

SUPPLEMENTARY DATA

NUMEROUS GENES IN LOCI ASSOCIATED WITH BODY FAT DISTRIBUTION ARE LINKED TO ADIPOSE FUNCTION

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Supplementary Table 1. Analyzed candidate genes for body fat distribution^a

SNP	Locus	Chromosome	Start position	Phenotype	Affymetrix probeset
rs905938	<i>DCST2</i>	1	155017448	WHRadjBMI	7920616
	<i>ZBTB7B</i>	1	155002627	WHRadjBMI ^b	7905854
rs714515	<i>DNM3</i>	1	171841478	WHRadjBMI	7907370
	<i>PIGC</i>	1	172441457	WHRadjBMI	7922330
rs10919388	<i>GORAB</i>	1	170532122	WHRadjBMI	7907213
rs2820443	<i>LYPLAL1</i>	1	219173831	WHRadjBMI	7909801
rs10925060	<i>NLRP3</i>	1	247415904	WCadjBMI	7911178
	<i>OR2W5</i>	1	247491068	WCadjBMI	7911197
rs2645294	<i>TBX15</i>	1	118883043	WHRadjBMI	7919028
	<i>WARS2</i>	1	119031216	WHRadjBMI	7919028
rs10929925	<i>SOX11</i>	2	5692667	HIP	8040070
rs1385167	<i>MEIS1</i>	2	66435125	WHRadjBMI	8042356
rs2124969	<i>ITGB6</i>	2	160099666	WCadjBMI	8056184
rs10195252	<i>GRB14</i>	2	164492406	WHRadjBMI	8056327
	<i>COBLL1</i>	2	164680185	WHRadjBMI	8056343
rs1569135	<i>CALCRL</i>	2	187341963	WHRadjBMI	8057578
	<i>TFPI</i>	2	187464230	WHRadjBMI ^b	8057599

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rs17819328	<i>PPARG</i>	3	12287850	WHRadjBMI	8077899
rs2276824	<i>PBRM1</i>	3	52545352	WHRadjBMI	8087951
rs2371767	<i>ADAMTS9</i>	3	64515654	WHRadjBMI	8088560
rs10804591	<i>PLXND1</i>	3	129555213	WHRadjBMI	8090591
rs17451107	<i>TIPARP</i>	3	156674416	WHRadjBMI ^b	8083569
	<i>LEKR1</i>	3	156826307	WHRadjBMI	8083576
	<i>CCNL1</i>	3	157146364	WHRadjBMI ^b	8091658
rs3805389	<i>NMU</i>	4	55595229	WHRadjBMI	8100464
rs9991328	<i>FAM13A</i>	4	88725954	WHRadjBMI	8101728
rs303084	<i>FGF2</i>	4	122826708	WHRadjBMI	8097262
	<i>NUDT6</i>	4	122892644	WHRadjBMI ^b	8102713
	<i>SPATA5</i>	4	122923070	WHRadjBMI	8097262
	<i>SPRY1</i>	4	123396791	WHRadjBMI ^b	8097282
rs1664789	<i>ARL15</i>	5	53884748	WCadjBMI	8112033
rs9687846	<i>ANKRD55</i>	5	56099680	WHRadjBMI ^b	8112159
	<i>MAP3K1</i>	5	56815073	WHRadjBMI	8105436
rs1045241	<i>TNFAIP8</i>	5	119268692	WHRadjBMI	8107520
	<i>HSD17B4</i>	5	119452443	WHRadjBMI	8107532
rs17472426	<i>CCNJL</i>	5	160251659	WCadjBMI	8115584
rs7705502	<i>CPEB4</i>	5	173888328	WHRadjBMI	8110055
rs6556301	<i>FGFR4</i>	5	177086872	WHRadjBMI	8110265
	<i>MXD3</i>	5	177305500	WHRadjBMI ^b	8116012
rs722585	<i>GMDS</i>	6	1623800	HIPadjBMI	8123562

