HHS Public Access

Author manuscript

Diabetologia. Author manuscript; available in PMC 2019 July 01.

Published in final edited form as:

Diabetologia. 2018 July ; 61(7): 1603–1613. doi:10.1007/s00125-018-4623-1.

Discordant association of the *CREBRF* rs373863828 A-allele with increased body mass index and protection from type 2 diabetes in Māori and Pacific (Polynesian) people living in Aotearoa New Zealand

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Contribution statement

MK, TJM, PRS, RM, TRM contributed to the design of the study. OD, LM, JdZ, LKS, ND, JHH, NR, TN, MSR, RD, STM, SV contributed to data collection and RKT, LY, JMDT, WWHE, DEW, RLM, PW, DG, ANS to data analysis and interpretation. MK, RM, TRM drafted the manuscript and all other authors reviewed it. The manuscript was approved by all authors. TRM is the guarantor of this work.

Text for tweet: New research from the #MauriceWilkinsCentre shows a Pacific-specific CREBRF #gene variant protects against #type2diabetes in #IndigenousMāori #CookIslandMāori #Samoan #Tongan & #Niuean people in #NewZealand @HRCNewZealand @Otago @AucklandUni @Pasifik_Ocean

Data availability

The dataset generated during the current study is not publicly available owing to consent restrictions but can be requested from the corresponding author under an appropriate arrangement.

Duality of interest

The authors declare no duality of interest relevant to the material presented herein.

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Abstract

Aim/Hypotheses—The A (minor) allele of *CREBRF rs373863828* associates with increased body mass index (BMI) and reduced risk of type 2 diabetes (T2D) in the Samoan population of Samoa and American Samoa. Our aim was to test *rs373863828* for association with BMI and odds of T2D, gout and chronic kidney disease (CKD) in Māori and Pacific (Polynesian) people living in Aotearoa New Zealand.

Methods—Association analyses were performed by linear and logistic regression with BMI, log-transformed BMI, waist circumference, T2D, gout and CKD in 2,286 adults. The primary analyses were adjusted for age, sex, the first four genome-wide principal components, and (when appropriate) BMI, waist circumference and T2D. The primary analysis was conducted in ancestrally-defined groups and association effects combined by meta-analysis.

Results—For the A-allele of *rs373863828* the effect size for log-transformed BMI was 0.038 (95% CI [0.022, 0.055], p=4.8×10^{-6}) and for T2D was OR=0.59 (95% CI [0.47, 0.73], p=1.9×10^{-6}). There was no evidence for association of genotype with variance in BMI (p=0.13). Nor was there evidence for association with serum urate (β=0.012 mmol/l, $p_{corrected}$=0.10), gout (OR=1.00, p=0.98) or CKD (OR=0.91, p=0.59).

Conclusions/interpretation—Our results replicated, with very similar effect sizes, association of the A-allele of *rs373863828* with higher BMI but lower odds of T2D among New Zealand Polynesian adults, as in Samoan adults living in Samoa and American Samoa.

Keywords

association; BMI; CREBRF; genetic Maori; obesity; Pacific; Polynesian; type 2 diabetes

Introduction

A missense variant (*rs373863828*, p.(Arg457Gln)) in *CREBRF* (encoding CREB3 regulatory factor) has been associated with body mass index (BMI) in the Samoan and American Samoan populations [1]. The minor allele (c.1370A p.(457Gln)) associated with a 1.36kg/m^2 higher BMI (~4kg in body weight, at 1.7m tall),[1, 2]. In a small sample from the Kingdom of Tonga the A (minor) allele associated with a greater BMI of 3.1kg/m^2 [3]. The high A-allele frequency of *rs373863828* among Samoans in Samoa and American Samoa (0.26) and in Tonga (0.15) [1, 3], compared to an exceedingly rare frequency in the Genome Aggregation Database (www.gnomad.broadinstitute.org accessed 1 October 2017; 5.3×10^{-5} in East Asian, 3.3×10^{-5} in South Asian, 4.0×10^{-5} in European, absent in ~12K African individuals) [4], supports the hypothesis that *rs373863828* is an important risk factor for obesity unique to Samoa and Tonga and possibly other Polynesian populations.

Unlike most obesity risk variants in other populations, the BMI-increasing A-allele of *rs373863828* associates with lower odds of type 2 diabetes (OR=0.59 to 0.74 after adjustment for BMI) [1]. This is contrary to the established observational positive association between type 2 diabetes and increased BMI. However, "favourable adiposity" genetic variants have recently been identified, which associate with higher BMI but lower risk of type 2 diabetes, hypertension and coronary artery disease in European populations [5–7]. For example, a genetic score of eleven such alleles was associated with higher BMI (+0.12kg/m^2) and higher body fat percentage (+0.30%) [8]. However, for a given BMI, individuals carrying these alleles were at reduced odds for type 2 diabetes (OR=0.84), hypertension (OR=0.94) and heart disease (OR=0.92) [8]. These genetic variants are hypothesised to promote energy storage by expanding favourable subcutaneous adipose tissue depots, resulting in less accumulation of fat in non-adipose tissues with a reduction in risk of type 2 diabetes and other metabolic consequences of insulin resistance [8,9]. In contrast, aside from increasing BMI and both decreasing fasting glucose and the risk of type 2 diabetes the *CREBRF rs373863828* A-allele does not associate with other features of insulin resistance such as hypertension, lipids or homeostatic model assessment of insulin resistance [1].

In Aotearoa New Zealand, obesity and type 2 diabetes are both very prevalent in Māori and Pacific people [9] and associate with other very prevalent metabolic-based conditions, in particular gout and chronic kidney disease (CKD) [10–12]. Moreover increased BMI is causal of increased urate [13] and diabetic kidney disease is ascribed as the cause of renal failure in 69% of Polynesian people [14]. *CREBRF rs373863828* has not previously been tested for association with gout or CKD [1]. Polynesian populations include those from West Polynesia (Samoa, Tonga, Niue and Tokelau) and East Polynesia (Aotearoa New Zealand Māori and Cook Island Māori). Given different pathogenic allele frequencies and different linkage disequilibrium structure between East and West Polynesian populations [15, 16], investigating *CREBRF rs373863828* in other Polynesian population groups may provide further insights into its association with increasing BMI, yet apparent reduction in risk of type 2 diabetes.

We tested for association of *CREBRF rs373863828* with BMI, waist circumference, type 2 diabetes, gout and CKD in people of Polynesian ancestry living in Aotearoa New Zealand. In addition, based on association of *FTO* genotype with variance in BMI in Europeans [17], we tested the association of *rs373863828* with variance in log-transformed BMI to detect possible underlying genetic and/or environmental influences in phenotypic variability.

