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The protective effect of rs373863828 on type 2 diabetes does not operate through a body composition pathway in adult Samoans

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Abstract

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Objective—To understand whether the paradoxical association of missense variant rs373863828 in *CREBRF* with higher BMI but lower odds of diabetes is explained by either metabolically favorable body fat distribution or greater fat-free mass.

Methods—We explored the association of the minor allele with dual-energy x-ray absorptiometry (DXA)-derived body composition in n=421 Samoans and used path analysis to examine the mediating role of fat and fat-free mass on the relationship between rs373863828 and fasting glucose.

Results—Among females, the rs373863828 minor A allele was associated with greater BMI. There was no association of genotype with percent body fat, visceral adiposity, or fat distribution in either sex. In both females and males, lean mass was greater with each A allele: 2.16 kg/copy (p=0.0001) and 1.73 kg/copy (p=0.02), respectively. Path analysis showed a direct negative effect of rs373863828 genotype on fasting glucose (p=0.004) consistent with previous findings, but also an indirect positive effect on fasting glucose operating through fat-free mass (p=0.027).

Conclusions—The protective effect of rs373863828 in *CREBRF*, common among Pacific Islanders, on type 2 diabetes does not operate through body composition. Rather, the variant's effects on body size/composition and fasting glucose likely operate via different, tissue-specific mechanisms.

Keywords

Body composition; Dual-Energy X-Ray Absorptiometry; *CREBRF*; Samoans; Type 2 Diabetes

Introduction

In 2016, based on a genome-wide association study (GWAS) of body mass index (BMI) in adult Samoans, we reported the identification of a missense variant in CREB3 regulatory factor (*CREBRF*) that is paradoxically associated with higher BMI and odds of obesity but with lower fasting blood glucose and odds of type 2 diabetes [1]. The minor allele A of rs373863828, an arginine-to-glutamine missense variant, had a frequency of 0.259 in the discovery sample, indicating that >40% of Samoans have at least one copy of the risk allele.

Our discovery has been replicated in several other Pacific Islander populations (Māori, Tongans, Cook Islanders, Niueans, Chamorro, Chuukese) [2–6] and may partially explain their greater risk of obesity compared to other ethnic groups. The paradoxical associations of rs373863828 with BMI and diabetes, however, remain unexplained. In a murine 3T3L1 adipocyte model, ectopic expression of the human variant enhanced adipogenesis and lipid storage compared to control [1]. Therefore, one hypothesis is that human carriers of the variant have greater fat mass relative to fat-free mass but store that fat in a more metabolically favorable distribution. Having proportionally greater abdominal fat is more detrimental for health than higher total body fat and visceral/abdominal fat is positively associated with metabolic disease, independent of overall adiposity [7–9]. Those with the variant may store more fat subcutaneously rather than viscerally, or peripherally rather than centrally) compared to those without the variant, thereby explaining their lower odds of diabetes. An alternative hypothesis is that those with the A allele have greater BMI as a result of greater fat-free mass and may more effectively regulate blood glucose as a result.

Greater muscle and bone mass promote lower serum glucose, greater insulin sensitivity, and lower risk of diabetes via multiple mechanisms [10,11]. Skeletal muscle, in particular, is a major site for insulin-dependent and independent glucose uptake and disposal as well as secretion of autocrine, paracrine, and endocrine factors that influence metabolic homeostasis [12,13].

To test these hypotheses, we (1) examined associations between rs373863828 genotype, body composition, and fat distribution measured using dual-energy x-ray absorptiometry (DXA) and (2) used path analysis to examine the potential mediating effects of fat and fat-free mass on the relationship between rs373863828 and fasting glucose.

Methods

Between August 2017 and March 2019 participants from our original GWAS sample [1] were recruited into a follow-up study to examine body composition and cardiometabolic health. With the exception of rs373863828 genotype, all data presented here were collected during that follow-up study. Participants with AA, AG, and GG genotypes were targeted in a 1:2:2 ratio. Protocols for the original GWAS in 2010 and the follow-up study have been previously published [14,15].

Eligible participants were not attempting to control their weight through medication or surgery, were resident on the island of ’Upolu, and were part of a maximally unrelated sample [maximum kinship 6.01%]. Women were not pregnant or lactating [15]. Willing participants (n=519; aged 30.7–72.7 years) gave written informed consent. Protocols were approved by the Yale University Institutional Review Board (IRB #1604017547), the University of Pittsburgh (#PRO16040077), and the Health Research Committee of the Samoan Ministry of Health.

