Genetic studies of leptin concentrations implicate leptin in the regulation of early adiposity

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Abstract

Leptin influences food intake by informing the brain about the status of body fat stores. Rare *LEP* mutations associated with congenital leptin deficiency cause severe early-onset obesity that can be mitigated by administering leptin. However, the role of genetic regulation of leptin in polygenic obesity remains poorly understood. We performed an exome-based analysis in up to 57,232 individuals of diverse ancestries to identify genetic variants that influence adiposity-adjusted leptin concentrations. We identify five novel variants, including four missense variants, in *LEP, ZNF800, KLHL31*, and *ACTL9*, and one intergenic variant near *KLF14.* The missense variant Val94Met (rs17151919) in *LEP* was common in individuals of African ancestry only and its association with lower leptin concentrations was specific to this ancestry ($P=2x10^{-16}$, n=3,901). Using *in vitro* analyses, we show that the Met94 allele decreases leptin secretion. We also show that the Met94 allele is associated with higher BMI in young African-ancestry children but not in adults, suggesting leptin regulates early adiposity.

Introduction

Leptin is an adipocyte-derived hormone that helps maintain homeostatic control of fat tissue mass by signaling the status of body energy stores to the appetite-regulating circuits of the brain [1]. Rare homozygous mutations in the leptin (*LEP*) gene can cause complete leptin deficiency that results in hyperphagia and severe early-onset obesity, which can be treated effectively by exogenous leptin administration [2, 3]. Mice and patients heterozygous for these mutations show partial leptin deficiency and increased body weight [4-6].

In the general population, leptin concentrations correlate closely with body fat mass. However, there is wide inter-individual variability; about 10-20% of obese individuals have leptin concentrations that are similar to those observed in non-obese individuals, which is in part due to genetic differences [7, 8]. Twin and family studies suggest that 30-50% of variation in leptin at any given level of adiposity and across different ethnic groups is explained by genetic differences [8]. The implications of this variability for body weight regulation remain poorly understood.

Identification of genetic variants associated with circulating leptin may shed new light on the role of variability in leptin levels in the general population. In a recent genome-wide association study (GWAS) of leptin concentrations, we identified four loci associated with leptin concentrations independent of body mass index (BMI) [9]. The variant most strongly associated with leptin concentrations was rs10487505, located 21 kb upstream from *LEP,* in a region shown to harbor a long non-coding RNA (EST EL947753) that influences the transcriptional control of leptin expression [10]. The leptin-decreasing allele of rs10487505 was nominally associated with ~0.03 kg/m^2 higher BMI in adults and 1.05-fold increased risk of early-onset obesity [9]. More recently, the association of the leptin-decreasing allele of rs10487505 with higher adult BMI, body fat percentage, and risk of extreme obesity was replicated in the UK Biobank [10]. The most pronounced association, however, was observed for body size at 10 years of age; carriers of the leptin-decreasing allele reported being "plumper" at age 10 compared to peers" more frequently than carriers of the allele associated with higher leptin concentration. The association between rs10487505 and childhood body size was recently replicated in 14,521 Norwegian children, and the peak effect of rs10487505 on BMI was observed in 1.5-year-old children [11].

In the present study, we sought to elucidate the genetic basis of leptin concentrations through screening genetic variants with an exome-targeted array in up to 57,232 individuals of European, African, East Asian or Hispanic ancestry. We confirm five previously established and identify five novel variants associated with leptin concentrations, including four missense variants in *LEP, ZNF800, KLHL31*, and *ACTL9*, and one intergenic variant near *KLF14.* The novel *LEP* variant, Val94Met (rs17151919), is associated with leptin concentrations in adults of African ancestry only. The leptin-lowering Met94 allele of the rs17151919 variant is associated with higher BMI in young children, but shows a weak or no association with BMI in adulthood, suggesting leptin regulates early adiposity.

Research Design and Methods

Study design

We performed an exome-based association study using data from 35 cohorts comprising up to 57,232 adults (≥18 years) of whom 50,321 were of European descent, 4,387 of African descent, 2,036 of East Asian descent, and 488 of Hispanic descent. We carried out additional analyses in men and women separately. All analyses were performed in models combining studies of all ancestries and in European ancestry cohorts only, for both additive and recessive genetic models. All participating institutions and coordinating centers approved the project. Informed consent was obtained from all study participants. We have reported the study-specific design, sample quality control, and descriptive statistics in **Tables S1-S2**.

Outcome traits

The participating studies acquired residuals for leptin concentrations (in ng/mL) using linear regression, adjusting for age, genome-wide principal components, and any study-specific covariates (e.g. study center). The residuals were calculated with and without adjustment for BMI. Studies with unrelated individuals acquired the residuals in men and women separately, whereas family-based studies additionally acquired sex-combined residuals adjusting for sex as a covariate. Case-control studies acquired the residuals in cases and controls separately. Finally, we rank-transformed the residuals using inverse normal transformation to follow a distribution with a mean of 0 and a standard deviation of 1.

Genotyping

All participating studies performed genotyping using the Illumina HumanExome BeadChip. The genotype calling was performed using the designated manufacturer's software, followed by zCall. Study-specific quality control measures were implemented before the association analyses to remove poorly genotyped variants (**Table S3**).

Study-level association analyses

Associations of the exome-wide variants with the residuals of leptin concentrations were examined using linear mixed models implemented in either RAREMETALWORKER [12] or RVTEST [13] (**Table S3**). The model accounted for potential cryptic relatedness by incorporating a kinship matrix. We performed the single variant association analyses using both additive and recessive genotypic models. We also calculated covariance matrices capturing LD relationships between markers within 1 Mb for use in gene-level meta-analyses.

Quality control of study-level association results

We applied the EasyQC package in R to association summary statistics from each participating study to identify cohort-specific QC issues. This included (i) identifying issues with calculation of leptin residuals and transformation of the residuals, (ii) identifying strand issues by comparing allele frequencies against reference alleles from the 1000 Genomes Project phase 1, and (iii) identifying issues arising from population stratification.

Single variant meta-analyses

The meta-analyses of summary statistics from the participating studies were carried out using RAREMETAL [14] by two different analysts in parallel. We excluded all variants with a call rate <98%, Hardy Weinberg equilibrium *P*-value $<1x10^{-6}$, or an allele frequency that strongly deviated from the 1000 Genomes reference frequency (>0.60 for all-ancestry analyses and >0.30 for ancestry-specific analyses). To identify the leptin-associated variants, we used the array-wide Bonferroni-corrected threshold of $P<2x10^{-7}$ for ~250,000 variants in the single variant analyses.

Gene-based meta-analyses

We performed gene-based analyses using the sequence kernel association test [15] (SKAT) and variable threshold [16] (VT) methods in RAREMETAL. The analyses were performed with two different sets of criteria (broad and strict) to select predicted damaging rare and low-frequency variants with MAF<5% annotated using five prediction algorithms: PolyPhen-2, HumDiv, HumVar, LRT, MutationTaster, and SIFT. The broad gene-based tests included nonsense, stop-loss, splice-site, and missense variants that were annotated as damaging by at least one of the five algorithms whereas the strict tests only included variants predicted as damaging by all of the five algorithms. The statistical significance for the gene-based tests was set at a Bonferroni-corrected threshold of $P<2.5x10^{-6}$ for 20,000 genes.

Age-stratified BMI analyses of variants in and near *LEP*

To study the influence of age on the association of the Val94Met variant in *LEP* and the rs10487505 variant near *LEP* with childhood BMI, we performed age-stratified analyses in children with African and European ancestry from the Center for Applied Genomics at Children's Hospital of Philadelphia (CHOP) cohort recruited from 2006 to present [17]. The participants had multiple BMI measurements at different ages and analyses were performed with measurements in 1-year age bins. The number of BMI measurements in each age bin is shown in **Tables S9** and **S10**. Statistical significance was defined as P<0.05. The Val94Met and rs10487505 variants were genotyped using the Illumina Infinium II HumanHap550 and Human610 BeadChip and imputed to the HRC r1.1 reference panel using the Sanger Imputation Server. All participants were biologically unrelated, aged between 2 and 18 years, and between -3 and +3 standard deviations of CDC-corrected BMI. The study was approved by the Institutional Review Board of the Children's Hospital of Philadelphia. Parental informed consent was given for each study participant.

Additionally, we used information on comparative body size at age 10 (data field 1687) for 452,264 individuals of European ancestry and 8,154 individuals of African ancestry from the UK Biobank. The participants were asked to choose one of the three categories of "about average", "thinner", or "plumper" to describe their body size compared to average when they were 10 years old.

Pathway enrichment analyses

We utilized the EC-DEPICT [18, 19] gene set enrichment analysis method to evaluate nonsynonymous index variants (strongest nonsynonymous variant within ±1 Mb boundary) with $P<5\times10^{-4}$ for association with either i) leptin unadjusted for BMI, or ii) leptin adjusted for BMI. EC-DEPICT's primary innovation is the use of "reconstituted" gene sets, which consist of gene sets downloaded from several databases that have been extended based on publicly available large-scale co-expression data [18]. Two analyses were performed: (i) all coding variants (N=93 loci for leptin unadjusted for BMI and N=91 loci for leptin adjusted for BMI) and (ii) coding variants with MAF<5% only (N=77 loci for leptin unadjusted for BMI and N=65 loci for leptin adjusted for BMI).

We also utilized PASCAL [20] to study the enrichment of exome-wide association results in gene sets and pathways using two estimation approaches: MAX and SUM. The MAX estimation is more powerful for single variant-driven associations whereas the SUM estimation is more powerful when multiple variants are driving the signal [20]. We used reconstituted gene sets from DEPICT and the reference data from UK10K [TwinsUK [21] and ALSPAC [22]] to estimate LD. The PASCAL analyses were performed for all exome-chip variants (N_{all}=265,780 for leptin adjusted for BMI, N_{all}=265,780 for leptin unadjusted). and for coding variants only (N_{coding}=176,035 for leptin adjusted for BMI, N_{coding}=180,864 for leptin unadjusted). No allele frequency or *P*-value thresholds were used to select variants for the PASCAL analyses. The pathway scoring method used by PASCAL combines individual gene scores without the need for a tuneable threshold parameter to determine inclusion of genes in the enrichment analysis [20].

Leptin adjusted for BMI is correlated with body fat free mass (correlation with fat-free mass index in the Fenland cohort = -0.39). The initial pathway analyses for leptin adjusted for BMI using EC-DEPICT and PASCAL suggested enrichment of skeletal-muscle related pathways. To make sure that the gene set enrichment results were not due to correlation between leptin adjusted for BMI and fat-free mass index, we corrected the effect sizes using the following equation [23]: $Beta_{corrected} = beta_{leptin} – (beta_{FFMI} \times r_{FFMIvs.LEPTIN})$, where $r_{FFMIvs.LEPTIN} = -0.39$ (Pearson correlation coefficient in the Fenland Study). The $beta_{FFMI}$-coefficients were extracted from an ongoing exome-wide association study of fat-free mass index in ~500,000 individuals.

Collider bias

Given that we adjusted leptin concentrations for BMI in our exome-based analyses and leptin and BMI are strongly correlated (r~0.5-0.8) [9], we tested all exome-based significant loci for evidence of collider bias [23-25]. For each index we extracted the association results from our BMI-unadjusted leptin analyses and from the largest published exome-wide analysis for BMI [19]. We corrected BMI-adjusted associations for potential bias due to phenotypic correlation between leptin concentrations and BMI, and compared the strength and significance of association with leptin concentrations unadjusted for BMI, leptin adjusted for BMI, and association with BMI (**Table S6**).

eQTL colocalization analyses

The *cis*-expression quantitative trait locus (*cis*-eQTL) analyses were carried out by using abdominal subcutaneous adipose tissue from 770 participants of the METSIM (Metabolic Syndrome in Men) study who all were Finnish men from Kuopio, Finland [26]. The eQTL mapping in 770 METSIM individuals was performed by EPACTS implementing a linear mixed model to account for the population structure among the samples. The eQTLs were defined as *cis* (local) if the peak association was within 1 Mb on either side of the exon boundaries of the gene. We also identified variants most strongly associated with genes/transcripts from the index variant ("eSNP"). We used METSIM LD (based on n=770, HRC imputation) to assess LD r^2 between the index variant and the lead eSNP. If the pairwise LD was r^2>0.80, we performed a reciprocal conditional analysis. We tested association between the lead SNP and transcript level when the lead eSNP was included in the model, and *vice versa*.

Expression of the potential causal genes in preadipocytes and mature adipocytes

We compared the expression of the candidate causal genes in the novel leptin-associated loci, including *ZNF800, KLF14, KLHL31*, *ACTL9*, *CNTD1* and *DNAJC18* in preadipocytes and mature adipocytes, two major constituent cell types of adipose tissue. Human preadipocytes isolated from adipose tissue were induced to undergo adipocyte differentiation *in vitro* [27]. RNA samples were obtained from preadipocytes and lipid-laden mature adipocytes at post-differentiation day 12.

Impact of Val94Met variant in *LEP* on leptin protein stability

We used UCSF Chimera 1.13.1 to model the 3D protein structure and valine-to-methionine substitution in the leptin protein [28]. The Rotamers tool and the Dunbrack Rotamer Libary were used to view and evaluate amino acid sidechain rotamers. The displayed orientation of methionine was chosen based on the clashes and contacts observed in the protein and hydrogen bonds [29]. To predict protein stability, we used SDM [30], iStable [31], Cupsat [32] and iMutant 2.0 [33]. All analyses applied the 3D structure for leptin (ID 1AX8) from the RSCCP Protein Data Bank as the reference data set.

Effect of the Val94Met variant in *LEP* on leptin protein stability and secretion rate

We tested the effect of the Val94Met variant on leptin protein stability and secretion rate in HEK293 cells *in vitro*. Human leptin cDNA clone was obtained from Open Biosystems Inc (Huntsville, AL) and subcloned into pcDNA3.1 vector. The original cDNA clone encodes the Val94 variant. The 94Met variant was created using Quikchange II site-directed Mutagenesis kit (Agilent, Santa Clara, CA), with the Val94 plasmid as template and the following mutagenesis primers (forward: 5'-atgccttccagaaacatgatccaaa tatccaac-3', reverse: 5'-gttggatatttggatcatgtttctggaaggcat-3'). Plasmids carrying Val94 or 94Met cDNA (0.05 μg) were introduced into HEK293 cells (0.65 million cells/well in 12-well plate) using Lipofectamine 2000 as previously described [34]. To measure intracellular leptin protein turnover and secretion rates, cells were treated with protein synthesis inhibitor cycloheximide (CHX, 20 μg/ml) in fresh media for 0.5 and 1 hr at 72 hr post-transfection. Cells incubated with fresh media for 1 hr without CHX were used as untreated controls. Conditioned media were saved for leptin assay, and cell lysate were prepared using NP-40 lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 0.5% NP-40, 10% glycerol, and 1x Roche protein inhibitor mixture). Leptin concentrations in cell lysates and the amount of leptin in conditioned media were determined using a human leptin ELISA kit (R&D Systems, Minneapolis, MN). Little or no cell debris was observed in the conditioned media after centrifugation, suggesting little or no cell breakage during the incubation. The experiments were carried in duplicates or triplicates and repeated four times.

Results

Five novel genetic variants show association with leptin concentrations independent of adiposity

To identify genetic variants associated with leptin concentrations, we tested the associations of 246,328 single nucleotide variants (SNVs), genotyped on an exome-targeted genotyping array, with leptin concentrations in up to 57,232 individuals of European (n=50,321), African (n=4,387), East Asian (n=2,036) or Hispanic ancestry (n=488) from 35 studies (**Tables S1-S3**). The exome-array provides a detailed coverage of gene-coding regions and includes tags for variants identified in previously published GWASs for human complex traits. Given the strong correlation between leptin and BMI (r~0.5-0.8) [9], we examined associations with leptin concentrations with and without adjustment for BMI. Additional analyses were performed in men (n=23,862) and women (n=32,940) separately. All the analyses were performed in all ancestries combined and in European-ancestry individuals only.

We confirmed five previously established [9] and identified five novel variants associated with leptin concentrations. The novel associations include four missense variants, in *LEP, ZNF800*, *KLHL31*, and *ACTL9*, and one intergenic variant near *KLF14* (**Table 1, Table S4**). The associations at already established loci include intergenic variants near *LEP* and *CCNL1*, a missense variant in *GCKR*, and intronic variants in *COBLL1* and *FTO* (**Table 1, Table S4**). To detect additional independent signals at the 10 leptin-associated loci, we performed conditional analyses, but no further signals were identified.

The association between rs1121980 near *FTO* and leptin concentrations became non-significant after adjustment for BMI (P_{unadj}=8x10^{-17}; P_{adjBMI}=0.45). The effects of all other known and novel loci were independent of BMI (**Table S5**). We tested whether the adjustment for BMI, a strongly correlated covariate [23], may have introduced collider bias, but found no evidence of such bias (**Table S6**).

The strongest variant associated with leptin concentrations was rs791600, an intergenic variant near the *LEP* gene. The rs791600 variant is in linkage disequilibrium (LD) (EUR r^2=0.70) with the rs10487505 variant identified in our previously published GWAS [9], which is not included in the exome array and was therefore not available for analyses in the present study. In the prior GWAS study, the rs10487505 variant showed a more significant association with BMI-adjusted leptin concentrations (beta=0.034 per allele, P=2.7x10^{-11}, n=29,252) than rs791600 (beta=0.029 per allele, P=3.0x10^{-9}, n=31,800) and thus is still considered the lead variant at this locus (**Figure S1**).

Nine of the 10 identified loci showed an association with leptin concentrations in all ancestries combined and in European ancestry only analyses. However, the novel *LEP* variant Val94Met (rs17151919) only showed a significant association in all ancestries combined (P=2x10^{-16}) and not in European-ancestry individuals alone (P=0.47, **Table S7**). In further ancestry-stratified analyses, we observed that the Met94-coding allele is common in populations of African ancestry (MAF=8%), less common in those with Hispanic ancestry (MAF=2%), very rare in those with European ancestry (MAF=0.02%) and monomorphic in people with East Asian ancestry [35]. In individuals of African descent, each Met94-coding allele was associated with 0.34 standard deviations (SD) lower leptin concentrations (*P*=$2x10^{-16}$, n=3,901) (**Figure S2**). The direction of effect was consistent in individuals with Hispanic (-0.21 SD effect per allele, *P*=0.29, n=488) and European ancestry (-0.19 SD effect per allele, *P*=0.47, n=44,401), but did not reach statistical significance, most likely because very few carriers were available (N_{HIS}=24, N_{EUR}=15) (**Table S7**).

Gene-based analysis identifies two novel genes with sex-specific effect on leptin

In addition to single variant-based association tests, we performed gene-based tests using rare and low-frequency coding variants in aggregate [15, 16] (**Methods**). We identified two genes associated with leptin concentrations. *CNTD1* showed association with leptin concentrations unadjusted for BMI in men (*P*=1×10^{-7}) but not in women (*P*=0.27) (**Table 2, Table S8**). The association in men was driven by five coding variants and was strongly attenuated by adjusting for BMI (*P*=0.007), suggesting that the association of *CNTD1* with leptin concentrations may be due to a link between *CNTD1* and adiposity, although no such connection has been previously reported. The *CNTD1* gene encodes cyclin N-terminal domain-containing 1, which is critical for meiotic crossover maturation and deselection of excess pre-crossover sites.

Another gene, *DNAJC18*, showed association with BMI-adjusted leptin concentrations in women (*P*=6×10^{-8}), but not men (P=0.02). The association in women was driven by two coding variants (**Table 2, Table S8**). *DNAJC18* is part of the Dnaj heat shock protein family. However, no function has yet been described to C18 subfamily.