Methods

Study population

Individuals, primarily from the Auckland, Waikato, and Christchurch regions of Aotearoa New Zealand ≥16, were recruited as participants of the Genetics of Gout, Diabetes and Kidney Disease in Aotearoa New Zealand Study [18]. A separate Māori sample-set from the *rohe* (area) of the Ngāti Porou *iwi* (tribe) of the Tairāwhiti (East Coast of the North Island) region was also included in the Aotearoa New Zealand Māori analysis. This sample-set was recruited in collaboration with Ngati Porou Hauora (Health Service) Charitable Trust. A

Pukapuka Island sample-set was recruited in collaboration with the Pukapuka Community of New Zealand Inc. in Mangere, South Auckland.

Information obtained at recruitment included age (years), sex, height (cm), weight (kg) and waist circumference (cm) measured by trained assessors. BMI was calculated by dividing weight by the square of height in metres. Participants were also asked the ancestry of each of their grandparents. Type 2 diabetes was ascertained by physician-diagnosis and/or participant reports and/or use of glucose lowering therapy. Biochemical measurements were performed at the Southern Community Laboratories (www.sclabs.co.nz). Estimated glomerular filtration rates (eGFR) were derived from participants' serum creatinine, age and sex using the Chronic Kidney Disease Epidemiology Collaboration equation [19]. Stage 4 and 5 CKD was defined by eGFR <30ml/min/1.73m^2. Obesity was defined as BMI >32 kg/m^2 [20]. Ethical approval was given by the NZ Multi-Region Ethics Committee (MEC/05/10/130; MEC/10/09/092; MEC/11/04/036) and the Northern Y Region Health Research Ethics Committee (NPHCT study; NTY07/07/074). All participants provided written informed consent for the collection of samples and subsequent analysis.

Participants were separated into sample-sets based on self-reported Pacific nation of ancestry of their grandparents. Those participants who also reported non-Polynesian ancestry were grouped according to their Polynesian ancestry. This resulted in seven sample-sets; Aotearoa New Zealand Māori (*n*=1,296, including 270 people from the Ngāti Porou Hauora Charitable Trust study), Cook Island Māori (*n*=205), Samoan (*n*=387), Tongan (*n*=181), Niuean (*n*=47), Pukapukan (*n*=75) and an 'Other' Polynesian group (*n*=271), which included individuals of Tahitian (*n*=3), Tokelauan (*n*=6) and Tuvaluan (*n*=5) ancestry, along with individuals who self-reported grandparental ancestry from more than one Pacific nation (*n*=257). Pukapuka is part of the Cook Islands but geographically located within West Polynesia. These analysis groups were further refined based on clustering of genome-wide principal component vectors one to four (details of calculation below), resulting in the exclusion of 176 people (ESM Figure 1) for one of three reasons. One, a clear mismatch between self-reported and principal component-defined ancestry (n=16; potentially indicating an error in DNA or phenotype sample ID alignment during processing); two, clustering outside of any of the principal component clusters (n=26); and three, individuals self-reported as Aotearoa New Zealand Māori and Cook Island Māori who clustered within the European and between the European and Eastern Polynesian clusters (n=135). The final groups used in all analyses were; Aotearoa New Zealand Māori (*n*=1,154), Cook Island Māori (*n*=197), Samoan (*n*=378), Tongan (*n*=175), Niuean (*n*=47), Pukapukan (*n*=70) and the 'Other' Polynesian group (*n*=265). Baseline characteristics for the final groupings are presented in Table 1.

Whole genome Illumina Infinium CoreExome

The Illumina Infinium CoreExome v24 bead chip platform was used to genotype participants for ~500,000 variants across the whole-genome. Bead chip genotyping batches were auto-clustered using GenomeStudio v2011.1 software (Illumina, San Diego, CA, United States). The Illumina GenomeStudio best practice guidelines and other quality

control protocols were applied [21, 22]. The genotyping batches were then merged and relevant quality control steps repeated in the full dataset.

Determination of principal components

Whole-genome principal component analysis vectors were calculated using a subset of 2,858 ancestry informative markers (as identified by Illumina) extracted from the CoreExome whole-genome genotypes. The SmartPCA (EIGENSOFT v6.0.1) [23]) program was used, with an output of 10 eigenvectors, no outlier removal, and no population size limit. Individuals of non-Polynesian ancestry were included, and the first four vectors plotted against each other to view the clustering of ancestral groupings (Asian, European, Eastern Polynesian, and Western Polynesian) (ESM Figure 1). The first four vectors, explaining 97.1% of the proportion of the variability within the sample-sets, were included as covariates in the linear and logistic regression to account for population stratification and cryptic relatedness.

CREBRF rs373863828 genotyping

Rs373863828 was directly genotyped because this variant was not present on the CoreExome platform and we were unable to impute the region owing to the unavailability of Māori and Pacific reference haplotypes. A custom designed TaqMan™ probe-set (Applied Biosystems, Foster City, CA, United States) was created for *rs373863828* using a custom Python script (snp_design; DOI: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 Polymerase Chain Reaction System (Roche Applied Science, Indianapolis, IN, United States) in 384-well plates. There was a 99% successful genotyping call rate. Re-genotyping of 25% of the sample-set demonstrated 100% concordance.

Association testing

Analyses were performed using the R v3.3.2 statistical software within RStudio v0.99.902 (https://www.rstudio.com). A multivariable linear regression model was used to test for association between the *rs373863828* minor allele (c.1370A p.(457Gln)) and the continuous variables log-transformed BMI (a Box-Cox normality plot for the BMI data yielded $\lambda = -0.39$), untransformed BMI, waist circumference and serum urate), with the ß-coefficient representing the estimated effect of each copy of the *rs373863828* A-allele. For binary outcomes (obesity, type 2 diabetes, gout and CKD), a multivariable binomial logistic regression model was used in a similar manner, with the allelic odds ratio (OR) representing the estimated effect of each copy of the A-allele. All analyses were adjusted for sex, age, the first four whole-genome principal component vectors, and BMI, type 2 diabetes or waist circumference where appropriate. Analyses were also performed in groups stratified by type

2 diabetes status. Each Polynesian population sample-set was analysed separately, and the effects combined using an inverse-variance-weighted fixed effect meta-analysis. Heterogeneity between sample-sets was assessed during the meta-analysis using Cochran's heterogeneity (Q) statistic, with a random-effects analysis used when there was evidence for heterogeneity ($p<0.05$). For the BMI, waist circumference and type 2 diabetes association analyses $p<0.05$ was set as the significance value, given the prior probability of detecting association [1]. For the other outcomes (n=4; serum urate, gout, CKD, variance in BMI) $p<0.0125$ was set as the significance value, accounting that none of these outcomes had previously been tested for association with *rs373863828* [1].

