Plasma Glycated CD59, a Novel Biomarker for Detection of Pregnancy-Induced Glucose Intolerance

Running Title: pGCD59 predicts pregnancy induced glucose intolerance

Pamela Ghosh[*1] PhD, Miguel A. Luque-Fernandez[*2] PhD, Anand Vaidya[3] MD, Dongdong Ma[1] PhD, Rupam Sahoo[1] PhD, Michael Chorev[1] PhD, Chloe Zera[4] MD, Thomas F. McElrath[4] MD, Michelle A. Williams[5] ScD, Ellen W. Seely[3] MD, Jose A. Halperin[1] MD.

[1]Division of Hematology, Brigham & Women's Hospital, Boston, MA, USA; [2]Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK and Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA; [3]Division of Endocrinology, Diabetes and Hypertension, Brigham & Women's Hospital, Boston, MA, USA; [4]Division of Maternal Fetal Medicine, Brigham & Women's Hospital, Boston, MA, USA; [5]Department of Epidemiology, Harvard T.H. Chan School of Public Health. Boston, MA, USA.

* These authors contributed equally to this paper

Corresponding Author: Jose A. Halperin, MD, 20 Shattuck Street Thorn 712, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA Email: jhalperin@partners.org Ph: 617-525-3140 Fax: 617-582-6069

Abstract

Objective

Plasma glycated CD59 (pGCD59) is an emerging biomarker in diabetes. We assessed whether pGCD59 could predict: the results of the glucose challenge test (GCT) for screening of gestational diabetes (GDM) (primary analysis); and the diagnosis of GDM and prevalence of large for gestational age (LGA) newborns (secondary analyses).

Research Design and Methods

Case-control study of 1,000 plasma samples from women receiving standard prenatal care: 500 with a normal GCT (controls) and 500 with a failed GCT and a subsequent OGTT (cases).

Results

Compared to controls, median pGCD59 was 8.5-fold higher in cases and 10-fold higher in GDM; median (IQR): controls: 0.33 (0.19); cases: 2.79 (1.4); GDM 3.23 (1.43) ($p<0.001$); AUROCs: 0.92. LGA prevalence was 4.3% in the lowest and 13.5% in the highest quartiles of pGCD59.

Conclusion

One pGCD59 measurement at week 24-28 identifies pregnancy-induced glucose intolerance with high sensitivity and specificity and can potentially identify risk for LGA.

Screening for Gestational Diabetes (GDM) with an oral glucose challenge test (GCT) is a standard of care for all non-diabetic pregnant women (1; 2) because the adverse pregnancy outcomes associated with GDM can be mitigated with appropriate therapy (3; 4). Screening (GCT) and diagnosis glucose tolerance tests (OGTT) are time consuming, uncomfortable and have poor reproducibility (5). Other tests such as HbA1c or fructosamine are not routinely measured during pre-natal care because of their low sensitivity and specificity to identify women at risk of GDM (6; 7).

The complement system and its regulators reportedly play a role in the pathogenesis of diabetes complications (8). In diabetes, non-enzymatic glycation inactivates the complement inhibitor CD59, forming glycated CD59 (GCD59) (9). Using a sensitive and specific ELISA for GCD59 in blood, we have shown that plasma GCD59 (pGCD59) levels are significantly higher in individuals with type 2 diabetes, and independently predict the response to OGTT (10).

Our primary objective was to assess the accuracy of pGCD59 in predicting the results of the GCT. As secondary aims we assessed the accuracy of pGCD59 in predicting the diagnosis of GDM by OGTT, and explored the association of pGCD59 with the prevalence of large for gestational age (LGA) newborns at delivery.

RESEARCH DESIGN AND METHODS

We performed a case-control study measuring pGCD59 in 1,000 samples from women undergoing routine two-step gestational diabetes screening and diagnosis at Brigham and Women's Hospital (BWH; 2012-2014). Two sets of 500 samples each were collected randomly from women that either passed the 50-gram GCT and

therefore did not undergo 3hr-OGTT (controls) or failed the GCT and therefore underwent standard of care 100-grams, 3-hr OGTT (cases) at BWH. Pregnancy week at sample collection was the same for controls and cases (26.5 ± 3.3 and 26 ± 1.8, respectively). Samples for pGCD59 measurement were separated from the same tubes used to measure plasma glucose and stored (-80°C) by Partners' Crimson Biorepository Core (CBC)(10), a clinical investigation facility that anonymously collects discarded materials from the clinical laboratories of Partners Healthcare Hospitals. Medical information was retrieved from electronic records before samples were de-identified; only coded samples were delivered for pGCD59 measurement. pGCD59 was measured using the specific ELISA described in (11); test operators were blind to the women's glucose status. Inter-assay coefficient of variation was **<**10.0%. Partners Healthcare IRB approved this study (Protocol: 2011P002254/BWH). We followed STARD guidelines for study design and reporting.

