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ASSOCIATION STUDIES ARTICLE

A trans-ancestral meta-analysis of genome-wide association studies reveals loci associated with childhood obesity

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Abstract

Although hundreds of genome-wide association studies-implicated loci have been reported for adult obesity-related traits, less is known about the genetics specific for early-onset obesity and with only a few studies conducted in non-European populations to date. Searching for additional genetic variants associated with childhood obesity, we performed a trans-ancestral meta-analysis of 30 studies consisting of up to 13 005 cases (≥95th percentile of body mass index (BMI) achieved 2–18 years old) and 15 599 controls (consistently <50th percentile of BMI) of European, African, North/South American and East Asian ancestry. Suggestive loci were taken forward for replication in a sample of 1888 cases and 4689 controls from seven cohorts of European and North/South American ancestry. In addition to observing 18 previously implicated BMI or obesity loci, for both early and late onset, we uncovered one completely novel locus in this trans-ancestral analysis (nearest gene, *METTL15*). The variant was nominally associated with only the European subgroup analysis but had a consistent direction of effect in other ethnicities. We then utilized trans-ancestral Bayesian analysis to narrow down the location of the probable causal variant at each genome-wide significant signal. Of all the fine-mapped loci, we were able to narrow down the causative variant at four known loci to fewer than 10 single nucleotide polymorphisms (SNPs) (*FAIM2*, *GNPDA2*, *MC4R* and *SEC16B* loci). In conclusion, an ethnically diverse setting has enabled us to both identify an additional pediatric obesity locus and further fine-map existing loci.

Introduction

Obesity is having a dramatic impact on modern societies, leading to substantial health issues, with an overall prevalence among children already >20% in many populations, including the USA (1). Obesity considerably contributes to mortality in the USA, representing a key risk factor for cardiometabolic and other chronic diseases.

The complex trait of obesity is the outcome of an interaction between environmental and genetic risk components (2). An excess in adipose tissue is commonly seen as an imbalance between energy uptake and utilization, and although now viewed as a disease may have historically conferred an advantage when food availability was restricted and high levels of physical activity were normal (3). Overall, obesity affects approximately 50 million girls and 74 million boys worldwide (1); most crucially, the prevalence of childhood obesity is on the increase worldwide (1), meaning that the known comorbidities are also on the rise across many ethnicities (2).

While environmental factors clearly play a role in the pathogenesis of childhood obesity, there is also strong evidence for a genetic component to obesity risk from twin and family studies, with heritability estimates for BMI being as high as 70% (4). Large-scale genome-wide association studies (GWAS) have now reported many hundreds of loci associated with BMI/obesity in adults and principally in populations of European ancestry (5). However, some studies have investigated the genome-wide genetics of obesity and/or BMI in children (6–11), but these did not address sex-specific or trans-ancestral associations.

In childhood and adolescence, BMI varies widely with age. To that end, working with the Center for Disease Control and