The association analyses for the major outcomes (BMI, log-transformed BMI and type 2 diabetes) were repeated using two different approaches that account for relatedness within the analysis group. First, a kinship coefficient matrix was calculated in PLINK v1.9 using 40,156 independent autosomal markers in the pooled dataset. Participant pairs with a kinship coefficient ($\hat{\pi}$) ≥0.125 (equivalent to first cousins or closer) were identified and one individual excluded (at random) to create a maximal set of unrelated individuals (n=1,665) for analysis. Multivariate linear (or logistic) regressions testing the *CREBRF rs373863828* association were done in this group, adjusting for sex, age, the first four principal component vectors, membership of the seven previously described ancestral groups, and BMI, type 2 diabetes or waist circumference where appropriate. Second, for BMI a linear mixed model regression analysis, which included the kinship coefficient matrix calculated above to adjust for relatedness, was performed in the entire analysis group (not excluding related individuals) using GenABEL [24,25] and the same adjusting variables as above.

Models of inheritance were investigated by formulating the genotype predictor in the linear and logistic regression models (adjusting by age, sex, principal components 1–4) in different ways: additive model (0, 1, 2) - one OR; dominant model (0, 1, 1) - one OR; or recessive model (0, 0, 1) - for each model the coded genotypes were treated as a continuous variable, producing a single effect estimate (ß or OR). A model selection tool (Akaike Information Criterion [26]) was used to select the most likely model. The smaller AIC value indicates the best model, but where the difference was less than two the simplest model was chosen.

Power

Based on estimates from ref. [1] and $\alpha=0.05$ the power to detect an effect size of 1.36 kg/m^2 per A-allele was >80% in the combined sample-set for a minor allele frequency ≥0.15 (ESM Figure 2). The power to detect a moderate protective effect for type 2 diabetes (OR=0.59) of the minor allele was >90% for a minor allele frequency ≥0.10 (ESM Figure 2). Power calculations for gout and CKD as outcomes with $\alpha=0.0125$ showed that power was adequate only to detect effect sizes of OR≥1.75 for gout and CKD and ≥0.032 mmol/l for serum urate (ESM Figure 2).

Testing for association of rs373863828 with variance in log-transformed BMI

Association of a genetic variant with variance in phenotype can detect a locus interacting in a non-additive way without prior knowledge of the interacting factor (environmental, intrinsic, genetic). Testing for association of *rs373863828* with variance in log-transformed

BMI was performed as previously described [27]. The variable used as a measure of variance was produced from residuals obtained from cohort-specific analyses regressing age, age^2 and age-by-sex. An independent ranked inverse normal transformation of absolute residuals generated z-scores, with squared z-scores (z^2) being the variance variable. To account for the influence of *rs373863828* mean-effect on the variance, the mean log-transformed BMI (per genotype) was subtracted from the log-transformed BMI of each participant and the z-scores re-calculated. Linear models associating *CREBRF* genotype with both the unadjusted and mean-effect adjusted variance z-scores were performed (Equation 1). This analysis was also done on the Samoan *rs373863828* genotype data [1], with age, age^2, age-by-sex and polity as the adjusting variables in the z-score calculation steps. The Aotearoa New Zealand sample-sets and the two Samoan sample-sets [1] were combined by an inverse-variance weighted fixed-effect meta-analysis.

Equation 1: A) unadjusted variance analysis. B) mean-effect adjusted variance analysis.

$$\text{A.}$$

$$\log(\text{BMI}) \text{, adjusting variables} + \text{residuals}$$

$$\text{unadjusted } z^2 = \left(\text{ranked inverse-normal transformed residuals}\right)^2$$

$$z^2, \textit{rs}373863828$$

$$\text{B.}$$

$$\log(\text{BMI} - \text{mean}(\text{BMI per } \textit{rs}373863828 \text{ genotype})) \text{, adjusting variables} + \text{residuals}$$

$$\text{adjusted } z^2 = \left(\text{ranked inverse-normal transformed residuals}\right)^2$$

$$z^2, \textit{rs}373863828$$

Results

Prevalence of rs373863828

The allele and genotype frequencies of *rs373863828* in each Polynesian sample-set are presented in Table 1 and Figure 1. There was no evidence for deviation from Hardy Weinberg equilibrium in the various sample-sets (Table 1; p for all datasets was ≥0.049). The relative frequencies of the minor (c.1370A) allele differed among the Polynesian groups, with the Samoan (MAF=0.236) and Pukapukan (MAF=0.243) groups exhibiting the highest frequency and the Niuean (MAF=0.096) group exhibiting the lowest frequency.

Association analysis with adiposity measures

A fixed-effect meta-analysis of the Polynesian samples showed significant association of *rs373863828* with log-transformed BMI (ß=0.038, $p=4.8\times10^{-6}$) with no evidence for heterogeneity between sample sets (p=0.19) (Tables 2 and ESM 1; Figure 2). Association analysis with untransformed BMI revealed similar results (Tables 2 and ESM 2; Figure 2). Analysis of the pooled dataset removing related individuals yielded very similar effect sizes (ß=0.037 [95% CI: 0.017, 0.057], $p=2.3\times10^{-4}$ for log-transformed BMI and ß=1.38 [0.66, 2.09], $p=1.6\times10^{-4}$ for untransformed BMI). Analysis adjusting for relatedness using

GenABEL also yielded very similar effect sizes (ß=0.038 [0.021, 0.054], p=1.2×10^{-5} for log-transformed BMI and ß=1.34 [0.73, 1.94], p=1.6×10^{-5} for untransformed BMI). The proportion of variance explained by *rs373863828* in the pooled dataset was 1.1%. One copy of the minor allele was sufficient to confer the effect (Table 2; ß=1.80 for the heterozygote group and ß=1.49 for the A-allele homozygote group compared to the major allele homozygotes in untransformed BMI analysis). This was supported by Akaike Information Criteria analysis where the dominant model had a difference of 3.3 less than the additive model. In the combined group there was association with higher odds of obesity (Table 2; OR=1.33, p=8×10^{-4} for >32 kg/m^2 and OR=1.54, p=1×10^{-5} for >40 kg/m^2). There was significant association with untransformed BMI in both the type 2 diabetes-positive and –negative groups (ß=1.62, p=2.5×10^{-3} and ß=1.21, p=5.0×10^{-4}, respectively, with no evidence for heterogeneity in effect size (p_{Het}=0.48)), indicating no evidence for a type 2 diabetes-specific effect on BMI. There was no indication of sex-specific effects (Table 2, Figure 3) and a sex-by-*rs373863828* interaction analysis of the pooled sample-set showed no evidence of interaction with BMI (p=0.67).

A fixed effect meta-analysis showed evidence of association between *rs373863828* and increased waist circumference in the full Polynesian sample-set (ß=2.98 cm per minor allele, p=1.4×10^{-5}) with no evidence for heterogeneity between sample-sets (p=0.089) (Figure 2; ESM Table 3**)**. Adjustment of the waist circumference analysis by BMI abrogated the association with waist circumference (Table 2; ß lowered from 2.98 to 0.66, p=0.092).