Statistical analysis

Patients' characteristics were described using medians and interquartile ranges for continuous variables and count proportions for categorical variables. Sensitivity and specificity of pGCD59 to predict the results of the GCT were assessed using non-parametric estimates of the receiver operating characteristic (ROC) curves and respective area under the curve (AUROC)(12). Positive and negative predictive values (PPV and NPV) and positive likelihood ratio (LR+) were calculated as in (13). Following WHO recommendations, LGA was defined as ≥90th percentile birth weight adjusted for gestational age at delivery and determined from the latest gender-specific reference curves derived from a large sample that reflects the ethnic distribution of the US

population(14). Statistical analyses were performed using Stata software, version 13.1 (Statacorp, College Station, TX, USA).

RESULTS

Among the 500 cases, 127 met Carpenter and Coustan (C&C) criteria for GDM(15). Supplementary Table 1 summarizes maternal and infant characteristics; the ethnic/racial composition of the women was comparable to that of the United States(14). Median pGCD59 levels were: 8.5-fold higher in the 500 cases than in the 500 controls and 10-fold higher in the 127 cases diagnosed with GDM by 3hr-OGTT (Figure 1, Supplementary Figure 1 and Supplementary Table 2). The probability density function (Figures 1A and 1B) and AUROCs (Figures 1C and 1D) show that pGCD59 independently discriminated cases from controls with high sensitivity and specificity, even after adjustment for covariates such as maternal age, BMI, race/ethnicity, multiplicity, gestational age and previous history of diabetes (adjusted AUROC controls vs. cases = 0.92, 95%CI: 0.88, 0.93; adjusted AUROCs controls vs. GDM = 0.92, 95%CI: 0.87, 0.96). PPV and NPV for the overall distribution of GCD59 values to identify cases were 99.3% (95%CI: 97.9, 99.8) and 87.5% (95%CI: 84.5, 90.1); and 99.1% (95%CI: 94.9, 99.9) and 95.6% (95%CI: 93.8, 97.4) to identify women with GDM. Women with pGCD59 values $\geq 6^{th}$ decile had a likelihood of having a failed GLT 8-fold higher than for those below the 6^{th} decile (LR+ 7.97 Supplementary Table 3).

Among the 852 singletons who had recorded birth weight and gender, 86 (10%) were identified as LGA, 28 born to controls and 58 to cases (Supplementary Table 1). Higher maternal pGCD59 was associated with a higher prevalence of LGA, which was 4.3% (9/207) in the lowest and 13.5% (29/214) in the highest quartile of pGCD59 (chi-square p-

value = <0.0001; Supplementary Figure 2). This result was not affected by adjustment for maternal age, race/ethnicity and BMI. Notably, 45/58 (78%) LGA infants in the case population were born to mothers who did not meet C&C OGTT threshold criteria for GDM but had median pGCD59 values 7-fold higher than controls (Supplementary Table 4).

CONCLUSION

This study explored the clinical utility of pGCD59 to screen/diagnose GDM. One maternal pGCD59 measurement at a mean gestational week ≈26 predicted the results of the GCT with high sensitivity and specificity and independently of covariates such as age, BMI, race/ethnicity, multiplicity, gestational age and previous history of diabetes (Figure 1, Supplementary Figure 1). Median pGCD59 values were progressively higher across the categories of maternal glucose tolerance (Supplementary Table 2). These findings indicate that pGCD59 potentially represents a convenient and effective alternative to the cumbersome glucose challenge methods currently used to screen/diagnose GDM.

Glucose challenge tests fail to recognize the continuous association between maternal hyperglycemia and abnormal pregnancy outcomes, and exclude milder forms of glucose intolerance that may still impart perinatal risk (16; 17). The progressively higher pGCD59 levels observed across the GCT-OGTT categories (Supplementary Table 2) suggest that pGCD59 may reflect the continuum of pregnancy-induced glucose intolerance described by the HAPO study(16).

pGCD59 levels at gestational week ≈26 were associated with higher prevalence of LGA at birth. Among cases, 22% of LGA newborns were born to women diagnosed with GDM

while 78% were born to women that failed the GCT but did not meet C&C criteria for GDM. This likely reflects the effect of treatment on women with formal diagnosis of GDM, and is consistent with conclusions of the HAPO and other studies showing that women in an intermediate category between “normal” and “abnormal” glucose tolerance are at higher risk of abnormal pregnancy outcomes(18). Currently, there are no guidelines for the management of women in the “intermediate” category and, therefore, their management is the same as that of women with a normal GCT. The fact the 45 cases who did not meet C&C criteria for GDM but delivered LGA newborns had median pGCD59 levels 7-fold higher than controls provides additional evidence for the potential clinical utility of pGCD59 for screening/diagnose GDM (16).

