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Genome-wide association study of anthropometric traits and evidence of interactions with age and study year in Filipino women

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Abstract

Increased values of multiple adiposity-related anthropometric traits are important risk factors for many common complex diseases. We performed a genome-wide association (GWA) study for four quantitative traits related to body size and adiposity (body mass index [BMI], weight, waist circumference, and height) in a cohort of 1,792 adult Filipino women from the Cebu Longitudinal Health and Nutrition Survey. This is the first GWA study of anthropometric traits in Filipinos, a population experiencing a rapid transition into a more obesogenic environment. In addition to identifying suggestive evidence of additional SNP association signals ($P < 10^{-5}$), we replicated ($P < 0.05$, same direction of additive effect) associations previously reported in European populations of both BMI and weight with *MC4R* and *FTO*, of BMI with *BDNF*, and of height with *EFEMP1*, *ZBTB38*, and *NPPC*, but none with waist circumference. We also replicated loci reported in Japanese or Korean populations as associated with BMI (*OTOL1*) and height (*HIST1H1PS2*, *C14orf145*, *GPC5*). A difference in local linkage disequilibrium between European and Asian populations suggests a narrowed association region for *BDNF*, while still including a proposed functional non-synonymous amino acid substitution variant (rs6265, Val66Met). Finally, we observed significant evidence ($P < 0.0042$) for age-by-genotype interactions influencing BMI for rs17782313 (*MC4R*) and rs9939609 (*FTO*), and for a study year-by-genotype interaction for rs4923461 (*BDNF*). Our results show that several genetic risk factors are associated with anthropometric traits in Filipinos and provide further insight into the effects of *BDNF*, *FTO*, and *MC4R* on BMI.

Introduction

Increased values of adiposity-related traits are important risk factors for many common complex diseases. Understanding the basis for increased human body size may lead to

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insights into disease etiologies. Higher body mass index (BMI), weight, and waist circumference are associated with type 2 diabetes, cardiovascular disease (CVD), hypertension, and cancer (1,2). Increased height, however, is inversely associated with CVDs (3).

Genome-wide association (GWA) studies have been used to identify multiple loci associated with variation in anthropometric measures (4–22), often with more than one correlated trait. For signals that are replicated across world populations, differences in population history provide potential to further localize the associated regions. Some signals may be population-specific due to differing allele frequencies and environmental contexts.

Over the last few decades, the adoption in Asian populations of Western-style diets of increased fats and carbohydrates and of more sedentary habits has led to a marked increase in obesity (23,24). In particular, a cohort of women from the ongoing Cebu Longitudinal Health and Nutrition Survey (CLHNS) based in the Philippines showed a six-fold increase in prevalence of overweight and obesity associated with nearly two decades of substantial and continuing socio-economic modernization (also illustrated by a increase in mean weight of 6.8 ± 7.1 kg) (24). The portion of increased prevalence due to the changes in environment versus increased age of these women is unclear.

We performed a GWA study to test for main effect SNP associations with measures of BMI, weight, waist circumference, and height in 1,792 adult Filipino women from the CLHNS. The longitudinal nature of this cohort also allowed us to examine the interactive effect of age and genotype on BMI over a 22-year period from 1983 to 2005.

Methods and Procedures

We initially evaluated 1,895 female participants from the ongoing CLHNS, mothers of a 1983 to 1984 birth cohort (25). Trained field staff conducted in-home interviews and collected anthropometrics, blood samples, and comprehensive environmental data (publicly available at www.cpc.unc.edu/projects/cebu/). Informed consent was obtained from all CLHNS subjects, and the University of North Carolina Institutional Review Board for the Protection of Human Subjects approved the study protocol.

Most outcome and covariate measures reported in this current study were taken from the 2005 survey, except height, which is the average of the measurement during the pregnancy of the birth cohort in 1983–1984 and the first post-partum measurement. BMI, weight, and waist circumference were highly correlated with each other (Pearson $r > 0.88$), but not with average height ($r < 0.44$) (Supplementary Table S1). For the longitudinal analyses, we used BMI from seven different time points in the study. The first BMI value was measured four months after birth of the index child; if data were missing, the measurement at six months or two months post-partum was substituted. Six additional measurements were taken at 1, 8, 11, 15, 19, and 22 years after the baseline survey (the final measurement is from the 2005 survey). Observations were excluded from the analysis if women were pregnant at the time of the survey. The phenotypes weight, waist circumference, and height were approximately normally distributed, and BMI values were natural log-transformed to satisfy model assumptions.

