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ARTICLE

The Pacific-specific *CREBRF* rs373863828 allele protects against gestational diabetes mellitus in Māori and Pacific women with obesity

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Abstract

Aims/hypothesis The *CREBRF* rs373863828 minor (A) allele is associated with increased BMI but reduced prevalence of type 2 diabetes in Māori and Pacific people. Given the shared aetiology of type 2 diabetes and gestational diabetes mellitus (GDM), we tested for an association between the *CREBRF* rs373863828 variant and GDM.

Methods We conducted a prospective cohort study of Māori and Pacific women nested within a nutritional intervention study for pregnant women with obesity. Women were enrolled at 12–17 weeks' gestation and underwent anthropometry and collection of buffy coats for later genetic testing. GDM was diagnosed by 75 g OGTT at 24–28 weeks' gestation using the International Association of Diabetes and Pregnancy Study Groups criteria. Genotyping was performed by real-time PCR with a custom *CREBRF* rs373863828 probe-set. The association between *CREBRF* rs373863828 and GDM was analysed separately by ethnic group using logistic regression, with effect estimates combined in a meta-analysis.

Results Of 112 Māori and Pacific pregnant women with obesity, 31 (28%) carried the *CREBRF* rs373863828 A allele (A/G or A/A) and 35 (31%) developed GDM. Women who carried the *CREBRF* rs373863828 A allele did not differ in BMI when compared with non-carriers (G/G). There was a fivefold reduction in the likelihood of GDM per *CREBRF* rs373863828 A allele (OR 0.19 [95% CI 0.05, 0.69], $p = 0.01$), independent of age, BMI and family history of diabetes (adjusted OR 0.13 [95% CI 0.03, 0.53], $p = 0.004$). GDM was diagnosed in 10% and 40% of women with and without the *CREBRF* rs373863828 A allele, respectively (no woman with the A/A genotype developed GDM).

Conclusions/interpretation The *CREBRF* rs373863828 (A) allele is associated with reduced likelihood of GDM in Māori and Pacific women with obesity and may improve GDM risk prediction.

Keywords *CREBRF* · Gestational diabetes mellitus · Maternal obesity · Type 2 diabetes mellitus

Abbreviations

GDM	Gestational diabetes mellitus
GWAS	Genome wide association studies
GWG	Gestational weight gain
HUMBA	Healthy Mums and Babies
IADPSG	International Association of Diabetes and Pregnancy Study Groups
ROC	Receiver operating characteristic

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Research in context

What is already known about this subject?

- The *CREBRF* rs373863828 A (minor) allele is associated with increased BMI but reduced risk of type 2 diabetes in Māori and Pacific people
- Gestational diabetes mellitus (GDM) and type 2 diabetes have a shared pathophysiology
- New approaches are needed to identify women at higher or lower risk of GDM so that pre-conceptual and early pregnancy interventions can be employed to prevent glucose intolerance

What is the key question?

- Is the *CREBRF* rs373863828 A (minor) allele associated with risk of GDM in Māori and Pacific women with obesity?

What are the new findings?

- The *CREBRF* rs373863828 A (minor) allele is carried by 28% of Māori and Pacific women with obesity
- This allele is associated with a fivefold reduction in the likelihood of GDM in Māori and Pacific women with obesity
- The addition of *CREBRF* rs373863828 status improved the predictive value of known clinical risk factors for GDM

How might this impact on clinical practice in the foreseeable future?

- Knowledge of *CREBRF* rs373863828 genotype may improve GDM risk prediction in Māori and Pacific women

Introduction

Gestational diabetes mellitus (GDM) is an increasing health problem worldwide and is associated with short- and long-term health risks for women and their offspring [1]. GDM is a state of impaired glucose tolerance and/or increased fasting blood glucose concentrations first recognised in pregnancy [2]. Normal pregnancy is characterised by increasing peripheral insulin resistance, thereby promoting transfer of glucose and fatty acids from the mother to the fetus. These changes are exaggerated in women with GDM; raised postprandial glucose concentration and/or fasting hyperglycaemia ensue when pancreatic insulin secretion is insufficient to achieve adequate glucose clearance and suppression of hepatic glucose output, respectively [2]. This may reflect an underlying defect in insulin signalling or pancreatic function [3].