Limitations: a) the study was observational, b) clinical and demographic characteristics were limited to those available in medical records, c) we could not adjust for the time of day when GCT was performed since all testing was done per routine clinical care (19), d) the study was not aimed at establishing a clinically useful cut-off value or assessing how pGCD59 measures might influence clinical care in real-time, the impact of treatment on the prevalence of LGA.

In summary, this is the first study showing that a single measurement of pGCD59 at gestational week ≈26 represents a simplified method to identify women who would have failed a GCT, are at higher risk of GDM and possibly of having an LGA newborn. Validation of pGCD59 as a biomarker for detection of pregnancy induced glucose intolerance and determination of clinically useful cut off values will require multi-center studies and “consensus” expert committees that will take into account relative risks, cost-benefits and other individual and public health considerations, as has been the norm with currently used

methodologies for screening and diagnosis of GDM. Future studies should also assess whether pGCD59 1) similarly classifies pregnant women with normal or abnormal glucose tolerance as defined by the 2hr, 75 grams OGTT recommended by the IADPSG, 2) is a predictor of adverse outcomes in pregnant women in the intermediary category glucose tolerance who might benefit from treatment, and 3) detects glucose intolerance earlier in pregnancy than current practice prompting earlier interventions that may mitigate further the risks associated with maternal hyperglycemia.

Author Contributions: PG, JAH, AV, ES, TM, CZ participated in study concept and design. PG, MAL, AV, JAH participated in acquisition, analysis or interpretation of the data. PG, MAL, AV, MC, JAH drafted the manuscript. PG, MAL, AV, ES, TM, MW, MC, CZ, DM, RS, JAH critically revised the manuscript for important intellectual content. MAL and MW performed the statistical analysis. The study was supervised by JAH. PG and JAH are the guarantors of this work and as such, had full access to all the data in the study and take responsibility for the integrity of the data.

ACKOWLEDGEMENT

Funding Sources: Research was supported by National Institutes of Health Grants DK095424 (JAH), DK62294 (JAH), DK089206 (JAH), DK101442 (JAH). AV was supported by the National Institutes of Health grants DK107407 and HL111771 and from the Doris Duke Charitable Foundation.

Duality of interests: JAH and MC have a financial interest in Mellitus. LLC. Mellitus is developing diagnostic tools for diabetes. JAH's and MC's interests were reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with

their conflict of interest policies. PG, MAL, AV, ES, TM, MW, CZ, DM and RS did not report any potential conflict of interest relevant to this article.

REFERENCES

1. Standards of Medical Care in Diabetes—2017: Summary of Revisions. Diabetes Care 2017;40:S4-S5
2. Gestational diabetes mellitus. Practice Bulletin No. 137. American College of Obstetricians and Gynecologists. Obstet Gynecol 2013; 122:406 –16.
3. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, Wapner RJ, Varner MW, Rouse DJ, Thorp JM, Jr., Sciscione A, Catalano P, Harper M, Saade G, Lain KY, Sorokin Y, Peaceman AM, Tolosa JE, Anderson GB: A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med 2009;361:1339-1348
4. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS: Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 2005;352:2477-2486
5. Catalano PM, Avallone DA, Drago NM, Amini SB: Reproducibility of the oral glucose tolerance test in pregnant women. Am J Obstet Gynecol 1993;169:874-881
6. Agarwal MM, Dhatt GS, Punnose J, Koster G: Gestational diabetes: a reappraisal of HBA1c as a screening test. Acta Obstet Gynecol Scand 2005;84:1159-1163
7. Cefalu WT, Prather KL, Chester DL, Wheeler CJ, Biswas M, Pernoll ML: Total serum glycosylated proteins in detection and monitoring of gestational diabetes. Diabetes Care 1990;13:872-875