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rs1294410	<i>LY86</i>	6	6588701	WHRadjBMI	8116734
rs7759742	<i>BTNL2</i>	6	32393339	WHRadjBMI	8125426
	<i>HLA-DRA</i>	6	32439842	WHRadjBMI ^b	8118548
rs1776897	<i>HMGA1</i>	6	34236800	WHRadjBMI	8118794
	<i>C6orf106</i>	6	34587280	WHRadjBMI ^b	8125850
rs1358980	<i>VEGFA</i>	6	43770209	WHRadjBMI	8119898
rs7739232	<i>KLHL31</i>	6	53647901	HIPadjBMI	8127177
s1936805	<i>RSPO3</i>	6	127118903	WHRadjBMI	8121916
rs10245353	<i>NFE2L3</i>	7	26152227	WHRadjBMI	8131944
rs1534696	<i>CBX3</i>	7	26201211	WHRadjBMI ^b	8131949
	<i>SNX10</i>	7	26291895	WHRadjBMI	8131957
rs7801581	<i>HOXA11</i>	7	27181157	WHRadjBMI	8138765
rs1144	<i>SRPK2</i>	7	105113601	WCadjBMI	8142036
rs13241538	<i>KLF14</i>	7	130732554	HIPadjBMI	8142971
rs7830933	<i>NKX2-6</i>	8	23702451	WHRadjBMI	8149820
	<i>STC1</i>	8	23841921	WHRadjBMI ^b	8149825
rs12679556	<i>EYA1</i>	8	71197433	WHRadjBMI ^b	8151310
rs12679556	<i>MSC</i>	8	71841542	WHRadjBMI	8151334
rs2398893	<i>PTPDC1</i>	9	94030794	WHR	8156506
rs10991437	<i>ABCA1</i>	9	104781002	WHRadjBMI	8162940
rs7044106	<i>C5</i>	9	120952335	HIPadjBMI	8163839
rs7917772	<i>SFXN2</i>	10	102714508	WHRadjBMI	7930148
rs11231693	<i>MACROD1</i>	11	63998556	WHRadjBMI	7949033

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	<i>VEGFB</i>	11	64234584	WHRadjBMI	7940904
rs11607976	<i>MYEOV</i>	11	69294138	HIP	7942118
rs1784203	<i>KIAA1731</i>	11	93394877	WCadjBMI	7943126
rs1394461	<i>CNTN5</i>	11	99020976	WHR	7943319
rs10842707	<i>SSPN</i>	12	26121951	WHRadjBMI	7954481
	<i>ITPR2</i>	12	26335352	WHRadjBMI	7961900
rs1443512	<i>HOXC13</i>	12	53938792	WHRadjBMI	7955845
rs4765219	<i>CCDC92</i>	12	123936409	WHRadjBMI	7967486
	<i>ZNF664</i>	12	123973215	WHRadjBMI ^b	7959751
	<i>FAM101A</i>	12	124289164	WHRadjBMI ^b	7959761
rs319564	<i>GPC6</i>	13	93226825	WHR	7969613
rs8042543	<i>KLF13</i>	15	31326855	WHRadjBMI	7982326
rs8030605	<i>RFX7</i>	15	56090533	WHRadjBMI	7989132
rs1440372	<i>SMAD6</i>	15	66702100	WHRadjBMI	7984353
rs4985155	<i>PDXDC1</i>	16	14974591	HIP	7993433
rs2047937	<i>ZNF423</i>	16	49487531	WCadjBMI	8001337
rs2925979	<i>CMIP</i>	16	81445170	WHRadjBMI	7997427
	<i>PLCG2</i>	16	81779258	WHRadjBMI ^b	7997453
rs2034088	<i>VPS53</i>	17	508668	HIPadjBMI	8010918
rs4646404	<i>PEMT</i>	17	17505563	WHRadjBMI	8013120
rs8066985	<i>KCNJ2</i>	17	70168616	WHRadjBMI	8009502
rs12454712	<i>BCL2</i>	18	63123346	WHRadjBMI	8023646
rs12608504	<i>KIAA1683</i>	19	18257096	WHRadjBMI ^b	8035435

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rs12608504	<i>JUND</i>	19	18279694	WHRadjBMI	8035445
rs4081724	<i>CEBPA</i>	19	33299934	WHRadjBMI	8036004
	<i>CEBPG</i>	19	33373669	WHRadjBMI ^b	8027566
rs979012	<i>BMP2</i>	20	6767694	WHRadjBMI	8060850
rs224333	<i>UQCC</i>	20	35302566	WHRadjBMI ^b	8065889
	<i>GDF5</i>	20	35433347	WHRadjBMI	8065905
	<i>CEP250</i>	20	35455139	WHRadjBMI ^b	8062137
rs6090583	<i>EYA2</i>	20	46894624	WHRadjBMI ^b	8063187
rs2294239	<i>ZNRF3</i>	22	28800654	WHRadjBMI	8072160
rs1053593	<i>HMGXB4</i>	22	35241535	HIPadjBMI	8072645

a. Genes are from Tables 1, 2 and 3 in Shungin et al (1)

b. Additional candidate gene from eQTL and bioinformatic analysis as presented in Table 2 by Shungin (1).