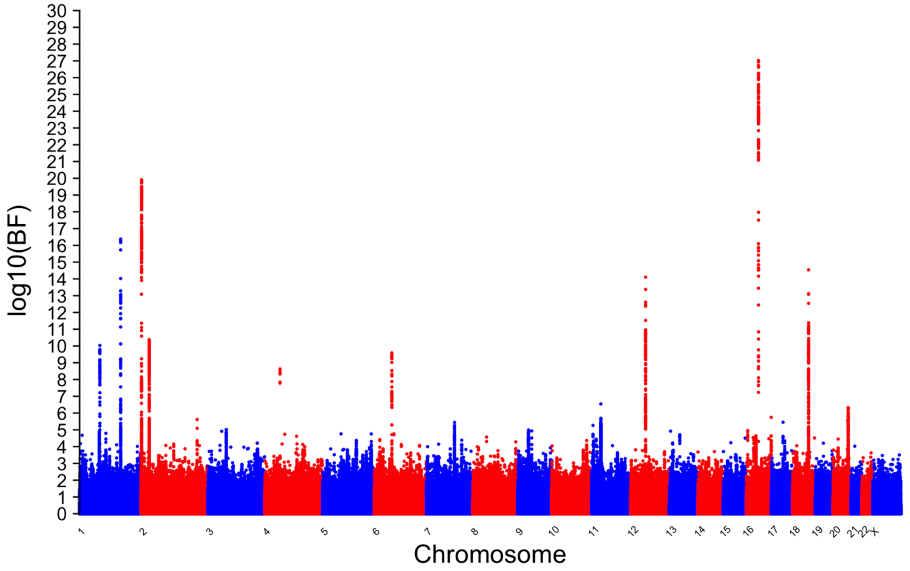

Figure 1. Manhattan plot of the trans-ancestral meta-analysis of the childhood obesity Stage 1 results. BF <0 have been represented by a value of 0. The y-axis is the $\log_{10}$ of the BF. Sentinel SNPs from loci that achieved at least $\log_{10}$ BF ≥ 4 were taken forward to Stage 2.

Prevention (CDC) definition of childhood obesity as being at or above the 95th percentile of BMI for age (12), we conducted a large-scale trans-ancestral GWAS meta-analysis of the trait to uncover additional loci in order to provide further biological insight into this condition.

Results

In order to identify novel genetic variants associated with childhood obesity, we performed a two-stage trans-ancestral meta-analysis consisting of the following: Stage 1, 30 genome-wide genotyped cohorts augmented with genetic data imputed to the 1000G-reference panel for discovery efforts; and Stage 2, 7 genotyped cohorts queried for SNPs that attained suggestive association in Stage 1 for the replication effort. The Stage 1 effort consisted of 13 005 cases (≥95th percentile of BMI achieved between 2 and 18 years old) and 15 599 controls (<50th percentile of BMI consistent throughout all measures during childhood). Stage 2 consisted of 1888 cases and 4489 controls. Each cohort was classified into four different groups based on ancestral makeup (either self-report or determined by PCA): European (Stage 1, 8613 cases and 12 696 controls; Stage 2, 921 cases and 1930 controls), African (Stage 1, 3282 cases and 1456 controls), American/Hispanic (Stage 1, 986 cases and 993 controls; Stage 2, 967 cases and 2759 controls) and East Asian group (Stage 1, 124 cases and 454 controls—consisting of East Asian ancestry samples from the USA and Singapore). The study characteristics are outlined in Supplementary Material, Table S1.

Stage 1: primary meta-analysis

Inverse variance weighted fixed-effects meta-analyses, as implemented with METAL, within each of the four major continental ancestries was used to estimate effect sizes for the input into the trans-ancestral analysis using MANTRA. Sentinel SNPs were chosen by examining blocks of associated SNPs and choosing the SNP with the maximum Bayes factor (BF) in each block. New blocks were determined by distance >100 kb between successive SNPs with a $\log_{10}$ BF ≥ 4. The trans-ancestral analysis yielded a total of 82 independent loci reaching suggestive association ($\log_{10}$ BF ≥ 4.0) while there were 11 independent loci reaching genome-wide association ($\log_{10}$ BF ≥ 6.0) (Supplementary Material, Table S2). A $\log_{10}$ BF of 6.0 is equivalent to a *P*-value of 5.0×10^{-8}. A $\log_{10}$ BF of 4.0 is equivalent to a *P*-value of 5.0×10^{-6}. The Manhattan plot of the trans-ancestral meta-analysis is shown in Fig. 1.

