Loss-of-function variants in *ADCY3* increase risk of obesity and type 2 diabetes

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Supplementary Tables

Supplementary Table 2. Association of *ADCY3* c.2433-1G>A with a range of metabolic and anthropometric traits in Greenlandic cohorts.

		Recessive model				Additive model			
Trait	*N*	β_{SD}	se_{SD}	β	*P*	β_{SD}	se_{SD}	β	*P*
Height (cm)	4,018	-0.354	0.264	-2.55	0.18	-0.056	0.059	-0.41	0.34
Weight (kg)	4,002	0.922	0.345	15.6	**0.0076**	0.148	0.073	2.13	0.044
Waist circumference (cm)	3,964	1.083	0.346	16.59	**0.0017**	0.138	0.073	2.12	0.060
Hip circumference (cm)	3,962	0.746	0.354	7.981	**0.035**	0.091	0.075	1.00	0.225
Waist-hip ratio	3,961	0.995	0.328	0.086	**0.0024**	0.13	0.069	0.012	0.060
VAT (cm)	2,681	0.858	0.352	2.161	**0.019**	0.149	0.08	0.30	0.063
SAT (cm)	2,671	0.293	0.363	0.562	0.42	0.044	0.083	0.086	0.59
SAT-VAT ratio	2,663	-0.421	0.364	-0.079	0.25	-0.032	0.082	-0.0090	0.70
Fasting serum insulin (pmol/L)	3,620	0.804	0.367	27.0	**0.028**	0.042	0.078	1.70	0.59
2-h serum insulin (pmol/L)	3,387	0.442	0.364	129	0.22	0.126	0.078	34.5	0.11
HbA_{1C} (%)	4,024	0.575	0.285	0.316	**0.044**	0.066	0.061	0.025	0.28
HOMA-IR (mmol/L×pmol/L)	3,613	0.909	0.37	1.25	**0.014**	0.064	0.079	0.125	0.42
$ISI_{0,120}$	3,354	-0.785	0.357	-0.136	**0.028**	-0.172	0.076	-0.128	**0.024**
Fasting serum HDL-cholesterol (mmol/L)	4,035	-0.812	0.334	-0.282	**0.015**	-0.125	0.072	-0.047	0.082
Fasting serum total cholesterol (mmol/L)	3,895	0.08	0.352	0.112	0.82	0.127	0.071	0.151	0.074
Fasting serum triglyceride (mmol/L)	4,035	0.815	0.358	1.13	**0.023**	0.266	0.076	0.219	**0.00046**

Results are shown for a recessive and an additive model. β_{SD} is the effect size estimated using traits values quantile transformed to a normal distribution and β is the effect size estimated

using untransformed values. The *P*-values are obtained from the quantile transformed value based analyses. *P*-values shown have not been corrected for multiple testing and nominally significant *P*-values are highlighted in bold. ISI, Insulin sensitivity index; SAT, Subcutaneous adipose tissue; VAT, Visceral adipose tissue.

Supplementary Table 3. Association with burden of heterozygous loss-of-function variants in *ADCY3* in trans-ethnic cohorts.

	Type 2 diabetes	Controls	Quantile	*P*	OR (95% CI)
Loss-of-function variants in ADCY3 (excluding rs146165057)	HE=7 WT=8,845	HE=1 WT=9,323	0.034	0.044	8.6 (1.1 - 69.5)
Loss-of-function variants in ADCY3 (AMP-T2D annotation incl. rs146165057)	HE=12 WT=8,840	HE=4 WT=9,320	0.032	0.037	3.4 (1.1 - 10.4)

