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Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity

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Obesity results from the interaction of genetic and environmental factors. To search for sequence variants that affect variation in two common measures of obesity, weight and body mass index (BMI), both of which are highly heritable, we performed a genome-wide association (GWA) study with 305,846 SNPs typed in 25,344 Icelandic, 2,998 Dutch, 1,890 European Americans and 1,160 African American subjects and combined the results with previously published results from the Diabetes Genetics Initiative (DGI) on 3,024 Scandinavians. We selected 43 variants in 19 regions for follow-up in 5,586 Danish individuals and compared the results to a genome-wide study on obesity-related traits from the GIANT consortium. In total, 29 variants, some correlated, in 11 chromosomal regions reached a genome-wide significance threshold of $P < 1.6 \times 10^{-7}$. This includes previously identified variants close to or in the *FTO, MC4R, BDNF* and *SH2B1* genes, in addition to variants at seven loci not previously connected with obesity.

Obesity results from a greater intake of calories than the body requires. The prevalence of obesity, defined as a BMI greater than 30 kg/m^2, has seen a dramatic increase worldwide in the last decades for both sexes and for all ages and ethnic groups. Obesity has tripled in the last five decades in the United States[1], with over 31% of males and 30% of females now classified as obese[2]. According to the World Health Organization, over 400 million people are obese (see URLs section). This poses a serious healthcare problem, as obesity is a major risk factor for several diseases including type 2 diabetes, cardiovascular diseases, dyslipidemia, hypertension, sleep apnea and some forms of cancer[3].

Twin, adoption and family studies have demonstrated the substantial heritability of obesity[4,5], and linkage and association studies have located obesity loci to numerous parts of the genome[6]. There are several, predominantly severe and of early onset, monogenic and syndromic forms of obesity described, although collectively they account for only a small percentage of cases[7]. Despite substantial heritability, the extensive efforts toward the search for obesity-related genes have met with modest success; *INSIG2*, *ENPP1* and *GAD2*, for example, have shown some promise but are not undisputed[7]. This may be due, in part, to the polygenic nature of obesity, whereby several sequence variants exert small effects and for which very large number of samples are required for detection, such as is the case with sequence variants affecting diversity in normal human height[8]. Recently, through GWA studies, common variants at the *FTO* (fat mass and obesity associated)[9] and *MC4R* (melanocortin 4 receptor)[10,11] loci were found to associate with fat mass, weight and obesity in several populations. Here we report results of a large GWA study for BMI and weight using a discovery sample comprising over 30,000 individuals.

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RESULTS

Genome-wide association for BMI and weight

To search for sequence variants that influence variance in weight and BMI, we analyzed genome-wide SNP data from 25,344 Icelanders, all of whom had weight and BMI information available (**Supplementary Table 1** online). Using these individuals as well as another 9,106 genotyped individuals without weight and BMI information allowed us to augment the analysis with a further 39,342 Icelanders who had weight and BMI information and whose genetic information could be partially inferred from the genotyped individuals. The Icelandic data were combined with data from 2,998 Dutch individuals, 1,890 European Americans and 1,160 African Americans. All these samples were genotyped using the 317K, 370K or 1M Illumina SNP chips. After exclusion of SNPs that failed quality control, 305,846 SNPs common to all chip types were selected for analysis and tested for association with standardized sex- and age-adjusted BMI and weight (see Methods). In addition, we combined our results with previously published analysis of 3,024 Swedish and Finnish individuals from the Diabetes Genetics Initiative (DGI)[12] typed with the Affymetrix GeneChip Human Mapping 500K platform. Of the 305,846 SNPs on the Illumina chips, 149,694 had a surrogate SNP with $r^2 > 0.8$ in the DGI dataset (on the basis of HapMap data[13]) and were used in the combined analysis. These five groups combined represent our discovery sample set.

In the discovery analysis, we observed a substantial excess of signals in a quantile-quantile plot compared to what was expected, even after excluding SNPs from the most significant locus at 16q12 (**Supplementary Fig. 1** online). Many SNPs at 2p25 and 16q12 reached genome-wide significance ($P < 1.6 \times 10^{-7}$) for BMI and weight, in addition to two SNPs at 1p31 for BMI, and one at 6p21 for weight (see **Tables 1** and **2** and **Supplementary Tables 2**, **3** and **4** online for results for individual sample sets). The 16q12 locus harbors the previously reported *FTO* gene.

Follow-up of the strongest signals in additional populations

Forty-three SNPs with a $P < 10^{-5}$ for BMI and/or weight were chosen for further analysis in two large sample sets from Denmark ($n = 5,586$, plus an additional 5,450 for three of the variants) and the GIANT consortium ($n = 32,615$)[14] with BMI and weight information. The follow-up analysis was achieved using genotypes from the Danish samples obtained by direct genotyping with individual SNP assays (**Supplementary Methods** and **Supplementary Table 5** online) and by *in silico* comparison of GWA data from the GIANT consortium (**Supplementary Table 6** online). Thirty-seven of the SNPs tested at 14 loci (1p31, 1p21, 1q25, 2p25, 3q27, 5q32, 6p21, 11p14, 12q13, 13q12, 16p11, 16q12, 18q21 and 19q13) were nominally significant (two-sided P value < 0.05) and with a consistent direction of the effect for either BMI or weight in the combined Danish and GIANT sample sets. Eleven of these loci, excluding 1p21, 5q32 and 13q12, were genome-wide significant after combination of the results from the discovery set with those from the Danish and the GIANT sample sets (combined $P < 1.6 \times 10^{-7}$; **Tables 1** and **2**). Ten of these loci (1p31, 1q25, 2p25, 3q27, 11p14, 12q13, 16p11, 16q12, 18q21 and 19q13) were genome-wide significant for both BMI and weight, whereas the 6p21 locus was genome-wide significant for weight only.