Association analysis with type 2 diabetes

A fixed-effect meta-analysis of the various sample sets revealed significant association with reduced odds of type 2 diabetes (OR=0.65, p=3.4×10^{-5}) that was strengthened after adjustment by BMI (OR=0.59, p=1.9×10^{-6}) with no evidence for heterogeneity between sample sets (p=0.069) (Tables 2 and ESM 4; Figure 4). Association analysis of the pooled dataset removing related individuals yielded very similar effect sizes (OR=0.56 [95% CI: 0.43, 0.72], p=7.9×10^{-6}). Similar to the observation for BMI, one copy of the minor allele appeared to be sufficient to confer the effect (Table 2: OR=0.55 for the heterozygote group compared to the major allele homozygote group). The Akaike Information Criteria analysis showed the dominant model to be similar to the additive model (0.21 less) thus the mode of inheritance for type 2 diabetes was concluded to be more consistent with an additive model.

A fixed effect meta-analysis that included data presented here, data from the Samoan [1] and Tongan [3] studies and that indicated no evidence for heterogeneity yielded for BMI β=1.43 [95% CI: 1.17,1.68], p=3.8×10^{-28} and for type 2 diabetes OR=0.62 [0.55,0.70], p=1.7×10^{-14}.

Association analysis of rs373863828 with serum urate, gout and CKD

We tested for association with serum urate (Table 2; ESM Figure 3). Unadjusted there was evidence for association of the A-allele with higher levels (ß=0.015 mmol/l, P=0.005, $p_{corrected}$=0.020), however this effect was mitigated after adjusting for BMI (ß=0.012 mmol/l, P=0.026, $P_{corrected}$=0.10). Similarly there was no evidence for association with gout (OR=1.00, p=0.98) (Tables 2 and ESM 5; ESM Figure 3). There was no statistically

significant (p<0.0125) evidence for association with CKD either before (OR=0.72, p=0.030, $p_{corrected}$=0.12) or after adjustment by type 2 diabetes and BMI (OR=0.91, p=0.59) (Tables 2 and ESM 6, ESM Figure 3). Excluding individuals with type 2 diabetes from the CKD analysis did not provide evidence for association with CKD in a single analysis of all samples pooled and adjusted for the first 4 PCA vectors, age, sex and BMI (OR=1.07 [0.65, 1.69], p=0.79). Finally, analysis in type 2 diabetes-positive and –negative groups yielded evidence for association only for serum urate in the type 2 diabetes-negative group (Table 2). However there was no evidence for a difference in effect size between the type 2 diabetes-positive and –negative groups for serum urate (p>0.13).

Association analysis of rs373863828 with variance in phenotype

The *rs373863828* variant was not significantly associated with log-transformed BMI variance at the *CREBRF* locus for any of the New Zealand sample-sets nor the two previously published Samoan cohorts [1]. Fixed effect meta-analysis of the New Zealand (n=2282), 1990s Samoan (n=1020) and discovery Samoan (n=1876) cohorts showed no evidence of association of *rs373863828* with variance in log-transformed BMI in either the unadjusted (ß=−0.053, p=0.15) or adjusted models (ß=−0.047, p=0.15) (ESM Table 7, ESM Figure 4).

Discussion

We confirmed the association of *CREBRF rs373863828* with higher BMI (1.38 kg/m^2 per minor allele), and higher waist circumference (2.98 cm), but lower risk of type 2 diabetes (OR=0.59) in adults of Polynesian (Māori and Pacific) ancestry living in Aotearoa New Zealand. These results are very similar to that of Samoans living in Samoa and American Samoa, where each copy of the minor allele associated with a 1.36 kg/m^2 increase in BMI and an OR for type 2 diabetes of 0.59 [1]. The percent variation in phenotype explained was also similar (1.9% in Samoan discovery sample, 1.1% in Samoan replication sample vs 1.1% in Aotearoa New Zealand), suggesting that the main effect of the rs373863828 A-allele is relatively impervious to environment. Consistent also with the Samoan data [1], adjustment by BMI strengthened the association with type 2 diabetes in the Aotearoa New Zealand sample set (Table 2; OR=0.65 to 0.59; Samoan discovery OR=0.64 to 0.59 [1]; Samoan replication OR=0.83 to 0.74 [1]). However, adjustment by BMI diminished the association with waist circumference (Table 2), indicating that the waist circumference association was driven by overall body mass distribution rather than central adiposity.

Precisely how these genetic epidemiological findings relate to the actual CREBRF-mediated molecular pathogenesis of obesity and type 2 diabetes is unclear in the absence of detailed knowledge of the molecular pathways involving CREBRF and in the absence of genetic association data with detailed body composition measures as outcome. Most population genetic variants associated with generalised obesity also associate with insulin resistance, hypertension, dyslipidemia and type 2 diabetes compatible with the degree of adiposity. However, there are some genetic variants which are associated with higher BMI and percentage body fat, but lower odds of type 2 diabetes along with lower insulin resistance, hypertension, circulating triglycerides, and LDL-cholesterol. These variants are also known

as ‘favourable adiposity’ variants [8] due to higher subcutaneous-to-visceral adipose tissue that suggests preferential fat storage away from visceral organs. The association of the A-allele of *rs373863828* with a higher BMI but reduced odds of type 2 diabetes is not entirely compatible with ‘favourable adiposity’, due to the lack of association with hypertension or lipids [1]. Consistent with the reduced odds of type 2 diabetes there was weak association of the A-allele with increased insulin sensitivity by homeostatic model assessment insulin resistance in the Samoan and American Samoan populations [1] (we did not have HOMA-IR data available). Cellular bioenergetics models show that the *rs373863828* A-allele promotes lipid and triacylglycerol storage at a reduced energy cost in the adipocyte suggesting that the metabolic activity of *CREBRF* in fat is important [1]. Detailed clinical studies are required to clarify whether visceral and subcutaneous body fat storage depots are altered among carriers of this variant.

It is notable that, from what is understood about the physiological role of CREBRF, there is no obvious role in regulation of appetite, which is seen at *FTO-IRX3* and other loci regulating BMI in Europeans. However, *CREBRF* is widely expressed (www.gtex.org), including throughout the brain and the *CREBRF* gene is known to regulate the CREB3/Luman protein, which is localised to the endoplasmic reticulum and plays an important role in axonal regeneration [28]. Interestingly, the CREB3/Luman protein was identified through its association with herpes simplex virus-related host cell factor 1, which has led to the hypothesis that Luman may play a role in the viral emergence from latency [29]. It will be important to explore the relationship between *CREBRF* expression in the hypothalamic nuclei and the role of CREB3/Luman in the intra–axonal translation and retrograde trafficking to promote neuronal survival in response to viral stimuli and how this may relate to BMI and type 2 diabetes.

Very recently a study was published reporting association of the *CREBRF rs373863828* A-allele with higher BMI in a pooled multi-ethnic sample of 4,572 New Zealand children of Māori, Pacific, European and Asian ethnicity [30]. Given that the association analysis was pooled and population-specific association analyses were not reported we are unable to directly compare our data with that study. The A-allele was reported at a prevalence of 0.015 in European children and 0.011 in Asian children [30], contrary to the very low prevalence in the Genome Aggregation Database (MAF=5.3×10^{-5} in East Asian, 3.3×10^{-5} in South Asian, 4.0×10^{-5} in European).