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Supplementary Table 2. Genes displaying association between adipose tissue expression and WHR or adipocyte phenotypes in global transcriptome analysis[#]

WHR	WAT Morphology	Lipolysis	Insulin-stimulated lipogenesis	
<i>CALCRL</i> [§]	<i>ARL15</i>	<i>CALCRL</i>	<i>ARL15</i> [§]	<i>MAP3K1</i> [§]
<i>EYA1</i>	<i>C6orf106</i>	<i>EYA1</i> [§]	<i>BCL2</i> [§]	<i>NFE2L3</i>
<i>FAM13A</i> [§]	<i>CCNL1</i>	<i>FAM13A</i>	<i>CBX3</i> [§]	<i>NKX2-6</i> [§]
<i>FGF2</i> [§]	<i>CMIP</i>	<i>HMGXB4</i> [§]	<i>CEP250</i> [§]	<i>NLRP3</i>
<i>HLA-DRA</i> [§]	<i>DNM3</i>	<i>MSC</i>	<i>CMIP</i> [§]	<i>PDXDC1</i> [§]
<i>HMGXB4</i>	<i>FAM13A</i> [§]	<i>TBX15</i> [§]	<i>DNM3</i> [§]	<i>PIGC</i> [§]
<i>KCNJ2</i>	<i>FGF2</i>		<i>FGF2</i> [§]	<i>PLCG2</i> [§]
<i>LY86</i>	<i>GMDS</i>		<i>GMDS</i> [§]	<i>PLXND1</i> [§]
<i>MAP3K1</i>	<i>GORAB</i>		<i>GORAB</i> [§]	<i>PTPDC1</i> [§]
<i>MSC</i>	<i>HLA-DRA</i> [§]		<i>GRB14</i> [§]	<i>RFX7</i> [§]
<i>MYEOV</i>	<i>KLHL31</i>		<i>HLA-DRA</i> [§]	<i>SPATA5</i> [§]
<i>NLRP3</i>	<i>LY86</i>		<i>HOXA11</i> [§]	<i>TFPI</i> [§]
<i>PBRM1</i>	<i>MAP3K1</i>		<i>HOXC13</i>	<i>UQCC</i> [§]
<i>TNFAIP8</i>	<i>MSC</i> [§]		<i>ITGB6</i> [§]	<i>WARS2</i> [§]
<i>UQCC</i>	<i>PDXDC1</i>		<i>KCNJ2</i>	<i>VPS53</i> [§]
	<i>PEMT</i>		<i>KIAA1731</i> [§]	<i>ZBTB7B</i> [§]
	<i>PLCG2</i> [§]		<i>KLF14</i> [§]	<i>ZNF423</i>
	<i>PLXND1</i>		<i>MACROD1</i>	<i>ZNF664</i> [§]
	<i>RFX7</i>			
	<i>VPS53</i>			
	<i>ZBTB7B</i>			

22,168 probesets representing 20,507 genes were analyzed for association with the specified phenotypes using SAM (Significance Analysis of Microarrays, 500 permutations, otherwise default setting) (2). Total number of significant genes was 1,539 for WHR, 2,623 for WAT Morphology, 798 for Lipolysis, and 7,749 for Insulin-stimulated lipogenesis (FDR <0.1% except for Lipolysis where FDR <1% was applied). Shown are significant genes overlapping candidate genes for body fat distribution from GWAS.

§ Genes remaining significant after adjustment for batch and, when appropriate, BMI i.e. listed in Table 2 and 3 in the present paper.

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Supplementary Table 3. Relationship between gene expression and adipose phenotypes

<i>Gene ID</i>	<i>WAT morphology</i>		<i>WHR</i>		<i>Lipogenesis</i>	
	r-value	<i>P</i>	Standard coefficient	<i>P</i>	Standard coefficient	<i>P</i>
<i>CBX3</i>	-	-	-	-	0.38	0.01
<i>FAM13A</i>	-0.32	0.016	-0.37	0.005	-	-
<i>FGF2</i>	-	-	-0.36	0.010	0.26	0.04
<i>PLCG2</i>	0.37	0.004	-	-	-0.18	0.25
<i>SFXN2</i>	-	-	-	-	0.42	0.003

Subcutaneous adipose tissue was investigated in a previously studied cohort (3). RNA was extracted as described and gene expression was measured by Taqman quantitative real time PCR using the best coverage and inventoried assay from Applied Biosystems (Foster City, CA, US). Linear regression was used for analysis of WAT morphology and multiple regression with BMI as co-variate was used for the other two phenotypes. Gene expression was expressed as ratio to the reporter gene LRP10. We choose genes from Tables 2 and 3 that displayed the strongest correlation in multiple regressions.