Our full methods for direct SNP genotyping and quality control (QC) as well as SNP imputation have been described previously (26). Briefly, genotyping was performed with the Affymetrix Genome-Wide Human SNP Array 5.0. Samples that failed DNA fragmentation, failed an array QC check (DM algorithm), or had a genotype call rate < 97% were excluded. We also removed one member from any likely first-degree relative pair as determined from identity-by-descent and identity-by-state estimates. We discarded individual SNPs due to

poor mapping, call rates < 90%, deviation from Hardy-Weinberg equilibrium ($P < 10^{-6}$), and/or ≥ 3 discrepancies among 40 duplicate pairs. Five HapMap CEPH trios were also genotyped for QC purposes, and we dropped any SNPs that showed ≥ 3 Mendelian inheritance errors or genotype discrepancies with known HapMap genotypes. We then used CLHNS genotypes of 352,264 SNPs and pooled reference haplotypes of 60 CEU and 90 combined CHB+JPT HapMap samples to impute the genotypes of an additional 1,878,188 SNPs in MACH (27). Imputed values were substituted for all 352,264 directly genotyped SNPs, including any missing genotypes. We then discarded SNPs with low-quality imputations (Rsq ≤ 0.3) and estimated minor allele frequencies ≤ 0.01. In total, 2,073,674 SNPs were tested for association with the four quantitative anthropometric traits in 1,792 non-pregnant CLHNS women with complete trait outcome and covariate data.

To evaluate population substructure among our CLHNS subjects, we constructed principal components (PCs) using the software EIGENSOFT (28,29). We tested each of the first 10 PCs for association with each of the four anthropometric outcomes (Supplementary Table S2). We included all PCs for which association with any trait was significant at $P < 0.05$, hence five PCs were used as covariates in the final SNP association model for all traits.

Array Studio version 3.1 was used to perform the GWA statistical analyses (Omicsoft Corporation, Research Triangle Park, NC, USA). Assuming an additive mode of inheritance, multivariable linear regression models were used to test for an association between the phenotypes and each imputed SNP genotype, with covariate adjustment for the first five PCs, age, age^2, total assets, natural log-transformed income, number of pregnancies (categorized into three groups: 0–4, 5–10, ≥11), and menopausal status. Each of these predictors was significantly associated ($P < 0.05$) with at least one anthropometric trait in our samples (Supplementary Table S2). Quanto version 1.2.3 was used for statistical power calculations (available on-line at hydra.usc.edu/gxe/).

For loci previously reported in a GWA study at $P < 5 \times 10^{-8}$ in at least 1,000 samples, we chose a single representative SNP. If this SNP was not present in our dataset, we substituted a proxy SNP in high LD ($r^2 > 0.8$ in both CEU and CHB+JPT, HapMap Release 22) when possible. For one study of an Asian population cohort, we also evaluated additional loci reported with less significant evidence of association ($P < 10^{-4}$). Conditional analyses to search for independent secondary signals were performed for all SNPs within a 2 Mb region centered on the SNP with the strongest primary signal, including the primary signal SNP as an additional covariate in the linear regression.

To detect differences in local LD structure we identified the genomic positions of the SNPs bounding a 1 LD-map unit window centered on the most strongly associated SNP in a locus, using previously constructed LD maps made from the individual CEU, CHB, and JPT HapMap populations (30).

Selected SNPs were tested for additive genotype effects on BMI in longitudinal models using SAS version 9.3 (SAS Institute, Cary, NC). General linear mixed models were adjusted for the following time-varying covariates: age, actual time in years since baseline study visit, assets, income, urbanicity index (31), menopause status, months since the previous visit spent lactating or pregnant, current lactation status and activity level. Women who were pregnant at the time of measurement were excluded from that particular time point, but included for all other visits at which they were not pregnant. Examination of the model residuals indicated that natural log-transformation was not appropriate in this longitudinal setting, and we therefore analyzed untransformed BMI.

Results

We tested 2,073,674 SNPs for association with BMI, weight, waist circumference, and height in the CLHNS cohort (Table 1, Supplementary Figure S1). No evidence of residual population stratification or cryptic relatedness between samples was observed based on genomic control values (λ_{GC} = 1.00–1.03) and quantile-quantile plots (Supplementary Figure S2). The most significant main effect associations ($P < 10^{-5}$) had not been previously reported (Table 2). The SNP most strongly associated with BMI was rs17124318 ($P = 5.91 \times 10^{-7}$), located downstream of *ATG4C*. For weight, the most strongly associated SNP was rs16877106 ($P = 1.44 \times 10^{-6}$), located in an intron of *ANAPC4*. The SNP most strongly associated with waist circumference was rs1440072 ($P = 7.87 \times 10^{-7}$), an intergenic SNP located downstream of *KCNE4* and in perfect LD ($r^2 = 1$ [CEU, CHB+JPT]) with a SNP in the 3'-UTR of the gene (rs3795884, $P = 1.65 \times 10^{-6}$). Our strongest height association signal (rs17818399, $P = 2.74 \times 10^{-7}$) spans the *PIGF* and *CRIPT* genes. Our study had 80% power to detect novel SNP associations that explain > 2.2% of the variation in trait outcome at $P < 5 \times 10^{-8}$.