Stage 2: replication

The 82 independent SNPs found in the first stage of the analysis were taken forward and genotyped in the Stage 2 cohorts. In total, following the combined Stage 1 and Stage 2 effort, 18 loci achieved genome-wide significance ($\log_{10}$ BF ≥ 6.0) in the meta-analysis (Table 1). Of the 18 genome-wide significant loci found in the analysis, eight SNPs (*TNNI3K*, *SEC16B*, *TMEM18*, *ADCY3*, *FAIM2*, *FTO*, *HOXB5* and *MC4R*) were found to be in linkage disequilibrium (LD) ($r^2 \geq 0.2$, European 1000 genomes project phase 3) with variants previously shown to be associated with childhood obesity (6). Two SNPs at the *GNPDA2* and *TFAP2B* loci were in LD ($r^2 \geq 0.2$, European 1000 genomes project phase 3) with variants previously shown to be associated with childhood BMI (8). Six of the SNPs at loci (*RANBP17*, *CALCR*, *BDNF*, *ADCY9* and both variants near *CBLN4*) are in LD ($r^2 \geq 0.2$, European 1000 Genomes Project Phase 3) with variants associated in the most recent adult BMI meta-analysis (5). After a search of the GWAS catalog, we found that two of the SNPs at two loci (*GPR1* and *METTL15*) were not in LD ($r^2 < 0.2$) with any variant known to be associated with childhood or adult BMI or related traits in the GWAS catalogue. However, it is noted that the *GPR1* variant had an $r^2 = 0.19$ with a variant we reported on previously (8) (rs13387838) as associated with childhood BMI. To further assess the novelty of the *GPR1* variant, we performed an approximate conditional regression analysis of rs114670539 conditioning on rs13387838. The *P*-value of rs114670539 changed from 4.52×10^{-8} pre-conditioning to 5.94×10^{-8} post-conditioning in the Stage 1 European samples, suggesting that it is indeed independent of rs13387838. With a subsequent search of Phenoscanner, however, we found that the *GPR1* variant (rs114670539) yielded a genome-wide association to 'comparative body size at age 10' in an unpublished UK Biobank GWAS (https://www.nealelab.is/uk-biobank). The novel *METTL15* variant (rs10835310) showed a genome-wide significant association with 'comparative height size at age 10' in the same unpub-

lished UK BioBank GWAS, but no genome-wide association to any metabolic traits. A regional association plot for the novel locus in the European sub-analysis for the genome-wide Stage 1 analysis is shown in Supplementary Material, Fig. S1.

Subsequent conditional analyses revealed a novel independent signal at *TMEM18* [rs62104180, $r^2 = 0.0008$ with the previously reported rs7579427; minor allele frequency (MAF) < 5%; Table 1]. A review of Phenoscanner revealed this variant to be associated with a number of metabolic traits in the UK Biobank, including BMI.

Heritability and genetic correlation analyses

We sought to estimate the genome-wide common SNP heritability of childhood obesity and to calculate the genetic correlation of childhood obesity to other diseases. We used the LD score regression web interface called LD Hub (13) to measure the common SNP heritability of childhood obesity ($h^2 = 0.33$) in the European summary statistics only, given that it was the only dataset of sufficient sample size. Out of 219 traits with measured heritability, childhood obesity was ranked in the top 10% of traits. Childhood obesity had a similar common SNP heritability to three pubertal growth traits (difference in height between adolescence and adulthood, age 14, $h^2 = 0.45$; height, females at age 10 and males at age 12, $h^2 = 0.43$; difference in height between childhood and adulthood, age 8, $h^2 = 0.33$) but adult BMI, $h^2 = 0.19$, had a lower heritability. We also used LD score regression to assess the degree of genetic correlation between the European meta-analysis and other traits. The European meta-analysis summary statistics were uploaded to LD Hub and compared to 235 other traits that were present on the file server. Statistical significance and genetic correlation were assessed with LDSC. Out of the 235 traits comparisons, 32 were significant after Bonferroni correction ($P < 0.00021$). There were traits that were positively or negatively genetically correlated with childhood obesity. While the most significant positive genetic correlation was with adult BMI ($r_g = 0.84$, $P = 3.4 \times 10^{-91}$) and the most significant negative genetic correlation was with age at menarche ($r_g = -0.40$, $P = 1.5 \times 10^{-24}$; Supplementary Material, Table S3), there were other less obvious genetic correlations such as negative genetic correlations with college completion and years of schooling and positive genetic correlations with excessive daytime sleepiness and squamous cell lung carcinoma.