Associations at the 11 genome-wide significant loci for BMI and/or weight

The strongest association with both BMI and weight was observed at 16q12 for the highly correlated SNPs rs8050136 (combined $P = 1.1 \times 10^{-47}$ for BMI) and rs3751812 (combined $P = 2.6 \times 10^{-37}$ for weight) in *FTO*. At this locus, there are three other SNPs reaching genome-wide significance for BMI and weight. One of these (rs6499640) is only weakly correlated with the strongest signal ($r^2 < 0.2$ with rs8050136; **Supplementary Table 7** online), and association between BMI and rs6499640 remained significant when the association was tested conditional on the association of rs8050136 at this locus ($P = 0.00039$; **Supplementary Table 8** online). This suggests either that rs6499640, located in an adjacent LD block to rs8050136, represents a different variant associated with obesity, or that a variant capturing the effect of both rs8050136 and rs6499640 remains to be identified.

The second best association with BMI and weight was seen at 2p25, captured by three highly correlated SNPs (**Supplementary Table 7**). Of these three SNPs, rs7561317, located about 22 kb downstream of *TMEM18* (transmembrane protein 18), gave the strongest signal (combined $P = 4.2 \times 10^{-17}$ and 7.0×10^{-18} for BMI and weight, respectively; **Tables 1** and **2**). The association signal captured by rs7561317 has not previously been linked to obesity or obesity-related traits. The *TMEM18* gene has recently been identified as a modulator of glioma-directed stem cell migration and is perhaps involved in cell movement in general[15]. It is localized to the nucleus, widely expressed in fetal and adult tissues and well conserved among divergent species.

Of the remaining nine genome-wide significant regions we identified, four (18q21, 16p11, 6p21 and 11p14) have previously been associated with obesity and/or obesity-related traits. Of these, the 18q21 locus harboring the *MC4R* gene has been extensively replicated across different populations[6]. The 18q21 SNP rs12970134 identified here (**Tables 1** and **2**) is located about 154 kb downstream of *MC4R* and has previously been found to associate with waist circumference and insulin resistance[11] and is highly correlated with rs17782313 ($r^2 = 0.81$, $D' = 0.96$ in the HapMap CEU dataset), which was reported in a large study to associate with fat mass, weight and height, suggesting an overall influence on adult size as well as an increased risk of obesity at the population level[10]. At the 16p11 locus, the G allele (Ala484) of the nonsynonymous SNP rs7498665 (T484A) in *SH2B1* (Src-homology-2 (SH2) domain containing putative adaptor protein 1) has previously been shown to associate with increased serum leptin, total fat, waist circumference and body weight[16]. Here we demonstrated an association with BMI for that same allele.

At 6p21, there are SNPs in three genes that have previously been reported to associate with obesity-related traits. They are the coding SNP rs1061581 (Q353Q)[17] in *HSPA1B* (heat shock 70kDa protein 1B), the nonsynonymous rs1041981 (T60N)[18] in *LTA* (lymphotoxin alpha), and the promoter[19] and intronic[20] SNPs (rs1800629 and rs1800610, respectively) in *TNF* (tumor necrosis factor). The variant identified in our GWA analysis at the 6p21 locus, rs2844479, does not correlate with rs1041981, rs1800629 or rs1800610 (with $r^2 < 0.1$ in the HapMap CEU dataset; **Supplementary Table 9** online). As rs1061581 is not part of the HapMap data, its correlation to rs2844479 could not be estimated. However, rs1061581 is unlikely to correlate with rs2844479, as the two are separated by many linkage disequilibrium blocks. The rs2844479 variant therefore likely represents a new association signal to weight. The genes closest to rs2844479 are *AIF1* (allograft inflammatory factor 1) and *NCR3* (natural cytotoxicity triggering receptor 3 precursor).