Our study has confirmed in adults of Māori and Pacific (Polynesian) ancestry living in Aotearoa New Zealand that the presence of each additional *CREBRF rs373863828* A-allele associates with increased BMI yet reduced odds of type 2 diabetes. While the prevalence of both obesity and type 2 diabetes is increased amongst New Zealand Māori and those of Pacific ethnicity [9], compared to New Zealand Europeans, our study confirms that Pacific-specific genetic variation underpins some of the inter-individual heterogeneity observed in the discordant manifestation of obesity and type 2 diabetes. This study supports the need to conduct comprehensive gene-phenotype studies in populations currently under-represented in genomic studies, in which different genetically segregating pathways linking obesity and type 2 diabetes clearly exist. Such studies are not only important for these populations *per se*, but are also important in illuminating the molecular biology of the pathogenesis of

metabolic disease in the wider human population and have the potential to lead to novel clinical interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors sincerely thank participants for generously donating their time and information to this study. The authors would like to thank J Drake (Department of Rheumatology, Canterbury District Health Board, New Zealand, J de Kwant, R Laurence, C Franklin, M House (all Department of Medicine, University of Auckland, New Zealand), N Aupouri, R Akuhata, Carol Ford (all of Ngati Porou Hauora Charitable Trust, Gisborne, New Zealand) and G Sexton (Counties Manukau District Health Board, Auckland, New Zealand) for recruitment.

Funding

The Health Research Council of New Zealand (Grant #'s 08/075, 10/548, 11/1075, 14/527) and the Maurice Wilkins Centre funded the New Zealand component, and the National Institute of Health funded the Samoa and American Samoa component (Grant # R01-HL093093 and R01-HL133040).

Abbreviations

AIC	Akaike Information Criteria
CKD	chronic kidney disease
CREBRF	cAMP-responsive element binding protein 3 regulatory factor
FTO	fat mass and obesity-associated
IRX3	iriquois-class homeodomain 3
MAF	minor allele frequency

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Research in context

What is already known about this subject?

- The A (minor) allele of *CREBRF rs373863828* (p.Arg457Gln) associates with increased body mass index yet reduced risk of type 2 diabetes in Samoans living in Samoa and American Samoa and with increased body mass index in Tongans living in the Kingdom of Tonga.
- The A-allele of *CREBRF rs373863828* is essentially unique to the Pacific population.

What is the key question?

- Does the A-allele of *rs373863828* associate with increased body mass index and reduced risk of type 2 diabetes in people of Māori and Pacific (Polynesian) ancestry living in Aotearoa New Zealand?

What are the new findings?

- The A-allele of *rs373863828* associates with increased body mass index and reduced risk of type 2 diabetes in people of Māori and Pacific (Polynesian) ancestry living in Aotearoa New Zealand.
- There was no association with serum urate levels, gout or chronic kidney disease.

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

- *CREBRF* participates in a newly discovered pathway of type 2 diabetes that may reveal new ways to manage type 2 diabetes.

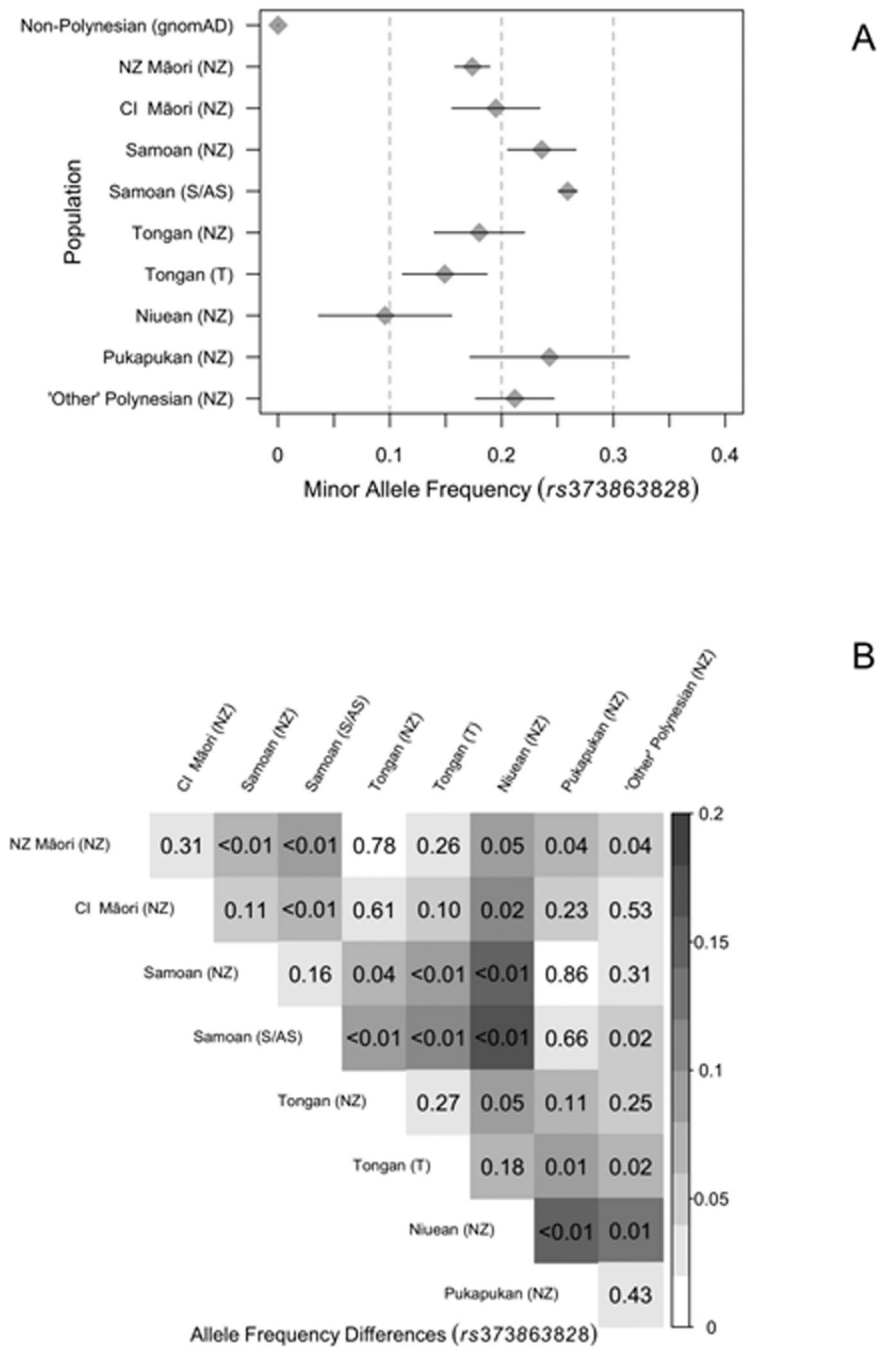

Figure 1:

Rs373863828 A-allele frequencies in various Māori and Pacific ancestral groups (top) and comparison of allele frequencies between the Māori and Pacific ancestral groups (bottom). The *P* values in the bottom figure are derived from a difference in proportions parametric z-test. NZ – New Zealand. S / AS – Samoa / American Samoa (1). T – Kingdom of Tonga (3). Non-Polynesian includes all populations in the Genome Aggregation Database (gnomAD; gnomad.broadinstitute.org).