8. Ghosh P, Sahoo R, Vaidya A, Chorev M, Halperin JA: Role of complement and complement regulatory proteins in the complications of diabetes. Endocr Rev 2015;36:272-288
9. Acosta J, Hettinga J, Fluckiger R, Krumrei N, Goldfine A, Angarita L, Halperin J: Molecular basis for a link between complement and the vascular complications of diabetes. Proc Natl Acad Sci U S A 2000;97:5450-5455
10. Ghosh P, Vaidya A, Sahoo R, Goldfine A, Herring N, Bry L, Chorev M, Halperin JA: Glycation of the complement regulatory protein CD59 is a novel biomarker for glucose handling in humans. J Clin Endocrinol Metab 2014:jc20134232
11. Ghosh P, Sahoo R, Vaidya A, Cantel S, Kavishwar A, Goldfine A, Herring N, Bry L, Chorev M, Halperin JA: A specific and sensitive assay for blood levels of glycated CD59: a novel biomarker for diabetes. Am J Hematol 2013;88:670-676
12. Pepe MS: *The statistical evaluation of medical tests for classification and prediction*. Oxford, Oxford University Press, 2004
13. Altman DG, Bland JM: Diagnostic tests 2: Predictive values. Bmj 1994;309:102
14. Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS: New intrauterine growth curves based on United States data. Pediatrics 2010;125:e214-224
15. Carpenter MW, Coustan DR: Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982;144:768-773
16. Group HSCR, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA: Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991-2002

17. Landon MB, Mele L, Spong CY, Carpenter MW, Ramin SM, Casey B, Wapner RJ, Varner MW, Rouse DJ, Thorp JM, Jr., Sciscione A, Catalano P, Harper M, Saade G, Caritis SN, Sorokin Y, Peaceman AM, Tolosa JE, Anderson GD: The relationship between maternal glycemia and perinatal outcome. Obstet Gynecol 2011;117:218-224
18. Tallarigo L, Giampietro O, Penno G, Miccoli R, Gregori G, Navalesi R: Relation of glucose tolerance to complications of pregnancy in nondiabetic women. N Engl J Med 1986;315:989-992
19. Goldberg RJ, Ye C, Sermer M, Connelly PW, Hanley AJ, Zinman B, Retnakaran R: Circadian variation in the response to the glucose challenge test in pregnancy: implications for screening for gestational diabetes mellitus. Diabetes Care 2012;35:1578-1584
20. DeLong ER, DeLong DM, Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837-845

FIGURE LEGENDS

Figure 1. A-B: p**GCD59 probability density functions by case-control status and between controls vs GDM.** Glucose challange tests were adjudicated using ACOG guidelines: failed 50-grams GCT $\geq$ 140mg/dL; 100 grams, 3-hr OGTT: No-GDM: 0 or 1 abnormal glucose value; GDM: 2+ abnormal glucose values based on Carpenter and Coustan criteria (C&C). **A:** Controls vs. Cases; **B:** Controls vs. GDM. The red dotted lines indicate the median pGCD59 values for the respective groups; the difference in median values between two groups and 95% confidence interval are mentioned on the figure (n=

1,000). **C-D: ROC curve AUCs by case-control status and controls vs GDM. C:** Controls vs. Cases; **D:** Controls vs. GDM. Marginal and conditional ROC curves were computed and adjusted for maternal age, BMI, race/ethnicity, multiplicity and gestational age at GCD59 determination and previous history of diabetes. AUROCs were derived using the DeLong, DeLong and Clarke-Pearson non-parametric tied corrected estimator(20) and the percentile values of the case observations with respect to the control distribution were used to derive the tied corrected estimator(20). Under non-parametric estimation, standard errors and derived AUROCs 95%CI were estimated using cross validation and bootstrapping procedures with 1,000 replications. *Solid lines*: unadjusted ROC curves. *Dashed lines*: ROC curves adjusted for maternal age, race/ethnicity, BMI, gestation week at pGCD59 determination and previous history of diabetes (n=1,000). *Insets* show adjusted AUC, sensitivity and specificity with 95% CI.