Weight and height were measured using a Tanita HD 351 digital weighing scale (Tanita Corporation of America, IL) and SECA 213 portable stadiometer (Seca GmbH & Co., Germany), respectively. DXA outcomes (assessed using a Lunar iDXA, GE Healthcare Medicine, Encore Version 17) included total body fat, lean, and bone mass. Visceral fat mass (estimated using GE CoreScan™) was also measured along with subcutaneous fat mass in the android (central) and gynoid (hip and thigh) regions and the limbs. To assess fat distribution we used DXA data to derive two commonly-used measures: Android-to-gynoid fat ratio (a measure of abdominal vs. gluteal fat distribution) was calculated, as well as trunk-to-peripheral fat ratio, a measure of centrality of fat deposition (fat mass in the trunk region divided by the sum of arm and leg fat [peripheral fat]). In both cases, lower values indicated more metabolically favorable, less central fat distribution. When a participant’s width exceeded the scan area, a right side scan was ‘mirrored’ and used to estimate total body composition [16]. Contraindications to DXA included exposure to additional X-rays or computed tomography (CT) in the prior 12 months; therefore, this analysis was restricted to n=421 participants (AA n=72, AG n=161, GG n=188). For analyses of visceral fat mass, the sample was further reduced by n=1 participant whose body size prevented the capture of the core region in a single scan. Venous blood samples were collected in sodium fluoride vacutainers, after an at least 10-hour overnight fast. Fasting plasma glucose levels were

obtained in triplicate using an Analox GM9 Glucose Analyzer (Analox Instruments Ltd., United Kingdom) and the three values averaged for use in analysis.

Associations between body size and composition outcomes and rs373863828 genotype were performed using linear models that were sex-stratified, to account for sexual dimorphism of the body size and composition traits, and combined. Sex-stratified models were adjusted for age and age^2 (sex and age were self-reported by participants; sex was validated with genetic information); combined models were adjusted for age, age^2, and sex. Age was mean-centered to avoid multicollinearity issues. Body composition outcomes were adjusted for height [17]. We also calculated height-independent body composition indices: Fat Mass Index (FMI): fat mass (kg)/height (m)^2 and Fat-Free Mass Index (FFMI): fat-free mass (kg)/height (m)^2, respectively) [18]. For analyses including fasting glucose, participants with fasting glucose ≥126 mg/dL (indicative of diabetes) or self-reported diabetes medication use were excluded (n=27 females, n=11 males). Genotype was modeled additively (the number of A alleles a participant carries), consistent with previous work [1]. Combined (male and female) effect estimates were obtained adjusting for sex. Sex-specific genotype effects were examined by testing a sex-by-genotype interaction term in the combined models. For ease of interpretation, effect sizes are presented on their original scale; sensitivity analyses using inverse-normally transformed traits following the method of Sofer et al. [19] gave similar results (Table S1).

Path analyses were used to examine the mediating effects of FMI and FFMI on the relationship between rs373863828 genotype and fasting glucose using maximum-likelihood estimation with conventional standard errors using the *sem* function in the *lavaan* package in R [20]. Due to the high correlation between FMI and FFMI reflecting general body size (Pearson r=0.714 [Pearson correlation test p=1.13×10^{-66}] after adjusting for sex, age, and genotype), they were evaluated for mediating effects in separate models by formally testing the indirect effect of rs373863828 genotype on fasting glucose through FMI or FFMI. All path analyses adjusted for age in predicting FMI/FFMI and fasting glucose. Analyses were performed overall (adjusting for the effect of sex on FMI/FFMI; average fasting glucose did not differ by sex) and in sex-stratified models. Fasting glucose was inverse-normally transformed for analyses (separately among males/females for use in sex-stratified analyses). Both unstandardized (β) and standardized ($\beta^* = \frac{s_x}{s_y}\beta$)estimated effects for path analysis are presented.

All analyses were conducted in R Version 3.6.0 (R Foundation for Statistical Computing, Austria) with the threshold for statistical significance set at p<0.05 due to the highly correlated nature of the traits being tested.

Results

Average BMI was estimated to be higher with each minor A allele of rs373863828 (1.31 kg/m^2 [p=0.0025] combined; 1.95 kg/m^2 [p=0.0014] in females; 0.60 kg/m^2 [p=0.32] in males; Table 1). Similarly, average total fat mass (p=0.02), android fat mass (p=0.009), trunk fat mass (p=0.01), and FMI (p=0.02) were estimated to be higher with each copy of the minor allele among females, but not in males (Table 1, Figure 1). In both sexes, average lean

mass was greater with each copy of the minor allele: by 2.16 kg/copy in females (p=0.0001) and 1.73 kg/copy in males (p=0.02) after adjusting for age, age^2, and height. Consistent with this, average FFMI was also greater per copy of the A allele (0.86 kg/m^2 [p=0.0001] in females and 0.61 kg/m^2 [p=0.01] in males), adjusting for age and age^2. Height was greater per copy of the minor allele in males only (p=0.003). There were no differences by genotype observed among either sex in visceral adiposity, percent body fat, or distribution of body fat based on android-to-gynoid ratio and trunk-to-peripheral fat ratio (Table 1). Additional plots illustrating distribution of body size and composition outcomes by genotype as well as fasting glucose by genotype are provided in Figure S1.