***LEP* Val94Met regulates leptin secretion and early adiposity**

The Val94Met (rs17151919) *LEP* variant was associated with BMI-adjusted leptin concentrations in individuals of African ancestry. A previous study in 2,129 African Americans in the CARDIA study (not included in the present meta-analyses) reported a significant association between the leptin-decreasing Met94 allele of the Var94Met (rs17151919) variant in *LEP* and up to 1.12 kg/m^2 higher BMI in adulthood (P=0.018) [36]. However, results from two larger studies of BMI by the African Ancestry Anthropometry Genetics Consortium (n=42,752; *P*=0.88) [37] and the African ancestry population of the UK Biobank study (N=7,820, *P*=0.17), did not replicate the association. Nevertheless, among the African ancestry population in the UK Biobank, carriers of the leptin-decreasing Met94 allele reported more often that, at age 10, they were "plumper" (compared to peers) (OR=1.11, *P*=0.04), suggesting that the effect of this variant may be age-dependent. To study the influence of age, we performed age-stratified analyses in up to 2,726 children with African-ancestry from the Center for Applied Genomics at Children's Hospital of Philadelphia (CHOP) cohort [17]. Comparing the effect sizes across different age points revealed that each leptin-decreasing Met94 allele was associated with 0.12-0.20 units higher BMI z-score between the ages 3 and 7 (P<0.05). The most pronounced effect was reached at age 6 years (**Figure 1, Table S9**), and no association with BMI was observed after age 8 years (betas -0.04 to 0.05) (**Figure 1, Table S9**), suggesting that the BMI-increasing effect of the Met94 allele wanes shortly before puberty. The rs10487575 variant near *LEP* showed a similar trajectory of association with childhood BMI as the Val94Met variant but the effect sizes were much more modest (**Figure 1, Table S10**), consistent with the five-fold smaller effect of rs10487505 on leptin concentrations compared to Val94Met in adults (**Table 1**).

The Val94Met variant is located at position 94 in the 167 amino acid leptin precursor protein and results in a valine to methionine change at position 73 of the mature protein (**Figure 2A**). Position 73 is situated at the leptin protein surface and is not believed to be involved in binding of leptin to its receptor. Nevertheless, structural prediction tools [30-33] suggested that the substitution of valine with methionine at this position is likely to lead to reduced stability of the mature leptin protein (**Figure 2A, Figures S3-4, Table S11**). This is consistent with our observation that the methionine-coding allele is associated with lower leptin concentrations.

To study the impact of the Val94Met variant on the intracellular turnover of the leptin protein and its secretion rate, we performed *in vitro* experiments in HEK293 cells. Leptin secretion rate – calculated as the amount of leptin secreted in 1 hour normalized to the respective cellular leptin content – was 20.4% lower in Met94 than in Val94 cells (P=0.0007 by repeated measures 1-way ANOVA) 72 hours post-transfection (**Figure 2B**). Leptin secretion rates between 48-72 hours post-transfection and during a 1-hour treatment with cycloheximide were 11.8% (P=0.0005) and 17.9% (P=0.0002) lower, respectively, in Met94 compared to Val94 (**Figure S5**). Notably, no difference was found in the intracellular turnover rate of leptin between Val94 and Met94 cells during a 0.5 or 1-hour incubation with cycloheximide to impair protein synthesis (**Figure 2C**). The unchanged turnover rate incorporates protein secretion and degradation, suggesting that decreased leptin secretion rate was likely associated with increased intracellular leptin degradation in Met94 cells. Overall, these *in vitro* experiments suggest that methionine substitution in position 73 of the mature leptin protein decreases the rate of leptin secretion from the cells, which may contribute to the association of the Met94 allele with lower leptin concentrations.

***ZNF800* locus regulates adipose gene expression and body composition**

The Pro103Ser (rs62621812) variant in *ZNF800* changes the amino acid sequence of CH2 zinc finger protein, a putative transcription factor [38]. We found that the Ser103 allele (frequency=2.8%) is associated with lower BMI-adjusted leptin concentrations (P=2.0x10^{-12}). As shown before [26], Pro103Ser is the lead variant associated with expression of *ZNF800* in subcutaneous adipose tissue in the Finnish METSIM Study (P=2.4x10^{-16}); the Ser103 allele is associated with higher *ZNF800* expression levels (**Table S12**). *ZNF800* is a master regulator in subcutaneous adipose tissue, as the Pro103Ser variant has also been associated with adipose tissue expression of nine other genes [26]. In the eQTL data, the leptin-decreasing Ser103 allele was not significantly associated with the expression of *LEP* (P=0.20), located 866 kb downstream, and the observed direction of the effect on *LEP* expression was opposite to that observed for leptin concentrations (beta=0.14 SD/allele vs. beta= -0.13 SD/allele, respectively), suggesting that the leptin-lowering effect of the Ser103 allele on leptin concentrations is unlikely to be mediated by direct transcriptional regulation of *LEP*.

In the UK Biobank study, we found that each leptin-decreasing Ser103 allele is associated with 0.14 kg/m^2 higher BMI (P=8.1x10^{-6}). However, there was no association between Ser103 allele and body fat percentage (-0.045% per allele, P=0.25), indicating that the variant impacts BMI primarily by increasing fat free body mass. Indeed, the leptin-decreasing Ser103 allele was associated with a 0.33 kg higher fat free mass (P=4.6x10^{-}

[20]) and only 0.13 kg higher fat mass (P=0.023). The Ser103 allele is associated with higher expression of the *ZNF800* gene in the tibial nerve (GTEx v8, P=1.4x10^{-6}, n=532) that innervates the muscles of the leg, and has been previously identified for association with increased appendicular lean mass [39]. There was no association between the Ser103 allele and self-reported body size at age 10 (P=0.75).

The *KLF14* locus regulates adipogenesis and fat distribution

The rs972283 variant (MAF_{EUR}=48%), associated with leptin concentrations, is located 51 kb upstream from *KLF14* and 2.5 Mb downstream from *LEP*, and is in near-perfect LD with previously reported GWAS variants for type 2 diabetes [40], insulin-resistance [41], HDL cholesterol [42], and body fat distribution [43]. As reported earlier [26], rs972283 is associated with *KLF14* expression in subcutaneous adipose tissue (**Table S12**). As *KLF14* is a master regulator in adipose tissue, rs972283 is also associated with the expression of multiple other genes in *trans* [44]. No significant association was observed between rs972283 and *LEP* expression in the METSIM eQTL study [44], suggesting that *KLF14* may not regulate leptin production at the transcriptional level, at least not in men. Lower expression of *KLF14* has been implicated in impaired adipogenesis due to defective adipocyte glucose uptake in women, characterized by the presence of fewer but larger adipocytes and a shift in fat distribution from gynoid stores to abdominal tissues [44]. However, while the effects of *KLF14* on adipogenesis and adipose redistribution have been found to be specific to women, there was no difference in the association of rs972283 with leptin levels between men and women (**Table S4**).

Interestingly, the carriers of the rs972283-G allele reported more frequently being plumper ($P=2.8x10^{-5}$) and shorter (P=0.014) than average at age 10 in the UK Biobank than non-carriers, whereas the same allele was associated with a lower BMI ($P=6.8x10^{-9}$) and increased height (P=0.010) in adults, suggesting that the effect of the rs972283 variant on body size may change during life course. In previous GWAS of adults, the rs972283-G allele has been identified to be associated with higher risk of type 2 diabetes [40] and insulin resistance [41], and lower hip circumference (adjusted for BMI) [43] and HDL cholesterol [42]. In the UK Biobank study, the rs972283-G allele was associated with lower body fat percentage in adults ($P=5.9x10^{-22}$).

The *KLHL31* locus is implicated in adipogenesis in adult females

The Val156Ile (rs3799260) variant (MAF_{EUR}=18%) in *KLHL31*, associated with leptin concentrations in female-only analyses, changes the amino acid sequence of the kelch-like family member 31 protein. *KLHL31* suppresses Wnt-β-catenin signaling that is involved in promoting adipocyte differentiation and suppressing oxidative metabolism in adipocytes. The Val156Ile variant is predicted to be benign/tolerated by SIFT/Polyphen [45, 46]. Previous genetic associations have identified a variant in low LD (rs7739232; EUR $r^2=0.27$) to be associated with BMI-adjusted hip circumference, also specific to women [43]. The rs7739232 variant was not included in the exome-array and was thus not analyzed in the present study. Our *in vitro* experiments showed that *KLHL31* is only expressed in mature adipocytes, but not in preadipocytes (**Figure S6**), suggesting that the gene is developmentally regulated.

In the UK Biobank, similar to the variants in and near *LEP*, the carriers of the leptin-decreasing Ile156 allele reported more often being plumper than average at age 10 ($P=5.6x10^{-6}$), but there was a weaker association with higher BMI (P=0.045) in adulthood.

In men, the *ACTL9* locus may regulate leptin concentrations in a cell non-autonomous fashion

Homozygosity for the minor allele of the Ser37Phe (rs2340550) variant in *ACTL9* was associated with leptin concentrations in men only in a recessive genetic model. While the Ser37Phe variant is predicted to be benign/tolerated by SIFT/Polyphen, another missense variant, Ala51Val (rs10410943), in high LD (EUR $r^2=0.99$) is predicted to be deleterious/probably damaging and could be the causal variant at the locus. The Ser37Phe variant is also in high LD ($r^2>0.8$) with several nearby non-coding variants (**Figure S7**). However, none of these overlaps with regulatory elements in adipocytes. The expression of *ACTL9* is restricted to the testis and it is therefore likely to act in an adipocyte non-autonomous fashion to influence leptin concentrations. Actin proteins have cytoskeletal functions and have also been implicated in signaling and nuclear activities.

Gene-set analyses implicate adipocyte-related pathways

We performed gene-set enrichment analyses using EC-DEPICT [18, 19, 47] and PASCAL [20] to identify biological processes and candidate pathways enriched for loci associated with leptin unadjusted or adjusted for BMI. Among coding variants associated with BMI-unadjusted leptin concentrations, PASCAL identified significant enrichment of the gene-set for “positive regulation of reproductive success” ($P_{empirical}=1.6x10^{-5}$) (**Table S13**), consistent with the crucial permissive role of leptin in the integrity of the gonadal axis [48]. Among coding variants associated with leptin adjusted for BMI, we found enrichment of the immune-related TRIM39 protein-protein interaction subnetwork [49, 50] ($P_{empirical}$=8.4x10^{-6}) (**Table S14**). No gene sets were found to be significantly enriched in PASCAL analyses where all exome-wide variants (coding and non-coding) for leptin adjusted for BMI were included, nor in the EC-DEPICT analyses.

Discussion

We identified 10 genetic variants associated with leptin concentrations and two gene-based associations using an exome-based genotyping array in up to 57,232 individuals with varying ancestries. The two independent variants most strongly associated with leptin concentrations were located in and near the *LEP* gene. The African ancestry-driven variant within *LEP*, Val94Met (rs17151919), was found to decrease leptin secretion in HEK293 cells whereas rs10487505 located near *LEP* overlaps a lncRNA that regulates *LEP* expression [10]. Both variants showed significant association with increased adiposity in children, whereas only a nominal or no association was observed in adults.

Previous analyses have shown that the leptin-lowering allele of rs10487505 is only weakly associated with higher BMI in adulthood but shows a pronounced association with BMI in early childhood [10, 11]. Similarly, we showed that the *LEP* Met94 allele, associated with lower leptin concentration, is associated with early childhood BMI. Our results suggest that leptin has an impact specifically on early adiposity, encouraging further studies to uncover the molecular mechanisms that underlie this age-dependent relationship between leptin and BMI.

The Val94Met and rs10487505 variants in and near *LEP* are likely to influence leptin concentrations by different molecular mechanisms. The novel African ancestry-driven variant Val94Met may affect circulating levels of leptin by reducing leptin secretion. The rs10487505 variant is associated with leptin mRNA levels in adipose tissue. Located upstream of *LEP* within a lncRNA (EL947753), we hypothesize that this variant interacts with enhancer regions to regulate the expression of *LEP*. Defects in *LEP* regulation in mice lead to a relative hypoleptinemic form of obesity that is responsive to leptin administration [10].

We identified four new loci associated with leptin concentrations, located in or near the *ZNF800*, *KLF14*, *KLHL31* and *ACTL9* genes. Two additional genes, *CNTD1* and *DNAJC18* were identified in gene-based analyses. The *ZNF800* and *KLF14* genes are master *trans*-regulators of adipose tissue gene expression [26] and located in the proximity of the *LEP* gene (866 kb and 2.5 Mb away, respectively). The variants in *ZNF800* and near *KLF14* were not associated with *LEP* mRNA levels, however, suggesting that they may be involved in translational or post-translational rather than transcriptional regulation of leptin production. *KLHL31* has been shown to promote adipocyte differentiation and suppress oxidative metabolism in adipocytes, whereas *ACTL9* is not expressed in adipocytes and could affect circulating levels by a non-cell autonomous mechanism. The *KLHL31* and *ACTL9* loci, and the *CNTD1* and *DNAJC18* genes, were only identified in sex-specific models and narrowly passed the array-wide significance threshold. Further validation of the association of these loci with leptin concentrations is warranted.

In summary, we identified a new genetic association of an African ancestry-specific missense variant rs17151919 in *LEP* with leptin concentrations and replicated the association of the rs10487505 variant near *LEP*. The pronounced association of these variants with BMI in early childhood implicates genetic regulation of *LEP* in early growth and suggests that young children may be particularly sensitive to the metabolic/behavioral effects of leptin. We also identified novel loci at *ZNF800*, *KLF14*, *KLHL31*, *ACTL9*, *CNTD1* and *DNAJC18* associated with leptin concentrations, providing additional insights into leptin physiology.

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Guarantor Statement

HY and TOK are the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest statement

This work was conducted prior to M.E.G's current affiliation with the National Heart, Lung, and Blood Institute, and, as such, the views expressed in this article do not represent the views of the NHLBI, NIH, or other government entity. D.M.-K. is a part-time clinical research consultant for Metabolon, Inc. M.A.N's participation is supported by a consulting contract between Data Tecnica International and the National Institute on Aging, National Institutes of Health. V.S. has served in advisory boards for Novo Nordisk and Sanofi and received honoraria from these companies. He also has ongoing research collaboration with Bayer Ltd (all unrelated to the present study). B.M.P. serves on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. J.R.K. reports stock ownership in Bristol Myers Squibb, Johnson & Johnson, Merck, and Pfizer.

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Tables

Table 1. Leptin-associated loci identified in exome-based association analyses

SNP	Chr	Position	Nearest gene	Trait	Most significant model	Annotation	EA	OA	EAF	Beta	SE	*P* value	N
Novel variants													
rs3799260	6	53,519,605	*KLHL31*	LeptinAdjBMI	Additive / All ancestries / Women	missense	C	T	0.175	0.055	0.010	1.0E-07	32,886
rs62621812	7	127,015,083	*ZNF800*	LeptinAdjBMI	Additive / All ancestries	missense	A	G	0.028	-0.127	0.018	2.0E-12	56,708
rs17151919	7	127,894,592	*LEP*	LeptinAdjBMI	Additive / All ancestries	missense	A	G	0.007	-0.333	0.040	1.5E-16	49,034
rs972283	7	130,466,854	*KLF14*	LeptinAdjBMI	Additive / European	intergenic	A	G	0.479	0.056	0.006	3.8E-18	49,830
rs2340550	19	8,808,942	*ACTL9*	LeptinAdjBMI	Recessive / European / Men	missense	A	G	0.316	0.071	0.014	2.0E-07	21,883
Previously identified variants													
rs1260326	2	27,730,940	*GCKR*	LeptinAdjBMI	Additive / All ancestries	missense	T	C	0.375	-0.050	0.006	2.7E-15	56,708
rs13389219	2	165,528,876	*COBLL1*	LeptinAdjBMI	Additive / All ancestries	intronic	T	C	0.410	0.053	0.007	3.0E-15	50,297
rs900399	3	156,798,732	*CCNL1*	LeptinAdjBMI	Additive / All ancestries / Women	intergenic	G	A	0.391	-0.054	0.008	1.2E-10	29,510
rs791600	7	127,865,816	*LEP*	LeptinAdjBMI	Additive / All ancestries	intergenic	A	G	0.422	-0.066	0.007	1.1E-23	49,282
rs1121980	16	53,809,247	*FTO*	Leptin	Additive / European	intronic	A	G	0.432	0.055	0.007	7.7E-17	49,909

The chromosomal positions are based on hg19.

Chr, chromosome; EA, Effect allele; OA, Other allele; EAF, Effect allele frequency; LeptinAdjBMI, leptin adjusted for body mass index

Table 2. Leptin-associated genes identified by gene-based exome-wide association analyses

Gene	Chr	Position	Trait	Most significant model	Method	N	P value	Beta	SE	N variants
CNTD1	17	40,950,810-40,963,605	Leptin	Additive / European / Men	SKAT broad	18,882	1.3E-07	0.898	0.165	5
DNAJC18	5	138,743,559-198,780,898	LeptinAdjBMI	Additive / All ancestries / Women	SKAT strict	29,510	5.5E-08	0.757	0.169	2

The chromosomal positions are based on hg19.

Chr, chromosome; LeptinAdjBMI, leptin adjusted for body mass index

Figure 1. Association of the leptin-decreasing alleles of the *LEP* Val94Met (rs17151919) variant (on the left) and the rs10487505 variant near *LEP* (on the right) with BMI standard deviation score (SDS) in the CHOP cohort. The analyses for the Val94Met variant were performed in up to 2,726 African ancestry participants and the analyses for the rs10487505 variant in up to 3,681 African and European ancestry participants of the CHOP cohort. The y-axis reports the effect of each leptin-decreasing allele on BMI at each age year. The error bars indicate 1 standard error of the mean (SEM).

Figure 2. Impact of Val94Met transversion at LEP rs17151919 on leptin secretion rate in HEK293 cells. The rs17151919 variant changes valine to methionine in position 73 of the mature leptin protein. A) The 3D illustration of leptin structure derived from RSCCB Protein Data Bank and modified with UCSF Chimera1.13.1. The prediction of protein stability is derived from the SDM2 server [30]. B) Leptin secretion rates for Val94 and Met94 expressed as the amount of leptin secreted in ng during a 1 hr incubation (72-73 hr post-transfection) (LEPs/hr) normalized by the respective cellular leptin content (LEPc) in untreated control cells at the end of incubation. Individual data points from four separate experiments (each with 2-3 technical replicates) are plotted. The normality of data distribution was examined using D'Agostino & Pearson normality test (p=0.65 and 0.54 for LEPV94 and LEPM94, respectively) and repeated measures one-way ANOVA was performed to assess the difference in secretion rate between the genotypes. Mean ± SD and AVOVA results (F and p values) are reported in the table below the graph. C) Intracellular leptin turnover rates for Val94 and Met94 alleles, obtained by measuring the relative cellular leptin contents in the untreated control cells

(defined as 1 for the respective *LEP* variant) and in samples treated with the protein synthesis inhibitor cycloheximide (CHX, 20 μg/ml) for 0.5 and 1.0 hour. Mean ± SD at each time point from four separate experiments (each with 2-3 technical replicates) are plotted. Paired t-test was used to assess the genotype effect on the fractions of cellular LEP remained after 0.5 hr and 1 hr of CHX treatment (p values are reported in the table below the graph). The average hourly turnover rates for Val94 and Met94 were 61±2%, and 60±3%, respectively, calculated by subtracting the percent cellular LEP remained after one hour of CHX treatment from those of the respective untreated controls (defined as 100%).