We also compared our results to the largest adult BMI GWAS dataset currently available. We used 698 independently associated SNPs from Yengo *et al.* (5) to compare the effect sizes between adult BMI and childhood obesity. We leveraged SNPs that were genome-wide significant in single SNP analyses. We extracted the effect sizes for these SNPs from our European Stage 1 analysis and compared them to the adult BMI effect sizes (correlation = 0.76; Supplementary Material, Fig. S2. A total of 562 out of 698 SNPs associated with adult BMI had the same direction of effect in childhood obesity.

Functional analysis and fine mapping

The trans-ancestral meta-analysis results were subsequently used to fine map the genome-wide significant loci through credible set analysis. A total of 4 loci had 99% credible sets with fewer than 10 SNPs (*FAIM2*, *GNPDA2*, *MC4R* and *SEC16B* loci). Even though the non-European samples formed a minority in the analysis, they enabled refinement of the interval within each of the 99% credible sets; indeed, none of the 4 loci with 99% credible sets of fewer than 10 SNPs in the trans-ancestral analysis had credible sets fewer than 10 SNPs in the European-only analysis. The *FAIM2* locus was refined to six SNPs, two of which are in the 3′ untranslated region of the gene and all residing within a 17 kb region on chromosome 12 (hg19: 50,246,252-50,263,148). The *GNPDA2* locus also yielded six SNPs in the 99% credible set, all residing within 12 kb of each other on chromosome 4 (hg19: 4,175,691-45,187,622). The signal near *MC4R* yielded four SNPs in the 99% credible set residing within 31 kb of each other on chromosome 18 (hg19: 57,824,038-57,854,694). Finally, the *SEC16B* locus had five SNPs in the 99% credible set, which were all within 11 kb of each other on chromosome 1 (hg19: 177,889,025-177,899,121; Supplementary Material, Table S4).

All 21 of the variants in the four 99% credible sets were analyzed with the Ensembl Variant Effect Predictor (14) to assess the enrichment of various functional groups in these sets. Intergenic variants were the most common predicted category with 43% of variants; 21% of variants were labeled as downstream gene variants that lie 3′ of a gene. The downstream variants were concentrated around *SEC16B* and *FAIM2*. Variants located in regulatory regions accounted 15% of the variants intronic variants represented 9% of variants. 3′ untranslated region variants of *FAIM2* represented 9% of variants and one variant was in a transcription factor binding site.

Lastly, in order to attempt to place these signals in to a functional context, we investigated whether the suggestively associated variants were likely to share the same causal variant as an expression quantitative trait loci (eQTLs) of a nearby gene. We conducted colocalization analyses with GTEx v7 for all loci with $\log_{10}\text{BF} \geq 4$ (Supplementary Material, Table S5). This analysis yielded significant colocalizations at two loci across a range of tissues. The sentinel variant rs2206277 yielded a colocalization with an eQTL of *TFAP2B* in tibial nerve tissue, while rs4077678 showed significant colocalizations in numerous tissues. The most significant eQTL and tissue pair for rs4077678 was *DNAJC27* in whole blood, *ADCY3* in whole blood, *CENPO* in whole blood and *DNAJC27-AS1* in brain cerebellum. The additional significant colocalizations can be found in Supplementary Material, Table S5.

Discussion

Our trans-ancestral GWAS meta-analysis represents a large genome-wide survey of childhood obesity and allowed for the detection of loci not readily picked up in European only ancestral populations. We confirmed 18 loci previously reported for childhood obesity or other metabolic phenotypes and identified one novel locus, namely at *METTL15*, associated with childhood obesity. Furthermore, the large overlap of at least nominally significant SNPs in both meta-analyses of pediatric obesity and adult BMI points to a shared genetic basis of these traits, at different times in the life course. The genetic correlation between childhood obesity and adult BMI was confirmed using LD score regression, along with a negative genetic correlation between childhood obesity and age at menarche.