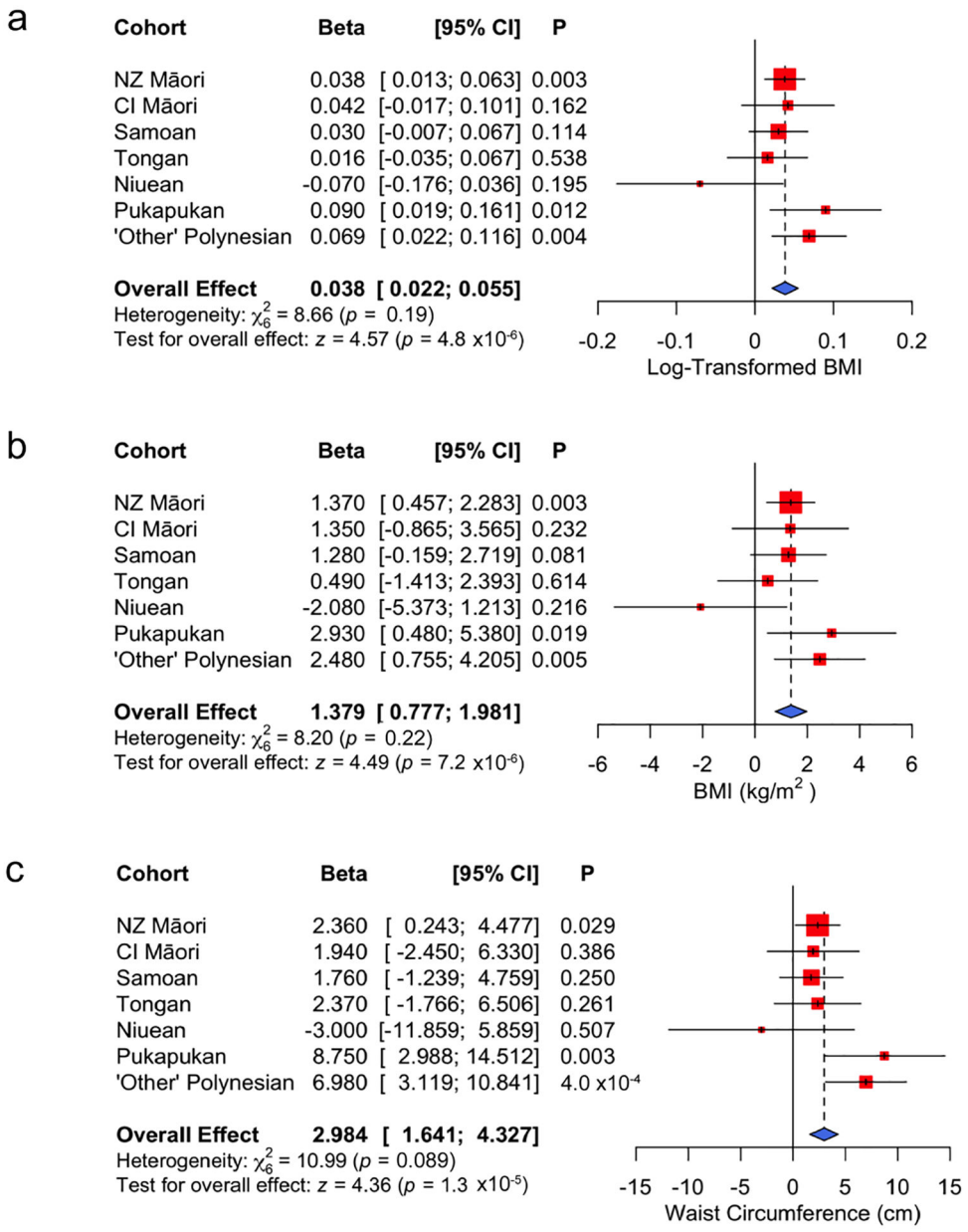

Figure 2:

Forest plot of fixed effect meta-analysis for *rs373863828* with log-transformed BMI (A), untransformed BMI (B), and waist circumference (C). Association adjusted for age, sex, first four PCA vectors, and type 2 diabetes.