Figure 1

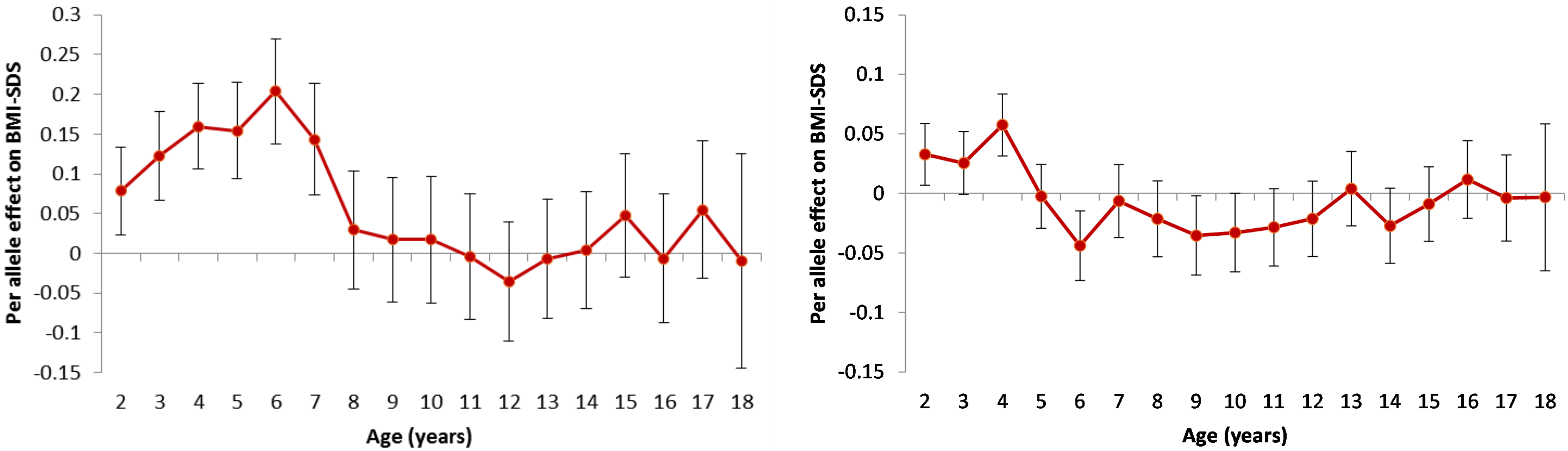
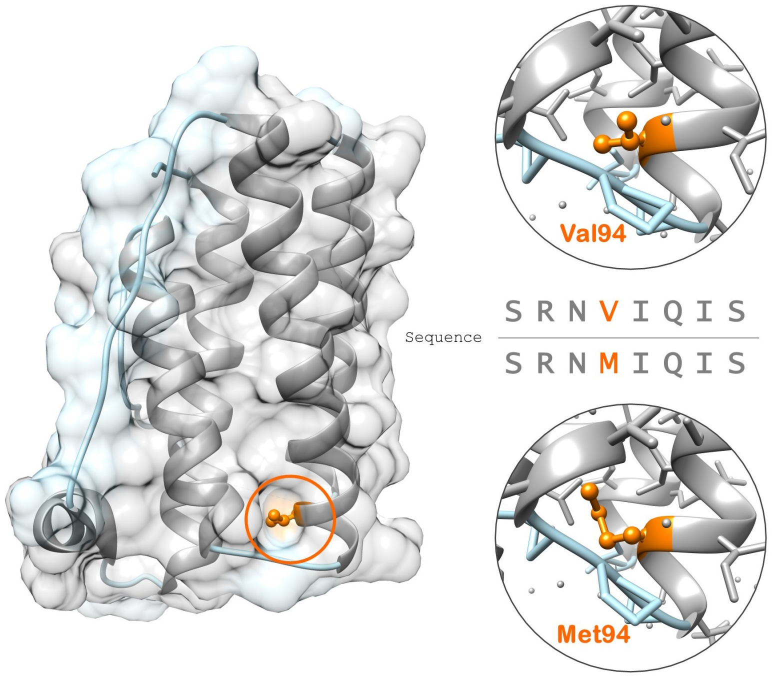

0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
Per allele effect on BMI-SDS
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
Per allele effect on BMI-SDS
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Age (years)

Figure 2

A)



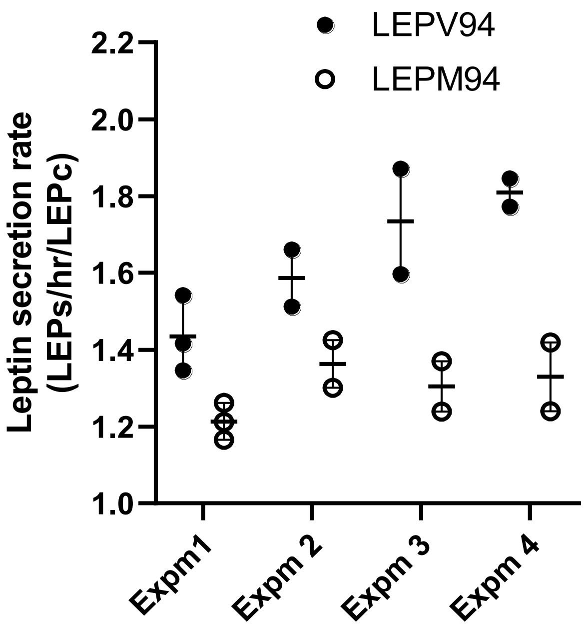
Predicted pseudo ΔΔG

- 0.72 (Reduced stability)

Mutation:

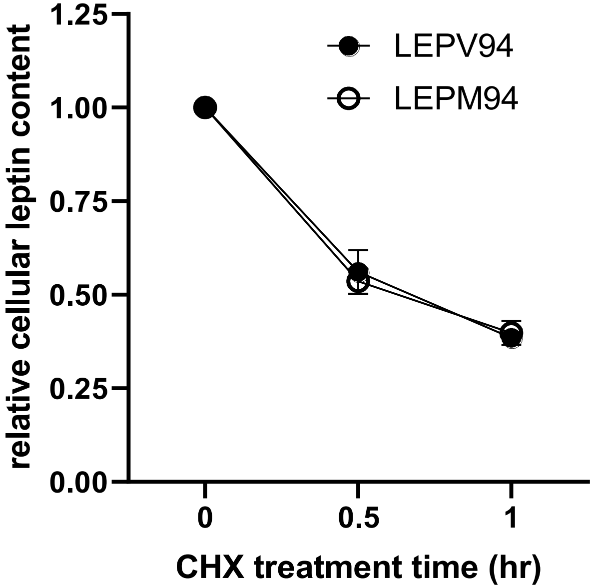
PDB ID:	1AX8
Chain:	A
WT:	VAL
Position:	73 (94)
SNP:	MET

B)



LEPV94	1.62±0.18
LEPM94	1.29±0.09
F (1,8)	28.82
p	0.0007

C)



	0.5 hr	1 hr
LEPV94	0.56±0.06	0.39±0.02
LEPM94	0.54±0.03	0.40±0.03
P (V94 vs M94)	0.316	0.366

Table S1. Study design, number of individuals and sample quality control for ExomeChip study cohorts

Study		Study design	Ethnicity	Total sample size (N)	Sample QC		Samples in analyses (N)	BMI assessment method	References
Short name	Full name				Call rate*	Other exclusions			
ARIC	Atherosclerosis Risk in Communities Study	Population-based	European American (EA) African American (AA)	462	≥ 95%	1) call rate <95%, 2) PCA outliers, 3)sex mismatch , 4) inbreeding coefficient +/-6SD from mean of ancestry distribution, 5) first degree relatedness; 6) comparison with GWAS data, exclude if >40% mismatch, 7) (p10GC) genotype quality score, representing the 10th percentile of the distribution of GenCall scores across all SNPs, 8) missing leptin, adiponectin, or BMI measures (only exclude from analyses missing respective phentoype trait)	340	Measured	**PMID: 2646917 PMID: 2387450 PMCID: PMC3709915 PMID: 12829649**
CHS EA and AA	Cardiovascular Health Study	Population-based	European American (EA) African-American (AA)	5088	≥ 95%	Following the central QC and joint variant calling, additional QC steps were applied to the CHS data using PLINK. SNPs with a missingness rate of >95% were removed and individuals meeting the following criteria were excluded from analysis. We further excluded individuals with low P10GC call, a missing genotype rate of > 97%, gender mis-matches identified by X chromosome homozygosity rates. The sample was limited to those of self-described European-ancestry (EA) and African-American (AA) participants. Principal components analysis was performed using a subset of common LD-pruned variants from the Exome Chip both for the full sample as well as in EA and AA strata. Individuals whose full-sample first principal component suggested a different ancestry from their self-reported ancestry were excluded as were individuals who were outliers for the first 10 ancestry-specific principal components. Pair-wise IBD measures were calculated and outliers with high levels of IBD were removed.	5044	Measured	**PMID: 23874508 PMID: 1669507**
CLHNS	Cebu Longitudinal Health and Nutrition Survey	Population Based Longitudinal	Filipino	1799	≥98%	1) Missing study specific covariates (household assets or household income)	1,792	Measured	**PMID: 20507864**
Ely	Ely study	Longitudinal cohort study	European ancestry	1592	> 98%	1) Heterozygosity check, 2) Ethnic outliers, 3) Duplicate individuals, 4) Sex discrepancy, 5) Unusually high number of singleton genotypes, 6) impossible IBD values, 7) phenotype missing	1,432	Measured	**PMID:17257284**
ERF study	Erasmus Rucphen Family study	Family-based	White European	2963	≥ 95%	--	1146	Measured	http://www.erasmusmc.nl/klinische_genetica/research/intro/genepi/
FAMHS	Family Heart Study	Family-based	White European	--	≥ 98%	1) Variants with missing rate > 5% (based on aggregate data) 2) pHWE<1e-6 3) Mendelian errors 4) minor allele count (MAC)<5 for variant-wise tests	1505	Measured	**PMID:8651220**
Fenland-CE	Fenland Study	Population-based	European ancestry	1077	> 98%	Heterozygosity check; Ethnic outliers; sex discrepancy; unusually high number of singleton genotypes; impossible IBD values; phenotype missing; excluding overlap exomechip samples	368	Measured	**PMID: 20519560**

Fenland-Exomechip	Fenland Study	Population-based	White European	1650	> 98%	1) heterozygosity outliers (>3.5 SDs), 2) ethnic outliers, 3) sex discrepancy, 4) unusually high number of singleton genotypes, 5) related (IBD > 0.1875)	1342	Measured	**PMID: 20519560**
FHS	Framingham Heart Study	Family-based	White European	8153	≥ 97%	1) Missing GWAS PCs, 2) Ethnic outlier, 3) Missing trait or covariate	7458	Measured	**PMID: 23874508**
FINRISK 1997	Finland National FINRISK Health Survey 1997	Population-based	White European	8325 (4006)	≥ 95%	1) Missing leptin or adiponectine levels, 2) Missing BMI, 3)Pregnancy	3917	Measured	**PMID: 29165699**
FINRISK 2007	Finland National FINRISK Health Survey 2007	Population-based	White European	6086 (3465)	≥ 95%	1) Missing leptin or adiponectin levels, 2) Missing BMI, height or weight, 3) Missing fat free mass or fat mass, 4) Pregnancy	2945	Measured	**PMID: 29158543**
HABC AA	Health, aging and body composition study	Population-based	African American ancestry	1139	> 95%	1) missing data, 2) relatedness, 3) acestry outliers, 4) heterozygosity outliers	1060	Measured	--
HABC EA	Health, aging and body composition study	Population-based	European ancestry	1663	> 95%	1) missing data, 2) relatedness , 3) acestry outliers, 4) heterozygosity outliers	1572	Measured	--
Inter99	Inter99	Population-based	European	6141	≥ 98%	1) Missing body weight and height. 2) Heterozygosity were calculated separately for maf < 1% and maf > 1% and samples were dropped judged by plots, 3) Cryptic relatedness (related to 20 or more individuals), 3) Technical duplicates , 4) Non-European population outliers from PCA plot (based on AIM SNPs), 5) Sex discrepancy	5594	Measured	**PMID: 14663300**
JHS	Jackson Heart Study	Population-based cohort with subset of families	African American	2803	≥ 95%	1) Missing outcome or covariate, 2) Heterozygosity, 3) PC outlier 4) Half of overlap with ARIC African Americans (coordinated with ARIC)	2312	Measured	**PMID: 16320381**
KORA	Kooperative Gesundheitsforschung in der Region Augsburg (Cooperative Health Research in the Region of Augsburg)	Population-based	White European	2921	≥98%	1) excess heterozygosity [i.e. |het_rate| > |mean+/-5sd|], 2) sex-check based on y-chromosome (remove men with <50% and women with >50% calls on y-chromosome), 3) remove of HAPMAP-samples 4) remove duplicates (keep sample with higher callrate), 5) remove samples with genetic inconsistencies with other genotyping / indication for contamination / population outliers	2916	Measured	--
Leipzig-adults	Leipzig Adults Study	Population-based	White European	902	≥ 99%	1) Missing phenotype, 2) Heterozygosity, 3) Non-European population outliers, 4) Technical duplicates with lower call rate 5) Sex discrepancy	902	Measured	**PMID: 20935630**
MESA CAU, CHN, AFA and HIS	Multi-Ethnic Study of Atherosclerosis (MESA) Cohort	Population-based	Caucasian;Chinese;Hispanic;African-American were recruited from six field centers	6375	≥ 95%	1) Ethnic outliers, 2) duplicates, 3) gender mismatch, 4) Phenoty outliers	CAU 2497 AFA 1655 CHN 769 HIS 1435	Measured	--
NEO Study	The Netherlands Epidemiology of Obesity Study	Population-based	European ancestry	6.604	≥ 98%	1) remove duplicate/swap samples, 2) remove samples with gender mismatch, 3) remove outliers in PCA	6.127	Measured	**PMID: 23576214]**

OMICS-Fenland	Fenland Study	Population-based	White European	8994	> 97%	1) Heterozygosity check, 2) Ethnic outliers, 3) sex discrepancy, 4) unusually high number of singleton genotypes, 5) impossible IBD values, 6) phenotype missing, 7) excluding overlap exomechip samples	7845	Measured	**PMID: 20519560**
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors	Population-based	White European	961	≥ 99%	1) Missing phenotype, 2) Heterozygosity, 3) Non-European population outliers, 4) technical duplicates with lower call rate 5) Sex discrepancy	961	Measured	**PMID: 16141402**
RAINE Study	Western Australian Pregnancy Cohort (RAINE) Study	Population-based	White European	1527	>=95%	1) Samepl disconcordance with GWAS data, 2) Heterozygosity 3) Missing body weight and height, 4) Did not participant in DEXA scan	1006	Measured	--
RISC	Relationship between Insulin Sensitivity and Cardiovascular disease	Population-based	White European	313	0.99	1) heterozygosity, 2) duplicates, 3) relatedness	313	Measured	**PMID:14968294**
RSI	Rotterdam Study	Population-based	White European	3163	≥ 98%	1) Heterozygosity, 2) gender-check	554	Measured	**PMID: 29064009**
SHIP-TREND	Study of Health in Pomerania - TREND	Population-based	White European	4270	≥ 98%	1) missing data, 2) duplicate samples (by estimated IBD), 3) reported and genotyped sex mismatch, 4) Heterozygosity	4149	Measured	**PMID: 20167617**
TwinsUK	TwinsUK	twin study	White European	4081	≥ 95%	1) missing phenotype, 2) sample call rate	1864	Measured	
WGHS	Women's Genome Health Study	population based trial	European	22618	>98%	1) Heterozygosity, 2) Batch effects, 3) see also Grove et al. (PLoS One (2013) doi: 10.1371/journal.pone.0068095)	789	Self-reported	**PMID: 18070814**
WHI	Women's Health Initiative	Cohort	European	21,857	≥ 95%	1) Unexpected Duplicates, 2) PC ancestry outliers, 3) Missing body weight and height	5886	Measured	**PMID: 9492970**
WHI	Women's Health Initiative	Cohort	African American	3,516	≥ 95%	1) Unexpected Duplicates, 2) PC ancestry outliers, 3) Missing body weight and height	884	Measured	**PMID: 9492970**
YFS	The Cardiovascular Risk in Young Finns Study	Population-based	White European	1998	≥ 95%	1) Pregnancy, 2) Heterozygosity, 3) Gender discrepancy, 4) MDS outliers	1681	Measured	**PMID: 18263651**

* Call rate to exclude individuals for whom genotyping success rate is less than a certain percentage (to exclude 'bad' samples/DNA)

**Exome-chip samples from this study

Table S2. Study-specific descriptive statistics of ExomeChip cohorts.

Study[a]	Trait	Men						Women					
		n	mean	SD	median	min	max	n	mean	SD	median	min	max
ARIC	Age (yrs)	249	53.8	5.7	53	45	65	342	53.2	5.6	53	44	65
	BMI (kg/m²)	249	28.7	4.04	28.2	20.4	44.9	342	28.1	5.6	26.8	18.1	49.5
	Leptin levels (ng/ml)	249	8.4	9.6	5.9	0.5	105.3	342	25.6	22.1	18.7	0.7	147.3
CHS-EA	Age (yrs)	484	72.9	5.4	72	65	91	533	72.6	4.9	72	65	92
	BMI (kg/m²)	482	26.4	3.6	26	16.9	39.4	531	26.3	4.9	25.5	15.6	47.7
	Leptin levels (ng/ml)	484	9.5	10.4	7.2	1.3	100	533	27.2	22.6	19.2	1.4	100
CHS-AA	Age (yrs)	88	73.6	5.7	73	65	89	121	73.7	5.4	73	66	90
	BMI (kg/m²)	88	26.4	3.8	26.1	18.2	37.7	121	29.6	5.3	29.3	18.3	44.5
	Leptin levels (ng/ml)	88	9.6	8.8	7.1	1.3	46.8	121	41.7	26.6	36.1	1.4	100
CLHNS	Age (yrs)	-	-	-	-	-	-	1792	48.5	6.1	47.7	35.7	69.3
	BMI (kg/m²)	-	-	-	-	-	-	1780	21.3	4.4	24.1	12.3	42.1
	Leptin levels (ng/ml)	-	-	-	-	-	-	1792	25.5	19.4	21.3	0	154.2
Ely	Age (yrs)	742	61.5	9.1	61.6	35.7	77.4	849	60.8	9.3	60.2	36.3	78.9
	BMI (kg/m²)	742	27.4	3.9	26.8	16	45.8	849	27.3	5.4	26.3	16.9	59.3
	Leptin levels (ng/ml)	658	9.2	8.1	7.1	0.1	63.1	769	33	26.7	25.7	0.7	198
ERF study	Age (yrs)	262	49.4	14.2	49.6	17.6	81.8	316	50.0	15.4	51.0	18.6	81.4
	BMI (kg/m²)	262	27.5	5.0	26.9	17.4	50.8	316	27.1	5.2	26.5	17.7	61.8
	Leptin levels (ng/ml)	262	27.7	43.8	16.8	0.6	535.9	316	91.3	89.1	60.0	0.0	599.3
FAMHS EA	Age (yrs)	737	52.5	13.9	53.9	25.2	91.0	768	52.8	13.1	53.9	25.2	88.7
	BMI (kg/m²)	737	27.8	5.0	27.0	16.0	49.6	768	28.1	6.9	26.4	16.1	55.1
	Leptin levels (ng/ml)	737	8.5	7.0	6.6	1.1	77.1	768	23.4	17.9	18.4	2.2	123.6
Fenland-CE	Age (yrs)	164	49.6	7.0	50.4	36.1	61.6	204	49.3	7.6	50.1	30.7	62.3
	BMI (kg/m²)	164	27.4	4.1	26.9	18.2	42.3	204	26.6	4.8	25.4	19.1	45.5
	Leptin levels (ng/ml)	164	7.46	7.26	5.70	0.50	57.90	204	23.69	19.80	16.75	2.20	112.00
Fenland-Exomechip	Age (yrs)	621	48.5	7.2	48.5	31.3	61.5	713	48.6	7.2	49.0	33.7	61.1
	BMI (kg/m²)	621	27.5	4.0	27.1	18.0	46.6	713	26.6	5.5	25.2	16.6	59.9