In our study, seven SNPs located within or downstream of *BDNF* (brain derived neurotrophic factor) at 11p14 showed strong association with BMI (**Supplementary Table 6**). One of these SNPs is the nonsynonymous rs6265 (V66M), which has mainly been associated with neurological and psychiatric disorders, although recent candidate gene studies have suggested its role in eating behavior[21] and BMI[22]. On the basis of the estimated pair-wise correlation among these SNPs,

Table 1 Variants that associate with variation in BMI

				Discovery set		Replication set		Combined		
Locus	SNP	Position	Allele tested	Effect	*P*	Effect	*P*	Effect (s.e.m.)	*P*	Nearest gene(s)
1p31	rs3101336	72523773	G	4.56	1.1×10^{-7}	3.01	1.8×10^{-5}	3.67 (0.55)	2.5×10^{-11}	*NEGR1*
"	rs2568958	72537704	A	4.66	9.9×10^{-8}	3.11	1.0×10^{-5}	3.77 (0.56)	1.2×10^{-11}	"
1q25	rs10913469	176180142	C	4.29	4.2×10^{-6}	2.55	0.002	3.36 (0.62)	6.2×10^{-8}	*SEC16B, RASAL2*
2p25	rs2867125	612827	G	6.83	1.1×10^{-10}	5.59	1.5×10^{-8}	6.11 (0.74)	1.7×10^{-16}	*TMEM18*
"	rs4854344	628144	T	6.33	2.9×10^{-10}	5.86	3.2×10^{-9}	6.05 (0.72)	6.8×10^{-17}	"
"	rs7561317	634953	G	6.42	2.4×10^{-10}	5.90	2.3×10^{-9}	6.12 (0.73)	4.2×10^{-17}	"
3q27	rs7647305[a]	187316984	C	4.66	3.1×10^{-6}	4.29	1.5×10^{-6}	4.42 (0.68)	7.2×10^{-11}	*SFRS10, ETV5, DGKG*
11p14	rs4074134	27603861	G	5.77	1.8×10^{-6}	4.27	5.0×10^{-6}	5.01 (0.76)	4.4×10^{-11}	*LGR4, LIN7C, BDNF*
"	rs4923461	27613486	A	5.89	1.1×10^{-6}	4.21	4.4×10^{-6}	5.03 (0.76)	3.2×10^{-11}	"
"	rs925946	27623778	T	4.9	2.0×10^{-7}	3.01	0.00021	3.85 (0.63)	8.5×10^{-10}	"
"	rs10501087	27626684	T	5.68	4.2×10^{-6}	4.32	4.0×10^{-6}	5.01 (0.77)	8.7×10^{-11}	"
"	rs6265	27636492	G	5.67	7.2×10^{-6}	3.64	9.9×10^{-6}	4.58 (0.73)	5.1×10^{-10}	"
12q13	rs7138803	48533735	A	4.89	9.6×10^{-7}	1.96	0.0051	3.28 (0.62)	1.2×10^{-7}	*BCDIN3D, FAIM2*
16p11	rs8049439	28745016	C	3.92	6.0×10^{-6}	3.41	3.7×10^{-6}	3.42 (0.56)	1.4×10^{-9}	*SH2B1, ATP2A1*
"	rs4788102	28780899	A	4.03	3.5×10^{-6}	3.48	2.8×10^{-6}	3.51 (0.57)	6.4×10^{-10}	"
"	rs7498665	28790742	G	4.25	1.7×10^{-6}	3.53	2.6×10^{-6}	3.63 (0.58)	3.2×10^{-10}	"
16q12	rs6499640	52327178	A	5.55	6.0×10^{-8}	5.13	9.2×10^{-7}	5.26 (0.73)	4.0×10^{-13}	*RPGRIP1L, FTO*
"	rs8050136	52373776	A	8.28	4.4×10^{-24}	7.66	7.6×10^{-26}	8.04 (0.55)	1.1×10^{-47}	"
"	rs3751812	52375961	T	8.92	3.3×10^{-24}	7.54	5.3×10^{-25}	8.09 (0.56)	4.1×10^{-47}	"
"	rs7190492	52386253	G	6.85	2.0×10^{-12}	5.39	3.6×10^{-12}	5.98 (0.61)	8.1×10^{-23}	"
"	rs8044769	52396636	C	6.85	7.9×10^{-16}	6.11	8.1×10^{-17}	6.40 (0.55)	7.6×10^{-31}	"
18q21	rs12970134	56035730	A	4.23	2.6×10^{-6}	4.34	1.6×10^{-7}	4.38 (0.62)	1.2×10^{-12}	*MC4R*
19q13	rs29941	39001372	C	4.25	5.6×10^{-6}	4.01	1.3×10^{-7}	4.18 (0.61)	7.3×10^{-12}	*CHST8, KCTD15*

Twenty-three variants, located in ten distinct genomic regions, showing association with variation in BMI. All variants shown have $P < 1.6 \times 10^{-7}$ after combination of the results from the five groups in the GWA study (Iceland, The Netherlands, European and African Americans, and DGI) with the results from two replication sets (the Danish sample set and results from the GIANT consortium; see **Supplementary Tables 5** and **6** for the effect and *P* value for replication sets separately). For comparison, the table shows the effect (in percentile of s.d.) and *P* value for the GWA study, the combined replication set and the combined analysis for the discovery and replication sets. Prior to combination, *P* values from individual GWA study groups were corrected using genomic control. When combining all groups, we excluded results from the DGI study from the GWA discovery group. The positions of the SNPs are reported in Build 36 coordinates. The last column lists the nearest genes to the associated variants (see **Supplementary Note** for a detailed list of genes in each of the associated regions).