Consistent with previous findings, the rs373863828 A allele was associated with lower fasting glucose, on average. The effect was stronger in women (-14.57 mg/dL/copy, p=0.006) than in men (-7.08 mg/dL/copy, p=0.15). Path analyses examining the mediating effect of fat mass index (FMI) on the relationship between rs373863828 and fasting glucose in the overall sample show a direct negative effect of the variant on fasting glucose (direct β=-0.128, p=0.011; Table 2 and Figure 2) but no significant indirect (mediated by FMI) effect (p=0.13). In contrast, when examining the mediating effect of FFMI on the relationship between rs373863828 and fasting glucose, we observed both direct and indirect (mediated by FFMI) effects of the variant (direct β=-0.146, p=0.004, indirect β=0.026, p=0.027, respectively; Table 3 and Figure 3). The estimated direct effects of the variant on fasting glucose in both FMI- and FFMI-mediated models were negative and similar in magnitude. In contrast, the variant's effect mediated via FFMI was positive and much smaller in magnitude than the direct effects. Sex-stratified path analyses (Figures S2 and S3; Tables S2 and S3) showed similar direct and FFMI-mediated effects of rs373863828 on fasting glucose in females (direct β=-0.255, p=0.00015, indirect β=0.069, p=0.006, respectively); however, in males, effect estimates were close to zero.

Discussion

In this study we demonstrate that the original association between the rs373863828 minor A allele and BMI persisted after 8 years. We also show that the positive effect of the allele on BMI is primarily a function of greater lean mass rather than fat mass among males, and a combination of greater lean and fat mass among females. Although we did observe greater fat-free mass among those with the missense variant, our findings do not support either of our original hypotheses: the inverse association between the A allele and type 2 diabetes does not appear to be related to more metabolically favorable fat distribution (based on the near-zero estimated effect sizes of rs373863828 genotype on average visceral fat, trunk-to-peripheral fat ratio, and android-to-gynoid fat ratio) nor to greater fat-free mass (based on the persisting direct effect of rs373863828 on fasting glucose after accounting for body composition in our path analysis). Notably, the indirect effect through FFMI was contrary to its hypothesized effect due to an estimated positive effect of FFMI on fasting glucose in our sample.

Many studies have provided evidence to support a role for fat distribution in either promoting or preventing insulin resistance. Although it may not be a causal factor [21,22], the volume of visceral adipose tissue is a strong predictor of insulin resistance, both

as a proportion of total body fat and independent of subcutaneous fat quantity [23–26]. Conversely, lower body subcutaneous adiposity may be metabolically protective [27–29]. Gluteofemoral adipose tissue mass is positively associated with insulin sensitivity as well as slower rates of lipolysis and free fatty acid release [27]. Insulin sensitivity is, however, one of two potential explanations for the chronic hyperglycemia that characterizes type 2 diabetes, the other being deficits in insulin secretion [30]. The fact that we did not observe associations between the minor rs373863828 allele and our chosen measures of fat distribution suggests that examination of insulin secretion rather than sensitivity/resistance may offer insight into the fasting glucose lowering effect of this missense variant.

In line with our findings, Burden and colleagues recently reported results of mixed-meal tolerance tests, intravenous glucose tolerance tests (IV-GTT), and hyperinsulinemic-euglycemic clamp studies completed on healthy men of Māori or Pacific ethnicity contrasting those with zero versus one or more copies of the rs373863828 A allele [31]. Based on a greater decline in blood glucose levels during the IV-GTT and a lack of observed differences in steady state glucose or insulin, glucose disposal rate, or insulin sensitivity index based on the hyperinsulinemic-euglycemic clamp studies, the authors concluded that the minor allele is not associated with insulin resistance. They did, however, observe an effect on insulin secretion, with those with the minor allele having higher plasma insulin levels 30 minutes after consuming a standardized meal. This finding was replicated in IV-GTT studies with higher glucose-induced increases in plasma insulin and C-peptide observed, particularly early after glucose administration [31]. While the overall findings require replication in a sample that includes women, they offer new avenues for exploration including whether the variant exerts its effect through β-cell mass, development, or secretory capacity. This knowledge may have important therapeutic value by providing novel mechanisms for enhancing functional β-cell mass to prevent or treat diabetes.