Data are from Accelerating Medicines Partnership Type 2 Diabetes Knowledge Portal (AMP-T2D; http://www.type2diabetesgenetics.org/) generated by the GoT2D, T2DGenes, SIGMA and LuCAMP consortia[6,7,8]. Variants annotated as stop-gained, frameshift or in a splice adapter/donor site were considered loss-of-function. All loss-of-function variants had a minor allele frequency (MAF) <5%. Burden association analyses were performed with principal components 1-4, age and sex as covariates and using data from individuals (*N*=18,176) that had no missing phenotype data. The counts of loss-of-function carriers stratified by ancestry are shown in Supplementary Table 5. At the AMP-T2D online site the rs146165057 (2:25048965-C/T) variant is annotated as a frameshift variant and is therefore included in the site's loss-of-function analyses. The second line of results in the table shows the results including this variant. However, rs146165057 is predicted to be a missense variant rather than a frameshift variant in dbSNP, so those results are not the relevant ones and are only included for completeness. The relevant results, and the one reported in the main paper, can be found in the first line of results in the table, where we manually analyzed the data excluding the rs146165057 missense carriers as loss-of-function carriers. Quantiles were calculated as the rank of *ADCY3* in the distribution of *P*-values for all genes in the dataset. No homozygous carriers were observed. HE, heterozygous; WT, wild type.

Supplementary Table 4. Loss-of-function variants in *ADCY3* in the Greenlandic and trans-ethnic cohorts.

Dataset	Position (hg19)	Exon	Variant ID	Consequence	Annotation	All (gnomAD) WT/HE/HO	Non-diabetic controls (Greenland or AMP-T2D) WT/HE/HO	Type 2 diabetes patients (Greenland or AMP-T2D) WT/HE/HO
Green-land	25050478	14	2:25050478-G/A	c.2433-1G>A	Splice acceptor	NA	3,823/171/7	293/20/3
AMP-T2D	25042887	21	2:25042887-AG/A	p.Phe1117SerfsTer3	Frameshift	243,698/1/0	9,324/0/0	8,851/1/0
AMP-T2D	25047374	16	2:25047374-C/T	p.Trp870Ter	Stop-gained	123,130/1/0	9,323/1/0	8,852/0/0
AMP-T2D	25048965	15	2:25048965-CAT/C	p.Met842AspfsTer31	Frameshift	122,987/1/0	9,324/0/0	8,851/1/0
AMP-T2D	25048965*	15	2:25048965-C/T*	p.Met842Ile	Missense*	138,356/92/0	9,321//3/0	8,847/5/0
AMP-T2D	25050893	13	2:25050893-G/A	c.1072-1G>A	Splice acceptor	138,588/13/0	9,324/0/0	8,851/1/0
AMP-T2D	25057661	9	2:25057661-T/C	c.1805+2T>C	Splice donor	123,024/2/0	9,324/0/0	8,851/1/0
AMP-T2D	25062828	6	2:25062828-G/GC	p.Val424ArgfsTer15	Frameshift	122,849/1/0	9,324/0/0	8,851/1/0
AMP-T2D	25141426	1	2:25141426-C/T	p.Trp144Ter	Stop-gained	123,012/2/0	9,324/0/0	8,850/2/0

Variants were annotated to canonical transcript ADCY3-001 (NM_004036) except c.1072-1G>A, which is annotated to alternative transcript ADCY3-201 (NM_001320613). Trans-ethnic cohort data were obtained from AMP-T2D (http://www.type2diabetesgenetics.org/), and only those tested for association are included. GnomAD genotype counts are from data obtained at http://gnomad.broadinstitute.org/gene/ENSG00000138031. HE, heterozygous; HO homozygous; WT, wildtype; T2D, type 2 diabetes. *This variant is annotated in AMP-T2D as frameshift but is a missense variant. This was therefore excluded from the final analysis of loss-of-function variants.