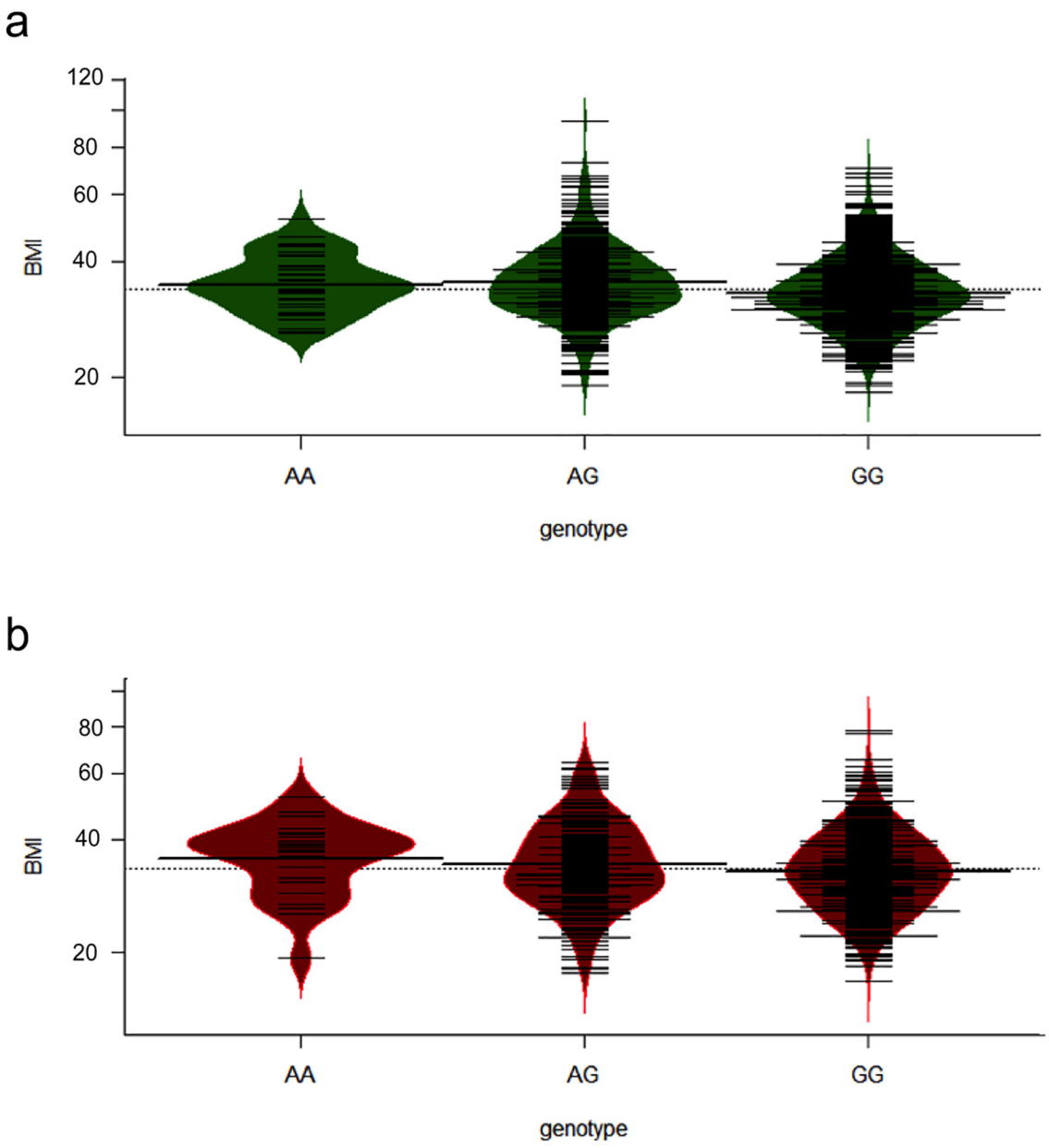

Figure 3:

Beanplots of BMI versus *rs373863828* genotype in men and women. A solid line shows the average for each group and the dotted line the overall average. Plots were generated using the R beanplot package [31].

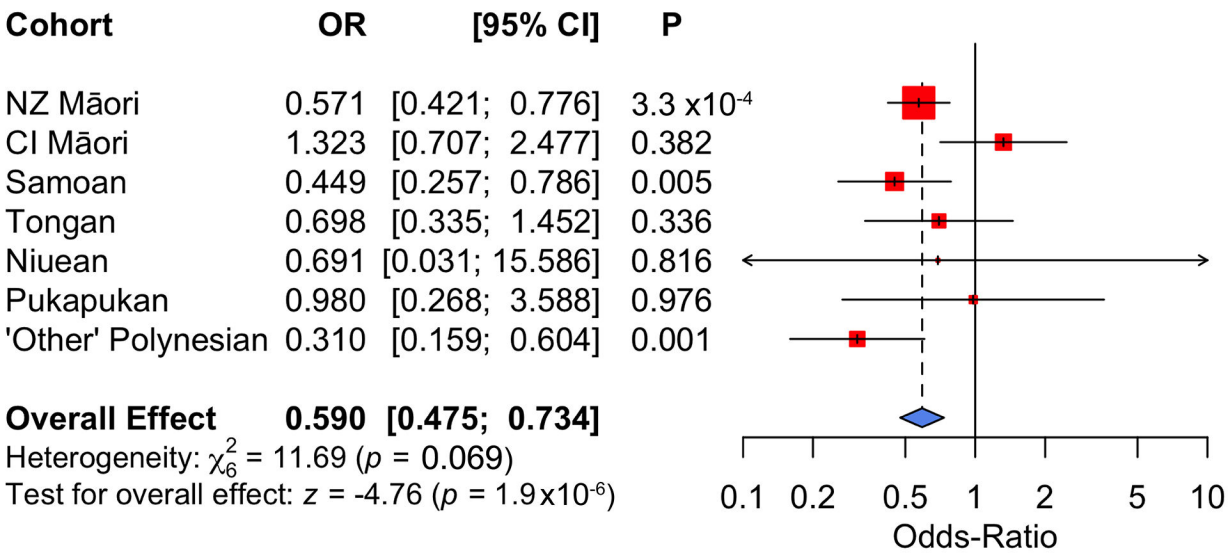

Figure 4:

Forest plot of fixed effect meta-analysis for *rs373863828* with type 2 diabetes. Association adjusted for age, sex, first four PCA vectors and type 2 diabetes (OR=0.59 [0.74,0.73]), P=1.9×10^{-6}).

Table 1:

Baseline characteristics

Characteristics	NZ Māori	Cook Island Māori	Samoan	Tongan	Niuean	Pukapukan	'Other' Polynesian
Number	1,154	197	378	175	47	70	265
SEX; n male(proportion)	666(0.577)	117(0.594)	280(0.741)	131(0.749)	36(0.766)	33(0.471)	162(0.611)
AGE; years±SD	52.25±14.66	52.16±14.28	45.25±13.50	43.61±14.67	48.28±12.72	44.14±17.28	42.38±15.41
WEIGHT; kg±SD	96.97±23.93	100.73±25.28	106.90±23.05	108.06±21.61	96.88±18.78	95.16±20.85	104.63±28.35
HEIGHT; cm±SD	169.57±9.55	169.03±9.99	173.39±9.84	174.18±8.53	171.73±8.16	165.56±9.34	171.59±8.30
BMI; kg/m^2±SD	33.79±7.96	35.37±8.56	35.63±7.84	35.55±6.88	32.77±5.09	34.60±6.61	35.43±8.42
WAIST CIRCUMFERENCE; cm±SD	108.04±18.24	110.75±15.85	111.80±16.28	112.53±14.72	106.06±12.78	108.20±16.07	110.06±18.81
[*]SU mmol/l)±SD	0.363±0.11	0.406±0.096	0.395±0.13	0.385±0.13	0.413±0.11	0.405±0.092	0.387±0.11
eGFR ml/min/1.73m^2±SD	71.23±28.42	69.36±28.70	72.49±27.31	73.00±31.76	70.94±26.68	79.79±22.23	76.25±28.25
[#]TRIACYLGLYCEROL mmol/l±SD	2.25±1.53	2.37±1.96	2.13±1.40	2.21±1.43	2.52±1.92	2.09±1.40	2.10±1.43
[#]HDL mmol/l±SD	1.16±0.364	1.17±0.393	1.10±0.340	1.09±0.373	1.10±0.332	1.21±0.227	1.13±0.366
[#]LDL mmol/l±SD	2.80 (1.01)	2.88 (0.99)	2.87 (1.05)	2.82 (1.06)	3.03 (1.21)	2.71 (1.05)	2.76 (0.93)
TYPE 2 DIABETES n(proportion)	301(0.273)	63(0.330)	83(0.226)	49(0.290)	10(0.213)	16(0.229)	61(0.234)
GOUT n(proportion)	456(0.434)	96(0.513)	207(0.570)	92(0.532)	21(0.500)	11(0.164)	100(0.407)
CHRONIC KIDNEY DISEASE n(proportion)	115(0.119)	23(0.139)	31(0.095)	23(0.146)	4(0.111)	0(0.000)	24(0.101)
rs373863828 GENOTYPE							
G/G n(proportion)	772(0.674)	127(0.651)	212(0.568)	120(0.686)	39(0.830)	42(0.600)	168(0.641)
G/A n(proportion)	348(0.304)	60(0.308)	146(0.391)	47(0.269)	7(0.149)	22(0.314)	77(0.294)
A/A n(proportion)	25(0.022)	8(0.041)	15(0.040)	8(0.046)	1(0.021)	6(0.086)	17(0.065)
HWE *p*-value	0.049	0.79	0.098	0.23	0.34	0.22	0.052
ALLELE							
A n(proportion)	398(0.174)	76(0.195)	176(0.236)	63(0.180)	9(0.096)	34(0.243)	111(0.212)

[*] Serum urate concentrations are reported from individuals not taking urate-lowering therapy.