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Supplementary Table 4. eQTL-analysis of SNPs associated with body fat distribution^a

CHR	SNP	BP	A1	N	Trait	Gene	<i>P</i> ^b	<i>P</i> ^c
1	rs984222	119503843	G	76	WHRadjBMI	<i>TBX15</i>	0.3909	
						<i>WARS2</i>	0.8790	
1	rs1011731	172346548	G	75	WHRadjBMI	<i>PIGC</i>	0.2288	
						<i>DNM3</i>	0.2715	
2	rs10195252	165513091	T	74	WHRadjBMI	<i>GRB14</i>	0.2396	
3	rs4684854	12488882	C	76	WHRadjBMI	<i>PPARG</i>	0.2268	
3	rs10804591	129334233	A	98	WHRadjBMI	<i>PLXND1</i>	0.0912	
4	rs9991328	89713121	T	86	WHRadjBMI	<i>FAM13A</i>	0.2533	
						<i>FGF2</i>	0.6818	
4	rs303084	124066948	A	97	WHRadjBMI	<i>SPATA5</i>	0.2850	
						<i>NUDT6</i>	0.9507	
5	rs11743303	55859952	G	76	WHRadjBMI	<i>MAP3K1</i>	0.4365	
5	rs6556301	176527577	T	98	WHRadjBMI	<i>FGFR4</i>	0.3393	
						<i>HLA-DRA</i>	0.3413	
6	rs7759742	32381736	A	97	WHRadjBMI	<i>HLA-DRA</i>	0.5812	
						<i>HLA-DRA</i>	0.5654	
8	rs1550280	23601830	C	98	WHRadjBMI	<i>NKX2-6</i>	0.4865	
8	rs12679556	72514228	G	98	WHRadjBMI	<i>MSC</i>	0.8155	
						<i>EYA1</i>	0.0160	
10	rs7917772	104487443	A	90	WHRadjBMI	<i>SFXN2</i>	0.0448	
12	rs4765219	124440110	C	97	WHRadjBMI	<i>ZNF664</i>	1.80E-07	1.03E-07
15	rs8030605	56504598	A	98	WHRadjBMI	<i>RFX7</i>	0.6116	
16	rs2925979	81534790	T	99	WHRadjBMI	<i>CMIP</i>	0.0066	0.0029
						<i>PLCG2</i>	0.1380	
18	rs12454712	60845884	T	99	WHRadjBMI	<i>BCL2</i>	0.7335	
20	rs224333	34023962	G	98	WHRadjBMI	<i>CEP250</i>	0.2267	
						<i>UQCC</i>	0.7169	
20	rs6090583	45558831	A	85	WHRadjBMI	<i>EYA2</i>	0.1274	
2	rs2124969	160989486	C	113	WCadjBMI	<i>ITGB6</i>	0.7716	
5	rs1664789	53282649	C	113	WCadjBMI	<i>ARL15</i>	0.7322	
6	rs722585	1775863	G	113	HipadjBMI	<i>GMDS</i>	0.9061	
7	rs13241538	130439862	C	113	HipadjBMI	<i>KLF14</i>	0.5863	
9	rs2398893	96758342	A	113	WHR	<i>PTPDC1</i>	0.2795	
16	rs4985155	15129459	A	113	Hip	<i>PDXDC1</i>	0.0072	0.0069
17	rs2034088	423051	T	113	HipadjBMI	<i>VPS53</i>	0.1833	

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- a) Single nucleotide polymorphisms (SNPs) tagging loci robustly associated with central fat accumulation were identified from literature (1, 4, 5). Lead SNP was chosen from the first study in which the locus was reported. Due to the multiplex format used for genotyping, rs1550280 ($r^2=0.96$) was genotyped instead of rs7830933. Genotyping of WHRadjBMI associated SNPs was conducted using the MassARRAY system with the iPLEX chemistry (Sequenom, San Diego, CA). Samples were analyzed by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik, Leipzig, Germany). Genotyping of waist circumference (WC) and hip associated SNPs were performed by Taqman assays (Applied Biosystems) in a second round of genotyping. DNA from 99 women was available for iPLEX genotyping and from 113 women for Taqman. The allele A1 is the trait-increasing allele in all cases. It was not possible to design a genotyping assay, or genotyping failed (i.e. results not in HWE) for SNPs tagging the *CALCRL*, *CBX3*, *CPEB4*, *HMGXB4*, *GORAB*, *HOXA11*, *KIAA1731*, *KLF13*, *TFPI*, and *ZBTB7B* genetic loci. Linear regression assuming an additive genetic model was used to assess the effect of the SNPs on expression of nearby candidate genes. As genes are chosen due to prior probability, a relaxed P for eQTL associations was set at $P<0.01$ for significance.
- b) P adjusted for analysis batch
- c) P adjusted for analysis batch and BMI

References

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