

Table SI. PCR amplification primers for the coding regions of *ADCY3* and *MC4R*.

Gene/exon	Forward (5'-3')	Reverse (5'-3')
<i>ADCY3</i>		
1	CGTTCCCAGTCCACTTCTCT	GCAGCGTGATGAAGAAGGAG
1	GCTCATAACCGCCCAGATCT	ACACACTTAGACTGGGCACA
2	GGACATTGGGCAACTTGAGG	GGAGAGATCTGGGTCAAGCA
3	GGCAGATCTGTGAGGTCTCT	CCGCTCCAGGTGATATCTGT
4	GAGCCCTGACCTGTGATTCT	CAGAGGAAGGACAGTGGGAG
5	TTGTGAAGCTGCTCAACGAG	TGGGCTGTGAGGAAGGTAAG
6	AAGTGGGGATAGGGAGGAAA	TAGGGAGGCCTTCTCTGAGG
7	CAGAACTGCCTCCACTTGG	CCCCAGAAACCAGCTCTCC
8	TCATCCTTGACGCCTCACTT	TTTGACCTTACTACCCCCGG
9	CGGGGAGCCAGGAAAGAC	GAACAAGCCCCATGAATCCC
10	CTGAGCATCCCAAATACGGC	ATCTAGCCAAGGTCTCCAGC
11	CTCACCCCATCTCACTGTCC	ACCCAGACTCTGCCGTACTC
12	CAACACAGCGTCAGAACTCC	GGGAGAGGAGCTTCAAGGAA
13	ATTTCCCACATCTTTCCCGGT	AGGTCCTAGCCAAACAGTGA
14	TATFGTCTCCTTCCCTCCCGC	TTTCCTTCCCTCCTCACTGC
15	CAGCCCTGATGTGAATACCA	CATGCTTCCCTCACCATCCT
16	ACTGCAACCTCTGCCTCCT	GCCGGAGAAATTCCTCTTTT
17	TGCTTGTTTACTGCCACTGC	TTGCTTGCCAGTCCCTTATC
18	GGAAACAGGCCAGAAAGTT	GGGGGTTGTGTGTCTGTTCT
19	TGCCTAGACCTGCTACGTGA	CATCAGCTGGGTTTTTGGTT
20	ATGTGATTTCCACCCTCAA	GACGCCACTGAGACAGATCA
21	GGACCCATGGATCAGTGCT	AGAGCTCACACATCCACGAA
<i>MC4R</i> , 1	CTAAAACTCCATGTCAAGCTCTG	GGCCAAAAAAGTAGCATGACAT

Table SII. Auxology and laboratory data for patients identified with the novel c.349T>A/p.Leu117Met variation.

A, Auxology				
Item	Patient 1		Patient 2	
	At presentation	At last visit	At presentation	At last visit
Age, years	9.2	16.5	11.1	16.7
Body height SDS	+1.4	+1.8	+2.3	+0.3
Body weight SDS	+3.5	+2.8	+3.3	+3.5
BMI SDS	+3.0	+3.0	+3.6	+3.2
WC SDS	NA	NA	+3.1	+3.0
BP, mmHg	122/70 (75th)	140/80 (97th)	120/55 (50th)	135/75 (75th)
Tanner stage	1	5	1	5

B, Laboratory OGTT test (75 mg glucose/m²)

Parameter	Patient 1		Patient 2	
	At presentation	At last visit	At presentation	At last visit
Glucose (0, 30, 60, 90, 120 min), mg/dl	77, 119, 88, 102, 94	73, 126, 154, 140, 131	91, 109, 99, 99, 98	106, 133, 128, 122, 115
Insulin (0, 30, 60, 90, 120 min), mIU/l	9.66	16, 52, 10, 9.4, 4.3	6.6, 89, 28, 29, 21	16, 54, 63.5, 43, 28
HOMA <2.9	1.9	2.9	1.3	4.2
Total cholesterol 125-200, (HDL >40/LDL <120), mg/dl	190 (40/104)	211 (39/139)	179 (53/78)	174 (48/111)
Triglycerides <150 mg/dl	231	166	72	75
SGOT/SGPT <15, IU/l	13/21	30/22	18/17	20/28
Creatinine 0.2-1.2 mg/dl	0.63	0.84	0.59	0.69
Uric acid 3.5-7.2 mg/dl	5.5	8.1	5.4	6.4
FT4 11.9-22.2 pmol/l	14.5	15.2	18.8	15.6
TSH 0.4-4.5 mIU/ml	1.71	1.9	1.83	1.58
Cortisol 140-690 nmol/l	113	154	279	306

For biochemical analysis, blood samples were collected after an overnight fast. BP centiles for age were evaluated according to national BP charts. Routine biochemistry was measured by using the usual enzymatic assays. Plasma insulin, thyroid function, prolactin and cortisol were determined by using the radioimmunoassay method according to manufacturers' instructions. SDS, standard deviation score; BMI, body mass index; WC, waist circumference; BP, blood pressure; OGTT, oral glucose tolerance test; HOMA, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SGOT, aspartate aminotransferase; SGPT, alanine aminotransferase; FT4, free thyroxine; TSH, thyroid stimulating hormone; NA, not available.

Table SIII. P-values from χ^2 test for association of between the presence of variant and sex or BMI SDS.

Presence of variant, dbSNP	Sex (n=33)	BMI SDS (n=33)
Novel p.Leu117Met	0.20 ^a	0.48 ^a
rs11676272 Hom	0.73	0.73
rs11676272 Het	0.52	0.86
rs2241758 Het	0.66	0.82
rs7604576 Hom	0.48	0.93
rs7604576 Het	0.80	0.41 ^a
rs1127568 Hom	0.74	0.80
rs1127568 Het	0.10	0.69

^aCalculated with Fisher's test due to low counts. Hom, homozygote; Het, heterozygote; BMI SDS, body mass index standard deviation score; SNP, single nucleotide polymorphism.