[#] Data were unavailable for lipid-lowering medications.

Table 2.

Rs373863828 association with weight measures, type 2 diabetes (T2D), gout and CKD in the full Polynesian sample-set meta-analysis.

Continuous trait	n	β (95% CI) continuous OR (95% CI) dichotomous	*p*	Covariables
Log-transformed BMI – All	2,125	0.038 (0.022, 0.055)	4.8×10^{-6}	4 PCA, sex, age, T2D
Male	1,335	0.042 (0.023, 0.062)	2.4×10^{-5}	4 PCA, age, T2D
Female	790	0.032 (0.0014, 0.062)	0.040	4 PCA, age, T2D
Untransformed BMI (kg/m^2) - All	2,125	1.38 (0.78, 1.98)	7.3×10^{-6}	4 PCA, sex, age, T2D
Untransformed BMI (kg/m^2) - All	1,890	0.16 (−0.18, 0.51)	0.35	4 PCA, sex, age, T2D, Waist
Male	1,335	1.51 (0.78, 2.23)	4.4×10^{-5}	4 PCA, age, T2D
Female	790	1.17 (0.089, 2.26)	0.034	4 PCA, age, T2D
Type 2 diabetes positive	557	1.62 (0.57, 2.67)	2.4×10^{-3}	4 PCA, sex, age
Type 2 diabetes negative	1,568	1.21 (0.53, 1.90)	5.0×10^{-4}	4 PCA, sex, age
Major allele homozygote	1,381	-	-	-
Heterozygote	670	1.80 (1.08, 2.53)	9.7×10^{-7}	4 PCA, sex, age, T2D
Minor allele homozygote	74	1.49 (−0.32, 3.30)	0.11	4 PCA, sex, age, T2D
Waist circumference (cm) - All	1,904	2.98 (1.64, 4.33)	1.4×10^{-5}	4 PCA, sex, age, T2D
Waist circumference (cm) - All	1,890	0.66 (−0.11, 1.42)	0.092	4 PCA, sex, age, T2D, BMI
Male	1,221	2.81 (1.23, 4.40)	5.0×10^{-4}	4 PCA, age, T2D
Female	683	3.29 (0.80, 5.78)	9.7×10^{-3}	4 PCA, age, T2D
Urate (mmol/l)[a]	1,237	0.015 (0.005, 0.026)	5.0×10^{-3}	4 PCA, sex, age
Urate (mmol/l)[a]	1,199	0.012 (0.0014, 0.022)	0.026	4 PCA, sex, age, BMI
Urate (mmol/l) - T2D positive[a]	266	0.0038 (−0.030, 0.038)	0.83	4 PCA, sex, age
Urate (mmol/l) -T2D negative[a]	944	0.012 (0.0014, 0.022)	0.026	4 PCA, sex, age
Urate (mmol/l) - T2D positive[a]	255	−0.018 (−0.049, 0.014)	0.28	4 PCA, sex, age, BMI
Urate (mmol/l) -T2D negative[a]	917	0.011 (0.0003, 0.021)	0.043	4 PCA, sex, age, BMI
Dichotomous trait				
Obesity (>32 kg/m^2)	2,125	1.33 (1.13, 1.58)	8×10^{-4}	4 PCA, sex, age, T2D
Obesity (>40 kg/m^2)	2,125	1.54 (1.27, 1.87)	1.0×10^{-5}	4 PCA, sex, age, T2D
Type 2 diabetes - All	2,190	0.65 (0.53, 0.80)	3.4×10^{-5}	4 PCA, sex, age
Type 2 diabetes - All	2,125	0.59 (0.47, 0.73)	1.9×10^{-6}	4 PCA, sex, age, BMI
Male	1,335	0.54 (0.41, 0.72)	1.5×10^{-5}	4 PCA, age, BMI
Female	779	0.66 (0.46, 0.95)	0.026	4 PCA, age, BMI
Major allele homozygote	1,381	-	-	-
Heterozygote	670	0.55 (0.43, 0.71)	3.2×10^{-6}	4 PCA, sex, age, BMI
Minor allele homozygote	74	0.72 (0.34, 1.53)	0.39	4 PCA, sex, age, BMI
Gout	2,114	1.10 (0.91, 1.32)	0.34	4 PCA, sex, age
Gout	2,009	1.00 (0.81, 1.22)	0.98	4 PCA, sex, age, BMI, T2D

Continuous trait	n	β (95% CI) continuous OR (95% CI) dichotomous	*p*	Covariables
Gout- T2D positive[b]	498	1.03 (0.71, 1.52)	0.86	4 PCA, sex, age
Gout- T2D negative	1,549	1.08 (0.86, 1.36)	0.50	4 PCA, sex, age
Gout- T2D positive[b]	480	0.89 (0.59, 1.33)	0.57	4 PCA, sex, age, BMI
Gout- T2D negative	1,510	0.99 (0.77, 1.26)	0.91	4 PCA, sex, age, BMI
CKD	1,849	0.72 (0.53, 0.97)	0.030	4 PCA, sex, age
CKD	1,795	0.72 (0.53, 0.99)	0.045	4 PCA, sex, age, BMI
CKD	1,810	0.86 (0.63, 1.15)	0.36	4 PCA, sex, age, T2D
CKD	1,756	0.91 (0.65, 1.28)	0.59	4 PCA, sex, age, BMI, T2D
CKD- T2D positive	488	0.71 (0.46, 1.09)	0.11	4 PCA, sex, age
CKD- T2D negative	1,322	1.12 (0.67, 1.87)	0.66	4 PCA, sex, age
CKD- T2D positive	470	0.80 (0.50, 1.27)	0.34	4 PCA, sex, age, BMI
CKD- T2D negative	1,286	1.12 (0.65, 1.93)	0.69	4 PCA, sex, age, BMI

[a]Analysis excluded individuals taking urate-lowering medication

[b]Analysis excluded individuals of Niuean and Pukapukan ancestry due to small sample-size