We found that the minor allele was associated with greater absolute fat-free mass in both sexes, suggesting a potential role for lean mass in mediating the variant's effect on glucose homeostasis. However, although the variant had a negative overall direct effect on fasting glucose, consistent with previous reports [1,4,6], the indirect effect of rs373863828 on fasting glucose mediated by FFMI was in the opposite direction (i.e., associated with higher fasting glucose). This is contrary to our hypothesis that the 'protective' effect of rs373863828 on type 2 diabetes operates through increased fat-free mass, however, it was not entirely unexpected given the correlation between FMI and FFMI observed among our sample: the greater the fat-free mass among our participants, the greater the fat mass. Among studies that have reported beneficial effects of fat-free mass on glucose metabolism [for example, 32–34], many expressed fat-free mass relative to body weight (as a ratio or percentage, rather than as a measure of body size as we did here); in such studies a high fat-free mass, when expressed as a percentage of body weight, would also represent low fat mass [35].

Independent of any role in improving glucose homeostasis, the association of the rs363863828 minor allele with greater fat-free mass observed here among Samoan adults is consistent with our previous findings of greater height in those with the A allele [36] (based on variation in fat-free mass attributable to height [37]) and our studies of body composition

in infants [38]. While the variant examined here has not been observed in individuals without Pacific Islander ancestry, associations of other *CREBRF* variants with lean mass traits (height, whole body, trunk and leg fat-free mass, for example) have been reported in UK Biobank data. [39, 40]. Notably, in our prospective study of the body composition of Samoan infants, in which we examined body composition using DXA within two weeks of birth and again at four months of age, we observed increased bone mass among those with AA/AG genotype compared to those with GG genotype in the first weeks of life [38]. At four months, both lean and bone mass were significantly greater among those with at least one copy of the minor allele compared to those without. Although it is not possible to obtain the same measures of fat distribution in infants as we explored here in adults, we also saw no effect on total or percent fat mass. The mechanism linking rs373863828 genotype and lean mass is unclear but the consistency among our findings suggest that it is established early in life.

While the minor allele was associated with greater lean mass in both males and females, and greater height in males, we observed a larger effect of the missense variant on BMI in females and a significant effect on fat mass in females only. Prior studies of this variant and its association with BMI, including our own, have adjusted for sex, rather than presenting sex-stratified analyses [1–6], so this phenomenon has not been noted previously. Our findings indicate that attempts to examine potential sex and tissue-specific effects of the variant are warranted.

The major strength of this study compared to other examinations of the rs373863828 genotype to date is the use of DXA-measured body composition, rather than BMI, which is unable to distinguish between fat and lean mass. We did, however, encounter many participants (19% of our recruited sample) who, because of our conservative safety criteria, could not participate due to recent high dose radiation exposure (most commonly from chest x-rays used in the investigation of respiratory illnesses). While those who could not receive a DXA scan were similar in age, BMI, and genotype distribution, this may limit generalizability of our findings. Furthermore, we note that the participants in our study sample had high average levels of body fat (approximately 30% in males and 45% in females) compared to other populations and that we limited our investigation of fat distribution to only two of several possible measures. Future studies are needed to explore the functional relationship between fat distribution and rs373863828 across the spectrum of adiposity, including examining the impact upon cardiometabolic disease risk factors.

Importantly, these findings do not suggest that the protective effect of the missense variant rs373863828 in *CREBRF*, common among Pacific Islanders, on type 2 diabetes operates through a body composition pathway among contemporary adult Samoans, characterized by large body sizes. Rather, the variant's effects on body size (BMI, fat-free and fat mass) and fasting glucose appear to be distinct from one another and likely operate via different, tissue-specific mechanisms. While those mechanisms remain unclear, the evidence presented here supports recent speculation that β-cell function may be an important next avenue of investigation [27]. As diabetes prevalence continues to increase among Samoans and other Pacific Islander populations, additional understanding of the mechanisms that underlie the protective effect of the *CREBRF* variant may be transformative for prevention and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known about this subject?

- The A allele of rs373863828, a missense variant in *CREBRF*, is paradoxically associated with both higher BMI and lower odds of Type 2 Diabetes in Pacific Islanders

What are the new findings in your manuscript?

- There is no association of rs373863828 genotype with percent body fat, visceral adiposity, or fat distribution. The A allele is, however, associated with greater lean mass.
- Path analyses show a significant negative effect of the A allele on fasting glucose but also an indirect positive effect operating through fat free mass.
- Differences in body composition do not explain the observation of lower fasting glucose among those with the rs3737863828 variant

How might your results change the direction of research or the focus of clinical practice?

- Lack of association between rs373863828 genotype and body composition suggests efforts to explain differences in glycemic control/diabetes risk by genotype should focus on insulin secretion, rather than resistance.