Although there is ongoing debate about optimal methods for screening and diagnosis of GDM, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) has recommended universal testing of pregnant women using an OGTT at 24–28 weeks of gestation [4]. However, the logistical challenges of completing an OGTT, with the required overnight fasting, blood sampling over 2 h and frequent nausea, impacts on uptake and adequacy of testing and GDM detection, while the timing of the OGTT results in a narrow window of opportunity for intervention in the third trimester. Further, there is increasing evidence that women with GDM may have metabolic derangement from early in pregnancy prior to clinically detected glucose intolerance [5]. Thus, an evidence-based strategy that enables risk stratification for GDM in early pregnancy or even prior to conception would be beneficial.

The genetic contribution to GDM is an emerging area of research that could potentially inform future risk stratification. Numerous genome wide association studies (GWAS) have identified strong reproducible susceptibility variants for type 2 diabetes across different populations [6–8]. In Korean women, GWAS have identified variants in *CDKAL1* and near *MTNR1B* that are associated with GDM [9]. Other studies have found that several variants (including *CDKAL1* and near *MTNR1B*) are associated with increased risk of both type 2 diabetes and GDM, suggesting that these conditions may have a shared genetic background [10, 11]. This is further supported by the fact that women affected by GDM have up to a sevenfold increased risk of subsequently developing type 2 diabetes [12].

Recently, a missense variant (rs373863828, Arg457Gln, c.1370G>A) in the *CREBRF* gene was identified as being strongly associated with higher BMI (+1.4 kg/m^2) and waist circumference (+3 cm), but lower risk of type 2 diabetes (OR 0.59) among adults of Polynesian [13, 14] and Micronesian [15] ancestry. The minor (A) allele is prevalent in New Zealand Māori and Pacific people (allelic frequency 10–27%) but exceedingly rare in other ethnic groups (0.01% in East Asians and 0.004% in Europeans) in the Genome Aggregation Database (http://gnomad.broadinstitute.org, accessed 1 October 2017) [14]. It is currently not known whether the *CREBRF* rs373863828 (A) variant is associated with reduced risk of GDM. Thus, our aim was to examine the association of the *CREBRF* missense variant with risk of

GDM in Māori and Pacific women with obesity and to investigate whether the *CREBRF* variant genotype improves clinical risk prediction for GDM in early pregnancy within this subgroup of Māori and Pacific women.

Methods

Study population This study was undertaken among women recruited to a nutritional intervention study known as the Healthy Mums and Babies (HUMBA) trial (www.anzctr.org.au registration no. ACTRN12615000400561) in South Auckland, New Zealand, where more than half of the maternity population is of Māori and Pacific descent [16]. The HUMBA trial is a 2 × 2 factorial randomised controlled trial that investigated whether excessive gestational weight gain in pregnant women with obesity and birthweight in their infants could be reduced by the following interventions: (1) a multi-faceted dietary intervention provided by community health workers that included text messaging compared with routine dietary advice; and/or (2) probiotics compared with placebo. The dietary intervention consisted of four home-based education sessions, reinforced with behaviour change techniques, personalised pregnancy weight gain targets and motivational text messaging three times per week. Women allocated to routine dietary advice received routine pamphlets on healthy eating in pregnancy available to all women in New Zealand. Women allocated to probiotics received a capsule of *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis BB12* (Chr.Hansen, Denmark) at a dose of 7×10^9 colony forming units daily until birth.

Women were eligible for this genetic substudy if they had a grandparent of New Zealand Māori or Pacific (Polynesian) ethnicity as determined by maternal self-report. If women had both Māori and Pacific ancestry, ethnicity was prioritised as Māori, in line with Statistics New Zealand guidelines. Women with a singleton pregnancy and BMI ≥30 kg/m^2 were recruited to the HUMBA trial between 12^{+0} and 17^{+6} weeks of gestation (gestational age is given as weeks^{days}). Women with pre-existing diabetes ($HbA_{1c} \geq 50$ mmol/mol [≥6.7%]) in early pregnancy were excluded [17].