[a]Data on 5,450 individuals from the Addition study were included in the analysis of this variant.

only four of the six are strongly correlated with rs6265 (with $r^2 > 0.8$, **Supplementary Table 7**). Two SNPs (rs925946 and rs7481311) are very weakly correlated to rs6265 ($r^2 < 0.2$) and probably represent an independent signal of association. The association between BMI and those two SNPs remained significant when it was tested conditional on the association to rs6265 ($P = 0.000082$ and 0.005, respectively; **Supplementary Table 8**).

In addition to the 2p25 (*TMEM18*) locus and the new signals discussed above for the 6p21 and 11p14 loci, we identified five new BMI- and weight-associated loci at 1p31, 1q25, 3q27, 12q13 and 19q13. Of these, the strongest association observed was to rs29941 (combined $P = 7.3 \times 10^{-12}$ and 3.2×10^{-9} for BMI and weight, respectively) at 19q13, about 4.4 kb downstream of *KCTD15* (potassium channel tetramerization domain containing 15), encoding a potential transcription factor[23,24]. This association was closely followed by rs2568958 (combined $P = 1.2 \times 10^{-11}$ and 2.1×10^{-8} for BMI and weight, respectively) at 1p31 and rs7647305 (combined $P = 7.2 \times 10^{-11}$ and 6.2×10^{-9} for BMI and weight, respectively) at 3q27. rs2568958 is located in the same LD block and about 16 kb upstream of exon 1 of *NEGR1* (neuronal growth regulator 1), which participates in the regulation of neurite outgrowth in the developing brain[25,26], and rs7647305 is located between *ETV5* (Ets variant gene 5), encoding a transcription factor, and *DGKG* (diacylglycerol kinase gamma), encoding an enzyme which regulates diacylglycerol by phosphorylating it to form phosphatidic acid. For a list and brief description of the genes located at each of the BMI- and weight-associated loci, see **Supplementary Table 10** and **Supplementary Note** online.

Effect of significant SNPs on T2D and physical traits

The combined effect of the variants at the 11 loci listed in **Tables 1** and **2** only explains a small fraction (less than 1%) of the population variance of weight and BMI. For BMI, this ranges from 0.31% for rs8050136 at 16q12 down to 0.04% for rs10913469 at 1q25, and for weight, the corresponding range is 0.25% for rs3751812 at 16q12 down to 0.05% for rs10913469 at 1q25. The average BMI and weight per genotype can be seen in **Supplementary Table 11** online. We tested the effect of the genome-wide significant variants on other related traits such as height, waist, hip and waist-hip ratio (WHR). For height, we used the sample sets previously described and used in a GWA study on height[8], combined with data from the Danish samples used here. Only two of the SNPs showed significant correlation to height: rs2844479[T] at 6p21 ($P = 3.3 \times 10^{-13}$) and rs7561317[G] at 2p25 ($P = 3.7 \times 10^{-5}$). Of note, the 6p21 variant shows stronger association with increased weight and height[8] than with BMI, which suggests that it has a general effect on body size rather than measures of obesity. In contrast, none of the tested variants showed strong association with waist, hip or WHR after adjustment for the effect of BMI or weight (data not shown).

To investigate to what extent the identified BMI- and weight-associated variants influenced the risk of being obese, we calculated the odds ratio (OR) for obese (BMI $\geq$ 30) versus normal-weight individuals (18.5 $<$ BMI $<$ 25) using data from four of the discovery, as well as the Danish, sample sets. For each of the independent signals at the 11 loci, the allele that associated with increased BMI and weight was associated with obesity (**Table 3** and **Supplementary Table 12**