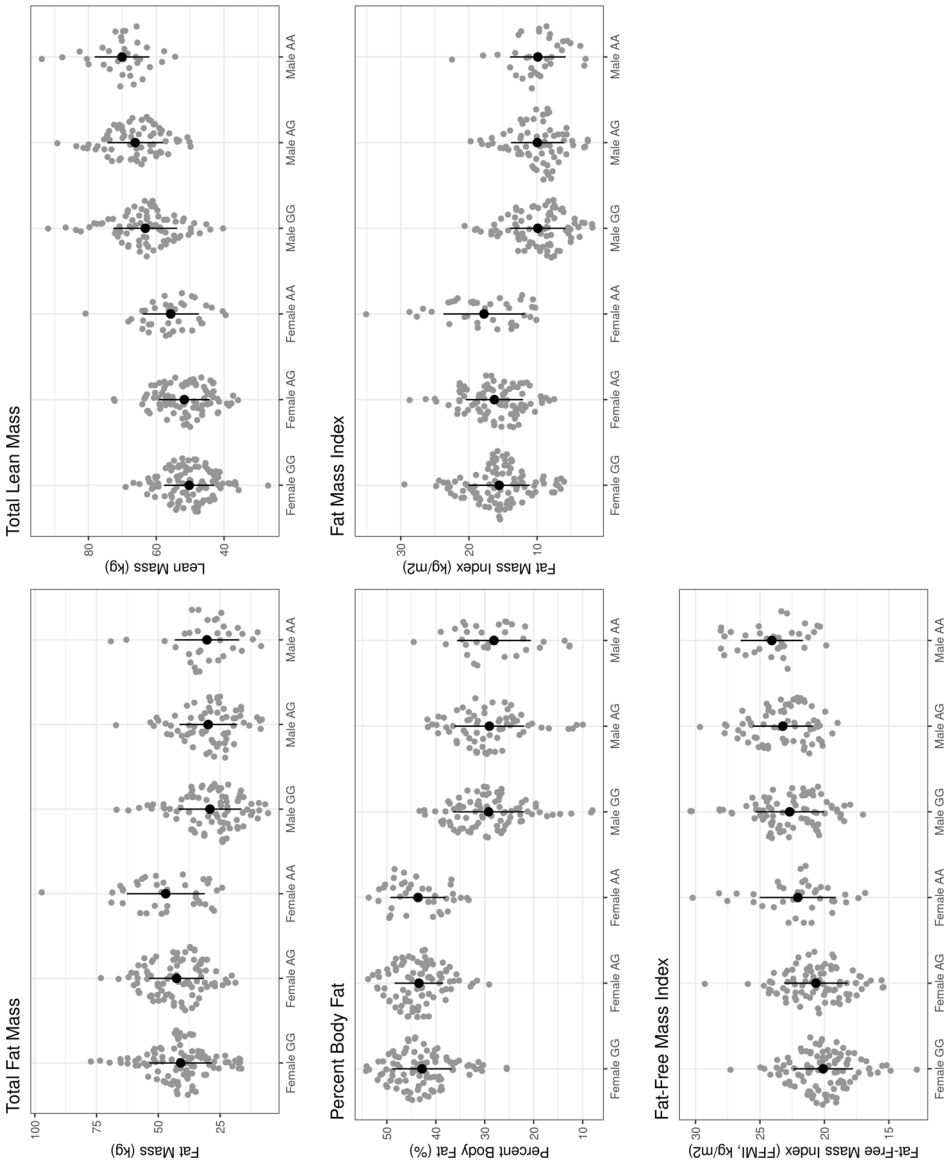

Figure 1.

Fat, lean, and bone mass and percent body fat by genotype. Gray dots indicate observed data. Black circles and connecting lines indicate genotype- and sex-specific mean ± SD values.