Ethics approval for this study was obtained from the Southern Health and Disability ethics committee, New Zealand (14/STH/205). All participants provided written informed consent for trial participation, the collection of samples and subsequent genetic analysis.

Measurement of variables Data obtained at the recruitment visit included demographic data, family history of diabetes, maternal anthropometric measures (height, weight, waist circumference and mid-arm circumference) and BP. Anthropometric measures were repeated at 28–30 weeks' and 36 weeks' gestation. Gestational weight gain (GWG) was defined as mean weekly weight gain between recruitment to 28 and 36 weeks' gestation by Institute of Medicine criteria [18]. Finger-prick non-fasting blood lipid and HbA_{1c} testing was conducted at each study visit (recruitment,) using the Roche Cobas b 101 point-of-care system [19]. A non-fasting blood (buffy coat) specimen was collected for genetic testing in consenting women.

Participants underwent a 75 g OGTT at 24–28 weeks' gestation before the second trial visit, including fasting, 1 h and 2 h venous blood glucose measurements. In this study, GDM was defined by IADPSG criteria (glucose concentrations: fasting ≥5.1 mmol/l; 1 h ≥10 mmol/l; or 2 h ≥8.5 mmol/l) [4]. It is unlikely that the women developed GDM after the 24–28 week timepoint, as pregnancy-related increases in insulin resistance peak by 24 weeks' gestation. Neither of the trial interventions (dietary intervention vs routine dietary advice nor probiotics vs placebo) altered the incidence rate of GDM, and so groups were combined for analysis.

SNP design and genotyping DNA extraction was conducted according to the manufacturer's recommendation using the PureLink Genomic DNA Mini Kit (Invitrogen, USA). A custom designed TaqMan probe-set (Applied Biosystems, USA) was created for rs373863828 using a custom Python script (snp_design; DOI: https://doi.org/10.5281/zenodo.56250) to annotate the human genome build 37 reference sequence (ftp://ftp.ensembl.org/pub/grch37, accessed 1 August 2016) with rs373863828 and any surrounding SNPs (obtained from the NCBI dbSNP build 147 common SNP list; ftp://ftp.ncbi.nlm.nih.gov/snp): forward primer: CAAGAGAGGATGCTGAGACCAT; reverse primer: ACCATGATGTAAGCCATTTTTCTGATACA; probe 1 (VIC): TGAGTGGAACC**G**AGATAC probe 2 (FAM): AGTGGAACC**A**AGATAC. Genotyping was performed using the LightCycler 480 Real-Time PCR System in 384-well plates (Roche Applied Science, USA). Quality control measures included genotyping of non-template controls (to ensure the absence of cross-contamination and primer cross-reactivity) and genotyping of samples set as technical replicates to evaluate consistency. There was 100% successful genotyping call rate. Re-genotyping of 25% of the samples demonstrated 100% concordance.

Analysis Statistical analyses were performed using the R v3.3.2 statistical software (within RStudio v0.99.902; www.rstudio.com). Univariable analysis was undertaken to compare clinical and biochemical risk factors among women with and without GDM and by *CREBRF* status (G/G vs G/A or A/A), using χ^2 test for categorical data and *t* test for continuous data. Logistic regression was used to test for an association between rs373863828 minor allele (c.1370A, p.457Gln) and GDM. Māori and Pacific women were

analysed separately and the effects were combined using an inverse-variance-weighted fixed-effect meta-analysis. Heterogeneity among sample sets was assessed using Cochran's heterogeneity (Q) statistics. A p value <0.05 in meta-analyses was considered statistically significant.

Results are presented as allelic ORs with 95% CIs, representing the estimated effect of each copy of the minor A allele on likelihood of GDM. Multivariable analysis was carried out to adjust for potential confounding (maternal age, BMI and family history of diabetes) and mediating factors (weekly weight change from recruitment to 36 weeks and HbA_{1c}, HDL-cholesterol and triacylglycerol concentrations at recruitment). None of the models adjusted for the HUMBA trial treatments. The predictive value of early pregnancy risk factors (maternal age, BMI and family history of diabetes) for GDM with and without *CREBRF* rs373863828 genotype status (A/G or A/A) was evaluated by comparing the area under the corresponding receiver operating characteristic (ROC) curves. Specificity, sensitivity, positive and negative predictive values and the negative likelihood ratio were calculated using the DAG stats software package (v2000, https://biostats.com.au/DAG_Stat) [20].