Eight SNP-trait associations previously reported for BMI, weight, waist circumference, and height were replicated ($P < 0.05$, effect in the same direction) in the CLHNS (Table 3, Table 4). Evidence of association with BMI was observed at the *BDNF* (rs4923461, $P = 0.00028$), *MC4R* (rs17782313, $P = 0.0073$), and *FTO* loci (rs9939609, $P = 0.0074$). Evidence from conditional analyses was consistent with a single BMI association signal at *MC4R*. For weight, we replicated previously reported associations at *FTO* (rs3751812, $P = 0.019$) and *MC4R* (rs12970134, $P = 0.041$). The CLHNS weight association for *KCTD15* (rs29941, $P = 0.034$) was in the opposite direction as previously reported, and did not meet our criteria for replication. For height, we replicated three previously reported associations with *EFEMP1* (rs3791679, $P = 0.0017$), *ZBTB38* (rs6440003, $P = 0.0048$), and *NPPC* (rs6718438, $P = 0.0096$). None of three previously reported associations with waist circumference replicated in the CLHNS. Together, these SNPs explain a small proportion of trait variation (R^2 = 1.9% [BMI], 1.1% [weight], 0.3% [waist circumference], and 2.9% [height]). Our study had 80% power to replicate ($P < 0.05$) SNPs that explained 0.44% of the total variation in anthropometric traits in 1,792 Filipino women after adjustment for covariates.

We additionally examined 20 SNPs reported in a Korean population cohort with suggestive evidence of association ($P < 10^{-4}$) with either BMI or height (12), and 10 SNPs reported in a Japanese cohort with suggestive evidence of association ($P < 10^{-5}$) with height (22). CLHNS data support evidence for association with BMI at the *OTOL1* locus (rs1399903, $P = 0.0097$) and three associations with height at the loci *GPC5* (rs8002779, $P = 0.016$), *HIST1H1PS2* (rs9393681, $P = 0.024$), and *C14orf145* (rs17110818, $P = 0.047$) (Supplementary Table S3).

Visually inspecting local HapMap LD for CEU and CHB+JPT at the eight replicated loci, we observed that an inter-population difference in LD appeared to narrow one of the association regions (Figure 1). Based on calculations from HapMap-based LD maps, the association signal at *BDNF* appears smaller in genomic size in Asian populations (115 kb [CHB], and 124 kb [JPT]) than in European ones (294 kb [CEU]). This association region contains a non-synonymous amino acid substitution SNP in *BDNF* (rs6265, Val66Met), which is in LD with the most associated CLHNS SNP rs4923461 (r^2 = 0.85 [CEU], 0.64 [CHB+JPT]).

To examine whether genetic effects changed over time due to age or increasingly obesogenic environmental conditions, SNPs at 12 loci previously reported as associated with BMI were further evaluated in longitudinal mixed models using data from seven visits

spanning 22 years. Of these 12 SNPs, three were nominally associated with BMI in CLHNS cross-sectional analysis (Table 3). We tested all 12 SNPs for genotype main effects and then for age-by-genotype and study year-by-genotype interactions, both individually and jointly. Due to confounding of age effects by study year (and vice-versa), when evaluating SNPs that showed genotype interactions involving either age or study year, we performed tests of genotype main effects and age-by-genotype interactions stratified by study year. The most significantly associated SNP from the longitudinal genotype main effect analysis was the *FTO* SNP rs9939609 ($P = 2.0 \times 10^{-5}$) (Supplementary Table S4). Three additional SNPs were nominally associated ($P < 0.05$) with BMI: rs4923461 (*BDNF*,$P = 0.0019$); rs17782313 (*MC4R*, $P = 0.0030$); and rs11084753 (*KCTD15*, $P = 0.027$). At all four loci, the directions of effects estimated from longitudinal models were consistent with the 2005 cross-sectional analysis. Only rs11084753 (*KCTD15*) had not shown at least nominal evidence for association in the cross-sectional analysis.

Four of the 12 SNPs showed statistically significant evidence ($P < 0.0042$, considering 12 tests) for an age-by-genotype interaction in the longitudinal analyses: rs4923461 (*BDNF*, $P = 2.4 \times 10^{-6}$); rs9939609 (*FTO*, $P = 3.8 \times 10^{-4}$); rs17782313 (*MC4R*, $P = 6.3 \times 10^{-4}$); and rs7498665 (*SH2B1*, $P = 8.2 \times 10^{-4}$) (Table 5). Three additional SNPs had nominally significant age-by-genotype interactions: rs6548238 (*TMEM18*, $P = 0.0053$), rs1093839 (*GNPDA2*, $P = 0.0086$), and rs2815752 (*NEGR1*, $P = 0.016$). Except for rs6548238 (*TMEM18*), the same pattern of significance occurred for all of these SNPs when tested for study year-by-genotype interactions in models without an age-by-genotype interaction term (Supplementary Table S5). In mixed models that included both age-by-genotype and study year-by-genotype interaction terms, only the age-by-genotype interaction for rs17782313 (*MC4R*) remained nominally significant ($P = 0.046$) (Supplementary Table S6).