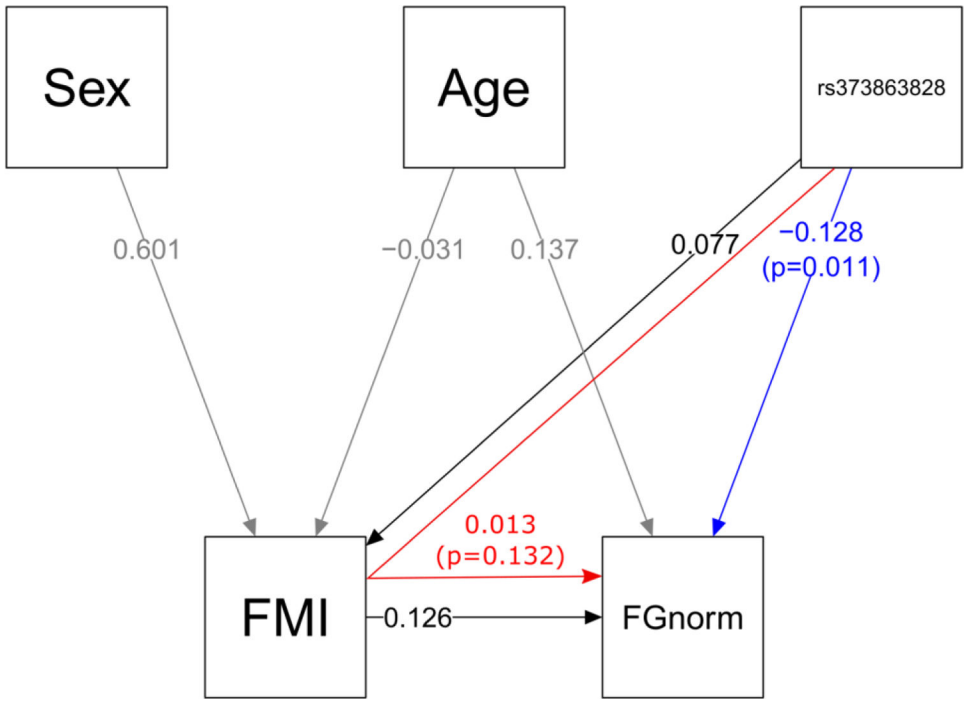

Figure 2.

Path diagram examining the mediating role of fat mass index (FMI) on the relationship between rs373863828 and inverse-normally-transformed fasting glucose (FGnorm). Standardized coefficients are plotted along each path. The direct effect of rs373863828 on FGnorm is shown in blue, the indirect (mediated by FMI) in red.

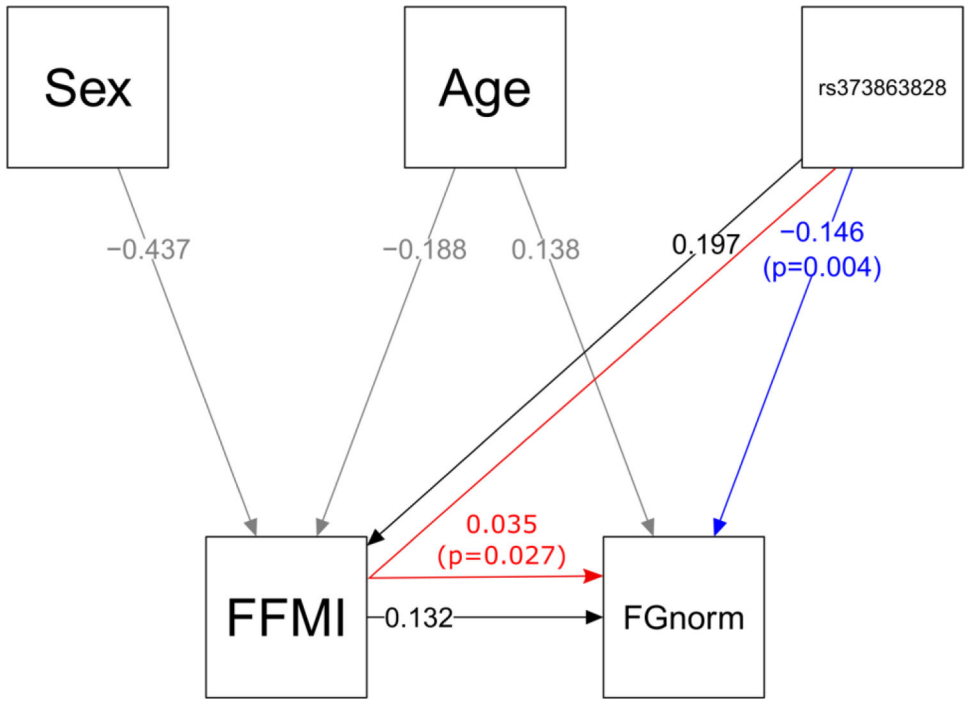

Figure 3.

Path diagram examining the mediating role of fat free mass index (FFMI) on the relationship between rs373863828 and inverse-normally-transformed fasting glucose (FGnorm). Standardized coefficients are plotted along each path. The direct effect of rs373863828 on FGnorm is shown in blue, the indirect (mediated by FFMI) in red.