Results

Of 230 women with obesity in the HUMBA trial, 166 (72%) were of Māori or Pacific ethnicity; 112 (67%) completed both OGTT and *CREBRF* testing and were included in the analysis (Fig. 1). The *CREBRF* rs373863828 (A/G or A/A) allele was carried by 31 (28%) women: 28/77 (36%) of the women without GDM and 3/35 (9%) of the women with GDM. GDM was diagnosed in 35 (31%) women: 3/27(11%) women with the A/G genotype and 32/81 (40%) women with the G/G genotype (Table 1); none of the four women with the A/A genotype developed GDM. In univariable analyses, women who developed GDM had higher BMI ($p = 0.02$), waist circumference ($p = 0.03$), and HbA_{1c} level at recruitment ($p < 0.001$) and higher fasting glucose concentration ($p < 0.001$) and 1 h and 2 h glucose concentrations (both $p < 0.001$) on OGTT (Table 1). There were no statistically significant differences in clinical and biochemical risk factors between women with and without the *CREBRF* rs373863828 (A) allele; carriers had lower fasting and 2 h glucose concentrations on OGTT (Table 1) but the differences did not reach statistical significance ($p = 0.05$ and $p = 0.07$, respectively).

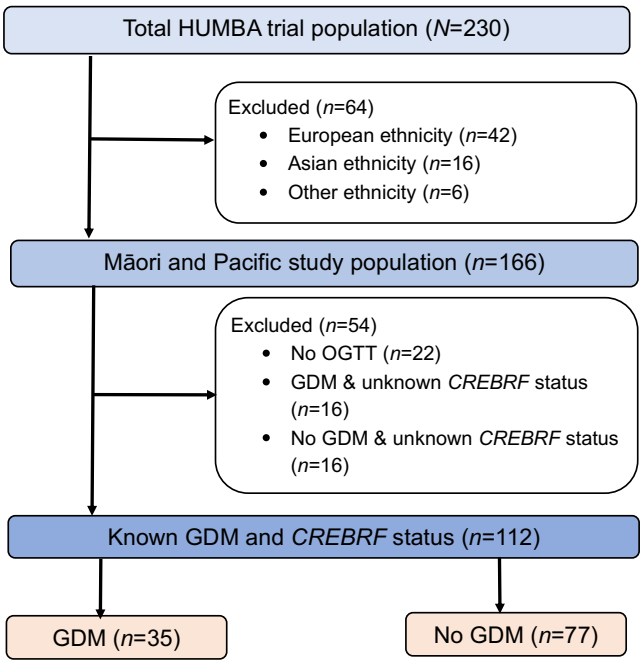

Fig. 1 Participant flow in the *CREBRF* study

The *CREBRF* rs373863828 (A) allele was associated with a statistically significant decrease in the likelihood of developing GDM in both univariable analysis (OR 0.19 [95% CI 0.05, 0.69], $p = 0.01$) and after adjustment for potential confounding by maternal age, BMI and family history of diabetes (adjusted OR 0.13 [95% CI 0.03, 0.53], $p = 0.004$) (Table 2). Adjustment for potential mediators, including GWG and early gestational HbA_{1c}, HDL-cholesterol and triacylglycerol concentrations, did not alter the association between *CREBRF* rs373863828 (A) allele and GDM (adjusted OR 0.18 [95% CI 0.04, 0.74], $p = 0.02$).

In ROC analysis, the AUC for clinical risk factors (maternal age, BMI and family history of diabetes) was 0.67 (95% CI 0.57, 0.78) (Fig. 2). Addition of rs373863828 status (A/G or A/A) increased the predictive value of the model, giving an AUC of 0.76 (95% CI 0.67, 0.85), an absolute increase of 8.8% (95% CI 1.0, 16.6, $p = 0.03$) (Fig. 2). Table 3 provides estimates of the specificity, sensitivity and positive and negative predictive value and negative likelihood ratio of rs373863828 status for GDM. Overall, the sensitivity and specificity of rs373863828 status for GDM was 91% and 36%, respectively, with a positive predictive value (G/G) of 40%, a negative predictive value (A/G or A/A) of 90% and a negative likelihood ratio of 0.24.