Further analyses stratified by study year only clearly supported an age-by-genotype interaction for rs17782313 (*MC4R*) (Figure 2). Specifically, the age-by-genotype interaction coefficients for rs17782313 were consistent across study visits, resulting in slightly increasing main effect estimates of genotype over time in models absent the age-by-genotype interaction term. In contrast, age-by-genotype interaction coefficients for rs4923461 (*BDNF*) were not significantly different from zero at any single study visit, but the effect of genotype, in main effects analyses only, consistently increased over the study visits (Figure 3). The *FTO* SNP rs9939609 showed no evidence for an age-by-genotype interaction at the first four study visits, but some evidence at the latter three (Supplementary Figure S3), and the main effects of genotype increased over the first four study visits, but decreased thereafter. The patterns were less clear for the other loci that exhibited evidence for age-by-genotype and study year-by-genotype interactions (Supplementary Figure S4).

Discussion

We have performed the first GWA scan for anthropometric traits in a cohort from the Philippines, a country undergoing socio-economic and nutrition transition. The strongest signals with suggestive evidence of association in the CLHNS ($P < 10^{-5}$) require confirmation in other studies. Among these signals, a SNP in the *KCNE4* locus (rs1440072) was associated with both BMI and waist circumference. *KCNE4* codes for the potassium voltage-gated channel, Isk-related family, member 4 protein, which acts as an inhibitory subunit to *KCNQ1* (potassium voltage-gated channel, subfamily Q, member 1) (32). *KCNQ1* is expressed in adipose tissue and has been associated with type 2 diabetes in both European and Asian populations (33–35).

We replicated ($P < 0.05$ and consistent direction of effect) 8 of 55 non-independent previously reported SNP-trait associations ($P < 5 \times 10^{-8}$) with BMI, weight, waist

circumference, and height, providing further evidence that these loci influence anthropometric trait variation across world populations. We also replicated three signals with suggestive evidence of association ($P < 10^{-4}$) with either BMI or height from another Asian population cohort (12,22). The longitudinal main effect results for the 12 previously reported BMI SNPs were consistent with the primary cross-sectional (2005 visit) results in that the same SNPs displayed nominal evidence across both approaches. Failure to replicate additional loci likely reflects modest power of the CLHNS study to detect the signals, but could also indicate that the loci are population-specific or influenced by environmental or dietary exposures that differ between populations.

Inter-population differences in local LD, which are especially pronounced between continental populations (36), may assist in fine-mapping the disease-causing variants in loci implicated across association studies. In some cases, the overlap of LD between two populations may correspond to an area of increased association signal strength, thus suggesting a narrower region of interest than in the discovery population alone (18). We observed an appreciable difference in LD between the European and Asian HapMap populations at the *BDNF* locus. Consistent with previous observations that the LD from the CHB and JPT populations is similar to the CLHNS (37), we observed a putatively smaller *BDNF* association region that still contained a non-synonymous amino acid substitution (rs6265, Val66Met) associated with obesity (38). Further suggestive evidence of *BDNF*'s functional relevance to BMI includes observations that heterozygous and conditional knockout mice develop hyperphagia and obesity (39–41).

Our longitudinal study also supports age-by-genotype and/or study year-by-genotype interactions for three loci previously identified as associated with BMI. Evidence for an age-bygenotype interaction for the *MC4R* SNP rs17782313 was consistent across all study visits, with additive effects of the C allele that increased with age. The effects of genotype did not appear to change over time due to study year, which would reflect the rapidly changing environment in the Philippines during the study period. Conversely, our observations in stratified analyses did not support an age-by-genotype interaction for the widely studied *BDNF* SNP rs4923461, but we found evidence suggesting increased main effects of genotype (specifically the A allele) over the study time period. Our results suggest that rs4923461 genotype likely interacts with a factor other than age that also changed over the 22-year study period. Dietary and other environmental factors changed considerably between 1983 and 2005 and one or more of these factors may modify the effect of the rs4923461 genotype. Rates of overweight and obesity were initially low, and increased over time, providing substantially more variation in levels of body fat.

The BMI association for the *FTO* SNP rs9939609 over time is even more multifaceted, as we found evidence in the models stratified by study year supporting decreased effects of genotype with age at the later study visits, but no evidence for such an effect during the first 11 years of the study when our subjects were younger. The main effects, absent the age-bygenotype interaction term, of rs9939609 appeared to increase during the first 11 years of the study, suggesting a positive interaction between genotype and study year during this period, but then to steadily decrease to levels near baseline most recently, reflecting the decreased effects of genotype with respect to age in the latter years. Similar to our *BDNF* result, neither the age-bygenotype or study year-by-genotype interaction term was significant in the longitudinal model when both effects were included together. Our previous report of an overall survey year-bygenotype interaction at rs9939609 influencing longitudinal BMI appears to incompletely represent the genetic complexity at this locus (37).

Recently, Hardy *et al.* reported evidence for age-by-genotype interactions influencing BMI for the same two *MC4R* and *FTO* SNPs (42). They observed increasing effects of genotype for both loci through childhood and adolescence up to age 20, and then decreasing effects through adulthood. Because their participants were all the same age at any given year of the study, they could not distinguish between the effects of age and changing environment over time. The CLHNS involved participants with wide ranging ages at baseline (15–48 years) followed for 22 years. While our stratified analyses can begin to separate the nature of these putative interactions, these analyses do not completely remove the mutual confounding of age and study year in our models because the participants necessarily aged during the course of the study.