	Leptin levels (ng/ml)	621	7.7	7.5	5.9	0.1	74.5	713	24.2	21.3	17.7	0.5	169.0
Fenland-OMICS	Age (yrs)	3035	48.3	7.4	48.6	30.9	62.3	3376	48.4	7.2	48.7	30.5	62.8
	BMI (kg/m²)	3035	27.3	4.2	26.8	15.3	50.6	3376	26.4	5.2	25.4	14.5	58.7
	Leptin levels (ng/ml)	3035	7.7	7.3	5.6	0.1	72.1	3376	23.3	20.3	17.3	0.1	199.0
FHS	Age (yrs)	1800	40.3	8.9	40.0	19.0	72.0	2034	40.0	8.8	40.0	19.0	70.0
	BMI (kg/m²)	1800	27.9	4.7	27.3	16.4	56.5	2030	26.0	6.1	24.4	15.6	60.6
	Leptin levels (ng/ml)	1800	6.1	6.2	4.3	0.2	64.2	2034	18.2	17.1	12.3	0.7	110.3
FINRISK97	Age (yrs)	1786	46.1	13.1	45.3	24.2	74.1	2134.0	44.8	12.4	44.2	24.2	73.8
	BMI (kg/m²)	1783	26.6	3.9	26.1	14.7	47.1	2133.0	26.0	4.9	25.1	16.6	51.6
	Leptin levels (ng/ml)	1761	6.2	6.2	4.3	1.6	76.2	2111.0	18.0	14.0	14.0	1.6	100.0
FINRISK07	Age (yrs)	1298	52.2	13.6	53.0	25.0	74.0	1647.0	51.0	15.5	51.0	25.0	74.0
	BMI (kg/m²)	1284	26.9	4.1	26.3	15.7	62.8	1635.0	26.6	5.4	25.4	15.9	52.7
	Leptin levels (ng/ml)	1284	7.8	8.2	5.3	0.1	89.1	1602.0	19.1	15.8	14.9	0.5	100.0
HABC AA	Age (yrs)	457	73.5	2.8	73.0	69.0	79.0	603	73.3	2.9	73.0	68.0	80.0
	BMI (kg/m²)	457	27.1	4.2	26.8	14.9	43.2	603	29.4	5.6	29.0	14.6	47.5
	Leptin levels (ng/ml)	457	8.1	7.2	6.4	0.0	60.3	603	24.8	15.0	22.3	0.3	99.3
HABC EA	Age (yrs)	825	73.9	2.9	74	69	80	747	73.6	2.8	73	69	80
	BMI (kg/m²)	825	27	3.7	26.6	17.6	44.2	747	26.1	4.5	25.6	15.6	44.7
	Leptin levels (ng/ml)	825	7.7	6.8	6	0.2	59.1	747	18.9	14	14.8	0.3	86.9
Inter99	Age (yrs)	2675	46.6	7.8	45.2	29.9	61.1	2828	45.8	8.0	45.1	29.7	61.3
	BMI (kg/m²)	2674	26.8	4.0	26.3	17.1	56.9	2825	25.8	5.0	24.7	15.2	55.7
	Leptin levels (ng/ml)	2675	4.6	5.1	3.2	0.2	70.7	2828	15.1	16.1	10.3	0.4	260.6
JHS	Age (yrs)	861	51.9	12.8	51.0	21.0	81.0	1434	53.8	12.6	53.0	21.0	91.0
	BMI (kg/m²)	861	30.4	30.4	29.2	16.4	66.1	1434	31.9	6.2	31.5	16.0	91.8
	Leptin levels (ng/ml)	861	12.0	11.5	8.8	0.8	106.9	1434	36.1	21.5	32.7	1.4	291.0
KORA	Age (yrs)	1415	49.6	13.4	50.0	25.0	74.0	1506	48.4	13.2	48.0	25.0	74.0
	BMI (kg/m²)	1411	27.4	3.8	26.9	16.3	55.1	1491	26.8	5.1	25.9	15.8	51.2
	Leptin levels (ng/ml)	1410	9.4	10.3	6.3	0.0	140.0	1506	27.9	23.6	20.5	0.3	212.0
Leipzig-adults	Age (yrs)	223	42.3	17.1	40.5	18.0	99.0	276	41.5	16.6	38.0	18.0	89.0
	BMI (kg/m²)	223	35.4	12.6	32.6	18.8	120.4	276	36.1	12.6	33.6	14.7	70.0
	Leptin levels (ng/ml)	223	14.3	13.8	10.1	0.2	62.1	276	35.1	23.5	34.1	0.2	142.9

MESA CAU	Age (yrs)	395	62.6	10.2	63.0	45.0	84.0	360	62.9	9.2	62.5	45.0	84.0
	BMI (kg/m²)	395	28.2	4.0	27.6	19.9	41.1	360	27.5	5.7	26.5	16.9	45.7
	Leptin levels (ng/ml)	395	10.4	10.6	7.1	0.2	79.9	360	27.2	23.0	20.7	1.1	156.5
MESA CHN	Age (yrs)	129	62.6	10.7	63.0	45.0	82.0	115	62.3	9.7	61.0	44.0	84.0
	BMI (kg/m²)	129	24.3	2.8	23.9	16.8	32.3	115	24.4	3.2	24.6	17.8	33.0
	Leptin levels (ng/ml)	129	5.8	5.8	3.7	0.4	36.5	115	18.7	16.4	13.2	1.2	113.9
MESA AFA	Age (yrs)	158	61.7	9.7	62	45	83	180	63.6	9.6	64	46	84
	BMI (kg/m²)	158	28.6	4.5	28.3	19	46.9	180	30.3	5.7	29.4	19.7	47.3
	Leptin levels (ng/ml)	158	15.3	17.3	9.3	0.2	150	180	41.6	29.4	37.3	2.8	190.9
MESA HIS	Age (yrs)	246	60.0	9.9	59.0	44.0	82.0	242	62.3	9.2	63.0	45.0	82.0
	BMI (kg/m²)	246	29.0	4.5	28.7	19.4	45.8	242	30.1	5.5	29.6	18.3	52.5
	Leptin levels (ng/ml)	246	11.0	11.0	7.1	0.0	66.8	242	33.8	25.9	27.8	0.9	224.9
NEO study	Age (yrs)	2941	56.2	6.0	57.0	44.0	66.0	3186	55.8	5.9	56.0	44.0	66.0
	BMI (kg/m²)	2941	29.8	3.9	29.3	19.3	54.4	3186	30.3	5.5	29.8	17.2	61.2
	Leptin levels (ng/ml)	2929	12.9	9.2	10.5	0.5	98.6	3172	36.0	23.1	31.9	0.5	262.0
PIVUS	Age (yrs)	479	70.1	0.2	70.1	69.8	72.3	466	70.3	0.1	70.3	69.9	70.8
	BMI (kg/m²)	479	27.0	3.7	26.8	17.7	43.4	466	27.1	4.9	26.5	16.6	49.8
	Leptin levels (ng/ml)	479	8.0	5.6	6.5	1.1	41.8	466	19.4	11.9	17.0	1.7	90.0
RAINE Study	Age (yrs)	467	20.1	0.4	20.0	19.4	22.1	412	20.0	0.4	19.9	18.3	21.9
	BMI (kg/m²)	467	24.5	4.3	23.8	16.9	48.9	412	24.2	5.0	23.0	15.4	46.5
	Leptin levels (ng/ml)	467	6.1	9.9	3.4	0.1	162.1	412	26.2	18.7	21.5	2.2	98.2
RISC	Age (yrs)	156	44.7	8.3	-	-	-	157	45.8	7.9	-	-	-
	BMI (kg/m²)	156	26.0	3.5	26.0	17.9	39.3	157	25.2	4.5	24.3	16.9	42.9
	Leptin levels (ng/ml)	156	5.5	5.6	4.1	0.0	35.7	157	20.9	16.6	16.1	0.9	110.0
RSI	Age (yrs)	273	66.7	7.1	66.1	55.2	88.7	279	69.2	7.6	69.4	55.1	90.8
	BMI (kg/m²)	268	25.7	2.8	25.8	18.4	35.3	272	26.8	4.6	26.0	18.2	59.5
	Leptin levels (ng/ml)	273	5.6	4.5	4.0	0.4	25.2	281	17.9	13.1	15.0	0.7	61.4
SHIP-TREND	Age (yrs)	410	50.5	14.1	51.0	22.0	80.0	545	50.0	13.3	50.0	20.0	81.0
	BMI (kg/m²)	410	28.1	3.7	28.0	19.2	43.9	545	27.0	5.1	26.3	18.5	53.7
	Leptin levels (ng/ml)	410	7.4	5.6	6.2	1.0	43.1	545	21.8	15.7	18.0	1.9	165.0
TwinsUK	Age (yrs)	-	-	-	-	-	-	1015	48.8	11.2	49.1	18.4	73.5

	BMI (kg/m²)	-	-	-	-	-	-	1015	25.2	4.5	24.3	15.1	46.0
	Leptin levels (ng/ml)	-	-	-	-	-	-	1015	16.9	12.0	13.6	1.1	79.4
WGHS	Age (yrs)	-	-	-	-	-	-	789	58.8	8.5	58.0	45.0	87.0
	BMI (kg/m²)	-	-	-	-	-	-	789	25.9	4.7	25.0	14.6	49.9
	Leptin levels (ng/ml)	-	-	-	-	-	-	789	22.8	16.9	19.1	1.4	145.0
WHI EA	Age (yrs)	-	-	-	-	-	-	1901	68.3	6.4	69.0	50.0	79.0
	BMI (kg/m²)	-	-	-	-	-	-	1901	27.7	6.6	26.5	15.7	159.8
	Leptin levels (ng/ml)	-	-	-	-	-	-	1901	20.9	18.6	16.2	0.2	148.8
WHI AA	Age (yrs)	-	-	-	-	-	-	468	65.5	6.8	66.0	50.0	79.0
	BMI (kg/m²)	-	-	-	-	-	-	468	30.2	7.7	29.1	17.2	141.0
	Leptin levels (ng/ml)	-	-	-	-	-	-	468	33.0	20.4	29.1	2.1	117.2
YFS	Age (yrs)	759	32	5	33	24	39	922	32.1	5	33	24	39
	BMI (kg/m²)	755	25.7	4	25.1	15.7	47.8	919	24.4	4.6	23.5	15.7	47.2
	Leptin levels (ng/ml)	759	5.4	4.2	4.3	0.8	32.1	922	15.2	9.7	13	1.5	63.3

* only report descrptives for the individuals included in each of the analyses

CHS NOTE: For age and BMI, I included all individuals who are included in one or more of the analyses

CHS NOTE: For leptin and adiponectin, I included all individuals in the biggest analysis (not adjusted for fat percentage or BMI)

Table S3. Information on genotyping methods, quality control of SNPs, imputation, and statistical analysis for ExomeChip study cohorts

Cohort	Genotyping Array	Genotype calling algorithm	Principal components		Inclusion criteria				Association analyses	
			Software	SNPs used from GWAS/ExomeCHIP/AIMS/Other	MAF	Call rate*	*P*-value for HWE	SNPs that met QC criteria	Polymorphic SNPs in meta-analysis	Analyses software
ARIC	Illumina ExomeChip V1.0	GenTrain 2.0 clustering algorithm	Eigensoft v3.0	Exomchip (MAF>5%)	≥ 0%	≥ 95%	> 10^{-6}	237898 **	163,162 (EA)	rvtests
CHS EA and AA	Illumina ExomeChip V1.0	--	R	ExomeChip	> 0%	≥ 97%	No filter	227061	--	raremetalworker
CLHNS	Affymetrix 500K	Birdseed v2	MACH	GWAS/ExomeCHIP	≥ 0%	≥95%	> 10^{-6}	2304702	28,560,246	mach2QTL
Ely	Illumina HumanCoreExome	GenCall	PLINK	GWAS	>0%	>95%	> $5x10^{-6}$	231349	231349	RAREMETALWORKER
ERF study	Illumina HumanExome chip v1.1	GenomeStudio v1.9. and zCall	--	--	>5%	>95%	> 10^{-5}	--	240017	rvtests
FAMHS	Illumina Human Exome 12v1.0 BeadChip	Genome Studio via central CHARGE-S genotyping	EIGENSTRAT	GWAS	≥0%	≥ 98%	> 10^{-6}	237373 **	--	raremetalworker
Fenland-CE	Illumina HumanCoreExome	GenCall	PLINK v1.9beta	GWAS	>0%	>95%	> 10^{-6}	15083259	234201	RAREMETALWORKER
Fenland-Exomechip	Illumina ExomeChip v1.0	Gencall + zcall	PLINK v1.07	ExomeChip	≥ 0%	>=97%	> 10^{-6}	241979	240859	RAREMETALWORKER
FHS	Illumina Infinium HumanExome BeadChip v1.0	Illumina issued cluster file HumanExome-12v1.egt + zCall + CHARGE best practices and joint calling	EIGENSOFT	GWAS	≥ 0%	≥ 97%	No filter	237767	--	raremetalworker

FINRISK 1997	Illumina HumanHap 610k		PLINK	ExomeCHIP	> 0%	≥ 90%	> 10^{-6}	509376	495420	rvtests
FINRISK 2007	Illumina HumanHap 610k		PLINK	ExomeCHIP	> 0%	≥ 90%	> 10^{-6}	509376	495420	rvtests
HABC AA	Illumina ExomeChip V1.0	CHARGE protocol	eignestrat	AIMs	≥ 0%	>95%	> 10^{-6}	228554	228554	rvtests
HABC EA	Illumina ExomeChip V1.0	CHARGE protocol	eignestrat	AIMs	≥ 0%	>95%	> 10^{-6}	228565	228565	rvtests
Inter99	Illumina HumanExome-12v1	GenCall + Zcall	PLINK	AIM SNPs for outlier detection, ExomeCHIP fo adjustment	> 0%	≥ 98%	> 10^{-4}	137187	137187	RMW
JHS	Illumina ExomeChip V1.0	CHARGE joint calling (Illumina GenomeStudio v2011.1 software was utilized with the GenTrain 2.0 clustering algorithm)	Eigenstrat smartpca	Bi-allelic ExomeChip SNPs with MAF > 0.05, HWE p > 0.000001, callrate > 99%, pruned to be pairwise independent with r = 0.3 in plink.	> 0%	≥ 95%	No filter	137716	--	rvtests
KORA	Illumina ExomeChip V1.0	GeneCall + Zcall (CHARGE Protocol)	genomest udio	ExomeCHIP	> 0%	≥98%	≥10^{-8}	1409	247868	rvtests
Leipzig-adults	Illumina HumanExome-12v1_A	GeneCall + Zcall (Oxford Protocol)	PLINK	ExomeCHIP MAF>1%	> 0%	≥ 99%	> 10^{-4}	231460	--	RareMetalWorker
MESA CAU, CHN, AFA, and HIS	Illumina Exome Chip v1.0	Illumina GenomeStudio2011.1	EIGENSTR AT	ExomeCHIP	> 0%	≥ 90%	> 10^{-6}	238876	238876	rvtests
NEO Study	Illumina HumanCoreExom eChip-24V1.0	GeneCall (SOP v5)	PLINK	Based on LD prune	> 0%	≥ 98%	> 10^{-6}	209874	209874	rvtests
OMICS-Fenland	Affymetrix Axiom UKBiobank	Axiom GT1	PLINK v1.9beta	GWAS	> 0%	≥ 95%	> 10^{-6}	719871	58240	RAREMETALWORKE R

PIVUS	Illumina HumanExome-12v1_A	GeneCall + Zcall (Oxford Protocol)	plink/MDS	AIMS	> 0%	≥ 99%	> 10^{-4}	233149	--	raremetalworker
RAINE Study	Illuminia HumanExome-12v1_A	Illumina GenomeStudio GenTrain Clustering algorithm + zCall	EIGENSOFT - smartpca	AIMS	>0%	>=95%	> 10^{-4}	240806	240062	rvtests
RISC	Illumina Human Exome Beadchip v1	GenCall followed by zCall	PLINK	ExomeCHIP	≥ 0%	0.99	> 10^{-4}	236875	236871	RMW
RSI	Illumina ExomeChip V1.1	GeneCall + Zcall (CHARGE Protocol)	PLINK	GWAS	>0%	≥ 90%	> 10^{-6}	237766	109402	rvtests
SHIP-TREND	Illumina ExomeChip V1.0	GeneCall (CHARGE JointCalling Clusterfile)	Illumina GenomeStudio v2011.1	AIMs	> 0%	≥ 98% (together with SHIP)	> 10^{-6}	238205	--	raremetalworker
TwinsUK	Illumina12v1-1_A	GeneCall	Plink	GWAS	> 0%	≥ 90%	> 10^{-6}	222804		raremetalworker 4.13.6
WGHS	Illumina HumanExome Beadchip v.1.1A	genomeStudio + zCall	EIGENSTRAT	GWAS	≥ 0%	>95%	> 10^{-6}	235667	234710	raremetal
WHI	Illumina Human Exome BeadChip v1.0	GenomeStudio v2010.3	SNPRelate	ExomeCHIP	> 0%	≥ 90%	> 10^{-6}	246470	246,303	rvtests
YFS	Illumina CoreExome v1.0b	GenCall	PLINK	ExomeCHIP	> 0%	≥ 95%	> 10^{-6}	238194	237,852	rvtest

* Call rate to exclude SNPs for which less than a certain percentage of individuals were successfully genotyped (i.e. to exclude 'bad' SNPs)

** Includes monomorphic SNPs

Table S4. Single-variant results in all statistical models for the leptin-associated loci

SNP	**Nearest Gene**	**EA**	**OA**	**EAF**	**Beta**	**SE**	***P* value**	**N**
Leptin / Additive / All ancestries								
rs1121980	*FTO*	A	G	0.424	0.050	0.006	9.4E-16	56,802
rs2340550	*ACTL9*	G	A	0.696	-0.005	0.007	4.6E-01	54,433
rs13389219	*COBLL1*	T	C	0.410	0.048	0.007	1.0E-12	50,386
rs1260326	*GCKR*	C	T	0.624	0.035	0.006	4.9E-08	56,802
rs900399	*CCNL1*	G	A	0.389	-0.036	0.007	2.5E-08	50,386
rs3799260	*KLHL31*	T	C	0.822	-0.023	0.008	3.7E-03	56,802
rs62621812	*ZNF800*	A	G	0.028	-0.097	0.018	8.0E-08	56,802
rs791600	*LEP*	A	G	0.422	-0.048	0.007	2.7E-13	49,371
rs17151919	*LEP*	A	G	0.007	-0.259	0.040	1.3E-10	49,111
rs972283	*KLF14*	G	A	0.551	-0.038	0.006	6.0E-10	56,802
Leptin / Additive / European								
rs1121980	*FTO*	A	G	0.432	0.055	0.007	7.7E-17	49,909
rs2340550	*ACTL9*	G	A	0.685	-0.008	0.007	2.8E-01	48,008
rs13389219	*COBLL1*	T	C	0.394	0.046	0.007	7.3E-11	43,493
rs1260326	*GCKR*	C	T	0.607	0.032	0.007	1.7E-06	49,909
rs900399	*CCNL1*	G	A	0.396	-0.033	0.007	2.4E-06	43,493
rs3799260	*KLHL31*	T	C	0.818	-0.024	0.008	3.8E-03	49,909
rs62621812	*ZNF800*	A	G	0.031	-0.098	0.018	8.2E-08	49,909
rs791600	*LEP*	A	G	0.411	-0.043	0.007	1.4E-09	42,478
rs17151919	*LEP*	A	G	0.000	0.134	0.261	6.1E-01	44,474
rs972283	*KLF14*	G	A	0.521	-0.041	0.006	1.1E-10	49,909
Leptin / Additive / All ancestries / Men								
rs1121980	*FTO*	A	G	0.433	0.075	0.009	9.7E-16	23,861
rs2340550	*ACTL9*	G	A	0.693	-0.027	0.010	7.5E-03	23,861
rs13389219	*COBLL1*	T	C	0.417	0.059	0.010	9.6E-09	20,822
rs1260326	*GCKR*	C	T	0.625	0.028	0.010	4.1E-03	23,861
rs900399	*CCNL1*	G	A	0.387	-0.030	0.010	2.8E-03	20,822
rs3799260	*KLHL31*	T	C	0.819	-0.007	0.012	5.7E-01	23,861
rs62621812	*ZNF800*	A	G	0.029	-0.100	0.027	2.6E-04	23,861
rs791600	*LEP*	A	G	0.406	-0.035	0.010	4.4E-04	20,822