Discussion

We have identified a novel protective biomarker, the minor (A) allele of *CREBRF* rs373863828, that is associated with a fivefold reduction in the likelihood of GDM (defined by IADPSG criteria) in Māori and Pacific women with obesity. This is an important finding, given the high rates of GDM and obesity in these ethnic groups, and the fact that GDM is a major risk factor for subsequent development of type 2 diabetes in women [12].

This decreased rate of GDM in women carrying the *CREBRF* rs373863828 minor (A) allele appeared to be due

Table 1 Cohort characteristics

Characteristic	Total ($N = 112$)	GDM			*CREBRF* rs373863828 (A) allele		
		No ($N = 77$)	Yes ($N = 35$)	p value	No ($N = 81$)	Yes ($N = 31$)	p value
Ethnicity, *n* (%)							
Māori	37 (33.0)	31 (40.3)	6 (17.1)	0.02	24 (29.6)	13 (41.9)	0.22
Pacific	75 (67.0)	46 (59.7)	29 (82.9)		57 (70.4)	18 (58.1)	
Maternal age, years	29.0 (6.1)	28.8 (6.1)	29.4 (6.2)	0.67	29.3 (6.3)	28.3 (5.7)	0.43
Family medical history, *n* (%)							
Hypertension[a]	55 (52.4)	38 (52.8)	17 (51.5)	0.91	37 (48.1)	18 (64.2)	0.14
Diabetes[b]	44 (40.4)	28 (36.8)	16 (48.5)	0.27	34 (43.0)	10 (33.3)	0.35
Body size at recruitment							
Height, cm	167.6 (5.2)	168.0 (5.5)	166.8 (4.4)	0.22	167.4 (5.1)	168.2 (5.5)	0.44
Weight, kg	110.8 (18.4)	108.7 (18.3)	115.5 (18.1)	0.07	109.6 (17.4)	113.9 (20.8)	0.31
BMI, kg/m^2	39.4 (6.4)	38.5 (6.1)	41.5 (6.6)	0.02	39.1 (6.0)	40.2 (7.3)	0.45
Waist circumference, cm	115.8 (12.8)	114.1 (12.8)	119.6 (11.9)	0.03	115.0 (11.9)	117.9 (14.9)	0.33
Metabolic status at recruitment							
HbA_{1c}, mmol/mol	35.3 (4.0)	34.2 (3.4)	37.7 (4.0)	<0.001	35.4 (4.1)	35.0 (3.5)	0.56
HbA_{1c}, %	5.4 (0.6)	5.3 (0.5)	5.5 (0.6)		5.4 (0.6)	5.4 (0.5)	
Total cholesterol, mmol/l	4.5 (0.7)	4.5 (0.7)	4.6 (0.8)	0.78	4.5 (0.7)	4.5 (0.7)	0.77
Triacylglycerol, mmol/l[c]	2.1 (0.6)	2.1 (0.6)	2.1 (0.6)	0.61	2.1 (0.6)	2.2 (0.6)	0.47
HDL-cholesterol, mmol/l[a]	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	0.37	1.5 (0.3)	1.5 (0.3)	0.88
LDL-cholesterol, mmol/l[a]	2.1 (0.8)	2.1 (0.7)	2.1 (0.9)	0.93	2.1 (0.8)	2.0 (0.7)	0.34
HUMBA trial treatments, *n* (%)							
Dietary intervention	57 (50.9)	39 (50.7)	18 (51.4)	0.93	42 (51.9)	15 (48.4)	0.74
Routine diet advice	55 (49.1)	38 (49.4)	17 (48.6)		39 (48.1)	16 (51.6)	
Probiotics	61 (54.5)	43 (55.8)	18 (51.4)	0.66	42 (51.9)	19 (61.3)	0.37
Placebo	51 (45.5)	34 (44.2)	17 (48.6)		39 (48.1)	12 (38.7)	
GWG, kg							
Mean weekly change recruitment to 28 weeks[d]	0.51 (0.32)	0.52 (0.34)	0.49 (0.28)	0.70	0.51 (0.34)	0.52 (0.30)	0.90
Mean weekly change recruitment to 36 weeks[a]	0.56 (0.31)	0.58 (0.34)	0.53 (0.25)	0.38	0.54 (0.32)	0.61 (0.30)	0.27
Hypertension in pregnancy, *n* (%)	15 (13.4)	10 (13.0)	5 (14.3)	0.86	11 (13.6)	4 (12.9)	0.93
OGTT glucose concentration, mmol/l							
Fasting	4.7 (0.5)	4.4 (0.3)	5.2 (0.5)	<0.001	4.7 (0.6)	4.5 (0.4)	0.05
1 h	8.0 (1.8)	7.2 (1.3)	9.6 (1.6)	<0.001	8.1 (1.9)	7.8 (1.7)	0.49
2 h	6.2 (1.2)	5.8 (0.9)	7.0 (1.4)	<0.001	6.3 (1.2)	5.8 (1.2)	0.07
CREBRF genotype, *n* (%)							
G/G	81 (72.3)	49 (63.6)	32 (91.4)	0.002			
A/G	27 (24.1)	24 (31.2)	3 (8.6)	0.01			
A/A	4 (3.6)	4 (5.2)	0 (0.0)				
Minor (A) allele	35 (15.6)	32 (20.8)	3 (4.3)	0.002			