In summary, we found suggestive evidence for additional association signals in a Filipino population cohort, and replicated several previously reported SNP associations with variation in BMI, weight, and height. We also further characterized in a longitudinal setting the *MC4R*, *BDNF*, and *FTO* loci associated with BMI. Together, these results show that multiple genetic risk factors identified in other populations are also associated with anthropometric traits in Filipinos despite a transitioning nutritional environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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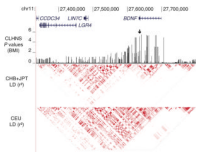

Figure 1. Further localization in the CLHNS of the BMI association signal at the *BDNF* locus

CLHNS association $-\log_{10}(P$ values) for BMI and nearby genes plotted against pair-wise HapMap Phase II linkage disequilibrium values from phased genotypes in the CEU and the CHB+JPT populations. Dark red indicates $r^2 = 1$ and white indicates $r^2 = 0$. An arrow marks the non-synonymous amino acid substitution variant Val66Met (rs6265).

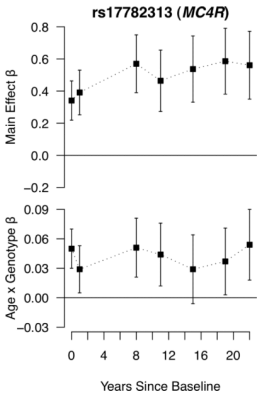

Figure 2.

Evidence for a consistent longitudinal age-by-genotype interaction influencing BMI at rs17782313 (*MC4R*). Cross-sectional main effect β coefficients (with no interaction term in the model) and age-by-genotype interaction β coefficients at seven time-points from baseline to 22 years afterward. Error bars represent standard errors. β coefficients are measured in untransformed BMI units (kg/m^2).

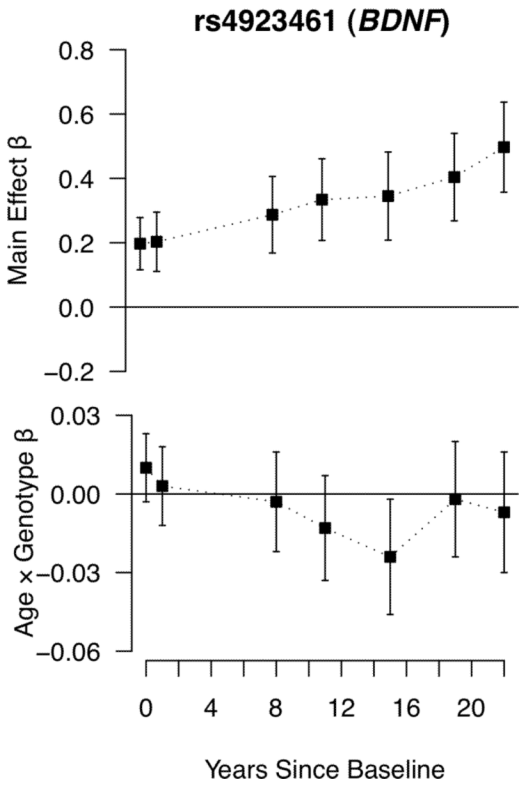

Figure 3.
No evidence for a longitudinal age-by-genotype interaction influencing BMI at rs4923461 (*BDNF*). Cross-sectional main effect β coefficients (with no interaction term in the model) and age-by-genotype interaction β coefficients at seven time-points from baseline to 22 years afterward. Error bars represent standard errors. β coefficients are measured in untransformed BMI units (kg/m^2).

Table 1

Demographic and descriptive statistics of the CLHNS cohort

Trait	Value	n
Body mass index (kg/m^2)	24.3 ± 4.4	1,780
Waist circumference (cm)	81.1 ± 10.9	1,779
Weight (kg)	55.2 ± 10.9	1,780
Average height (cm)	150.4 ± 4.9	1,792
Age (years)	48.4 ± 6.1	1,792
Number of pregnancies	6.5 ± 3.0	1,792
Menopausal status (yes/no)	687 / 1105	1,792

All values are mean ± s.d. unless specified otherwise.

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Table 2

SNPs with suggestive evidence of association ($P < 10^{-5}$) with four anthropometric traits in the CLHNS