Table 2 Variants that associate with variation in weight

				Discovery set		Replication set		Combined		
Locus	SNP	Position	Allele tested	Effect	*P*	Effect	*P*	Effect (s.e.m.)	*P*	Nearest gene(s)
1p31	rs3101336	72523773	G	4.00	8.7×10^{-6}	2.68	0.00067	3.23 (0.59)	5.0×10^{-8}	*NEGR1*
"	rs2568958	72537704	A	4.14	6.9×10^{-6}	2.79	0.00038	3.36 (0.60)	2.1×10^{-8}	"
1q25	rs10913469	176180142	C	4.87	4.3×10^{-7}	3.09	0.001	3.92 (0.67)	5.8×10^{-9}	*SEC16B, RASAL2*
2p25	rs2867125	612827	G	6.87	3.6×10^{-10}	5.83	4.1×10^{-9}	6.31 (0.76)	7.8×10^{-17}	*TMEM18*
"	rs4854344	628144	T	6.46	8.6×10^{-10}	6.10	$3.4 \times 1\ 0^{-10}$	6.27 (0.73)	1.3×10^{-17}	"
"	rs7561317	634953	G	6.59	6.2×10^{-10}	6.14	2.5×10^{-10}	6.36 (0.74)	7.0×10^{-18}	"
3q27	rs7647305[a]	187316984	C	4.34	1.7×10^{-5}	3.63	4.8×10^{-5}	3.95 (0.68)	6.2×10^{-9}	*SFRS10, ETV5, DGKG*
6p21	rs2844479	31680935	T	5.88	1.2×10^{-9}	1.91	0.018	3.66 (0.64)	9.0×10^{-8}	*NCR3, AIF1,BAT2*
"	rs2260000	31701455	T	4.37	1.4×10^{-6}	2.20	0.004	3.13 (0.59)	1.4×10^{-7}	"
"	rs1077393	31718508	T	4.80	5.0×10^{-7}	2.19	0.0032	3.21 (0.59)	4.4×10^{-8}	"
11p14	rs4074134	27603861	G	4.86	5.4×10^{-5}	3.32	0.00032	4.12 (0.76)	5.8×10^{-8}	*LGR4, LIN7C, BDNF*
"	rs4923461	27613486	A	4.98	3.8×10^{-5}	3.14	0.00069	4.06 (0.77)	1.1×10^{-7}	"
"	rs925946	27623778	T	4.84	1.5×10^{-6}	2.72	0.00077	3.74 (0.64)	6.2×10^{-8}	"
"	rs10501087	27626684	T	4.79	0.00012	3.29	0.00025	4.06 (0.76)	8.6×10^{-8}	"
"	rs10835211	27657941	A	4.53	6.3×10^{-6}	2.95	0.00069	3.65 (0.67)	5.2×10^{-8}	"
12q13	rs7138803	48533735	A	4.19	7.3×10^{-6}	2.60	0.00092	3.37 (0.62)	5.6×10^{-8}	*BCDIN3D, FAIM2*
16p11	rs8049439	28745016	C	3.90	2.1×10^{-5}	3.47	5.2×10^{-6}	3.50 (0.59)	3.7×10^{-9}	*SH2B1, ATP2A1*
"	rs4788102	28780899	A	4.09	8.2×10^{-6}	3.50	4.7×10^{-6}	3.60 (0.60)	1.4×10^{-9}	"
"	rs7498665	28790742	G	4.35	3.2×10^{-6}	3.52	4.4×10^{-6}	3.73 (0.60)	5.8×10^{-10}	"
16q12	rs6499640	52327178	A	4.76	5.3×10^{-6}	5.94	4.5×10^{-8}	5.33 (0.75)	1.3×10^{-12}	*RPGRIP1L, FTO*
"	rs8050136	52373776	A	7.15	1.6×10^{-18}	6.84	9.8×10^{-20}	7.10 (0.56)	9.3×10^{-37}	"
"	rs3751812	52375961	T	8.06	6.9×10^{-20}	6.77	1.8×10^{-19}	7.26 (0.57)	2.6×10^{-37}	"
"	rs7190492	52386253	G	5.74	2.1×10^{-9}	5.04	1.5×10^{-10}	5.30 (0.61)	2.3×10^{-18}	"
"	rs8044769	52396636	C	6.00	5.7×10^{-13}	5.66	5.8×10^{-14}	5.77 (0.56)	3.0×10^{-25}	"
18q21	rs633265	55982448	A	3.47	9.1×10^{-6}	2.97	6.7×10^{-5}	3.23 (0.55)	3.1×10^{-9}	*MC4R*
"	rs1350341	55993513	T	3.51	5.7×10^{-6}	2.90	0.0001	3.17 (0.54)	4.9×10^{-9}	"
"	rs12970134	56035730	A	4.45	2.3×10^{-6}	4.76	4.6×10^{-8}	4.68 (0.64)	3.6×10^{-13}	"
19q13	rs29941	39001372	C	4.13	2.3×10^{-5}	3.57	5.3×10^{-5}	3.76 (0.63)	3.2×10^{-9}	*CHST8, KCTD15*

Twenty-eight variants, located in eleven distinct genomic regions, showing association with variation in weight. All variants shown have $P < 1.6 \times 10^{-7}$ after combination of the results from the five groups in the GWA study (Iceland, The Netherlands, European and African Americans, and DGI) with the results from two replication sets (the Danish sample set and results from the GIANT consortium; see **Supplementary Tables 5** and **6** for the effect and *P* value for replication sets separately). For comparison, the table shows the effect (in percentile of s.d.) and *P* value for the GWA study, the combined replication set and the combined analysis for the discovery and replication sets. Prior to combination, *P* values from individual GWA study groups were corrected using genomic control. When combining all groups, we excluded results from the DGI study from the GWA discovery group. The positions of the SNPs are reported in Build 36 coordinates. The last column lists the nearest genes to the associated variants (see **Supplementary Note** for a detailed lists of genes in each of the associated regions).

[a]Data on 5,450 individuals from the Addition study were included in the analysis of this variant.

online). The effect was strongest for rs8050136 at the *FTO* locus (combined analysis OR = 1.27 and $P = 3.0 \times 10^{-26}$), followed by rs7561317 at the *TMEM18* locus (combined analysis OR = 1.2 and $P = 2.6 \times 10^{-9}$). As expected, the weakest association with obesity was observed for the locus specific to weight and height at 6p21 (OR = 1.07, $P = 0.0093$). We also carried out the same analysis with respect to risk of having type 2 diabetes (T2D) in Icelandic and Danish case-control sample sets. In the combined analysis of the Icelandic and Danish samples, we observed significant ORs for the two variants representing the signal in *FTO* on 16q12 as well as significant associations on 1p31, 3q27 and 12q13 (**Table 3** and **Supplementary Table 13** online). However, when we included standardized sex- and age-adjusted BMI values as covariates in the logistic regression, only 12q13 remained significant; 6p21 became significant as well.