Table 1:

Age, body size, body composition, and fasting glucose by genotype

	Female					Male					Combined *		Sex-by-genotype interaction
	GG	AG	AA	β [95% CI]	*p*	GG	AG	AA	β[95% CI]	*p*	β[95% CI]	*p*	*p*
	(n=103)	(n=92)	(n=38)			(n=85)	(n=69)	(n=34)					
Age (years)	50.84 (9.48)	50.63 (9.76)	50.03 (8.96)	−0.36[−2.05, 1.32]	0.67	53.14 (10.27)	53.68 (9.34)	49.04 (11.01)	−1.59 [−3.54 0.36]	0.11	−0.93 [−2.20, 0.34]	0.15	0.35
Body Mass Index (kg/m²) [a]	35.83 (6.39)	37.09 (6.24)	40.29 (9.22)	1.95 [0.76, 3.14]	**0.0014**	32.65 (6.44)	33.16 (5.71)	33.95 (5.92)	0.60 [−0.58, 1.79]	0.32	1.31 [0.46, 2.16]	**0.0025**	0.09
Weight (kg) [a]	94.20 (19.26)	97.28 (17.47)	106.54 (24.87)	5.21 [1.93, 8.50]	**0.002**	95.67 (21.24)	99.42 (18.13)	103.84 (19.76)	3.67 [−0.16 7.50]	0.06	4.43 [1.93, 6.92]	**0.0005**	0.45
Height (cm) [a]	161.82 (5.63)	161.83 (5.30)	162.64 (6.61)	0.24 [−0.70, 1.17]	0.62	170.90 (6.36)	172.95 (6.02)	174.77 (5.45)	1.71 [0.59, 2.83]	**0.003**	0.9 [0.18, 1.62]	**0.014**	0.05
Total Fat Mass (kg) [b]	40.93 (12.62)	42.62 (11.05)	47.02 (15.76)	2.51 [0.37, 4.65]	**0.02**	29.09 (12.71)	29.78 (11.66)	30.32 (13.04)	−0.11 [−2.54, 2.31]	0.93	125 [−0.35, 2.85]	0.13	0.07
Total Lean Mass (kg) [b]	50.27 (7.43)	51.71 (7.50)	55.73 (8.29)	2.16 [1.09, 3.23]	**0.0001**	63.22 (9.42)	66.21 (8.23)	70.08 (8.08)	1.73 [0.30, 3.15]	**0.02**	2.00 [1.14, 2.86]	**0.000007**	0.68
Total Bone Mass (kg) [b]	2.53 (0.41)	2.52 (0.40)	2.66 (0.36)	0 03 [−0.02, 0.07]	0.23	3.25 (0.46)	3.35 (0.44)	3.49 (0.44)	0.02 [−0.05, 0.09]	0.51	0.03 [−0.01, 0.07]	0.19	0.87
Visceral Fat Mass (kg) [b]	1.33 (0.57)	1.47 (0.63)	149 (0.63)	0.09 [−0.02, 0.19]	0.10	1.86 (1.08)	1.99 (1.11)	1.78 (1.07)	−0.01 [−0.22, 0.21]	0.96	0.04 [−0.07, 0.15]	0.45	0.35
Missing	0	0	0			0	1	0					
Percent Fat (%) [b]	42.80 (6.01)	43.46 (4.96)	43.63 (5.66)	0 46 [−0.53, 1.45]	0.36	29.21 (7.28)	29.06 (7.11)	28.11 (7.54)	−0.38 [−1.82, 1.06]	0.60	0.06 [−0.78, 0.90]	0 89	0.28
Fat Mass Index (FMI, kg/m²) [a]	15.57 (4.50)	16.27 (4.20)	17.79 (5.96)	0.99 [0.17, 1.81]	**0.02**	9.91 (4.08)	9.98 (3.85)	9.89 (4.08)	0.03 [−0.75, 0.80]	0.94	0.53 [−0.04, 1.10]	0 07	0.07
Fat-Free Mass Index (FFMI, kg/m²) [a]	20.10 (2.31)	20.66 (2.46)	22.07 (2.97)	0.86 [0.44, 1.28]	**0.0001**	22.70 (2.67)	23.23 (2.33)	24.08 (2.42)	0.61 [0.13, 1.09]	**0.01**	0.74 [0.42, 1.06]	**0.000005**	0.39