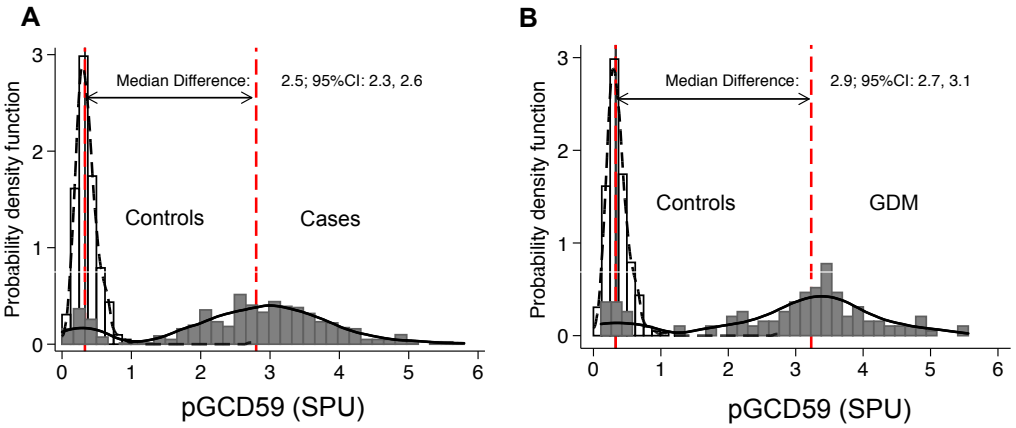
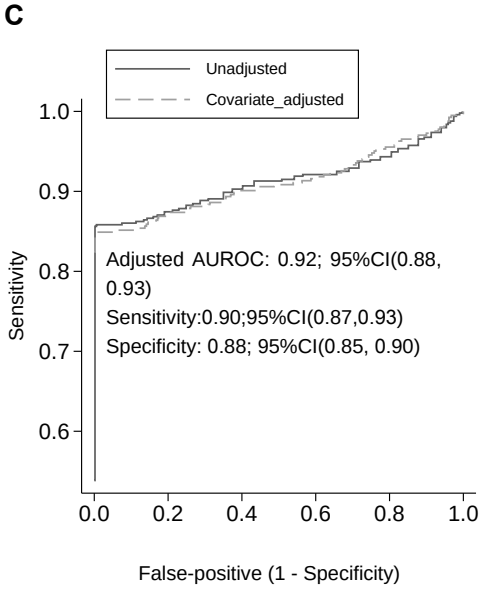
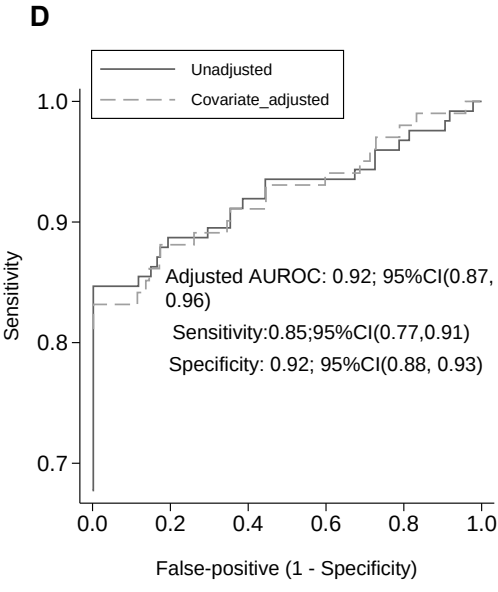

A
Median Difference:
2.5; 95%CI: 2.3, 2.6
Controls
Cases
Probability density function
pGCD59 (SPU)
B
Median Difference:
2.9; 95%CI: 2.7, 3.1
Controls
GDM
Probability density function
pGCD59 (SPU)