Although functional efforts are required to identify the actual effector genes at these loci, using similar approaches to what were applied to *FTO* locus that led to the implication of *IRX3* and *IRX5* (15–18), no inferences could be made from eQTLs for our novel childhood obesity loci. For the novel locus *METTL15*, the actual effector gene may be the well-established adult obesity *BDNF* gene that resides in the same topologically associating domain. Furthermore, rs2749808 near *CBLN4* gene is intergenic and may influence *MC3R*, given that it has already been strongly implicated in the pathogenesis of obesity (19,20). We also further

Table 1. Top independent novel and known SNPs that reached genome-wide significance ($\log_{10}$ BF ≥ 6) in the conditional or trans-ancestral meta-analyses

Chr	Position	Marker	Nearest gene	Analysis	Alleles	African			Asian			European			Hispanic			Trans-ancestral		Previously known
						Beta	SE	P	Beta	SE	P	Beta	SE	P	Beta	SE	P	BF	Het	Metabolic phenotype
1	74,983,835	rs10493544	*TNNI3K*	Full	t/c	0.18	0.06	3.86E-03	−0.36	0.26	1.62E-01	0.14	0.02	1.14E-13	0.02	0.05	6.45E-01	11.81	0.35	Childhood obesity
1	177,889,025	rs539515	*SEC16B*	Full	a/c	−0.19	0.05	2.77E-04	0.08	0.25	7.37E-01	−0.18	0.02	2.68E-14	−0.24	0.06	4.06E-05	18.07	0.16	Childhood obesity
2	466,003	rs62104180	*TMEM18*	Conditional	a/g	–	–	–	–	–	–	−0.32	0.06	4.52E-09	–	–	–	7.10	0.00	Adult BMI
2	631,183	rs7579427	*TMEM18*	Full	a/c	0.26	0.07	2.98E-04	−0.25	0.29	3.93E-01	0.21	0.02	8.54E-18	0.25	0.07	5.96E-04	20.25	0.20	Childhood obesity
2	25,122,840	rs4077678	*ADCY3*	Full	c/g	−0.16	0.06	1.58E-02	−0.13	0.17	4.35E-01	−0.14	0.02	1.44E-13	−0.11	0.06	7.42E-02	13.38	0.10	Childhood obesity
2	207,064,335	rs114670539	*GPR1*	Full	t/c	0.14	0.19	4.57E-01	–	–	–	0.26	0.05	2.14E-08	0.03	0.17	8.79E-01	6.12	0.23	Comp. body size at age 10
4	45,187,622	rs925494	*GNPDA2*	Full	t/c	0.24	0.06	4.21E-05	−0.02	0.21	9.25E-01	0.10	0.02	4.04E-07	0.19	0.08	1.50E-02	8.57	0.37	Childhood BMI
5	170,599,327	rs2053682	*RANBP17*	Full	a/c	0.15	0.05	1.94E-03	0.27	0.22	2.15E-01	0.09	0.02	6.76E-06	0.11	0.05	3.64E-02	6.73	0.13	Adult BMI
6	50,798,526	rs2206277	*TFAP2B*	Full	t/c	0.13	0.06	4.95E-02	0.02	0.20	9.15E-01	0.14	0.02	5.93E-10	0.21	0.05	5.39E-05	11.63	0.14	Childhood BMI
7	93,269,367	rs10224397	*CALCR*	Full	a/g	0.18	0.05	7.05E-04	0.07	0.18	6.83E-01	0.09	0.02	2.18E-06	0.08	0.07	2.51E-01	6.53	0.15	Adult BMI
11	27,667,236	rs17309874	*BDNF*	Full	a/g	0.12	0.08	1.13E-01	–	–	–	0.12	0.02	2.59E-08	0.20	0.07	2.82E-03	8.52	0.11	Adult BMI
11	28,355,657	rs10835310	*METTL15*	Full	t/c	0.10	0.05	5.41E-02	0.05	0.19	7.79E-01	0.10	0.02	3.90E-08	0.04	0.08	6.25E-01	6.26	0.13	Novel
12	50,263,148	rs7132908	*FAIM2*	Full	a/g	–	–	–	0.19	0.20	3.33E-01	0.15	0.02	4.00E-16	0.23	0.07	5.70E-04	16.39	0.14	Childhood obesity
16	4,017,567	rs2540031	*ADCY9*	Full	a/t	0.12	0.06	3.93E-02	0.46	0.19	1.51E-02	0.08	0.02	2.75E-05	0.17	0.06	2.64E-03	6.33	0.30	Adult BMI
16	53,806,453	rs56094641	*FTO*	Full	a/g	−0.17	0.07	2.02E-02	−0.48	0.24	4.16E-02	−0.21	0.02	1.31E-28	−0.28	0.06	6.55E-06	31.88	0.19	Childhood obesity
17	46,664,608	rs2740752	*HOXB5*	Full	t/c	0.18	0.05	1.06E-03	−0.07	0.22	7.52E-01	0.11	0.03	1.34E-04	0.20	0.06	7.89E-04	6.81	0.15	Childhood obesity
18	57,829,135	rs6567160	*MC4R*	Full	t/c	−0.22	0.06	8.66E-05	–	–	–	−0.15	0.02	1.16E-11	−0.19	0.11	6.97E-02	13.20	0.14	Childhood obesity
20	54,149,014	rs2749808	*CBLN4*	Full	t/c	−0.12	0.05	1.28E-02	−0.14	0.17	4.20E-01	−0.10	0.02	1.12E-06	−0.08	0.05	1.44E-01	6.47	0.09	Adult BMI
20	54,482,276	rs1437206	*CBLN4*	Full	t/c	0.18	0.05	9.67E-05	0.23	0.28	4.13E-01	−0.10	0.02	3.23E-06	−0.21	0.08	1.16E-02	7.43	1.00	Adult BMI