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	Female					Male					Combined*		Sex-by-genotype interaction
	GG	**AG**	**AA**	**β [95% CI]**	***p***	**GG**	**AG**	**AA**	**β[95% CI]**	***p***	**β[95% CI]**	***p***	***p***
	(n=103)	**(n=92)**	**(n=38)**			**(n=85)**	**(n=69)**	**(n=34)**					
Android Fat Mass (kg) [b]	3.72 (1.281	3.94 (1.22)	4.43 (1.63)	0.31 [0.08, 0.53]	**0.009**	3.01 (1.64)	3.14 (1.48)	3.03 (1.57)	−0.03 [−0.34, 0.28]	0.83	0.15 [−0.04, 0.33]	0.12	0.06
Gynoid Fat Mass (kg)[b]	6.14 (2.08)	6.33 (1.79)	6.91 (2.72)	0.30 [−0.04, 0.65]	0.09	4.22 (1.87)	4.24 (1.79)	4.39 (1.92)	−0.07 [−0.43, 0.29]	0.70	0.12 [−0.13, 0.37]	0.34	0.1
Android:Gvnoid Fat Ratio[b]	0.62 (0.12)	0.63 (0.11)	0.66 (0.14)	0.02 [−0.00, 0.04]	0.07	0.68 (0.16)	0.72 (0.17)	0.67 (0.17)	0.02 [−0.02, 0.05]	0.34	0.02 [−0.00, 0.03]	0.08	0.63
Trunk Fat Mass (kg) [b]	21.65 (6.58)	22.78 (6.02)	25.12 (8.33)	1.50 [0.35, 2.66]	**0.01**	16.80 (7.97)	17.46 (7.09)	17.09 (7.71)	−0.11 [−1.61, 1.39]	0.88	0.75 [−0.17, 1.67]	0.11	0.07
Peripheral Fat Mass (kg)[b,c]	18.17 (6.32)	18.69 (5.36)	20.68 (7.85)	0.97 [−0.08, 2.01]	0.07	11.15 (4.75)	11.17 (4.72)	12.02 (5.33)	−0.02 [−0.96, 0.93]	0.97	0.47 [−0.25, 1.19]	020	0.11
Trunk-to-Peripheral Fat Ratio[b]	1.23 (0.23)	1.24 (0.19)	1.26 (0.25)	0.02 [−0.02, 0.06]	0.34	1.47 (0.28)	1.56 (0.28)	1.41 (0.28)	0.01 [−0.04, 0.07]	0.62	0.01 [−0.02, 0.05]	0.36	0.81
Fasting Glucose (mg/dL) [a]	132.87 (72.00)	113.68 (40.86)	105.51 (25.16)	−14.57 [−24.86, −4.29]	**0.006**	124.13 (58.18)	117.05 (47.65)	106.81 (16.64)	−7.08 [−16.87, 2.72]	0.16	−11.1 [−18.18, −4.01]	**0.002**	0.31
Excluded[d]	11	14	2			5	4	2					

Values are mean (standard deviation). Beta values [95% confidence intervals] are the estimated difference in mean per copy of the minor (A) allele (between AA and AG and GG) from a linear model.

[a] Linear models were adjusted for age and age^2

[b] Linear models were adjusted for age , age^2, and height

[c] Pheripheral fat mass was calculated from the sum of limb (arm and leg) mass

[d] Individuals reporting diabetes diagnosis and/or taking medication for diabetes excluded

* all combined models were adjusted for sex

Table 2.

Path analysis results for examining the mediating role of fat mass index (FMI) on the relationship between rs373863828 and inverse-normally-transformed fasting glucose (FGnorm). Columns are Outcome (Y), Predictor (X), effect estimate (β), standard error (SE), standardized effect estimate ($\beta^* = \frac{s_x}{s_y}\beta$), and p-value (*p*).

Y	**X**	**β**	**SE**	**β***	***p***
FMI	Sex	6.617	0.452	0.601	1.30E-48
FMI	Age	−0.017	0.023	−0.031	4.51E-01
FMI	rs373863828	0.569	0.300	0.077	5.79E-02
FGnorm	FMI	0.023	0.009	0.126	1.30E-02
FGnorm	rs373863828 direct	−0.172	0.067	−0.128	1.07E-02
FGnorm	Age	0.014	0.005	0.137	6.67E-03
FGnorm	rs373863828 indirect (through FMI)	0.013	0.009	0.010	1.32E-01

Table 3.

Path analysis results for examining the mediating role of fat free mass index (FFMI) on the relationship between rs373863828 and inverse-normally-transformed fasting glucose (FGnorm). Columns are Outcome (Y), Predictor (X), effect estimate (β), standard error (SE), standardized effect estimate ($\beta^* = \frac{s_x}{s_y}\beta$), and p-value (*p*).

Y	X	β	SE	β*	*p*
FFMI	Sex	−2.458	0.253	−0.437	2.49E-22
FFMI	Age	−0.054	0.013	−0.188	2.98E-05
FFMI	rs373863828	0.744	0.168	0.197	9.51E-06
FGnorm	FFMI	0.047	0.018	0.132	1.06E-02
FGnorm	rs373863828 direct	−0.195	0.068	−0.146	4.35E-03
FGnorm	Age	0.014	0.005	0.138	6.25E-03
FGnorm	rs373863828 indirect (through FFMI)	0.035	0.016	0.026	2.70E-02