Figure 1



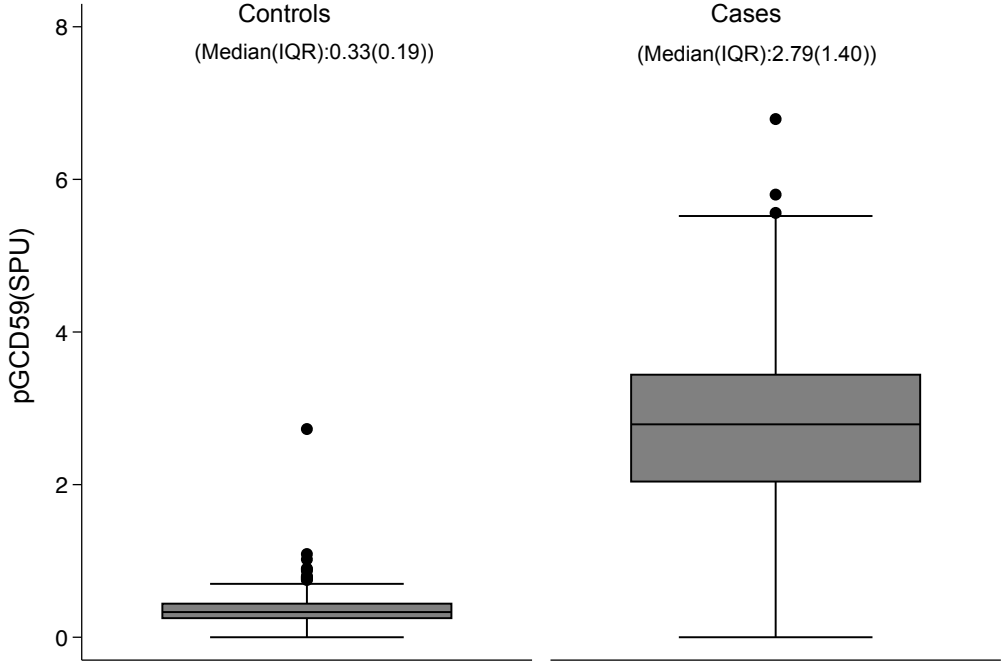
Controls vs. Cases



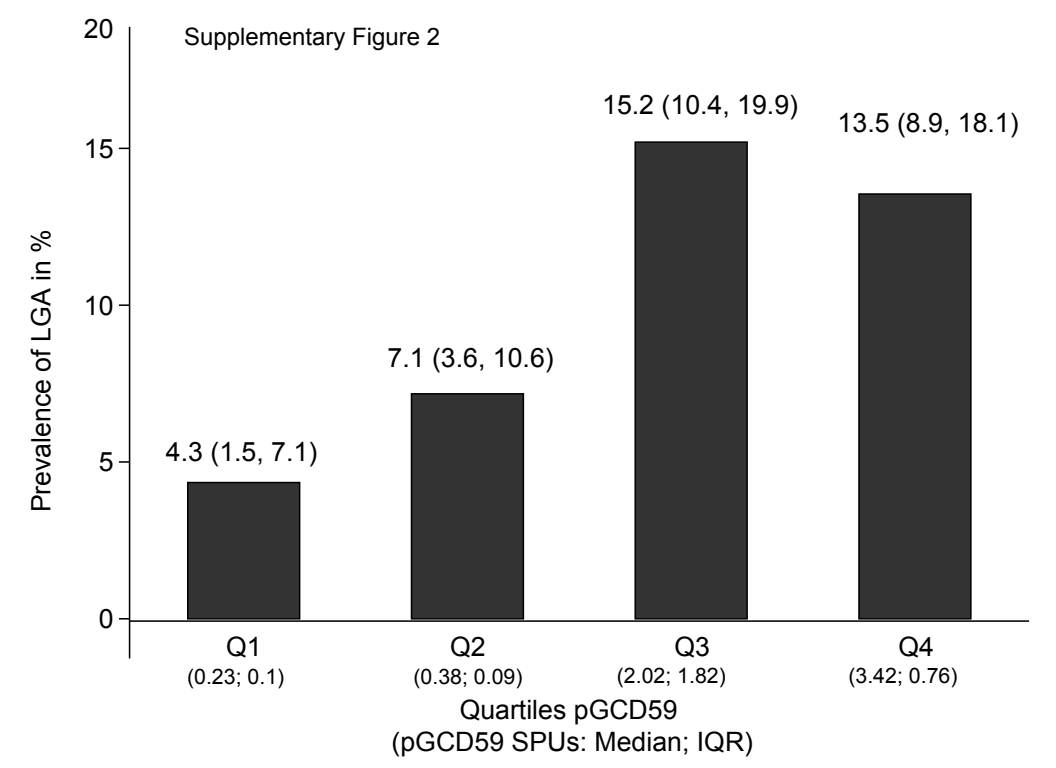
Controls vs. GDM

Figure 1

Supplementary Figure 1



Supplementary Figure 1. Box/whisker plots showing the distribution of pGCD59 values by case-control status (n = 1000): Median and IQR values are shown in the figure.


Supplementary Figure 2
20
15
10
5
0
Prevalence of LGA in %
4.3 (1.5, 7.1)
7.1 (3.6, 10.6)
15.2 (10.4, 19.9)
13.5 (8.9, 18.1)
Q1
(0.23; 0.1)
Q2
(0.38; 0.09)
Q3
(2.02; 1.82)
Q4
(3.42; 0.76)
Quartiles pGCD59
(pGCD59 SPUs: Median; IQR)

Supplementary Figure 2. Prevalence of LGA by quartiles of pGCD59 in the study population. The Figure shows the prevalence of LGA with 95% confidence intervals in parenthesis in the four quartiles of pGCD59 (n= 852). The median pGCD59 values with interquartile range (IQR) are shown below each quartile.