Betas and standard errors (SE) are shown for each ancestral-specific sub-analysis. The heterogeneity (Het) of the Bayes' Factor (BF) is also shown. If the variant [or in LD ($r2 > 0.2$)] was previously found in a metabolic phenotype, that phenotype is shown. '—' indicates that the variant did not pass quality control in that ancestral grouping. The first allele is the effect allele for which the beta applies.

implicated *TMEM18* as the effector gene at this locus given the independent signal plus the rarer variants (MAF < 5%) in the same neighborhood.

Trans-ancestral meta-analysis is particularly valuable in fine-mapping loci to narrow down the area harboring the causal variant. This is due to the different LD patterns present in different ancestral populations. Despite known limitations to various fine-mapping approaches (such as whether or not the same set of variants were present in all input datasets), using MANTRA and credible set analysis we were able to narrow down the potential causal variant to fewer than 10 variants at four different loci (*FAIM2*, *GNPDA2*, *MC4R* and *SEC16B*). Using the colocalization method, we were able to narrow down the putative causal variants and causal tissues for the *ADCY3* and *TFAP2B* loci. There are colocalized eQTLs for various tissues with these associated loci that will need to be followed up in the future. The *ADCY3* locus is interesting in that there seems to be multiple genes (*DNAJC2*, *ADCY3*, *CENPO* and *DNAJC27-AS1*) colocalizing with the rs4077678 locus in multiple tissues (whole blood, tibial nerve, skin, adipose, lung, pituitary, esophagus and cerebellum). Whether this is due to coordination in all the genes in these tissues is an open question.

As with our previous GWAS of childhood obesity, we continued to use CDC definition as at or above the 95th percentile of BMI for age (21) and indeed represents the general guide for clinical practice (22). This is driven by the fact that there is a complex relationship between BMI and body fat in childhood, where it varies over time and especially during puberty. The larger heritability of childhood obesity compared to adult BMI, along with the correlation of the effects of the two traits, suggests that childhood obesity is an effective proxy trait to find variants associated with adult BMI but at smaller sample sizes.