rs17151919	*LEP*	A	G	0.006	-0.310	0.066	3.1E-06	22,153
rs972283	*KLF14*	G	A	0.544	-0.036	0.009	1.3E-04	23,861
Leptin / Additive / European / Men								
rs1121980	*FTO*	A	G	0.433	0.077	0.010	1.8E-15	21,921
rs2340550	*ACTL9*	G	A	0.684	-0.029	0.010	5.9E-03	21,921
rs13389219	*COBLL1*	T	C	0.395	0.057	0.011	7.0E-08	18,882
rs1260326	*GCKR*	C	T	0.608	0.026	0.010	8.4E-03	21,921
rs900399	*CCNL1*	G	A	0.395	-0.026	0.011	1.5E-02	18,882
rs3799260	*KLHL31*	T	C	0.819	-0.007	0.012	5.6E-01	21,921
rs62621812	*ZNF800*	A	G	0.031	-0.100	0.027	2.7E-04	21,921
rs791600	*LEP*	A	G	0.410	-0.032	0.010	2.0E-03	18,882
rs17151919	*LEP*	A	G	0.000	0.155	0.349	6.6E-01	20,213
rs972283	*KLF14*	G	A	0.522	-0.037	0.010	1.1E-04	21,921
Leptin / Additive / All ancestries / Women								
rs1121980	*FTO*	A	G	0.417	0.035	0.008	1.5E-05	32,940
rs2340550	*ACTL9*	G	A	0.697	0.010	0.009	2.5E-01	30,571
rs13389219	*COBLL1*	T	C	0.405	0.040	0.009	5.6E-06	29,563
rs1260326	*GCKR*	C	T	0.624	0.043	0.008	2.1E-07	32,940
rs900399	*CCNL1*	G	A	0.391	-0.049	0.008	6.2E-09	29,563
rs3799260	*KLHL31*	T	C	0.825	-0.041	0.010	5.6E-05	32,940
rs62621812	*ZNF800*	A	G	0.027	-0.102	0.024	2.2E-05	32,940
rs791600	*LEP*	A	G	0.434	-0.060	0.009	2.9E-12	28,548
rs17151919	*LEP*	A	G	0.007	-0.233	0.049	1.8E-06	26,957
rs972283	*KLF14*	G	A	0.555	-0.043	0.008	6.5E-08	32,940
Leptin / Additive / European / Women								
rs1121980	*FTO*	A	G	0.431	0.044	0.009	6.2E-07	27,987
rs2340550	*ACTL9*	G	A	0.685	0.007	0.010	4.6E-01	26,086
rs13389219	*COBLL1*	T	C	0.392	0.038	0.009	6.1E-05	24,610
rs1260326	*GCKR*	C	T	0.606	0.040	0.009	6.0E-06	27,987
rs900399	*CCNL1*	G	A	0.397	-0.048	0.009	2.5E-07	24,610
rs3799260	*KLHL31*	T	C	0.818	-0.043	0.011	8.9E-05	27,987
rs62621812	*ZNF800*	A	G	0.032	-0.103	0.024	2.1E-05	27,987
rs791600	*LEP*	A	G	0.413	-0.054	0.009	8.3E-09	23,595
rs17151919	*LEP*	A	G	0.000	-0.039	0.380	9.2E-01	24,260

rs972283	*KLF14*	G	A	0.521	-0.048	0.009	1.6E-08	27,987
Leptin / Recessive / All ancestries								
rs1121980	*FTO*	A	G	0.424	0.071	0.011	1.1E-10	56,802
rs2340550	*ACTL9*	G	A	0.696	-0.006	0.009	4.6E-01	54,433
rs13389219	*COBLL1*	T	C	0.410	0.062	0.012	3.2E-07	50,386
rs1260326	*GCKR*	C	T	0.624	0.046	0.009	2.3E-07	56,802
rs900399	*CCNL1*	G	A	0.389	-0.042	0.012	6.4E-04	50,386
rs3799260	*KLHL31*	T	C	0.822	-0.029	0.009	1.5E-03	56,802
rs62621812	*ZNF800*	A	G	0.021	-0.037	0.124	7.7E-01	56,802
rs791600	*LEP*	A	G	0.422	-0.074	0.012	6.9E-10	49,371
rs17151919	*LEP*	A	G	0.007	-0.527	0.190	5.6E-03	49,111
rs972283	*KLF14*	G	A	0.551	-0.049	0.009	2.5E-07	56,802
Leptin / Recessive / European								
rs1121980	*FTO*	A	G	0.432	0.078	0.012	3.2E-11	49,909
rs2340550	*ACTL9*	G	A	0.685	-0.011	0.009	2.5E-01	48,008
rs13389219	*COBLL1*	T	C	0.394	0.061	0.013	4.5E-06	43,493
rs1260326	*GCKR*	C	T	0.607	0.041	0.009	1.2E-05	49,909
rs900399	*CCNL1*	G	A	0.396	-0.036	0.013	6.6E-03	43,493
rs3799260	*KLHL31*	T	C	0.818	-0.031	0.010	1.3E-03	49,909
rs62621812	*ZNF800*	A	G	0.023	-0.037	0.124	7.7E-01	49,909
rs791600	*LEP*	A	G	0.411	-0.070	0.013	5.8E-08	42,478
rs17151919	*LEP*	A	G	0.000	NA	NA	NA	44,474
rs972283	*KLF14*	G	A	0.521	-0.054	0.010	9.5E-08	49,909
Leptin / Recessive / All ancestries / Men								
rs1121980	*FTO*	A	G	0.433	0.096	0.017	8.5E-09	23,861
rs2340550	*ACTL9*	G	A	0.693	-0.031	0.013	1.7E-02	23,861
rs13389219	*COBLL1*	T	C	0.417	0.085	0.019	7.0E-06	20,822
rs1260326	*GCKR*	C	T	0.625	0.031	0.014	2.4E-02	23,861
rs900399	*CCNL1*	G	A	0.387	-0.029	0.019	1.3E-01	20,822
rs3799260	*KLHL31*	T	C	0.819	-0.014	0.014	3.3E-01	23,861
rs62621812	*ZNF800*	A	G	0.021	-0.067	0.192	7.3E-01	23,861
rs791600	*LEP*	A	G	0.406	-0.065	0.019	4.6E-04	20,822
rs17151919	*LEP*	A	G	0.005	-0.725	0.260	5.3E-03	22,153
rs972283	*KLF14*	G	A	0.544	-0.050	0.015	6.0E-04	23,861

Leptin / Recessive / European / Men								
rs1121980	*FTO*	A	G	0.433	0.100	0.017	7.6E-09	21,921
rs2340550	*ACTL9*	G	A	0.684	-0.035	0.014	1.1E-02	21,921
rs13389219	*COBLL1*	T	C	0.395	0.080	0.020	7.1E-05	18,882
rs1260326	*GCKR*	C	T	0.608	0.027	0.014	6.0E-02	21,921
rs900399	*CCNL1*	G	A	0.395	-0.023	0.020	2.6E-01	18,882
rs3799260	*KLHL31*	T	C	0.819	-0.015	0.014	2.9E-01	21,921
rs62621812	*ZNF800*	A	G	0.023	-0.067	0.192	7.3E-01	21,921
rs791600	*LEP*	A	G	0.410	-0.065	0.019	8.7E-04	18,882
rs17151919	*LEP*	A	G	0.000	NA	Inf	NA	20,213
rs972283	*KLF14*	G	A	0.522	-0.052	0.015	5.6E-04	21,921
Leptin / Recessive / All ancestries / Women								
rs1121980	*FTO*	A	G	0.417	0.058	0.015	6.8E-05	32,940
rs2340550	*ACTL9*	G	A	0.697	0.011	0.012	3.6E-01	30,571
rs13389219	*COBLL1*	T	C	0.405	0.052	0.016	9.4E-04	29,563
rs1260326	*GCKR*	C	T	0.624	0.059	0.012	3.9E-07	32,940
rs900399	*CCNL1*	G	A	0.391	-0.061	0.016	1.5E-04	29,563
rs3799260	*KLHL31*	T	C	0.825	-0.047	0.012	8.4E-05	32,940
rs62621812	*ZNF800*	A	G	0.019	-0.149	0.162	3.6E-01	32,940
rs791600	*LEP*	A	G	0.434	-0.088	0.016	1.9E-08	28,548
rs17151919	*LEP*	A	G	0.007	-0.195	0.280	4.9E-01	26,957
rs972283	*KLF14*	G	A	0.555	-0.055	0.012	8.5E-06	32,940
Leptin / Recessive / European / Women								
rs1121980	*FTO*	A	G	0.431	0.068	0.016	1.4E-05	27,987
rs2340550	*ACTL9*	G	A	0.685	0.006	0.013	6.5E-01	26,086
rs13389219	*COBLL1*	T	C	0.392	0.055	0.018	2.1E-03	24,610
rs1260326	*GCKR*	C	T	0.606	0.055	0.013	1.3E-05	27,987
rs900399	*CCNL1*	G	A	0.397	-0.057	0.017	9.9E-04	24,610
rs3799260	*KLHL31*	T	C	0.818	-0.049	0.013	1.5E-04	27,987
rs62621812	*ZNF800*	A	G	0.022	-0.149	0.162	3.6E-01	27,987
rs791600	*LEP*	A	G	0.413	-0.083	0.017	1.8E-06	23,595
rs17151919	*LEP*	A	G	0.000	NA	NA	NA	24,260
rs972283	*KLF14*	G	A	0.521	-0.062	0.014	5.6E-06	27,987
LeptinAdjBMI / Additive / All ancestries								

rs1121980	*FTO*	A	G	0.424	0.003	0.006	5.7E-01	56,708
rs2340550	*ACTL9*	G	A	0.695	-0.014	0.007	3.2E-02	54,339
rs13389219	*COBLL1*	T	C	0.410	0.053	0.007	3.0E-15	50,297
rs1260326	*GCKR*	C	T	0.624	0.050	0.006	2.7E-15	56,708
rs900399	*CCNL1*	G	A	0.389	-0.041	0.007	5.2E-10	50,297
rs3799260	*KLHL31*	T	C	0.822	-0.036	0.008	4.0E-06	56,708
rs62621812	*ZNF800*	A	G	0.028	-0.127	0.018	2.0E-12	56,708
rs791600	*LEP*	A	G	0.422	-0.066	0.007	1.1E-23	49,282
rs17151919	*LEP*	A	G	0.007	-0.333	0.040	1.5E-16	49,034
rs972283	*KLF14*	G	A	0.550	-0.053	0.006	6.3E-18	56,708
LeptinAdjBMI / Additive / European								
rs1121980	*FTO*	A	G	0.432	0.005	0.007	4.5E-01	49,830
rs2340550	*ACTL9*	G	A	0.685	-0.016	0.007	2.6E-02	47,929
rs13389219	*COBLL1*	T	C	0.394	0.053	0.007	1.1E-13	43,419
rs1260326	*GCKR*	C	T	0.607	0.048	0.007	4.3E-13	49,830
rs900399	*CCNL1*	G	A	0.396	-0.040	0.007	9.2E-09	43,419
rs3799260	*KLHL31*	T	C	0.818	-0.038	0.008	3.8E-06	49,830
rs62621812	*ZNF800*	A	G	0.031	-0.127	0.018	2.8E-12	49,830
rs791600	*LEP*	A	G	0.411	-0.063	0.007	5.4E-19	42,404
rs17151919	*LEP*	A	G	0.000	-0.187	0.261	4.7E-01	44,401
rs972283	*KLF14*	G	A	0.521	-0.056	0.006	3.8E-18	49,830
LeptinAdjBMI / Additive / All ancestries / Men								
rs1121980	*FTO*	A	G	0.433	0.028	0.009	2.6E-03	23,822
rs2340550	*ACTL9*	G	A	0.693	-0.050	0.010	8.5E-07	23,822
rs13389219	*COBLL1*	T	C	0.417	0.052	0.010	3.8E-07	20,787
rs1260326	*GCKR*	C	T	0.624	0.043	0.010	8.4E-06	23,822
rs900399	*CCNL1*	G	A	0.387	-0.036	0.010	4.3E-04	20,787
rs3799260	*KLHL31*	T	C	0.819	-0.023	0.012	6.0E-02	23,822
rs62621812	*ZNF800*	A	G	0.029	-0.148	0.027	7.0E-08	23,822
rs791600	*LEP*	A	G	0.406	-0.054	0.010	6.8E-08	20,787
rs17151919	*LEP*	A	G	0.006	-0.399	0.066	1.2E-09	22,119
rs972283	*KLF14*	G	A	0.544	-0.045	0.009	1.8E-06	23,822
LeptinAdjBMI / Additive / European / Men								
rs1121980	*FTO*	A	G	0.433	0.026	0.010	6.4E-03	21,883

rs2340550	*ACTL9*	G	A	0.684	-0.053	0.010	4.1E-07	21,883
rs13389219	*COBLL1*	T	C	0.395	0.048	0.011	5.2E-06	18,848
rs1260326	*GCKR*	C	T	0.608	0.042	0.010	2.1E-05	21,883
rs900399	*CCNL1*	G	A	0.395	-0.033	0.011	2.1E-03	18,848
rs3799260	*KLHL31*	T	C	0.819	-0.025	0.012	4.3E-02	21,883
rs62621812	*ZNF800*	A	G	0.031	-0.146	0.028	1.1E-07	21,883
rs791600	*LEP*	A	G	0.410	-0.049	0.010	2.5E-06	18,848
rs17151919	*LEP*	A	G	0.000	-0.225	0.352	5.2E-01	20,180
rs972283	*KLF14*	G	A	0.522	-0.048	0.010	4.8E-07	21,883
LeptinAdjBMI / Additive / All ancestries / Women								
rs1121980	*FTO*	A	G	0.417	-0.013	0.008	1.2E-01	32,886
rs2340550	*ACTL9*	G	A	0.697	0.007	0.009	4.2E-01	30,517
rs13389219	*COBLL1*	T	C	0.405	0.052	0.009	2.0E-09	29,510
rs1260326	*GCKR*	C	T	0.624	0.059	0.008	6.2E-13	32,886
rs900399	*CCNL1*	G	A	0.391	-0.054	0.008	1.2E-10	29,510
rs3799260	*KLHL31*	T	C	0.825	-0.055	0.010	1.0E-07	32,886
rs62621812	*ZNF800*	A	G	0.027	-0.125	0.024	2.2E-07	32,886
rs791600	*LEP*	A	G	0.434	-0.079	0.009	4.1E-20	28,495
rs17151919	*LEP*	A	G	0.007	-0.291	0.050	5.7E-09	26,915
rs972283	*KLF14*	G	A	0.555	-0.063	0.008	3.7E-15	32,886
LeptinAdjBMI / Additive / European / Women								
rs1121980	*FTO*	A	G	0.431	-0.009	0.009	3.2E-01	27,947
rs2340550	*ACTL9*	G	A	0.685	0.008	0.009	4.1E-01	26,046
rs13389219	*COBLL1*	T	C	0.392	0.055	0.009	3.8E-09	24,571
rs1260326	*GCKR*	C	T	0.606	0.057	0.009	9.4E-11	27,947
rs900399	*CCNL1*	G	A	0.398	-0.058	0.009	3.4E-10	24,571
rs3799260	*KLHL31*	T	C	0.818	-0.057	0.011	2.2E-07	27,947
rs62621812	*ZNF800*	A	G	0.032	-0.126	0.024	1.9E-07	27,947
rs791600	*LEP*	A	G	0.413	-0.080	0.009	2.9E-17	23,556
rs17151919	*LEP*	A	G	0.000	-0.310	0.375	4.1E-01	24,221
rs972283	*KLF14*	G	A	0.521	-0.066	0.009	1.3E-14	27,947
LeptinAdjBMI / Recessive / All ancestries								
rs1121980	*FTO*	A	G	0.424	0.000	0.011	9.8E-01	56,708
rs2340550	*ACTL9*	G	A	0.695	-0.014	0.009	9.8E-02	54,339

rs13389219	*COBLL1*	T	C	0.410	0.080	0.012	7.0E-11	50,297
rs1260326	*GCKR*	C	T	0.624	0.057	0.009	1.8E-10	56,708
rs900399	*CCNL1*	G	A	0.389	-0.057	0.012	4.3E-06	50,297
rs3799260	*KLHL31*	T	C	0.822	-0.044	0.009	1.7E-06	56,708
rs62621812	*ZNF800*	A	G	0.021	-0.145	0.124	2.4E-01	56,708
rs791600	*LEP*	A	G	0.422	-0.099	0.012	2.4E-16	49,282
rs17151919	*LEP*	A	G	0.007	-0.795	0.190	2.9E-05	49,034
rs972283	*KLF14*	G	A	0.550	-0.071	0.009	6.1E-14	56,708
LeptinAdjBMI / Recessive / European								
rs1121980	*FTO*	A	G	0.432	-0.005	0.012	6.8E-01	49,830
rs2340550	*ACTL9*	G	A	0.685	-0.018	0.009	5.7E-02	47,929
rs13389219	*COBLL1*	T	C	0.394	0.081	0.013	1.4E-09	43,419
rs1260326	*GCKR*	C	T	0.607	0.053	0.009	1.9E-08	49,830
rs900399	*CCNL1*	G	A	0.396	-0.054	0.013	3.8E-05	43,419
rs3799260	*KLHL31*	T	C	0.818	-0.047	0.010	1.3E-06	49,830
rs62621812	*ZNF800*	A	G	0.023	-0.145	0.124	2.4E-01	49,830
rs791600	*LEP*	A	G	0.411	-0.099	0.013	2.7E-14	42,404
rs17151919	*LEP*	A	G	0.000	NA	Inf	NA	44,401
rs972283	*KLF14*	G	A	0.521	-0.079	0.010	8.0E-15	49,830
LeptinAdjBMI / Recessive / All ancestries / Men								
rs1121980	*FTO*	A	G	0.433	0.024	0.017	1.6E-01	23,822
rs2340550	*ACTL9*	G	A	0.693	-0.065	0.013	6.5E-07	23,822
rs13389219	*COBLL1*	T	C	0.417	0.082	0.019	1.4E-05	20,787
rs1260326	*GCKR*	C	T	0.624	0.046	0.014	7.9E-04	23,822
rs900399	*CCNL1*	G	A	0.387	-0.036	0.019	6.2E-02	20,787
rs3799260	*KLHL31*	T	C	0.819	-0.030	0.014	2.9E-02	23,822
rs62621812	*ZNF800*	A	G	0.021	-0.293	0.192	1.3E-01	23,822
rs791600	*LEP*	A	G	0.406	-0.095	0.019	4.2E-07	20,787
rs17151919	*LEP*	A	G	0.005	-0.942	0.258	2.5E-04	22,119
rs972283	*KLF14*	G	A	0.544	-0.058	0.015	6.2E-05	23,822
LeptinAdjBMI / Recessive / European / Men								
rs1121980	*FTO*	A	G	0.433	0.021	0.017	2.2E-01	21,883
rs2340550	*ACTL9*	G	A	0.684	-0.071	0.014	2.0E-07	21,883
rs13389219	*COBLL1*	T	C	0.395	0.072	0.020	3.3E-04	18,848

rs1260326	*GCKR*	C	T	0.608	0.045	0.014	1.6E-03	21,883
rs900399	*CCNL1*	G	A	0.395	-0.032	0.020	1.1E-01	18,848
rs3799260	*KLHL31*	T	C	0.819	-0.034	0.015	2.0E-02	21,883
rs62621812	*ZNF800*	A	G	0.023	-0.293	0.192	1.3E-01	21,883
rs791600	*LEP*	A	G	0.410	-0.092	0.019	2.1E-06	18,848
rs17151919	*LEP*	A	G	0.000	NA	NA	NA	20,180
rs972283	*KLF14*	G	A	0.522	-0.065	0.015	2.0E-05	21,883
LeptinAdjBMI / Recessive / All ancestries / Women								
rs1121980	*FTO*	A	G	0.417	-0.016	0.015	2.6E-01	32,886
rs2340550	*ACTL9*	G	A	0.697	0.016	0.012	1.8E-01	30,517
rs13389219	*COBLL1*	T	C	0.405	0.080	0.016	4.3E-07	29,510
rs1260326	*GCKR*	C	T	0.624	0.068	0.012	4.4E-09	32,886
rs900399	*CCNL1*	G	A	0.391	-0.083	0.016	2.3E-07	29,510
rs3799260	*KLHL31*	T	C	0.825	-0.063	0.012	1.3E-07	32,886
rs62621812	*ZNF800*	A	G	0.019	-0.241	0.162	1.4E-01	32,886
rs791600	*LEP*	A	G	0.434	-0.112	0.016	5.7E-13	28,495
rs17151919	*LEP*	A	G	0.007	-0.570	0.284	4.5E-02	26,915
rs972283	*KLF14*	G	A	0.555	-0.086	0.012	2.5E-12	32,886
LeptinAdjBMI / Recessive / All ancestries / Women								
rs1121980	*FTO*	A	G	0.431	-0.022	0.015	1.5E-01	27,947
rs2340550	*ACTL9*	G	A	0.685	0.015	0.012	2.3E-01	26,046
rs13389219	*COBLL1*	T	C	0.392	0.093	0.018	1.7E-07	24,571
rs1260326	*GCKR*	C	T	0.606	0.062	0.013	7.0E-07	27,947
rs900399	*CCNL1*	G	A	0.398	-0.085	0.017	9.2E-07	24,571
rs3799260	*KLHL31*	T	C	0.818	-0.066	0.013	2.2E-07	27,947
rs62621812	*ZNF800*	A	G	0.022	-0.241	0.162	1.4E-01	27,947
rs791600	*LEP*	A	G	0.413	-0.118	0.017	1.4E-11	23,556
rs17151919	*LEP*	A	G	0.000	NA	NA	NA	24,221
rs972283	*KLF14*	G	A	0.521	-0.096	0.014	1.4E-12	27,947

Table S5. Comparison of BMI-adjusted and BMI-unadjusted results for leptin associated loci

SNP	Chr	Position	Gene	Meta-analysis	Annotation	EA	OA	Beta AdjBMI	Beta	SE AdjBMI	SE	P AdjBMI	P	N AdjBMI	N
Novel loci															
rs3799260	6	53519605	*KLHL31*	Additive / All ancestries /Women	missense	C	T	0.055	0.041	0.010	0.010	1.0E-07	5.6E-05	32,886	32,940
rs62621812	7	127015083	*ZNF800*	Additive / All ancestries	missense	G	A	0.127	0.097	0.018	0.018	2.0E-12	8.0E-08	56,708	56,802
rs17151919	7	127894592	*LEP*	Additive / All ancestries	missense	G	A	0.333	0.259	0.040	0.040	1.5E-16	1.1E-10	49,034	49,111
rs972283	7	130466854	*KLF14*	Additive / European	intergenic	A	G	0.056	0.041	0.006	0.006	3.8E-18	1.1E-10	49,830	49,909
rs2340550	19	8808942	*ACTL9*	Recessive / European / Men	missense	A	G	0.071	0.035	0.014	0.014	2.0E-07	1.1E-02	21,883	21,921
Previously identified loci															
rs1260326	2	27730940	*GCKR*	Additive / All ancestries	missense	C	T	0.050	0.035	0.006	0.006	2.7E-15	4.9E-08	56,708	56,802
rs13389219	2	165528876	*COBLL1*	Additive / All ancestries	intronic	T	C	0.053	0.048	0.007	0.007	3.0E-15	1.0E-12	50,297	50,386
rs900399	3	156798732	*CCNL1*	Additive / All ancestries /Women	intergenic	A	G	0.054	0.049	0.008	0.008	1.2E-10	6.2E-09	29,510	29,563
rs791600	7	127865816	*LEP*	Additive / All ancestries	intergenic	G	A	0.066	0.048	0.007	0.007	1.1E-23	2.7E-13	49,282	49,371
rs1121980	16	53809247	*FTO*	Additive / European	intronic	A	G	0.005	0.055	0.007	0.007	4.5E-01	7.7E-17	49,830	49,909

The chromosomal positions are based on hg19.