SNP	Nearby gene	Chr	Position	Allele 1/2	Allele 1 Freq	Trait	Effect size (β ± s.e.m.)	*P* value
rs17124318	*ATG4C*	1	63,253,318	C/G	0.87	BMI	0.072 ± 0.014	5.91×10^{-7}
rs1440072	*KCNE4*	2	223,644,982	C/T	0.06	BMI	0.055 ± 0.012	4.37×10^{-6}
rs817858	*RAD23B*	9	109,165,632	G/C	0.13	BMI	0.042 ± 0.009	7.42×10^{-6}
rs1406503	*ZNF804B*	7	88,398,814	C/G	0.05	BMI	0.073 ± 0.016	8.69×10^{-6}
rs2373011	*ANKS1B*	12	98,485,480	C/G	0.42	BMI	0.026 ± 0.006	9.00×10^{-6}
rs9906155	*SEPT9*	17	73,213,292	T/C	0.91	BMI	0.045 ± 0.010	9.20×10^{-6}
rs2662467	*LVRN*	5	115,381,567	C/T	0.79	BMI	0.051 ± 0.011	9.62×10^{-6}
rs10740609	*PCDH15*	10	56,460,975	T/A	0.07	BMI	0.055 ± 0.012	9.85×10^{-6}
rs9313296	N/A	5	165,310,277	C/G	0.01	weight	10.004 ± 2.007	6.83×10^{-7}
rs16877106	*ANAPC4*	4	25,012,364	C/T	0.96	weight	5.420 ± 1.121	1.44×10^{-6}
rs907121	N/A	8	123,032,396	C/T	0.57	weight	1.826 ± 0.382	1.87×10^{-6}
rs17124318	*ATG4C*	1	63,253,318	C/G	0.87	weight	3.984 ± 0.858	3.63×10^{-6}
rs11108495	*C12orf55*	12	95,332,048	T/C	0.45	weight	1.554 ± 0.337	4.28×10^{-6}
rs10740609	*PCDH15*	10	56,460,975	T/A	0.07	weight	3.405 ± 0.745	5.21×10^{-6}
rs12594515	*SQRDL*	15	43,772,363	C/G	0.69	weight	1.705 ± 0.373	5.28×10^{-6}
rs1440072	*KCNE4*	2	223,644,982	C/T	0.06	waist	3.662 ± 0.739	7.87×10^{-7}
rs2373011	*ANKS1B*	12	98,485,480	C/G	0.42	waist	1.713 ± 0.363	2.46×10^{-6}
rs9290936	*IL1RAP*	3	191,735,329	G/T	0.61	waist	1.791 ± 0.387	4.03×10^{-6}
rs11647936	*KLHL36*	16	83,243,010	A/T	0.77	waist	2.294 ± 0.497	4.16×10^{-6}
rs13156607	*CCDC99*	5	168,832,565	T/C	0.11	waist	3.909 ± 0.851	4.61×10^{-6}
rs7302017	*PPM1H*	12	61,290,850	G/A	0.46	waist	1.712 ± 0.375	5.47×10^{-6}
rs9313296	N/A	5	165,310,277	C/G	0.01	waist	9.396 ± 2.065	5.72×10^{-6}
rs12594515	*SQRDL*	15	43,772,363	C/G	0.69	waist	1.727 ± 0.384	7.16×10^{-6}
rs3773996	*IL1RAP*	3	191,718,778	A/G	0.63	waist	1.616 ± 0.365	9.96×10^{-6}
rs17818399	*PIGF-CRIPT*	2	46,679,530	C/A	0.17	height	1.127 ± 0.218	2.74×10^{-7}
rs17638464	*FSTL5*	4	162,821,659	G/A	0.83	height	1.382 ± 0.289	1.91×10^{-6}
rs11888559	*CYP20A1*	2	203,873,416	T/C	0.15	height	1.051 ± 0.221	2.08×10^{-6}

SNP	Nearby gene	Chr	Position	Allele 1/2	Allele 1 Freq	Trait	Effect size (β ± s.e.m.)	*P* value
rs6670655	*PBX1*	1	163,005,795	T/C	0.18	height	1.258 ± 0.267	2.73×10^{-6}
rs2660869	*LTA4H*	12	95,003,398	C/G	0.02	height	4.387 ± 0.939	3.19×10^{-6}
rs2277912	*FASTKD2*	2	207,342,481	G/T	0.15	height	1.066 ± 0.232	4.73×10^{-6}
rs2691543	*RPL13AP17*	7	77,802,724	T/C	0.97	height	3.449 ± 0.755	5.20×10^{-6}

Chromosomal positions are reported in NCBI Build 36 coordinates. Reported alleles are from the positive strand. The allele that increased the trait value is listed as allele 1. Effect sizes are reported as β coefficients per copy of allele 1 and their associated standard errors (s.e.m.). BMI is reported in natural log-transformed units.

Chr, chromosome; BMI, natural log-transformed body mass index; N/A, no protein-coding gene within 500 kb of the SNP.