We have conducted a large-scale transancestral two-stage GWAS for childhood obesity, where we robustly identified a novel childhood obesity locus. We have also shown that childhood obesity is genetically very similar to adult BMI and with far greater numbers of samples we would most likely see more significant loci in common with the two phenotypes. As such, we have gained greater insights in the biology of obesity in the pediatric setting and these loci warrant further functional follow up in order to provide greater potential therapeutic insights.

Materials and Methods

Research subjects

The Stage 1 dataset consisted of 30 genome-wide genotyped studies from various ethnicities with BMI measured in childhood (2–18 years old) except Genetics of Overweight Young Adults (GOYA), which included some time points between 18 and 19 years old. The participating cohorts in these analyses were the follwowing: the Children's Hospital of Philadelphia (CHOP) Study, the Generation R Study, the Singapore Cohort study Of the Risk factors for Myopia, the Avon Longitudinal Study of Parents and Children, the Western Australian Pregnancy Cohort (Raine) Study, the Amsterdam Born Children and their Development-Genetic Enrichment Study, the Copenhagen Prospective Study on Asthma in Childhood (COPSAC2000), the French Obesity of the Youth (OBE) Study, the German Infant Study on the influence of Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus)/the Influence of life-style factors on the development of the immune system and allergies in East and West Germany Study, the GOYA Study, the Helsinki Birth Cohort Study (HBCS), the HOLBAEK Study, the INfancia y Medio Ambiente [Environment and Childhood] Project, the Manchester Asthma and Allergy Study, Northern Finland Birth Cohort 1986, Northern Finland Birth Cohort 1966, the Physical Activity and Nutrition in Children Study, 1958 British Birth Cohort (1958BC), Young Finns Study, the Children's Health Study (CHS) and the MEXICO Study. Further information on the 1st stage cohorts is found in Supplementary Material, Table S1.

The Stage 2 dataset consisted of seven targeted genotype studies with BMI measured in childhood (ages 2–18 years) except the FAMILY study that included some time points <2 years of age. These studies were derived from the following participating cohorts: the CHS, the FAMILY study, The Norwegian Mother and Child Cohort Study, the Santiago Longitudinal Study, the American Indians from Arizona Study and the VIVA la Familia Study.

Trait definition

Case and control definitions were based on national standard growth curves of BMI versus age for children from 2 to 18 years old. For instance, CHOP used the CDC standard growth curves [as featured in previous papers (12,22)]. The exception to this is the HBCS and 1958BC, as pediatric measures were made over two or six decades ago, respectively, so contemporary curves are not appropriate—in this case they generated their own reference curves. Cases were defined as an individual whose BMI is greater than or equal to the 95th percentile at any point in childhood. Controls were defined as an individual whose BMI was less than or equal to the 50th percentile consistently throughout childhood for all available measures.

Statistical analysis

Each cohort was analyzed independently using a logistic regression framework (using an additive genetic model) where samples of different ancestry and samples genotyped on different SNP microarrays were analyzed separately. Eigenvectors calculated from principal components analysis were used as covariates in the logistic regression by each cohort where appropriate.

For the discovery stage of the meta-analysis, data from high-density SNP arrays in each cohort were imputed to the 1000 Genomes integrated variant Phase 1 release v3 reference panel. Individual cohorts were responsible for their own pre-imputation sample exclusion criteria. Pre-imputation SNP quality control was applied by each individual cohort and it was recommended to remove SNPs with call rate < 95%, Hardy–Weinberg equilibrium $P < 1 \times 10^{-4}$ and a MAF filter that incorporated the accuracy of the genotyping of lower frequency SNPs. Cohort specific quality control and deviations from the recommended analysis parameters can be found in Supplementary Material, Table S6. Post-imputation quality control consisted of removing SNPs with MAF < 0.01, minor allele count <10, r2_Hat <0.3, proper_info <0.4 or plink_info <0.8 (depending on the software used for the statistical association analysis), as well as removing insertions and deletions.