Chr, chromosome; EA, Effect allele; OA, Other allele; EAF, Effect allele frequency; LeptinAdjBMI, leptin adjusted for body mass index

Table S6. Examination of collider bias with BMI among the exome-array significant loci associated with leptin adjusted for BMI

Locus	MarkerName	EA	EAF	xL	pL	xLadjB	pLadjB	xLadjBa	xB	pB
FTO	rs1121980	A	0.4316428	0.05486291	7.71E-17	0.004952214	4.47E-01	0.04153	0.07481	6.70E-225
*ACTL9**	rs2340550	G	0.6846579	-0.007649348	2.79E-01	-0.01562951	2.62E-02	-0.01394	0.00345	1.51E-01
COBLL1	rs13389219	T	0.3938886	0.04618875	7.30E-11	0.05254237	1.13E-13	0.05871	0.01261	8.16E-08
GCKR	rs1260326	C	0.6070448	0.03182518	1.71E-06	0.04773787	4.32E-13	0.04993	0.00449	5.24E-02
CCNL1	rs900399	G	0.3961604	-0.03304733	2.43E-06	-0.04024503	9.25E-09	-0.04198	-0.00355	1.24E-01
*KLHL31**	rs3799260	T	0.8183081	-0.02397769	3.79E-03	-0.03820487	3.83E-06	-0.03499	0.00657	1.71E-02
ZNF800	rs62621812	A	0.03142557	-0.09769461	8.18E-08	-0.1273454	2.80E-12	-0.11685	0.02147	1.24E-03
LEP	rs791600	A	0.4110841	-0.04264698	1.36E-09	-0.06262022	5.35E-19	-0.06034	0.00466	4.54E-02
*LEP**	rs17151919	A	0.000166917	0.1342779	6.07E-01	-0.1868478	4.73E-01	-0.18299	0.00789	9.20E-01
KLF14	rs972283	G	0.5211696	-0.04137304	1.12E-10	-0.05554037	3.84E-18	-0.05942	-0.00793	2.68E-04

xL, Effect size for leptin

pL, P value for leptin

xLadjB, Effect size for leptin adjusted for BMI

pLadjB, P value for leptin adjusted for BMI

xLadjBa, Corrected effect size for leptin adjusted for BMI

xB, Effect size for BMI

pB, P value for BMI

* The *ACTL9*, *KLHL31*, and *LEP* rs17151919 loci reached array-wide significance ($P<2x10^{-7}$) in meta-analyses of European-ancestry men (recessive model), all-ancestry women, and African-ancestry men and women combined, respectively. The results shown are from meta-analyses of European ancestry individuals only.

Table S7. Ancestry-specific results for the Val94Met (rs17151919) missense variant in *LEP*

Ancestry	Trait	Chr:Position	EA	OA	N	EAF	N_{GG}	N_{GA+AG}	N_{AA}	beta	se	Pvalue	I2
All	LeptinAdjBMI	7:127894592	A (Met94)	G (Val94)	49034	0.0067	40075	609	28	-0.333	0.040	1.53E-16	76%
European	LeptinAdjBMI	7:127894592	A (Met94)	G (Val94)	44401	0.0002	36065	15	0	-0.187	0.261	4.73E-01	0%
African	LeptinAdjBMI	7:127894592	A (Met94)	G (Val94)	3901	0.0800	3302	571	27	-0.343	0.042	2.40E-16	94%
Hispanic	LeptinAdjBMI	7:127894592	A (Met94)	G (Val94)	488	0.0221	464	23	1	-0.209	NA	2.85E-01	NA
East Asian	LeptinAdjBMI	7:127894592	A (Met94)	G (Val94)	244	NA	NA	NA	NA	NA	NA	NA	NA

EA, effect allele; OA, other allele; EAF, effect allele frequency

Table S8. Gene-based results in all statistical models for leptin-associated genes

Gene	Method	N	P value	beta	se	N variants
Leptin / Additive / All ancestries						
CNTD1	SKAT broad	49,597	9.1E-04	0.350	0.094	6
CNTD1	SKAT strict	48,582	7.0E-02	1.043	0.330	1
CNTD1	VT broad	49,597	3.9E-06	0.746	0.149	4
CNTD1	VT strict	48,582	7.0E-02	1.043	0.330	1
DNAJC18	SKAT broad	56,013	2.2E-02	0.062	0.057	7
DNAJC18	SKAT strict	49,597	5.1E-05	0.466	0.135	2
DNAJC18	VT broad	56,013	4.3E-03	0.323	0.096	5
DNAJC18	VT strict	49,597	1.1E-03	0.466	0.135	2
Leptin / Additive / European						
CNTD1	SKAT broad	42,704	1.4E-05	0.580	0.126	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	42,704	1.1E-05	0.720	0.153	4
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	49,120	3.1E-02	0.045	0.060	7
DNAJC18	SKAT strict	42,704	5.3E-05	0.478	0.140	2
DNAJC18	VT broad	49,120	8.4E-03	0.360	0.112	5
DNAJC18	VT strict	42,704	1.3E-03	0.478	0.140	2
Leptin / Additive / All ancestries / Men						
CNTD1	SKAT broad	20,822	2.0E-05	0.580	0.137	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	20,822	6.4E-06	1.026	0.209	3
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	23,861	5.3E-01	-0.061	0.086	6
DNAJC18	SKAT strict	20,822	2.0E-01	0.034	0.223	2
DNAJC18	VT broad	23,861	3.4E-01	-0.569	0.360	2
DNAJC18	VT strict	20,822	1.3E-01	-0.896	0.499	1
Leptin / Additive / European / Men						
CNTD1	SKAT broad	18,882	1.3E-07	0.898	0.165	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	18,882	1.4E-07	0.898	0.165	5
CNTD1	VT strict	NA	NA	NA	NA	NA

DNAJC18	SKAT broad	21,921	5.3E-01	-0.059	0.087	6
DNAJC18	SKAT strict	18,882	2.0E-01	0.034	0.223	2
DNAJC18	VT broad	21,921	6.4E-01	-0.566	0.446	2
DNAJC18	VT strict	18,882	1.3E-01	-0.896	0.499	1
Leptin / Additive / All ancestries / Women						
CNTD1	SKAT broad	29,563	5.5E-01	0.178	0.123	6
CNTD1	SKAT strict	28,548	7.3E-02	0.981	0.297	1
CNTD1	VT broad	29,563	3.2E-02	0.553	0.211	4
CNTD1	VT strict	28,548	7.3E-02	0.981	0.297	1
DNAJC18	SKAT broad	32,940	1.3E-02	0.151	0.075	7
DNAJC18	SKAT strict	29,563	1.9E-05	0.717	0.166	2
DNAJC18	VT broad	32,940	5.8E-04	0.452	0.117	5
DNAJC18	VT strict	29,563	3.3E-05	0.717	0.166	2
Leptin / Additive / European / Women						
CNTD1	SKAT broad	24,610	2.7E-01	0.233	0.188	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	24,610	1.3E-01	0.478	0.240	4
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	27,987	1.9E-02	0.132	0.081	6
DNAJC18	SKAT strict	24,610	1.3E-05	0.767	0.177	2
DNAJC18	VT broad	27,987	8.1E-04	0.557	0.146	4
DNAJC18	VT strict	24,610	2.8E-05	0.767	0.177	2
LeptinAdjBMI / Additive / All ancestries						
CNTD1	SKAT broad	49,508	4.6E-02	0.242	0.093	6
CNTD1	SKAT strict	48,493	9.1E-02	0.969	0.330	1
CNTD1	VT broad	49,508	9.0E-04	0.560	0.149	4
CNTD1	VT strict	48,493	9.1E-02	0.969	0.330	1
DNAJC18	SKAT broad	55,919	4.3E-03	0.083	0.057	7
DNAJC18	SKAT strict	49,508	1.2E-07	0.485	0.136	2
DNAJC18	VT broad	55,919	1.8E-02	0.279	0.096	5
DNAJC18	VT strict	49,508	7.1E-04	0.485	0.136	2
LeptinAdjBMI / Additive / European						
CNTD1	SKAT broad	42,630	3.8E-03	0.430	0.126	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	42,630	2.0E-03	0.525	0.153	4

CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	49,041	8.4E-03	0.063	0.060	7
DNAJC18	SKAT strict	42,630	2.3E-07	0.474	0.141	2
DNAJC18	VT broad	49,041	6.4E-02	0.286	0.113	5
DNAJC18	VT strict	42,630	1.6E-03	0.474	0.141	2
LeptinAdjBMI / Additive / All ancestries / Men						
CNTD1	SKAT broad	20,787	7.1E-02	0.313	0.138	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	20,787	1.5E-02	0.606	0.210	3
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	23,822	2.6E-01	-0.124	0.086	6
DNAJC18	VT broad	23,822	1.6E-01	-0.713	0.359	2
DNAJC18	SKAT strict	20,787	9.6E-02	0.036	0.223	2
DNAJC18	VT strict	20,787	4.2E-02	-1.138	0.498	1
LeptinAdjBMI / Additive / European / Men						
CNTD1	SKAT broad	18,848	7.4E-03	0.565	0.165	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA
CNTD1	VT broad	18,848	2.6E-03	0.565	0.165	5
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	21,883	2.6E-01	-0.109	0.087	6
DNAJC18	SKAT strict	18,848	9.6E-02	0.036	0.223	2
DNAJC18	VT broad	21,883	2.5E-01	-0.838	0.446	2
DNAJC18	VT strict	18,848	4.2E-02	-1.138	0.498	1
LeptinAdjBMI / Additive / All ancestries / Women						
CNTD1	SKAT broad	29,510	3.0E-01	0.238	0.124	6
CNTD1	SKAT strict	28,495	7.7E-02	0.972	0.305	1
CNTD1	VT broad	29,510	1.3E-02	0.620	0.212	4
CNTD1	VT strict	28,495	7.7E-02	0.972	0.305	1
DNAJC18	SKAT broad	32,886	7.6E-04	0.234	0.075	7
DNAJC18	SKAT strict	29,510	5.5E-08	0.757	0.169	2
DNAJC18	VT broad	32,886	4.4E-04	0.460	0.118	5
DNAJC18	VT strict	29,510	1.5E-05	0.757	0.169	2
LeptinAdjBMI / Additive / European / Women						
CNTD1	SKAT broad	24,571	1.7E-01	0.373	0.186	5
CNTD1	SKAT strict	NA	NA	NA	NA	NA

CNTD1	VT broad	24,571	6.3E-02	0.554	0.239	4
CNTD1	VT strict	NA	NA	NA	NA	NA
DNAJC18	SKAT broad	27,947	2.4E-03	0.207	0.081	6
DNAJC18	SKAT strict	24,571	7.9E-08	0.774	0.179	2
DNAJC18	VT broad	27,947	4.1E-03	0.496	0.147	4
DNAJC18	VT strict	24,571	3.2E-05	0.774	0.179	2

Table S9. Association of the leptin-decreasing Met94 allele of *LEP* Val94Met (rs1715919) with BMI z-score in African-ancestry children from the CHOP cohort.

Age Bin	N	Allele freq.	Beta	SE	*P*
2	2726	0.089	0.079	0.055	0.153
3	2570	0.089	0.123	0.056	0.029
4	2572	0.093	0.160	0.054	0.003
5	2381	0.089	0.154	0.060	0.010
6	2030	0.091	0.204	0.066	0.002
7	1769	0.092	0.143	0.070	0.041
8	1583	0.092	0.029	0.074	0.694
9	1476	0.099	0.017	0.078	0.824
10	1446	0.095	0.017	0.080	0.832
11	1500	0.095	-0.004	0.079	0.964
12	1455	0.096	-0.036	0.075	0.631
13	1460	0.101	-0.007	0.075	0.928
14	1417	0.104	0.004	0.074	0.959
15	1355	0.099	0.048	0.077	0.537
16	1287	0.093	-0.006	0.081	0.937
17	1098	0.102	0.055	0.087	0.527
18	451	0.085	-0.009	0.135	0.946

Table S10. Association of the leptin-decreasing C allele of rs10487505 near *LEP* with BMI z-score in a meta-analysis of African-ancestry and European ancestry children from the CHOP cohort.

Age Bin	N	Allele freq.	Beta	SE	*P*
2	3681	0.462	0.033	0.026	0.203
3	3618	0.467	0.026	0.026	0.334
4	3681	0.469	0.058	0.026	0.027
5	3557	0.471	-0.002	0.027	0.929
6	3166	0.473	-0.044	0.029	0.132
7	2869	0.469	-0.006	0.031	0.835
8	2711	0.465	-0.021	0.032	0.504
9	2571	0.465	-0.035	0.033	0.290
10	2608	0.468	-0.033	0.033	0.317
11	2705	0.462	-0.028	0.032	0.380
12	2685	0.454	-0.021	0.032	0.502
13	2697	0.459	0.004	0.031	0.898
14	2679	0.454	-0.027	0.032	0.389
15	2604	0.451	-0.009	0.031	0.777
16	2463	0.458	0.012	0.033	0.719
17	2130	0.465	-0.004	0.036	0.917
18	663	0.456	-0.003	0.062	0.959

Table S11. Predicted change in leptin protein stability upon the Val94Met change (Val73Met in the mature leptin protein) in the amino acid sequence

Tool	Protein (PDB-ID)	WT/MT	Chain	Overall stability	Predicted ΔΔG
CUPSAT	LEP (1AX8)	VAL/MET	A	Decreased	-0.22
I-Mutant v2.0	LEP (1AX8)	VAL/MET	A	Decreased	--
SDM	LEP (1AX8)	VAL/MET	A	Decreased	-0.72

Table S12. Colocalization of METSIM subcutaneous adipose tissue eQTLs at GWAS loci for leptin

SNP	Chr	Position	MAF	Probeset	Allele 1 / EA	Allele 2	eQTL gene	GWAS variant association with expression level				Lead eSNP association with expression level						LD r^2
								Beta initial	P initial	Beta conditional	P conditional	Lead eSNP	Allele 1/ Allele 2	Beta initial	P initial	Beta conditional	P conditional	
rs62621812	7	127,015,083	0.06	11736419_a_at	G	A	*ZNF800*	-0.871	2.40E-16	0.000	3.18E-01	rs62621812	A/G	0.871	2.40E-16	0.000	3.2E-01	1.00
rs972283	7	130,466,854	0.45	11737563_at	A	G	*KLF14*	0.233	4.14E-06	-0.322	4.46E-01	rs6467315	G/C	-0.238	2.26E-06	-0.552	1.9E-01	0.98
rs1260326	2	27,730,940	0.36	11729870_x_at	C	T	*EMILIN1*	-0.230	9.22E-06	0.166	5.23E-01	rs780094	C/T	-0.240	3.33E-06	-0.407	1.1E-01	0.96
rs900399	3	156,798,732	0.32	11717399_a_at	A	G	*TIPARP*	-0.905	2.99E-72	-0.213	1.57E-01	rs13322435	G/A	0.922	9.57E-77	0.715	2.0E-06	0.91

LD r2 calculated using 770 METSIM samples (Finnish males) included in eQTL data

A1 (column E) is the leptin raising allele from the Exome Chip analysis. A1 is also the effect allele for the effect sizes listed in columns H and J. Allele 1 in column O is the effect allele for the effect in columns O/Q.

FDR<1% ($P < 2.37 \times 10^{-4}$)

Table S13. PASCAL gene set enrichment analysis results for leptin unadjusted for BMI using coding variants only.

(A) Leptin not adjusted for BMI, European, additive model, sex-combined analysis. Coding variants included. SUM method used (Bonferroni correction for 1000 gene sets and 2 traits: P<2.5E-05 for both chi2Pvalue and empPvalue)

Name	chi2Pvalue	empPvalue	Annotation
GO:2000243	1.30E-04	8.80E-05	positive regulation of reproductive process
MP:0005501	3.40E-04	0.000284	abnormal skin physiology
ENSG00000204713	4.31E-04	0.000389	TRIM27 PPI subnetwork
ENSG00000112448	4.31E-04	0.000397	ENSG00000112448 PPI subnetwork
ENSG00000215641	4.31E-04	0.000404	TRIM27 PPI subnetwork
GO:0032769	4.85E-04	0.000335	negative regulation of monooxygenase activity
MP:0002769	5.05E-04	0.000492	abnormal vas deferens morphology
ENSG00000008853	8.38E-04	0.000432	RHOBTB2 PPI subnetwork
ENSG00000081019	9.58E-04	0.00058	RSBN1 PPI subnetwork
GO:0072527	1.37E-03	9.70E-04	pyrimidine-containing compound metabolic process
ENSG00000143344	1.56E-03	0.00076	RGL1 PPI subnetwork

(B) Leptin not adjusted for BMI, European, additive model, sex-combined analysis. Coding variants included. MAX method used (Bonferroni correction for 1000 gene sets and 2 traits: P<2.5E-05 for both chi2Pvalue and empPvalue)

Name	chi2Pvalue	empPvalue	Annotation
GO:2000243	1.53E-05	1.59E-05	positive regulation of reproductive process
ENSG00000143344	4.85E-04	1.56E-04	RGL1 PPI subnetwork
ENSG00000215641	1.95E-04	1.69E-04	TRIM27 PPI subnetwork
ENSG00000204713	1.95E-04	1.94E-04	TRIM27 PPI subnetwork
ENSG00000112448	1.95E-04	1.96E-04	ENSG00000112448 PPI subnetwork
MP:0002769	2.23E-04	2.33E-04	abnormal vas deferens morphology
GO:0032769	2.57E-04	2.93E-04	negative regulation of monooxygenase activity
ENSG00000074211	3.57E-04	4.03E-04	PPP2R2C PPI subnetwork
ENSG00000008853	1.30E-03	4.40E-04	RHOBTB2 PPI subnetwork
ENSG00000169682	6.59E-04	5.50E-04	SPNS1 PPI subnetwork
ENSG00000081019	8.20E-04	5.60E-04	RSBN1 PPI subnetwork
GO:0004715	7.77E-04	5.70E-04	non-membrane spanning protein tyrosine kinase activity
GO:0010458	7.32E-04	6.00E-04	exit from mitosis
ENSG00000090054	2.10E-03	9.00E-04	SPTLC1 PPI subnetwork
ENSG00000113578	6.06E-04	9.50E-04	FGF1 PPI subnetwork
MP:0008347	1.09E-03	9.70E-04	decreased gamma-delta T cell number

Table S14. PASCAL gene set enrichment analysis for leptin adjusted for BMI using coding variants only.