Supplementary Table 5. Loss-of-function variants in trans-ethnic cohorts stratified by ancestry

		Type 2 diabetic individuals			Non-diabetic individuals		
	N (all)	*N* (total)	*N* (HE carriers)	Allele frequency	*N* (total)	*N* (HE carriers)	Allele frequency
European	6,356	3,214	1	0.016%	3,142	0	0%
African-American	1,741	734	4	0.27%	1,007	1	0.050%
East Asian	2,158	1,009	0	0%	1,149	0	0%
Hispanic	5,722	2,811	2	0.036%	2,911	0	0%
South Asian	2,199	1,084	0	0%	1,115	0	0%
Total	18,176	8,852	7	0.040%	9,324	1	0.0054%

No homozygous carriers were observed. HE, heterozygous.

Supplementary Table 6. Clinical descriptive data of Greenlandic individuals in all individuals and stratified according to disease state

	All individuals	Non-diabetic individuals	Type 2 diabetic individuals	Obese (BMI>30 kg/m^2)
N (men/women)	4,038 (1,794/2,244)	2,585 (1,100/1,485)	301 (136/165)	861 (315/546)
Age (yrs)	44.1 (14.7)	41.0 (13.1)	57.8 (12.8)	47.2 (13.6)
Height (cm)	162 (9.3)	163 (9.1)	159 (9.0)	161 (9.0)
Weight (kg)	69.2 (15.4)	68.4 (14.5)	73.8 (20)	88 (12.9)
BMI (kg/m^2)	26.3 (5.1)	25.7 (4.7)	29.1 (6.8)	33.9 (3.5)
Waist circumference (cm)	90.8 (13.4)	89.1 (12.3)	99.3 (16.2)	109.0 (9.3)
Hip circumference (cm)	98.7 (9.5)	98.2 (9.0)	102.0 (11.5)	110.0 (7.8)
Waist-hip ratio	0.918 (0.082)	0.906 (0.078)	0.974 (0.088)	0.988 (0.075)
VAT (cm)	7.04 (2.26)	6.70 (2.01)	8.34 (2.89)	9.34 (2.38)
SAT (cm)	3.01 (1.52)	2.99 (1.53)	3.14 (1.54)	4.48 (1.34)
SAT-VAT ratio	0.448 (0.241)	0.464 (0.249)	0.401 (0.219)	0.527 (0.24)
F-p glucose (mmol/L)	5.70 (0.93)	5.39 (0.41)	7.54 (2.3)	6.05 (1.1)
2-h-p glucose (mmol/L)	5.90 (2.5)	5.00 (1.3)	11.1 (4.8)	6.90 (2.9)
Fasting serum insulin (pmol/L)	38 (25-56)	36 (25-52)	50 (32.5-89)	65 (46-92)
2-h serum insulin (pmol/L)	109 (47-211)	87 (39-164)	233 (124-412)	180 (87-326)
HbA1C (%)	5.79 (0.55)	5.67 (0.40)	6.42 (1.14)	5.95 (0.65)
HOMA-IR (mmol/L×pmol/L)	1.35 (0.88-2.07)	1.23 (0.82-1.82)	2.36 (1.5-4.5)	2.46 (1.73-3.54)
$ISI_{0,120}$	2.63 (1.91-3.94)	3.03 (2.3-4.39)	1.19 (0.955-1.61)	1.98 (1.45-2.66)
Fasting serum HDL-cholesterol (mmol/L)	1.66 (0.53)	1.65 (0.49)	1.77 (0.78)	1.43 (0.44)
Fasting serum total cholesterol (mmol/L)	5.88 (1.21)	5.79 (1.2)	6.32 (1.28)	6.15 (1.19)
Fasting serum triglyceride (mmol/L)	1.01 (0.75-1.39)	0.96 (0.73-1.29)	1.22 (0.85-1.9)	1.34 (0.97-1.89)
Type 2 diabetes (% cases)	7.5	0	100	14

Clinical descriptive data for the individuals included in the Greenlandic association studies. Data are mean (SD) for normally distributed traits or median (interquartile range) for non-normally distributed traits unless otherwise stated. Data are shown in all, in type 2 diabetes controls and cases and for obese (BMI>30 kg/m^2) individuals. ISI, insulin sensitivity index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.