Role of genes at genome-wide significant loci

To investigate how the loci might affect BMI or weight, we looked for common biological function of genes neighboring (within 200 kb) the association signals using the biological-process classification of the Panther database[27] (data not shown). Although no significant clustering was observed, we note the presence of genes involved in apoptosis and neural development or activity, and fewer genes involved in metabolic function than would be expected given the phenotype. A role for leptin in adipocyte apoptosis[28] has been demonstrated, and so other initiators and inhibitors of apoptosis could similarly be involved, such as *LTA* or *TNF* (6p21) and *FAIM2* or *TMBIM6* (also known as *TEGT*, 12q13). Several of the BMI- and weight-associated variants are near genes expressed in the brain and affecting neuronal development or activity. This includes variants close to *MC4R* (18q21) and *SH2B1* (16p11), involved in neural signaling, *BDNF* (11p14), involved in neural development, and *FTO* (16q12), expressed in regions of the brain affecting feeding regulation. Furthermore, some of the newly identified genes such as *NEGR1* (1p31), *LIN7C* (11p14) and potentially *TMEM18* (2p25) are involved in neural development and may therefore also influence the development and function of brain centers involved in the regulation of feeding.

Correlation of genome-wide significant variants with gene expression

We previously measured the expression of 23,720 transcripts using an Agilent microarray and RNA from both adipose tissue and blood from 674 and 1,002 Icelandic individuals, respectively[29] (see **Supplementary Methods**). Of those individuals, 603 with adipose tissue data and 745 with data from blood have been genotyped with

Table 3 Association of the BMI and weight variants at the 11 loci with obesity and T2D

			Obesity		T2D		
Loci	SNP	Allele	OR (95% CI)	*P*	OR (95% CI)	*P*	P_{adj}
1p31	rs2568958	A	1.07 (1.02–1.12)	0.0046	1.06 (1.00–1.12)	0.038	0.15
1q25	rs10913469	C	1.11 (1.05–1.18)	0.00011	0.99 (0.93–1.06)	0.87	0.27
2p25	rs7561317	G	1.20 (1.13–1.27)	2.6×10^{-9}	1.05 (0.97–1.13)	0.22	0.9
3q27	rs7647305	C	1.11 (1.05–1.17)	0.00018	1.08 (1.00–1.15)	0.036	0.35
6p21	rs2844479	T	1.07 (1.02–1.12)	0.0093	0.95 (0.90–1.01)	0.11	0.028
11p14	rs6265	G	1.12 (1.06–1.19)	0.00022	0.98 (0.92–1.06)	0.64	0.12
"	rs925946	T	1.11 (1.05–1.16)	3.5×10^{-5}	1.04 (0.98–1.11)	0.15	0.73
12q13	rs7138803	A	1.14 (1.09–1.19)	2.5×10^{-8}	1.11 (1.05–1.17)	0.00023	0.0090
16p11	rs7498665	G	1.08 (1.03–1.13)	0.00076	1.05 (1.00–1.11)	0.069	0.25
16q12	rs6499640	A	1.16 (1.10–1.21)	1.1×10^{-9}	1.07 (1.01–1.13)	0.027	0.48
"	rs8050136	A	1.27 (1.21–1.32)	3.0×10^{-26}	1.13 (1.07–1.19)	1.6×10^{-5}	0.086
18q21	rs12970134	A	1.12 (1.06–1.17)	9.9×10^{-6}	1.04 (0.98–1.10)	0.18	0.91
19q13	rs29941	C	1.10 (1.04–1.15)	0.00021	1.01 (0.95–1.07)	0.7	0.96

The association with obesity and T2D for 13 SNPs located in the 11 loci showing significant association with weight or BMI. For each locus, the SNP that shows the most significant association is tested, except for 11p14 and 16q12, where two SNPs, possibly representing two distinct signals of association, are selected. In all cases, the allele that correlates with increased weight or BMI is tested. For obesity (BMI $\geq$ 30), the association is tested relative to normal weight individuals (18.5 $<$ BMI $<$ 25). The analysis is done on the sample sets from Iceland (9,255 normal weight; 5,996 obese), The Netherlands (1,432; 331), Denmark (2,413; 949), and the European (472; 591) and African-American (213; 625) sample sets from John Hopkins, USA. Shown are the results for all five sample sets combined using the Mantel-Haenzel method. For T2D, the analysis is done for 1,405 Icelandic T2D cases compared to 23,190 Icelandic controls and for 2,471 Danish T2D cases and 4,841 controls. The ORs and *P* values presented are for the two sample sets combined using a Mantel-Haenzel model. Also included is a *P* value, P_{adj}, for the association with T2D adjusted for BMI by including standardized sex- and age-adjusted BMI values as covariates in the logistic regression. All *P* values presented, except for the Danish sample set, were adjusted using the method of genomic control.