(A) Leptin adjusted for BMI, European, additive model, sex-combined analysis. Coding variants included. SUM method used (Bonferroni correction for 1000 gene sets and 2 traits: P<2.5E-05 for both chi2Pvalue and empPvalue)			
Name	**chi2Pvalue**	**empPvalue**	**Pathway/Gene-set**
ENSG00000175575	3.69E-05	7.90E-06	TRIM39PPI subnetwork
ENSG00000204599	3.69E-05	7.90E-06	PAAF1 PPI subnetwork
ENSG00000206495	3.69E-05	8.80E-06	ENSG00000206419 PPI subnetwork
ENSG00000206419	3.69E-05	1.03E-05	ENSG00000105972 PPI subnetwork
ENSG00000105972	7.14E-05	1.13E-05	mitochondrial large ribosomal subunit
GO:0005762	1.62E-04	2.66E-05	organellar large ribosomal subunit
GO:0000315	1.62E-04	2.76E-05	KLF1 PPI subnetwork
ENSG00000105610	1.96E-04	2.78E-05	negative regulation of monooxygenase activity
GO:0032769	1.58E-04	5.20E-05	BCL10 PPI subnetwork
ENSG00000142867	6.84E-05	6.10E-05	CHD2 PPI subnetwork
ENSG00000173575	1.20E-04	6.20E-05	UBE3B PPI subnetwork
ENSG00000151148	6.91E-05	6.70E-05	abnormal skin physiology
MP:0005501	8.97E-05	6.70E-05	CCDC33 PPI subnetwork
ENSG00000140481	3.61E-04	9.70E-05	abnormal cell migration
ENSG00000198925	1.53E-04	2.13E-04	HSPA12A PPI subnetwork
MP:0003091	2.13E-04	1.59E-04	SV2A PPI subnetwork
ENSG00000159164	5.38E-04	1.61E-04	MTHFD1L PPI subnetwork
ENSG00000120254	4.09E-04	1.75E-04	REACTOME_REGULATION_OF_ACTIVATED_PAK:2P34_BY_PROTEASOME_MEDIATED_DEGRADATION
REACTOME_REGULATION_OF_ACTIVATED_PAK:2P34_BY_PROTEASOME_MEDIATED_DEGRADATION	3.32E-04	1.79E-04	ATG9A PPI subnetwork
ENSG00000165868	7.61E-04	2.16E-04	ENO2 PPI subnetwork
ENSG00000178363	2.21E-04	4.50E-04	EEF1A2 PPI subnetwork
ENSG00000111674	8.97E-04	2.31E-04	RHOBTB2 PPI subnetwork
ENSG00000008853	6.23E-04	2.49E-04	exit from mitosis
GO:0010458	5.32E-04	2.67E-04	ZNF462 PPI subnetwork
ENSG00000148143	8.18E-04	2.78E-04	TOP2B PPI subnetwork
ENSG00000077097	5.67E-04	2.82E-04	RSBN1 PPI subnetwork
ENSG00000081019	6.96E-04	3.13E-04	HLA-G PPI subnetwork
ENSG00000204632	1.21E-03	3.16E-04	ENSG00000206443 PPI subnetwork
ENSG00000206443	1.21E-03	3.21E-04	HLA-G PPI subnetwork
ENSG00000206506	1.21E-03	3.22E-04	acanthosis

MP:0001874	3.68E-04	3.41E-04	REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1APCC
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1APCC	5.20E-04	3.43E-04	SBF1 PPI subnetwork
ENSG00000100241	9.96E-04	3.67E-04	ENSG00000206413 PPI subnetwork
ENSG00000206413	1.46E-03	3.75E-04	NIPSNAP1 PPI subnetwork
ENSG00000184117	1.14E-03	3.78E-04	abnormal CD4-positive T cell differentiation
MP:0008076	7.62E-04	3.81E-04	HLA-E PPI subnetwork
ENSG00000206493	1.46E-03	3.85E-04	REACTOME_P53:INDEPENDENT_G1S_DNA_DAMAGE_CHECKPOINT
REACTOME_P53:INDEPENDENT_G1S_DNA_DAMAGE_CHECKPOINT	8.27E-04	4.13E-04	ZNF317 PPI subnetwork
ENSG00000130803	5.25E-04	4.35E-04	REACTOME_P53:INDEPENDENT_DNA_DAMAGE_RESPONSE
REACTOME_P53:INDEPENDENT_DNA_DAMAGE_RESPONSE	8.27E-04	4.40E-04	PDE1A PPI subnetwork
ENSG00000198838	4.45E-04	5.70E-04	TOMM34 PPI subnetwork
ENSG00000115252	1.34E-03	4.46E-04	FNBP1 PPI subnetwork
ENSG00000187239	1.01E-03	4.47E-04	CALML3 PPI subnetwork
ENSG00000101210	5.88E-04	4.50E-04	REACTOME_MYD88_DEPENDENT_CASCADE_INITIATED_ON_ENDOSOME
REACTOME_MYD88_DEPENDENT_CASCADE_INITIATED_ON_ENDOSOME	8.42E-04	4.55E-04	REACTOME_UBIQUITIN_MEDIATED_DEGRADATION_OF_PHOSPHORYLATED_CDC25A
REACTOME_UBIQUITIN_MEDIATED_DEGRADATION_OF_PHOSPHORYLATED_CDC25A	8.27E-04	4.62E-04	cellular defense response
GO:0006968	9.97E-04	4.76E-04	macrolide binding
GO:0005527	6.63E-04	4.91E-04	REACTOME_TOLL_LIKE_RECEPTOR_78_TLR78_CASCADE
REACTOME_TOLL_LIKE_RECEPTOR_78_TLR78_CASCADE	8.42E-04	5.10E-04	RPN1 PPI subnetwork
ENSG00000163902	1.62E-03	5.10E-04	FK506 binding
GO:0005528	6.63E-04	5.20E-04	ARID5B PPI subnetwork
ENSG00000150347	7.60E-04	5.20E-04	SEC31A PPI subnetwork
ENSG00000138674	1.23E-03	5.20E-04	SEPT3 PPI subnetwork
ENSG00000100167	1.37E-03	5.20E-04	ROGDI PPI subnetwork
ENSG00000067836	1.31E-03	5.30E-04	NAPB PPI subnetwork
MP:0004957	5.34E-04	8.00E-04	DCLK1 PPI subnetwork
ENSG00000125814	1.34E-03	5.50E-04	ADARB2 PPI subnetwork
ENSG00000185736	8.02E-04	5.60E-04	abnormal cardinal vein morphology
MP:0004783	1.81E-03	5.60E-04	RYR3 PPI subnetwork
ENSG00000025772	1.35E-03	5.80E-04	columnar/cuboidal epithelial cell differentiation
GO:0002065	1.43E-03	5.90E-04	RTN3 PPI subnetwork
ENSG00000133318	1.50E-03	6.00E-04	LIN7B PPI subnetwork
ENSG00000104863	1.80E-03	6.00E-04	AMOTL1 PPI subnetwork
ENSG00000166025	1.02E-03	6.10E-04	REACTOME_UBIQUITIN:DEPENDENT_DEGRADATION_OF_CYCLIN_D1
REACTOME_UBIQUITIN:DEPENDENT_DEGRADATION_OF_CYCLIN_D1	1.62E-03	6.10E-04	USP11 PPI subnetwork

ENSG00000102226	1.28E-03	6.20E-04	ATP2B1 PPI subnetwork
ENSG00000070961	9.08E-04	6.50E-04	ENSG00000186979 PPI subnetwork
ENSG00000186979	9.97E-04	6.70E-04	ZNF174 PPI subnetwork
ENSG00000103343	1.81E-03	6.90E-04	REACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC
REACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC	1.25E-03	7.10E-04	abnormal vascular development
MP:0000259	1.29E-03	7.10E-04	KIF21A PPI subnetwork
ENSG00000139116	1.80E-03	7.70E-04	REACTOME_CDK:MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6
REACTOME_CDK:MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6	1.59E-03	7.90E-04	testis tumor
MP:0006262	1.72E-03	7.90E-04	abnormal blastocyst morphology
ENSG00000133083	2.15E-03	8.40E-04	APPBP2 PPI subnetwork
ENSG00000062725	1.08E-03	8.60E-04	PDE1B PPI subnetwork
ENSG00000123360	1.95E-03	8.60E-04	abnormal body weight
MP:0001259	1.22E-03	8.70E-04	REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU:RICH_ELEMENTS
REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU:RICH_ELEMENTS	1.13E-03	8.90E-04	REACTOME_APCCCDC20_MEDIATED_DEGRADATION_OF_SECURIN
REACTOME_APCCCDC20_MEDIATED_DEGRADATION_OF_SECURIN	1.33E-03	8.90E-04	STXBP5 PPI subnetwork
ENSG00000164506	1.05E-03	9.10E-04	embryonic digestive tract morphogenesis
GO:0048557	1.86E-03	9.10E-04	REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0
REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0	1.94E-03	9.10E-04	HLA-F PPI subnetwork
ENSG00000204642	2.31E-03	9.50E-04	REACTOME_ACTIVATED_TLR4_SIGNALLING
REACTOME_ACTIVATED_TLR4_SIGNALLING	1.92E-03	9.60E-04	REACTOME_UBIQUITIN:DEPENDENT_DEGRADATION_OF_CYCLIN_D
REACTOME_UBIQUITIN:DEPENDENT_DEGRADATION_OF_CYCLIN_D	1.62E-03	9.90E-04	REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1ALPHA
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1ALPHA	2.20E-03	9.90E-04	CAMK1 PPI subnetwork
ENSG00000134072	3.14E-03	9.90E-04	COPE PPI subnetwork
(B) Leptin adjusted for BMI, European, additive model, sex-combined analysis. Coding variants included. MAX method used (Bonferroni correction for 1000 gene sets and 2 traits: P<2.5E-05 for both chi2Pvalue and empPvalue)			
Name	**chi2Pvalue**	**empPvalue**	**Pathway/Gene-set**
GO:0032769	6.37E-05	1.93E-05	negative regulation of monooxygenase activity
MP:0005501	3.04E-05	4.62E-05	abnormal skin physiology
ENSG00000206495	1.68E-04	4.71E-05	TRIM39 PPI subnetwork
ENSG00000204599	1.68E-04	5.40E-05	TRIM39 PPI subnetwork
ENSG00000206419	1.68E-04	6.00E-05	ENSG00000206419 PPI subnetwork
ENSG00000198925	7.30E-05	9.60E-05	ATG9A PPI subnetwork
ENSG00000105972	3.12E-04	1.70E-04	ENSG00000105972 PPI subnetwork
MP:0003091	3.86E-04	1.87E-04	abnormal cell migration
ENSG00000142867	8.77E-04	3.36E-04	BCL10 PPI subnetwork

GO:0033273	3.51E-04	5.01E-04	response to vitamin
ENSG00000163902	1.19E-03	4.91E-04	RPN1 PPI subnetwork
ENSG00000138674	1.06E-03	5.09E-04	SEC31A PPI subnetwork
GO:0002065	1.32E-03	6.10E-04	columnar/cuboidal epithelial cell differentiation
ENSG00000120254	9.48E-04	6.40E-04	MTHFD1L PPI subnetwork
REACTOME_TOLL_LIKE_RECEPTOR_78_TLR78_CASCADE	1.39E-03	6.40E-04	REACTOME_TOLL_LIKE_RECEPTOR_78_TLR78_CASCADE
REACTOME_MYD88_DEPENDENT_CASCADE_INITIATED_ON_ENDOSOME	1.39E-03	6.40E-04	REACTOME_MYD88_DEPENDENT_CASCADE_INITIATED_ON_ENDOSOME
ENSG00000165699	2.08E-03	7.60E-04	TSC1 PPI subnetwork
ENSG00000164506	1.09E-03	7.80E-04	STXBP5 PPI subnetwork
ENSG00000185825	1.38E-03	9.00E-04	BCAP31 PPI subnetwork
GO:0071299	2.22E-03	9.80E-04	cellular response to vitamin A

SUPPLEMENTARY INFORMATION

Yaghootkar H, Zhang Y, Spracklen CN, Karaderi T, Huang LO, Bradfield J, et al.

Genetic studies of leptin concentrations implicate leptin in the regulation of early adiposity

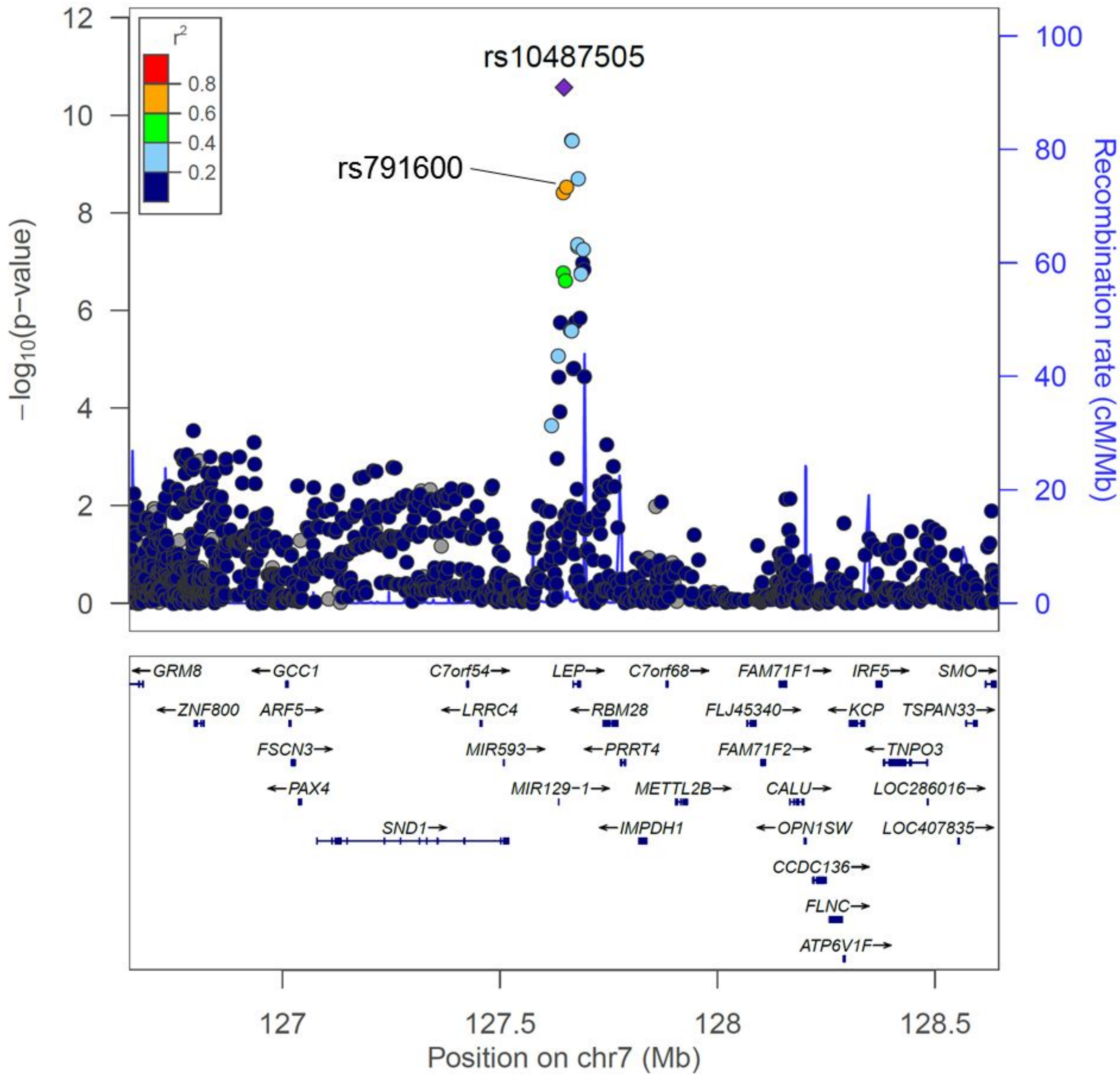


Figure S1. Association of rs10487505 and rs791600 variants near *LEP* with leptin concentrations adjusted for BMI in a genome-wide association study of up to 32,161 individuals of European ancestry (Kilpeläinen et al., 2016).

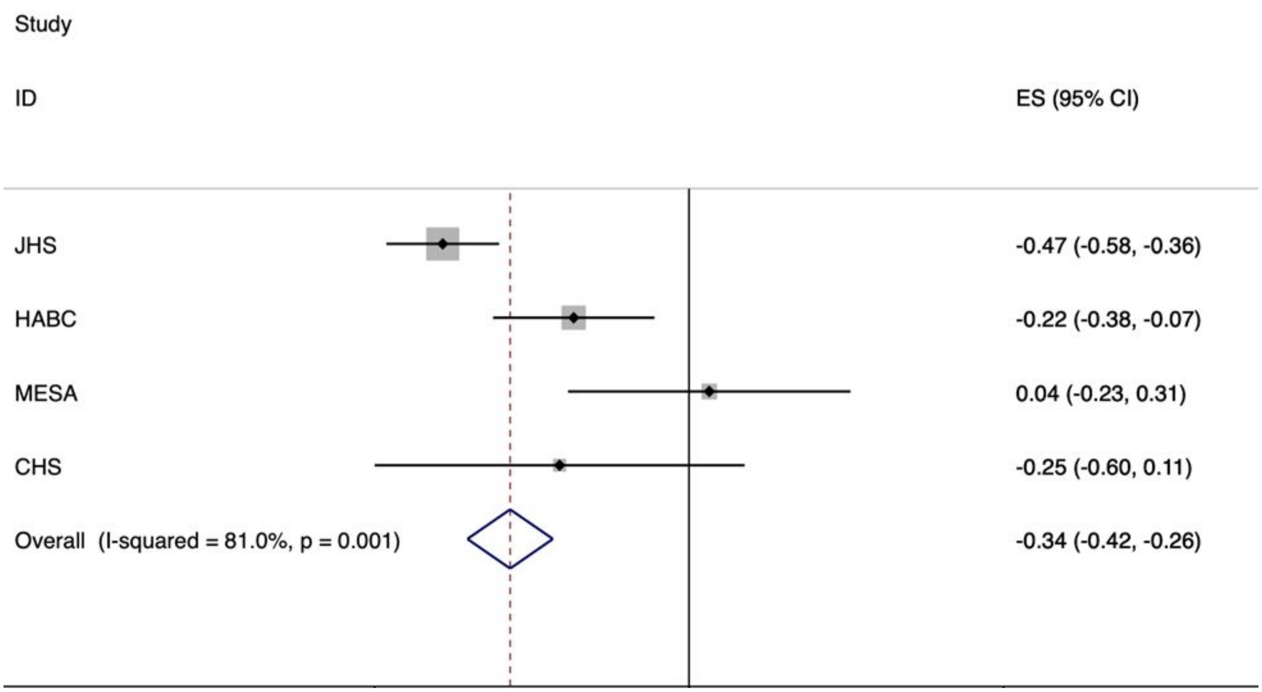


Figure S2. Meta-analysis of the association of the Met94 allele of rs17151919 with leptin concentrations adjusted for BMI in cohorts of African ancestry.