Table 3

CLHNS association for SNPs previously reported to be associated with BMI, weight, and waist circumference

SNP	Nearby gene	Chr	Position	Allele 1/2	Allele 1 Freq	Trait	Effect size (β ± s.e.m.)	*P* value	Ref
rs4923461	*BDNF*	11	27,613,486	A/G	0.52	BMI	0.021 ± 0.006	**2.8×10^{-4}**	(9)
rs17782313	*MC4R*	18	56,002,077	C/T	0.12	BMI	0.023 ± 0.009	**0.0073**	(8)
rs9939609	*FTO*	16	52,378,028	A/T	0.18	BMI	0.020 ± 0.007	**0.0074**	(21)
rs2815752	*NEGR1*	1	72,585,028	A/G	0.88	BMI	−0.015 ± 0.009	0.087	(10)
rs11084753	*KCTD15*	19	39,013,977	G/A	0.30	BMI	−0.014 ± 0.008	0.095	(10)
rs7647305	*DGKG*	3	187,316,984	C/T	0.90	BMI	0.017 ± 0.012	0.16	(9)
rs10938397	*GNPDA2*	4	44,877,284	G/A	0.19	BMI	0.007 ± 0.008	0.40	(10)
rs7498665	*SH2B1*	16	28,790,742	G/A	0.18	BMI	0.006 ± 0.008	0.42	(10)
rs6548238	*TMEM18*	2	624,905	C/T	0.92	BMI	0.007 ± 0.011	0.50	(10)
rs7566605	*INSIG2*	2	118,552,495	C/G	0.45	BMI	0.004 ± 0.006	0.54	(20)
rs1569019	*GPR133*	12	130,142,144	A/C	0.01	BMI	−0.012 ± 0.026	0.64	(19)
rs10838738	*MTCH2*	11	47,619,625	G/A	0.20	BMI	0.001 ± 0.007	0.90	(10)
rs3751812	*FTO*	16	52,375,961	T/G	0.18	weight	1.034 ± 0.442	**0.019**	(9)
rs29941	*KCTD15*	19	39,001,372	A/G	0.79	weight	−0.891 ± 0.421	0.034	(9)
rs12970134	*MC4R*	18	56,035,730	A/G	0.14	weight	0.983 ± 0.480	**0.041**	(9)
rs1077393	*BAT3*	6	31,718,508	A/G	0.44	weight	−0.453 ± 0.352	0.20	(9)
rs2568958	*NEGR1*	1	72,537,704	A/G	0.88	weight	−0.691 ± 0.538	0.20	(9)
rs7561317	*TMEM18*	2	634,953	G/A	0.92	weight	0.561 ± 0.629	0.37	(9)
rs7647305	*DGKG*	3	187,316,984	C/T	0.90	weight	0.324 ± 0.702	0.64	(9)
rs4788102	*SH2B1*	16	28,780,899	A/G	0.18	weight	0.203 ± 0.459	0.66	(9)
rs10913469	*SEC16B*	1	176,180,142	C/T	0.16	weight	0.146 ± 0.505	0.77	(9)
rs7826222	*MSRA*	8	9,897,490	G/C	0.39	waist	0.624 ± 0.392	0.11	(15)
rs12970134	*MC4R*	18	56,035,730	A/G	0.14	waist	0.658 ± 0.494	0.18	(7)
rs987237	*TFAP2B*	6	50,911,009	G/A	0.21	waist	−0.037 ± 0.447	0.93	(15)

Significant associations ($P < 0.05$, consistent direction of effect) are shown in boldface.

Ref, reference; other columns and abbreviations are as described in **Table 2**.

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Table 4

CLHNS association for SNPs previously reported to be associated with height

SNP	Nearby gene	Chr	Position	Allele 1/2	Allele 1 Freq	Effect size (β ± s.e.m.)	*P* value	Ref
rs3791679	*EFEMP1*	2	55,950,396	A/G	0.29	0.590 ± 0.188	**0.0017**	(6)
rs6440003	*ZBTB38*	3	142,576,899	A/G	0.13	0.672 ± 0.238	**0.0048**	(4)
rs6718438	*NPPC*	2	232,863,810	T/C	0.62	0.427 ± 0.165	**0.0096**	(17)
rs1042725	*HMGA2*	12	64,644,614	C/T	0.18	0.381 ± 0.210	0.07	(4)
rs7846385	*PXMP3*	8	78,322,734	C/T	0.15	0.327 ± 0.219	0.14	(6)
rs678962	*DNM3*	1	170,456,512	G/T	0.13	0.357 ± 0.240	0.14	(6)
rs10946808	*HIST1H1D*	6	26,341,366	A/G	0.48	0.257 ± 0.174	0.14	(5)
rs1046934[a]	*TSEN15*	1	182,290,152	C/A	0.46	0.288 ± 0.199	0.15	(6)
rs4842838	*ADAMTSL3*	15	82,373,128	G/T	0.46	−0.234 ± 0.167	0.16	(14)
rs3118914	*DLEU7*	13	50,014,902	G/T	0.99	1.117 ± 0.823	0.17	(14)
rs4743034	*ZNF462*	9	108,672,174	A/G	0.24	0.238 ± 0.186	0.20	(6)
rs6570508[b]	*GPR126*	6	142,755,535	G/A	0.28	0.310 ± 0.246	0.21	(5)
rs13273123	*PLAG1*	8	57,263,345	A/G	0.95	0.474 ± 0.398	0.23	(12)
rs7153027	*TRIP11*	14	91,496,975	A/C	0.73	0.203 ± 0.177	0.25	(6)
rs12214804[c]	*HMGA1*	6	34,296,844	C/T	0.13	0.338 ± 0.303	0.26	(6)
rs4800148	*CABLES1*	18	18,978,326	A/G	0.88	0.322 ± 0.322	0.32	(6)
rs2282978	*CDK6*	7	92,102,346	C/T	0.11	−0.232 ± 0.248	0.35	(4)
rs4533267	*ADAMTS17*	15	98,603,794	A/G	0.39	0.152 ± 0.168	0.37	(6)
rs11205277	*SF3B4*	1	148,159,496	G/A	0.36	0.277 ± 0.322	0.39	(6)
rs5742692	*IGF1*	12	101,323,728	A/G	0.80	0.159 ± 0.203	0.43	(22)
rs12338076	*QSOX2*	9	138,261,561	C/A	0.21	0.186 ± 0.239	0.43	(22)
rs6060369	*UQCC*	20	33,370,575	C/T	0.44	0.129 ± 0.167	0.44	(18)
rs967417	*BMP2*	20	6,568,893	G/A	0.17	0.165 ± 0.218	0.45	(6)
rs1812175	*HHIP*	4	145,794,294	G/A	0.48	−0.108 ± 0.157	0.49	(6)
rs2844479	HLA class III	6	31,680,935	A/C	0.53	0.110 ± 0.176	0.53	(6)
rs3760318	*ADAP2*	17	26,271,841	G/A	0.75	0.063 ± 0.184	0.73	(6)
rs4713858	*PPARD*	6	35,510,763	G/A	0.52	0.048 ± 0.163	0.77	(6)
rs1635852	*JAZF1*	7	28,155,936	T/C	0.79	−0.037 ± 0.204	0.86	(13)