Data are *n* (%) or mean (SD)

p values are for the comparison (χ^2 or *t* test) between women who have vs do not have GDM or who do vs do not carry the *CREBRF* rs373863828 minor (A) allele

[a] Data missing for 7 participants

[b] Data missing for 3 participants

[c] Data missing for 6 participants

[d] Data missing for 9 participants

to a reduction in both fasting and 2 h OGTT glucose concentrations. Adjustment for potential confounding strengthened this association, suggesting that the *CREBRF* rs373863828 minor (A) allele is associated with reduced likelihood of

Table 2 Association between *CREBRF* rs373863828 (A) allele and risk of GDM in Māori and Pacific women

Population	Model 1[a]			Model 2[b]			Model 3[c]		
	N	OR (95% CI)	*p* value	N	OR (95% CI)	*p* value	N	OR (95% CI)	*p* value
Total	112	0.19 (0.049, 0.69)	0.012	112	0.13 (0.033, 0.53)	0.004	102	0.18 (0.042, 0.74)	0.018
Māori	37	0.33 (0.040, 2.73)	0.30	37	0.19 (0.012, 2.85)	0.23	33	0.17 (0.01, 3.01)	0.23
Pacific	75	0.15 (0.033, 0.69)	0.015	75	0.12 (0.023, 0.59)	0.009	69	0.18 (0.034, 0.93)	0.04

[a] Model 1 is unadjusted

[b] Model 2 adjusts for potential confounding by maternal age, BMI at recruitment and family history of diabetes

[c] Model 3 adjusts for potential mediation by mean weekly weight change from baseline to 36 weeks' gestation, and HbA_{1c}, HDL-cholesterol and triacylglycerol concentrations at recruitment

GDM independent of maternal age, BMI and family history of diabetes. Adjustment for GWG and HbA_{1c}, HDL-cholesterol and triacylglycerol concentrations in early gestation did not alter the association between the *CREBRF* rs373863828 (A) minor allele and GDM, suggesting that these are not mediating factors.

The *CREBRF* gene is found on chromosome 5 and encodes a negative regulatory factor of the cyclic AMP-responsive element-binding protein 3 (CREB3), which in turn is involved in regulation of protein translation. The rs373863828 (A) allele variant was first associated with higher BMI (1.5 kg/m^2) and waist circumference (3 cm) but lower odds of type 2 diabetes (OR 0.59) among people of Polynesian ancestry [13, 14, 21]. The minor (A) allele was also weakly associated with increased insulin sensitivity by HOMA-IR in the Sāmoan and American Sāmoan populations [13]. The protective effect of the minor (A) allele for GDM is concordant with the findings of previous association studies among non-pregnant women, which suggested a strong genetic relationship between the *CREBRF* A allele and lower risk of both GDM and type 2 diabetes.