Supplementary Table 1: Women's and infant's socio-demographic and anthropometric characteristics, n= 1,000. The race/ethnicity composition of our study population closely resembles that of the United States population

	Controls, n(%)	Cases, n(%)	Cases (Failed-GLT), n(%)			
	Normal-GCT	Failed-GCT	No-GDM		GDM	
			OGTT with 0 abnormal value	OGTT with 1 abnormal value		
Maternal characteristics	**n=500**	**n=500**	**n=273**	**n=100**	**n=127**	**p-value***
Age in categories(yr)						0.001
<20	18(3.6)	8(1.6)	5(1.8)	1(1.0)	2(1.6)	
20-29	171(34.2)	119(23.8)	66(24.2)	25(25.0)	28(22.0)	
30-34	174(34.8)	182 (36.4)	106(38.8)	39(39.0)	37(29.1)	
35-39	99(19.8)	140(28.0)	73(26.8)	22(22.0)	45(35.4)	
>40	38(7.6)	51(10.2)	23(8.4)	13(13.0)	15(11.8)	
Race						<0.001
Asiatic	34(6.8)	55(12.6)	29(11.9)	9(10.5)	17(15.9)	
Black	56(11.3)	57(13.1)	33(13.6)	7(8.1)	17(15.9)	
Hispanic	98(19.7)	132(30.3)	73(30.4)	24(27.9)	35(32.7)	
Others	90(18.2)	3(0.7)	2(0.8)	0(0)	1(0.9)	
White	218(43.9)	189(43.3)	106(43.6)	46(54.5)	37(34.6)	
BMI at first prenatal visit(kg/m^2)						<0.001
<19	51(10.3)	42(8.7)	32(11.9)	6(6.5)	4(3.3)	
20-24	229(46.2)	144(29.8)	98(36.6)	21(22.8)	25(20.3)	
25-29	140(28.2)	144(29.8)	66(24.6)	30(32.6)	48(39.0)	
>30	76(15.3)	153(31.7)	72(26.9)	35(38.1)	46(37.4)	
Previous history of diabetes						0.151
Yes	2(0.4)	6(1.2)	5(1.9)	0(0)	1(0.8)	
No	489(99.6)	480(98.8)	263(98.1)	97(100)	120(99.2)	
Infant characterisitics						

Large for gestational age (singleton infants)**						0.001
Yes	28(6.7)	58(13.3)	33(13.8)	12(14.2)	13(11.7)	
No	388(93.3)	378(86.7)	207(86.2)	73(85.8)	98(88.3)	
Gender						0.145
Male	234(52.1)	254(56.7)	142(57.3)	38(43.2)	62(55.4)	
Female	215(47.9)	194(43.3)	106(42.7)	50(56.8)	50(44.6)	
Multiplicity						0.052
Yes	18(3.6)	31(6.3)	14(5.2)	6(6.2)	11(8.8)	
No	477(96.4)	459(94.7)	255(94.8)	91(93.8)	113(91.1)	

*Difference of proportions cases vs controls: Chi-square p-value

**Restricted to only singleton cases and defined as a birth-weight ≥90th percentile adjusted for gestational week at delivery and determined from the latest gender-specific reference curves derived from a large sample of infants reflecting the ethnic distribution of the US population

Supplementary Table 2: pGCD59 median, distribution and interquartile range by case control status and OGTT sub-groups. n= 1,000

		N	**Median**	**IQR**	**p-value of trend**
					<0.001
Controls- Normal-GCT		500	0.33	0.19	
Cases- Failed-GCT		500	2.79	1.40	
No-GDM	OGTT with 0 abnormal value	273	2.68	1.31	
	OGTT with1 abnormal value	100	2.77	1.27	
GDM		127	3.23	1.43	

Delta Normal-GCT vs Failed-GCT 2.46, 95%CI(2.34, 2.57) p-value <0.001
Delta Normal-GCT vs GDM 2.9 (2.72, 3.07) p-value <0.001
Delta Normal-GCT vs OGTT with 1 abnormal value 2.44 95%CI(2.22, 2.65) p-value <0.001
Delta Normal-GCT vs OGTT with 0 abnormal value 2.33 95%CI(2.2, 2.4) p-value <0.001
IQR: Interquartile Range

Supplementary Table 3: pGCD59 decile cutoffs to predict glucose challenge test

(GCT) results, n=1,000

Cutoff GD59 Deciles	Sensitivity	specificity	Correctly Classify	PPV	NPV	LR+	LR-	Youden Index
≥5	88.46%	72.20%	80.28%	76.09%	86.22%	3.18	0.16	0.61
≥6	86.03%	89.20%	87.63%	88.85%	86.46%	7.97	0.16	0.75
≥7	77.53%	99.80%	88.73%	99.74%	81.62%	387.66	0.23	0.77

Youden index = (sensitivity + specificity) - 1; its value ranges from 0 to 1, and has a zero value when a diagnostic test gives the same proportion of positive results for groups with and without the disease (i.e., the test is useless). A value of 1 indicates that there are no false positives or false negatives (i.e., the test is perfect).