SNP	Nearby gene	Chr	Position	Allele 1/2	Allele 1 Freq	Effect size (β ± s.e.m.)	*P* value	Ref
rs12986413	*DOT1L*	19	2,121,954	T/A	0.38	0.029 ± 0.184	0.87	(5)
rs12198986	*BMP6*	6	7,665,058	A/G	0.21	−0.033 ± 0.211	0.88	(6)
rs7664706[d]	*PRKG2*	4	82,437,781	T/C	0.10	0.020 ± 0.272	0.94	(14)
rs314277	*LIN28B*	6	105,514,355	A/C	0.05	0.029 ± 0.500	0.95	(5)
rs6830062	*LCORL*	4	17,626,828	T/C	0.84	0.011 ± 0.216	0.96	(6)

Columns and abbreviations are as described in **Table 2**.

[a] rs1046934 is a proxy for reported SNP rs2274432 (r^2 = 1 [CEU], 0.98 [CHB+JPT], HapMap Phase 2, Release 22).

[b] rs6570508 is a proxy for reported SNP rs4896582 (r^2 = 0.96 [CEU], 0.92 [CHB+JPT]).

[c] rs12214804 is a proxy for reported SNP rs1776897 (r^2 = 1 [CEU, CHB+JPT]).

[d] rs7664706 is a proxy for reported SNP rs2011962 (r^2 = 1 [CEU, CHB+JPT]).

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Table 5

Evidence of longitudinal SNP associations with BMI in the CLHNS

		Age	Genotype	Genotype × Age	
SNP	**Gene locus**	**Effect size (β ± s.e.m.)**	**Effect size (β ± s.e.m.)**	**Effect size (β ± s.e.m.)**	***P* value**
rs4923461	*BDNF*	0.277 ± 0.018	−0.185 ± 0.145	0.013 ± 0.003	**2.4×10^{-6}**
rs17782313	*MC4R*	0.288 ± 0.018	−0.093 ± 0.218	0.015 ± 0.004	**6.3×10^{-4}**
rs9939609	*FTO*	0.312 ± 0.018	0.070 ± 0.185	0.013 ± 0.004	**3.8×10^{-4}**
rs2815752	*NEGR1*	0.309 ± 0.019	0.238 ± 0.226	−0.011 ± 0.004	0.016
rs11084753	*KCTD15*	0.292 ± 0.018	−0.325 ± 0.209	0.000 ± 0.004	0.96
rs7647305	*DGKG*	0.277 ± 0.020	0.039 ± 0.293	0.008 ± 0.006	0.18
rs10938397	*GNPDA2*	0.287 ± 0.018	−0.264 ± 0.196	0.010 ± 0.004	0.0086
rs7498665	*SH2B1*	0.311 ± 0.018	−0.543 ± 0.192	0.013 ± 0.004	**8.2×10^{-4}**
rs6548238	*TMEM18*	0.289 ± 0.018	0.585 ± 0.272	−0.008 ± 0.005	0.0053
rs7566605	*INSIG2*	0.291 ± 0.018	0.012 ± 0.100	0.000 ± 0.004	0.99
rs1569019	*GPR133*	0.277 ± 0.031	−0.084 ± 0.660	−0.007 ± 0.013	0.58
rs1O838738	*MTCH2*	0.297 ± 0.019	−0.113 ± 0.181	0.004 ± 0.004	0.31

β coefficients are measured in untransformed BMI units (kg/m^2). Significant associations ($P < 0.0042$, considering 12 tests) are shown in boldface.