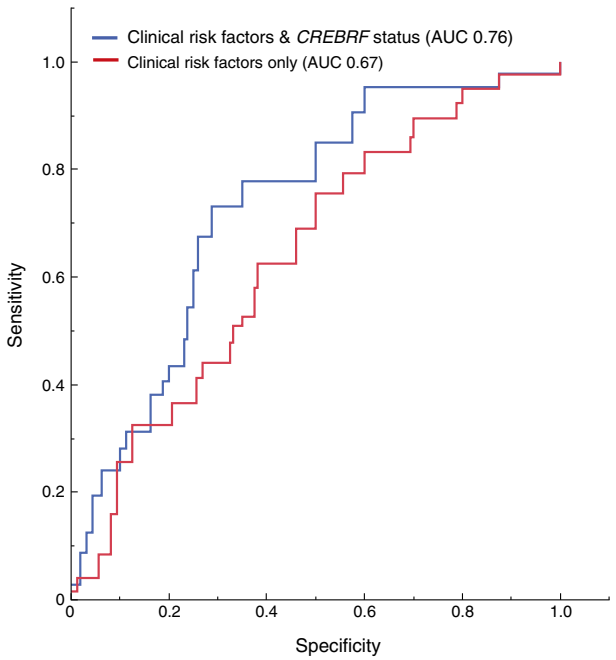

Fig. 2 ROC curves for prediction of GDM. Clinical risk factors include maternal age, BMI and family history of diabetes

In this cohort of pregnant women with obesity, when comparing those with and without the minor (A) allele there were no statistically significant differences in BMI, waist circumference or GWG. We speculate that the influence of the *CREBRF* rs373863828 variant may be associated with increased lean mass rather than any changes in adiposity manifested as GWG. *CREBRF* rs373863828 variant carriers have been shown to be taller [22, 23] and hence height-related increase in muscle mass and pancreatic beta cell mass may decrease the risk of GDM and type 2 diabetes without influencing GWG.

It remains unclear how the rs373863828 minor (A) allele contributes to higher BMI yet lower risk of type 2 diabetes. Cellular models have suggested that the minor (A) allele promotes lipid storage at a reduced energy cost in the adipocytes [13], although this does not explain the lower risk of type 2 diabetes or GDM. *CREBRF* may also have a role in modifying fat distribution (subcutaneous vs abdominal fat), which may influence flux of NEFA and disposal of glucose [24]. Interestingly, *CREBRF*-knockout mice have shown markedly reduced prolactin secretion and augmented glucocorticoid receptor signalling [25]. Given the key role of prolactin in the adaptation of insulin-secreting beta cells during pregnancy [26], and the known diabetogenic effects of glucocorticoids [27], this suggests that *CREBRF* has a role in adaptive changes in the pancreas and in the regulation of insulin resistance during pregnancy. However, further investigation of beta cell function, insulin sensitivity and glucose disposal levels are needed to fully understand the effect of the *CREBRF* rs373863828 minor (A) allele on glucose metabolism during pregnancy. Elucidating the underlying molecular mechanisms may provide opportunity for development of novel therapeutic interventions for GDM, particularly as *CREBRF* has been considered as a target gene in other conditions, such as gastric cancer and glioblastoma [28, 29].

In this population, detection of the *CREBRF* rs373863828 minor (A) allele had high (90%) negative predictive value for GDM, giving a negative likelihood ratio of 0.24. Although not

Table 3 Predictive value of the *CREBRF* status for GDM

Population	*N*	Sensitivity	Specificity	PPV	NPV	Negative likelihood ratio[a]
Total	112	0.91 (0.77, 0.98)	0.36 (0.26, 0.48)	0.40 (0.29, 0.51)	0.90 (0.74, 0.98)	0.24 (0.08, 0.72)
Māori	37	0.83 (0.36, 1.00)	0.39 (0.22, 0.58)	0.21 (0.07, 0.42)	0.92 (0.64, 1.00)	0.43 (0.07, 2.72)
Pacific	75	0.93 (0.77, 0.99)	0.35 (0.21, 0.50)	0.47 (0.34, 0.61)	0.89 (0.65, 0.99)	0.20 (0.05, 0.80)

