GWAS has investigated the relationship between genotypes and glucose measurements in pregnancy (17) and found genome-wide significant influence of four known T2D genes on FG (Glucokinase Regulator (*GCKR*), Glucose-6-phosphatase 2 (*G6PC2*), Protein Phosphatase 1 Regulatory Subunit 3B (*PPP1R3B*) and Melatonin Receptor 1B (*MTNR1B*)). A GWAS of GDM, conducted in a Korean population, found that known T2D-associated variants in the *CDK5* Regulatory Subunit Associated Protein 1 Like 1 (*CDKAL1*) and *MTNR1B* gene regions were also associated with GDM (16). However, although metabolic changes in pregnancy may look similar to those in the development of T2D, and although T2D and GDM share several genetic risk factors, the underlying mechanisms behind the diabetogenic development in pregnancy might be different.

Our study is the first to examine the genetics of OGTT data at several time points during pregnancy. In our GWAS, we found three novel SNPs (rs116745876 (Mitogen-Activated Protein Kinase Kinase Kinase 1 (*MAP3K1*)), rs11682804 (Protein Kinase C Epsilon (*PRKCE*)), rs11112715 (NUAK Family Kinase 1 (*NUAK1*)), which might potentially be related to glucose metabolism in pregnancy. However, our power calculations strongly suggest that these GWAS findings most likely represent type 1 errors, since the existence of SNPs with large effect size would be very atypical in the case of complex quantitative traits. We will therefore make our single marker test results available for future GWAS meta-

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analyses of these traits, and as a replication resource. The formation of large-scale consortia – as has been done for other glucose-related traits and diseases – will be necessary to understand glucose metabolism during pregnancy and detect novel genetic loci for GDM.

Our look-up of robustly associated glucose-related variants in our study of pregnant mothers showed some nominally significant associations in the expected directions, although most of the SNPs were not significantly associated with the outcomes due to the low power of our study with respect to single-variant associations. When these variants were subsequently combined into GRS, power increased and the combined effects of the SNPs show association with the glucose measures in pregnancy. Finding the expected association between GRS_{FG} and FG in early pregnancy confirmed that our sample had utility for using GRSs to test hypotheses about the genetic aetiology of glucose measures during pregnancy. We chose to present our main result without Bonferroni corrections, because the physiological traits the GRS are expected to reflect are highly correlated (13). However, due to the metabolic changes during pregnancy, all analyses might be of physiological interest.

Our previous findings indicated promising clinical relevance of the PC2 scores (13), and the negligible associations between the GRSs and PC2 scores were therefore disappointing. This may partly be explained by the fact that the GRSs in our analyses were all based on single measurements, whereas the dynamic curve feature reflected in the PC2 (a delayed glucose peak combined with a prolonged period of high postprandial values) will not be covered by one single measurement. Similarly, whereas the AUC is a simple and good substitute for the PC1 scores (13), the dynamic, temporal information in the PC2 scores is not as easily replaced by simple measures. Calculating the timing of the glucose peak from five OGTT glucose measurements, without smoothing of the measurements and estimating continuous curves, could result in very crude measurements of the timing of the peak and loss of information and precision. We therefore recommend that this curve characteristic is estimated by FDA. Validation of the physiological relevance of the PCs is important, but challenging, since few studies provide gold standard measurements of metabolic features in combination with FDA of OGTT. In non-pregnant populations, high correlations between HbA1c and FG and 2hG have been found (34, 35). It may therefore be speculated that the information in average measurements like the HbA1c may partly overlap with the information in the PC1 scores. Furthermore, insulin resistance in the

muscle can be shown as impaired glucose uptake after ingestion of a carbohydrate-rich meal and results in postprandial hyperglycaemia (36) and that stress on the pancreatic β -cells may lead to impaired insulin secretion (37). It is therefore reasonable to speculate that the PC2 might reflect aspects of insulin resistance or secretion. The genetic and environmental contribution to such mechanisms in pregnancy is not known, and these issues should be addressed in future studies, to gain more insight into the dynamics of glucose regulation.

In this study we showed that known SNPs for FG appeared to explain similar percentages of variance in FG at two time points during pregnancy as in the non-pregnant population. GRS for BMI and T2D explained some of the variation in the maternal glucose measurements, including the glucose curve shape component interpreted as the general glucose level. Interestingly, SNPs weakly associated ($P > 5 \times 10^{-8}$) with 2hG and T2D in GWAS data from the non-pregnant population seemed to explain more variation in the traits than the known genome-wide significant SNPs alone. These results highlight the importance of polygenic contributions to the glucose traits and suggest an overlap in the genetic aetiology of these traits in pregnant individuals and the non-pregnant population.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-18-0478>.

Declaration of interest

Kåre I Birkeland is an editor of the *European Journal of Endocrinology*. The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

G H M, E Q, K I B, M L, C S, D M E and K F F designed the research; T L, K R N contributed to data collection and lab analysis; G H M conducted the research and analysed data; G H M, D M E and K F F wrote the paper; M L, C S, R B P, T L, K R N, E Q, L G, K I B contributed to the discussions and edited the paper. All authors read and approved the final manuscript. K F F is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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