either the Illumina 317K or 370K SNP chips. We used this dataset to investigate the correlation between the variants at the 11 loci that showed significant association to BMI and/or weight and the expression of neighboring genes (**Supplementary Note**). At 1p31, we observed 12.1% increased expression of *NEGR1* in blood per copy of the A allele of rs2568958 ($P = 4.2 \times 10^{-13}$; **Supplementary Table 14** online). For rs2844479 at 6p21, we observed about an 11% increase in expression of *CSNK2B* per T allele carried both in adipose tissue and blood ($P < 10^{-60}$ and $P < 10^{-26}$, respectively). At 16p11, the G allele of rs7498665 is highly correlated with the expression of several genes, most significantly with *TUFM* in blood ($P < 10^{-41}$), but also with *SULT1A1*, *SULT1A2*, *SH2B1*, *APOB48R* and *EIF3C* (also known as *EIF3S8*). We note, however, that at this locus there seem to be several putative segmental duplications spanning some of the genes in the area (**Supplementary Note**). It is possible that rs7498665 correlates with the presence of some of those duplications and through that correlates with the expression of genes that are duplicated. None of the correlations between the SNPs and the expression described above changed substantially when we adjusted for BMI or weight in the regression, and no other SNPs at those loci showed stronger correlation with the expression of the corresponding genes. For the associated variants at the remaining eight loci, either no correlation was observed with the expression of genes in the region, or the observed correlation could be explained by other SNPs that showed much weaker association with BMI or weight.

DISCUSSION

Obesity is a major health concern in the developed as well as the developing world. Until recently, few variants had been discovered that affect the common forms of obesity. Initially, we carried out a GWA study with over 30,000 individuals to search for sequence variants that may have a role in the development of obesity and found both previously described variants and several additional variants that may shed some light on new pathways involved in obesity and, in particular, weight gain. This was followed by genotyping and *in silico* analysis in two large independent sample sets where several of those variants, representing 11 loci, met stringent criteria of genome-wide significance. The identified variants not only showed a significant, yet modest, effect on the two obesity-related traits studied, BMI and weight, but also on the risk of being obese.

Many of the associated variants are near genes that are highly expressed in the brain and/or have previously been shown to have a function in neuronal development or activity. This underscores the importance of genes that regulate food intake over those involved in metabolism. This is not unexpected, as a considerable part of the regulation of energy balance in humans is probably controlled by genes responsible for feeding behavior or energy sensing.

It is important to note that all of the variants reported are relatively common, and their combined effect only explains a small fraction of the variation in BMI and weight. Furthermore, apart from the *MC4R* locus, few of the other loci have been extensively fine mapped, and therefore, other variants with potentially greater effect on BMI and weight could be identified at these loci. Very rare mutations within those loci, as for the *MC4R* gene, could contribute to the more extreme forms of obesity. For two of the loci, 16q12 and 11p14, none of the tested variants can fully account for the observed association, suggesting that either two independent obesity variants reside within these loci or that a variant capturing the effect of both variants remains to be identified.

Of the 11 genome-wide significant loci identified, all but one showed strong association with both BMI and weight. The weight-specific locus 6p21, one of the two loci identified that also associated significantly with height, showed a relatively weak association with variations in BMI, and its effect is likely to be on variation in body size rather than on obesity. Apart from the 6p21 locus, the identified loci could thus be having an effect on obesity through adiposity-related weight gain.

It is expected that future GWA studies would benefit from even larger sample sizes or meta-analysis of such studies. However, analysis based on current genotyping platforms that are biased toward common variants will, most likely, add to the collection of common variants with modest risk that we expect will only explain a modest

fraction of the variation in BMI and weight. Developing and using tools that will allow us to identify rare variants with higher risk will become essential to be able to fully explain the heritability of BMI and weight variation.

METHODS

Study subjects and SNP genotyping. *Icelandic samples*. Weight and BMI information was available for 74,921 and 74,735 Icelanders, respectively, and of those, 25,344 were directly genotyped with either the Illumina 317K or 370K SNP chips, and a further 39,342 had imputed genotypes (see **Supplementary Methods**). The recruitment of the 1,405 T2D cases used in this study has been described previously[30], and the 23,190 controls used are described in **Supplementary Methods**. These studies were all approved by the Data Protection Commission of Iceland and the National Bioethics Committee of Iceland. Written informed consent was obtained from all participants, and personal identifiers were encrypted as previously described[31]. The Icelandic study participants were genotyped with the Illumina Human Hap300 or Human Hap300-duo+ (referred to as the 317K) or Human CNV370-Duo (referred to as the 370K) Bead Arrays (Illumina).

Dutch samples. Weight and BMI information was available from two studies by the Radboud University Nijmegen Medical Centre (RUNMC), the Nijmegen Bladder Cancer Study ($n = 1{,}164$) and the Nijmegen Biomedical Study ($n = 1{,}942$). Informed consent was obtained from the participants and the local institutional review board (IRB), and the IRB of the RUNMC approved the respective studies. Of the 3,106 available samples, 2,998 provided genotypes for the analysis. The Dutch study participants were genotyped with the Illumina Human Hap300-duo+ Bead Arrays.