Data are probability (95% CI) or likelihood ratio (95% CI)

CREBRF wild type (G/G) considered as positive screen and *CREBRF* rs373863828 variant (A/G or A/A) considered as negative screen

[a] Likelihood ratios of 0.1 have a large effect on post-test probability of disease, ratios of 0.2 have a moderate effect and ratios of 0.5 a slight effect [34]

NPV, negative predictive value; PPV, positive predictive value

sufficiently predictive to replace diagnostic testing for GDM [30], the *CREBRF* rs373863828 genotype could be used in Māori and Pacific women for risk stratification in early pregnancy, and there is increasing evidence that risk prediction models need to incorporate ethnic-specific biomarkers [31]. Māori and Pacific women carrying the minor allele exhibited a low incidence of GDM, despite clinically increased risk due to obesity. Given that 60% of Māori and Pacific women in the Counties Manukau region in New Zealand have obesity and nearly 30% carry the protective minor (A) allele, genotyping may have clinical utility by allowing targeting of resources for women at higher risk of GDM (absence of minor [A] allele). This could include preventative dietary interventions and pharmacotherapy, and earlier testing for GDM by OGTT, rather than by the current two-step GDM screening used in New Zealand (OGTT is reserved for women with an abnormal non-fasting polycose test). These data have relevance not only for Aotearoa/New Zealand but also for the Pacific Islands, which have even higher rates of type 2 diabetes and GDM [32].

Although the magnitude of association that we observed between the *CREBRF* rs373863828 minor (A) allele and GDM was large and not obviously confounded, the fact that our study was conducted only in women with obesity raises the possibility of selection bias. However, typical collider conditioning is unlikely as this requires both the exposure (*CREBRF* rs373863828 genotype) and outcome (GDM) to be causally linked to sample selection (obesity), and this does not seem plausible in the case of GDM. Nevertheless, replication of our findings in a broader population is warranted, particularly as we used meta-analysis of self-reported ethnicity to estimate exposure effects rather than genetic population structure estimates.

Overall, the combination of *CREBRF* rs373863828 genotype and clinical risk factors had greater predictive value for GDM than clinical risk factors alone. We were unable to explore whether there is an interaction between *CREBRF* rs373863828 genotype and clinical risk factors for prediction of GDM due to the limited sample size of our study. It is possible that clinical risk prediction may perform better in Māori and Pacific women with the rs373863828 GG genotype, allowing further optimisation of risk stratification, but this would require evaluation in larger cohorts, including women without obesity. Identifying ways to prevent development of GDM among Māori and Pacific women is a health priority [33].

In summary, we have shown that the *CREBRF* rs373863828 minor (A) allele is associated with substantially reduced likelihood of GDM in Māori and Pacific women with obesity. The *CREBRF* rs373863828 genotype could potentially improve risk stratification for GDM in early pregnancy in Māori and Pacific women.

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Data availability Published data are available to approved researchers under the data sharing arrangements provided by the Clinical Data Research Hub (CDRH), based at the Liggins Institute, University of Auckland (https://wiki.auckland.ac.nz/researchhub). Metadata, along with instructions for data access, are available at the University of Auckland's research data repository, Figshare (https://auckland.figshare.com). Data access requests are to be submitted to the Data Access Committee via researchhub@auckland.ac.nz. De-identified published data will be shared with researchers who provide a methodologically sound proposal and have appropriate ethical and institutional approval. Researchers must sign and adhere to the Data Access Agreement, which includes a commitment to using the data only for the specified proposal, to refrain from any attempt to identify individual participants, to store data securely and to destroy or return the data after completion of the project. The CDRH reserves the right to charge a fee to cover the costs of making data available, if required.

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Contribution statement MK, RM, JT, LM, KOG, RT and CM planned the study. KOG, RT, LM and CM supervised data collection. MK, TM and MJ performed genotyping. MK, JT and CM conducted analyses. MK and CM drafted the manuscript. All authors contributed to the discussion, critically appraised the manuscript and approved the final version for publication. CM and RM are the guarantors for this work and accept full responsibility for the conduct of the study, had access to the data, and controlled the decision to publish.

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