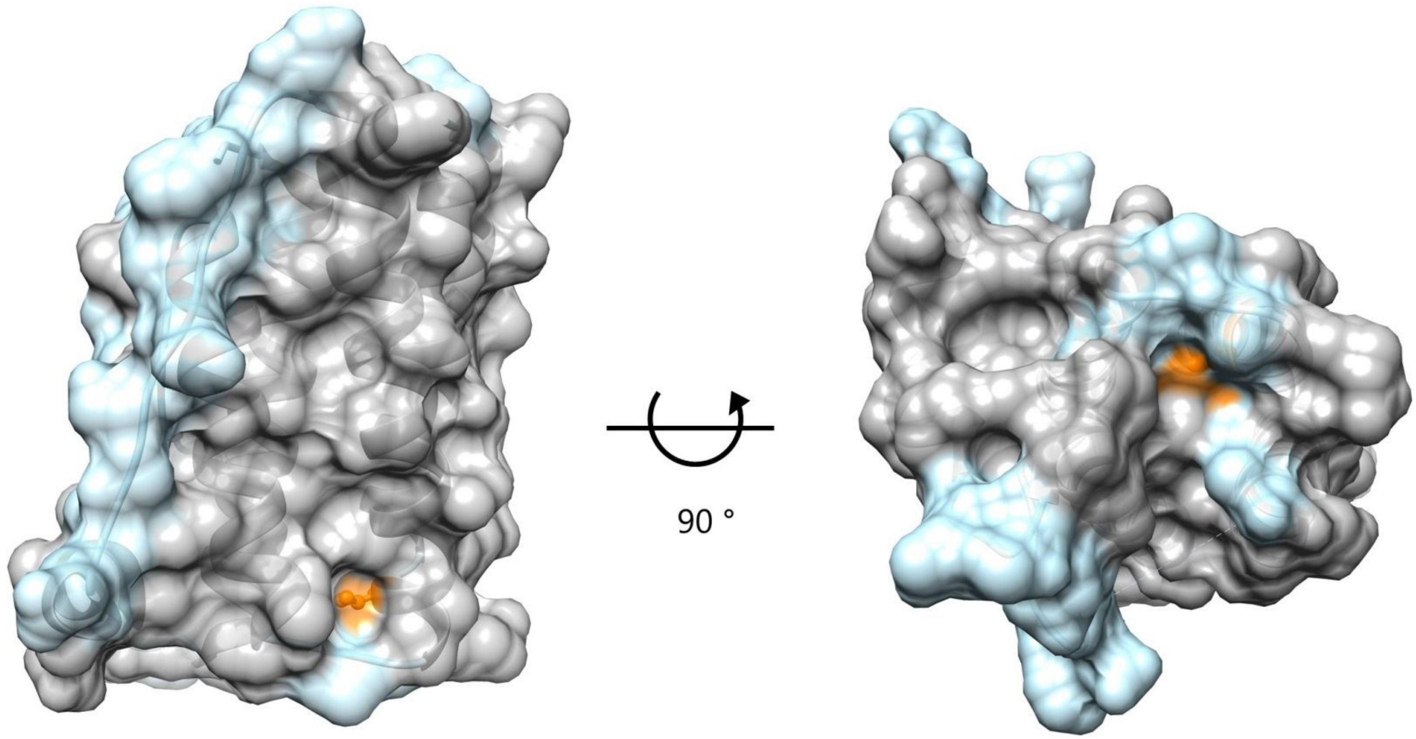
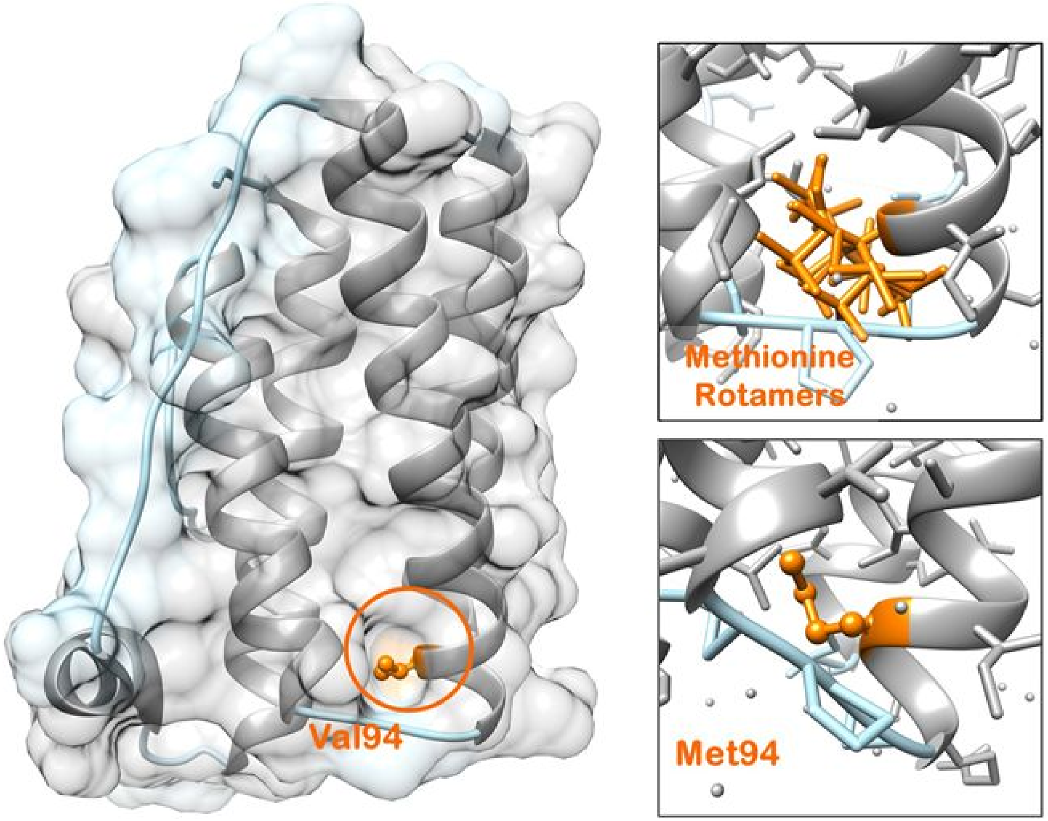
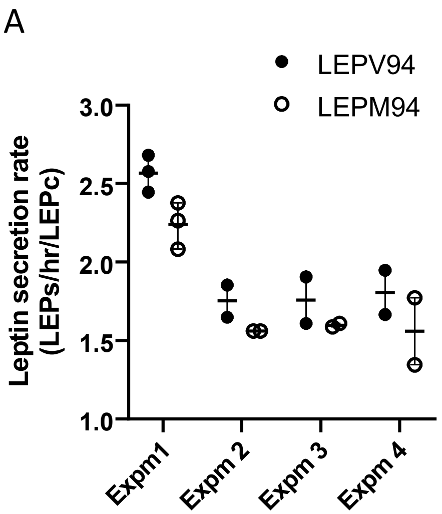


Figure S3: Surface region of the leptin protein with the Val94Met position (Val73Met in the mature protein) highlighted.

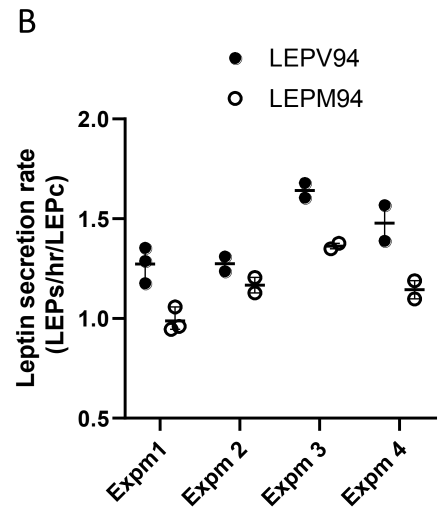


Chi 1	Chi 2	Probability	Clashes
-68.8	-59.1	0.2023	4
-68.3	175.6	0.1882	5
-70.1	-171.9	0.1331	3
-174.5	60.7	0.0947	5
-68.7	178.8	0.0923	2
-65.7	-60.3	0.0562	5
-179.2	-179.3	0.0534	9
-173.6	176.5	0.0512	5
-66.7	-63.1	0.0412	5
-170.7	65.2	0.0223	8
-175.7	176.7	0.0218	11
179.8	-84.1	0.0182	5
-169.7	67.0	0.0086	6
69.3	-176.2	0.0042	14

Figure S4: Leptin structure and the predicted impact of mutagenesis in position 73 from valine to methionine. The Rotamer list on the left shows sidechain torsions (Chi 1 and 2), with the probability and number of interatomic clashes, i.e. unfavourable interactions where atoms are too close together. On the right, the lower picture shows all possibilities for sidechain torsions when methionine is substituted with valine, whereas the upper picture displays the substitution with the highest probability (marked with red square in the Rotamer list).



LEPV94	2.04±0.42
LEPM94	1.80±0.36
F (1,8)	30.96
p	0.0005



LEPV94	1.40±0.18
LEPM94	1.15±0.15
F (1,8)	43.54
p	0.0002

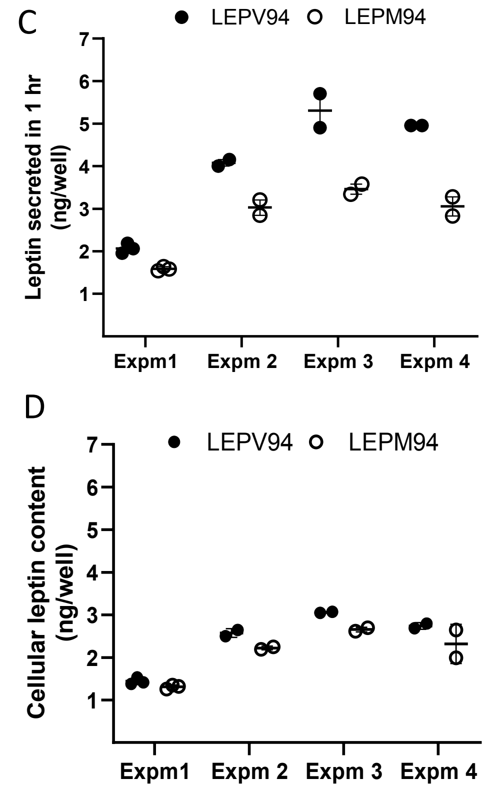


Fig S5. Impact of Val94Met transversion at *LEP* rs17151919 on leptin secretion rate in HEK293 cells in different conditions. A) Leptin secretion rates for Val94 and Met94 during a 24-hr incubation period (48-72 hr post-transfaction), expressed as the amount of leptin secreted in ng per hour over 24 hrs (LEPs/hr) normalized by the respective cellular leptin content (LEPc, ng) at the end of incubation. B) Leptin secretion rates for Val94 and Met94 during a 1-hour incubation (72-73 hr post-transfection) in the presence of cycloheximide (CHX, 20 µg/ml) expressed as the amount of leptin secreted in ng during the 1-hour incubation (LEPs/hr), normalized by the respective cellular leptin content (LEPc, ng). Individual data points from four separate experiments (each with 2-3 technical replicates) are plotted. All data passed D'Agostino & Pearson normality test and repeated measures one-way ANOVA was performed to assess the difference in secretion rate between the genotypes. Mean ± SD and AVOVA results (F and p values) are reported in the table below each graph. C-D. The amounts of leptin secreted (LEPs) during a 1 hr incubation (72-73 hr post-transfection) in untreated control cells (C), and the corresponding cellular leptin content (LEPc) at the end of the incubation (D). Leptin secretion rates shown in Fig 2B were ratios of the amounts of leptin secreted (LEPs) over the corresponding cellular leptin contents (LEPc) shown here.

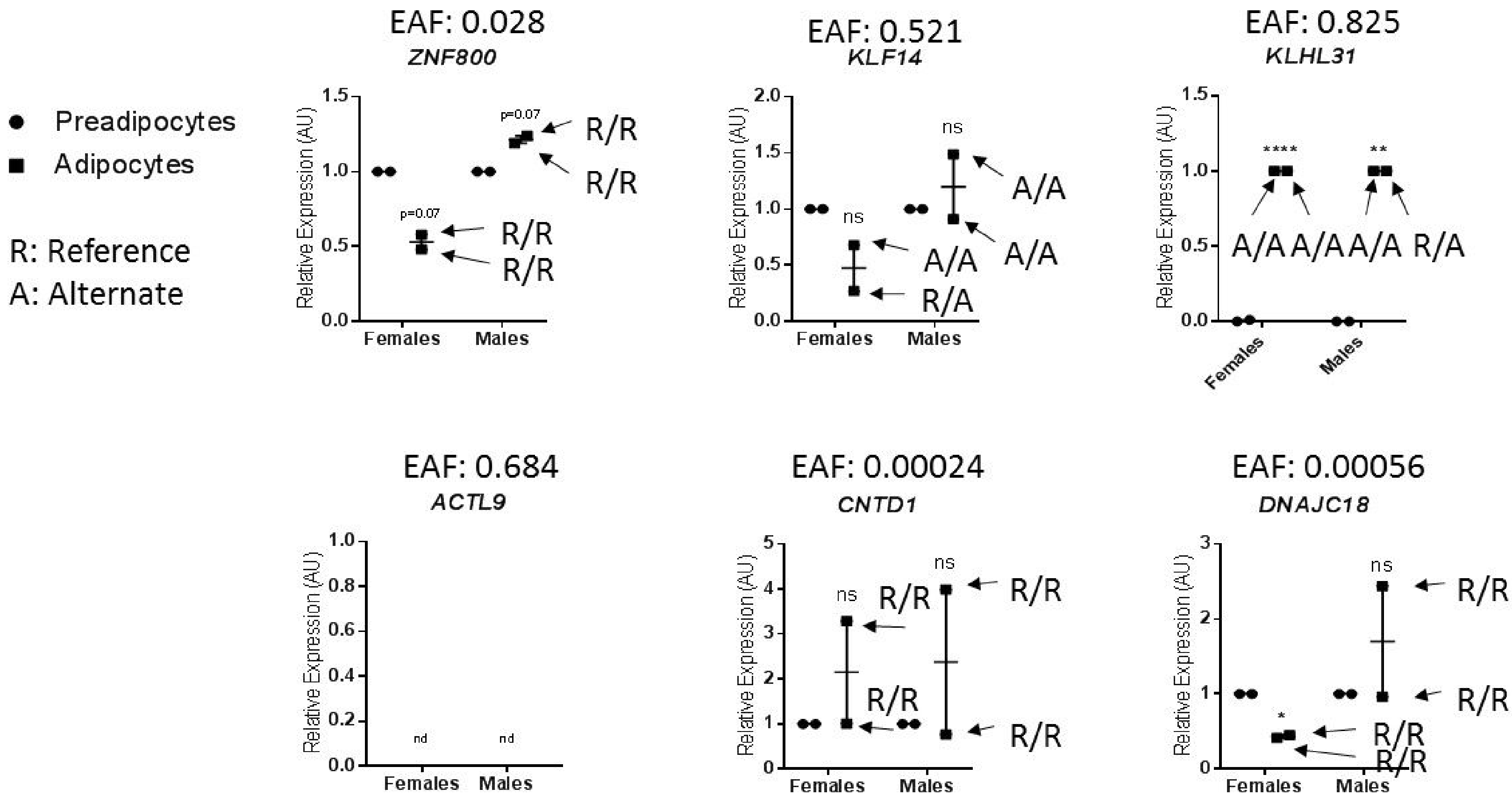


Figure S6. Expression of leptin modifiers in human preadipocytes and mature adipocytes. De-identified human subcutaneous adipose stromal cells were generously provided by the Boston NORC and were cultured and differentiated as previously described (Lee and Fried, 2014). Preadipocytes and *in vitro*-differentiated adipocytes from two females and two males were studied. Lipid-laden cells were assayed between 10-14 days after initial treatment with differentiation factors. Transcript levels were determined by RT-qPCR, normalized to the geometric mean of *RPLP0* and *PPIA*, and expressed relative to levels in preadipocytes. Two-way repeated measures ANOVA with post-hoc Sidak's multiple comparison tests were performed *: $p<0.05$, **: $p<0.01$, ****: $p<0.0001$, ns (no statistical difference) are indicated, comparing the transcript levels between preadipocytes and mature adipocytes. There was an interaction between sex and differentiation stage for ZNF800 ($p<0.01$). No *ACTL9* transcript was detected (nd: none detected). Genotypes of the individuals were marked as R-reference allele and A-alternative allele.

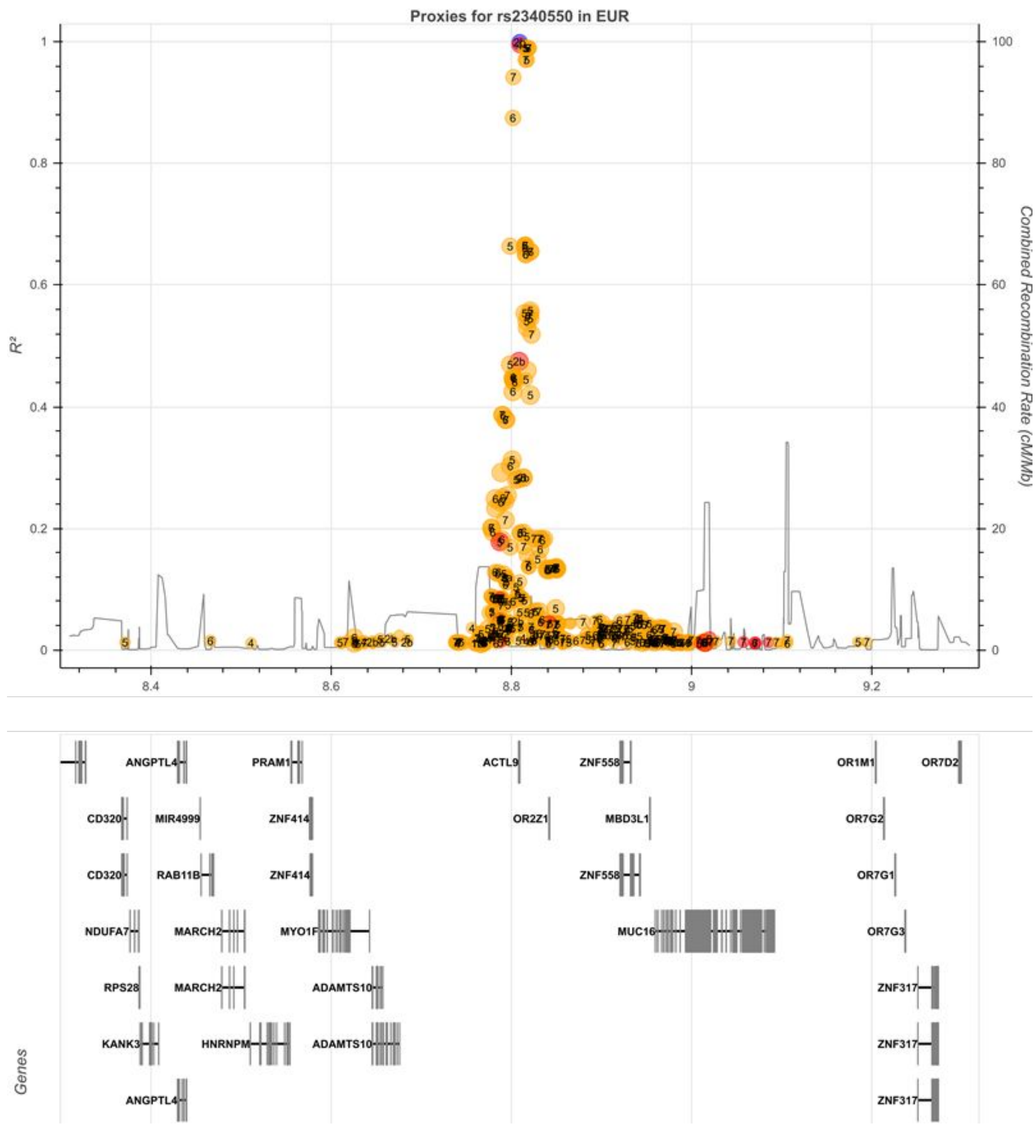


Figure S7. Linkage disequilibrium between the Ser37Phe (rs2340550) variant in *ACTL9* and variants within ±500 kb in the 1000 Genomes European ancestry reference panel. The numbering refers to Regulome DB score of the variants (www.regulomedb.org). Non-coding variants are marked in orange color and coding variants in red. The plot was produced using LDlink (https://ldlink.nci.nih.gov).

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The **Framingham Heart Study (FHS)** was initiated in 1948 and is comprised of 5,209 participants from Framingham, MA (US), who have undergone examinations every other year to evaluate cardiovascular disease and related risk factors. The Offspring cohort was recruited in 1971 and includes 5,124 children of the Original cohort and the children’s spouses. Participants from the Offspring cohort have attended exams roughly every four years. The current analysis includes 2,223 individuals with available phenotypic and genotypic information.

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