Supplementary Table 4: pGCD59 median and interquartile ranges by case control

status and relevant covariates. n= 1,000

	Controls, n(%)	Cases, n(%)	Cases (Failed-GLT), n(%)			
	Normal-GCT	Failed-GCT	No-GDM		GDM	
			OGTT with 0 abnormal value	OGTT with 1 abnormal value		
Maternal characteristics	n= 500	n= 500	n=273	n= 100	n= 127	p-value*
Age in categories(yr)						0.025
<20	0.39 (0.19)	3.48(1.65)	3.08 (0.59)	4.56 (0.00)	4.09 (1.22)	
20-29	0.31 (0.17)	2.78(1.28)	2.74 (1.30)	2.62 (1.07)	3.23 (0.93)	
30-34	0.35 (0.18)	2.65(1.38)	2.57 (1.41)	2.61 (1.12)	3.19 (0.88)	
35-39	0.33 (0.20)	2.95(1.48)	2.76 (1.34)	3.10 (1.13)	3.22 (1.87)	
>40	0.33 (0.21)	2.74 (2.10)	2.53 (1.22)	3.02 (2.96)	3.31 (3.50)	
Race						<0.001

Asian	0.32 (0.19)	2.77 (1.46)	2.63 (1.14)	2.95 (2.49)	3.01 (2.65)	
Black	0.29 (0.18)	3.09 (1.63)	2.95 (1.14)	1.81 (2.05)	3.54 (0.94)	
Hispanic	0.33 (0.16)	3.03 (1.04)	2.91 (1.05)	2.78 (0.98)	3.34 (0.77)	
Others	0.33 (0.20)	2.75 (3.01)	2.97 (0.43)	-	0.17 (0.00)	
White	0.34 (0.19)	2.47 (1.43)	2.23 (1.45)	2.65 (1.14)	2.68 (2.09)	
BMI at first prenatal visit (kg/m^2)						<0.001
<19	0.28 (0.17)	2.53 (0.94)	2.56 (0.85)	1.98 (2.18)	3.26 (3.24)	
20-24	0.35 (0.20)	2.72 (1.55)	2.54 (1.44)	2.95 (1.33)	3.00 (1.66)	
25-29	0.33 (0.18)	2.92 (1.28)	2.76 (1.17)	2.78 (1.17)	3.31 (1.15)	
>30	0.32 (0.18)	2.78 (1.35)	2.73 (1.43)	2.64 (1.05)	3.22 (1.65)	
Previous history of diabetes						<0.001
Yes	1.48 (2.48)	3.05 (0.79)	2.92 (0.57)	-	4.00 (0.00)	
No	0.33 (0.19)	2.77 (1.40)	2.68 (1.30)	2.76 (1.24)	3.22 (1.41)	
Infant characteristics						
Large for gestational age**						<0.001
Yes	0.39 (0.21)	2.78(0.93)	2.61 (0.85)	2.73 (1.10)	3.47 (0.34)	
No	0.33 (0.19)	2.77(1.39)	2.68 (1.32)	2.73 (1.26)	3.19 (1.53)	
Gender						0.052
Male	0.33 (0.20)	2.85 (1.39)	2.72 (1.30)	2.94 (1.41)	3.23 (1.50)	
Female	0.33 (0.18)	2.76 (1.39)	2.61 (1.26)	2.66 (1.11)	3.21 (1.24)	
Multiplicity						0.007
Yes	0.33 (0.34)	3.00 (1.49)	2.59 (1.34)	3.73 (1.29)	3.30 (1.33)	
No	0.33 (0.18)	2.78 (1.38)	2.68 (1.31)	2.74 (1.24)	3.23 (1.38)	

*Kruskal-Wallis test (complete case analysis)
**Restricted to only singleton cases
IQR: Interquartile Range

Median pGCD59 differences according to maternal socio-demographic and anthropometric characteristics were assessed using non-parametric tests.