US samples. Weight and height information was obtained from subjects recruited as part of the Johns Hopkins Sibling and Family Heart Study an ongoing prospective family study. Probands with documented coronary artery disease (CAD) before the age of 60 were asked to participate as well as their siblings, spouse and offspring. The US participants were genotyped with the Illumina Human Hap1000 (referred to as the 1M) Bead Array.

Danish samples. Weight and BMI information was obtained from subjects in two population cohorts: (i) the population-based Inter99 samples[32] recruited at the Research Centre for Prevention and Health and (ii) the ADDITION samples[33] recruited for a high-risk screening and intervention study for type 2 diabetes in general practice and sampled through the Department of General Practice at the University of Aarhus. The case-control study of type 2 diabetes included all individuals with type 2 diabetes from the Inter99 as well as from the outpatient clinic at the Steno Diabetes Center. The controls consisted of glucose-tolerant subjects from the Inter99 cohort. Informed written consent was obtained from all study subjects. The study was approved by the Ethical Committee of Copenhagen County and was in accordance with the principles of the Helsinki Declaration. The Danish samples were genotyped at deCODE genetics with the Centaurus (Nanogen) platform[34].

GIANT samples. The GIANT consortium samples are the combined datasets from 13 genome-wide association (GWA) studies, amounting to a total of 32,615 individuals[14]. BMI and weight association results for 43 SNPs were provided to us by the consortium.

Association testing. All information on weight and BMI was corrected for year of birth and standardized to have a standard normal distribution, within both sexes, for each study population separately. In Iceland, year of birth was rounded to five years and used as a factor variable in the correction, but in the other populations, a linear term in year of birth was used to correct the measurements. Measured and self-reported weight and BMI were corrected separately in Iceland. For each SNP, a classical linear regression, using the genotype as an additive covariate and weight or BMI as a response, was fit to test for association.

We scaled all test statistics by the method of genomic controls[35] using the s.d. of the z statistic as an estimate of the inflation factor. The estimated inflation factors for weight and BMI, respectively, were 1.223 and 1.184 in the Icelanders, 1.016 and 1.018 in the Dutch, 1.181 and 1.155 in the Americans of European descent, 1.147 and 1.150 in the African Americans, and 1.048 and 1.034 in the DGI data. Adjusted P values were calculated by dividing the corresponding z score by the inflation factor.

We combined data from all the samples by using an estimate of the effective sample size of each population. For the non-Icelandic samples, the actual sample size divided by the inflation factor squared was used as an estimate of the effective sample size used in combining weight and BMI, respectively: 2831.4 and 2822.07 for the Dutch, 1275.4 and 1225.0 for the Americans of European descent, 881.64 and 884.91 for the African Americans, and 2500.84 and 2570.66 for the DGI sample. For the Icelandic sample, a rough estimate of 20,000 was used as an estimate of the effective sample size, both for weight and BMI, because of the complexities introduced by the family-based association described below. Inappropriate effective sample size estimates will reduce power but will not affect the validity of the analysis. An overall z score was calculated by summing the z scores weighted by the square root of the effective sample size, overall populations, and dividing by the square root of the sum of the sample sizes. An overall estimate of the effect per allele was calculated by weighting together the effects in each population by the population's effective sample size.

The fraction of variance explained was calculated using the formula $2f(1-f)a^2$, where f is the frequency of the variant and a is its additive effect. As an estimate of the population frequency, we used a simple average of the frequency in the Icelandic, the Dutch, the Americans of European descent and the Danish sample sets. For the calculation of the fraction of variance explained by the SNPs in **Tables 1** and **2**, only one SNP was picked per locus.

For a description of the GIANT BMI meta-analysis, see ref. 14. In the GIANT weight meta-analysis, each of the 13 studies involved performed quality control on genotyped SNPs and samples, and subsequently imputed genotypes using the HapMap CEU reference panel. The weight phenotype was inverse-normal transformed separately in each gender, and the sex-stratified cohorts were tested for association using an additive model with age, sex and age^2 as covariates. All alleles were listed on the forward strand, and meta-analysis was done using METAL with genomic control correction enabled and using the standard error weighting scheme.

URLs. WHO, http://www.who.int/mediacentre/factsheets/fs311/en/index.html.

Accession codes. Genbank Entrez Gene: accession codes for the genes mentioned in this article are presented in **Supplementary Table 10**. NCBI GEO: gene expression data are available under the accession numbers GSE7965 and GPL3991.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

G.T., G.B.W., U.T. and K.S. wrote the first draft of the paper. G.B.W., V.S., A.H., U.S., S.G., S.T., I.J., E.J.O., G.H.O., T. Jonsson, L.T. and T.R. participated in the collection of the Icelandic data. K.B.-J., T.H., G.A., T. Jorgensen, T.L. and O.P. recruited and phenotyped the Danish study samples. K.K.A., A.L.M.V., N.R., E.K. and L.A.K. collected the Dutch data. D.M.B., L.R.Y. and L.C.B. collected the US data. G.T., G.B.W., D.F.G. and P.S. analyzed the data. G.B.W., T. Jonsdottir and F.J. carried out the genotyping. G.T., G.B.W., J.G., A.K., U.T. and K.S. planned and supervised the work. All authors contributed to the final version of the paper.

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