Ancestral-specific inverse variance weighted fixed-effect meta-analysis was performed using METAL. Genomic control was applied to each cohort prior to meta-analysis and to the final meta-analysis statistics. SNPs were filtered out of the ancestral-specific meta-analysis if the heterogeneity i-squared >0.5 or if they were present in fewer than 50% of the total samples in the meta-analysis. Trans-ancestral meta-analysis was performed using MANTRA on the summary statistics obtained from the

ancestral-specific meta-analyses (Supplementary Material, Fig. S3).

Sentinel SNPs were selected at each locus from the suggestively associated results ($\log_{10}$ Bayes' factor > 4) as the SNP at each locus with the largest BF in the trans-ancestral results to maximize reproducibility across ethnicities. A locus was defined as a collection of SNPs whose next physically closest suggestively associated SNP was within 100 kb. This collection of SNPs was tested for association in the Stage 2 dataset.

The Stage 2 dataset was then combined with the Stage 1 dataset to test for association in the ancestral-specific analyses and in the overall trans-ancestral analysis. The combined Stage 1 + Stage 2 results that resulted in genome-wide significant results ($\log_{10}$ Bayes' factor >6) are shown in Table 1. Stage 2 findings were only evaluated when combined with Stage 1 and not independently given the small sample size relative to Stage 1.

Sentinel SNPs that achieved genome-wide significance were queried against the GWAS catalogue and other available studies within Phenoscanner (23). A sentinel variant achieving $P < 5.0 \times 10^{-8}$ in a prior metabolic GWAS was considered already discovered.

Conditional regression

Genome-wide Complex Trait Analysis (GCTA) was used for pseudo-conditional regression analysis to identify variants independently associated with childhood obesity at the genome-wide significance level (trans-ancestral $\log_{10}$ BF > 6). The CHOP African American, European American, Hispanic and East Asian samples were used to estimate the LD in GCTA. The genome-wide significant sentinel SNPs from the Stage 1 analysis were used as conditioning variants for the Stage 1 summary statistics. The ancestral-specific conditional analysis results were then analyzed in MANTRA to identify trans-ancestral significance. The top genome-wide significant SNP in the resulting conditional analysis results was then added into the list of conditioning SNPs to be analyzed again. When there were no more genome-wide significant SNPs, the conditional regression was then halted. A separate pseudo-conditional regression analysis was carried out by conditioning rs114670539 on rs13387838 using the CHOP European American cohort to estimate LD.

LD score regression

LD score regression was performed using the LD Hub website interface (http://ldsc.broadinstitute.org/ldhub). The results from the European only meta-analysis were used for the LD score regression. Childhood obesity was compared against every phenotype available on LD Hub with the exception of the UK Biobank phenotypes and the previous childhood obesity meta-analysis.

eQTL analysis colocalization

We used *coloc* (with default parameters) to perform a Bayesian colocalization analysis comparing the meta-analysis results with GTEX version 7. We used variants with a $\log_{10}$ Bayes' factor ≥ 4 in the Stage 1 analysis with 47 tissues from GTEX in the colocalization analysis. GWAS BFs were used directly as input, while eQTL effect sizes and standard errors were used to estimate approximate BFs for input. A significant colocalization was defined as PP.H3.abf + PP.H4.abf >0.99 and PP.H4.abf/PP.H3.abf >5 (24). PP.H3.abf is defined as the posterior probability of two distinct causal variants. PP.H4.abf is defined as the posterior probability of one common causal variant.

Credible set analysis

The script credible_set_analysis.py located at https://github.com/edm1/Credible-set-analysis/blob/master/credible_set_analysis.py was used to calculate the 99% credible sets for every genome-wide significant locus. The sum of the posterior probabilities was calculated from a sorted list of the most significant Bayes' factors until the cumulative sum was equal to or greater than 0.99. This set of SNPs was then considered the 99% credible set.

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Conflict of Interest statement

Shana McCormack has participated in advisory boards for Rhythm Pharmaceuticals and Reata Pharmaceuticals. She is a site Principal Investigator (PI) for a clinical trial supported by Levo Pharmaceuticals.

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Cohort

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