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A genome-wide association meta-analysis identifies new childhood obesity loci

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Multiple genetic variants have been associated with adult obesity and a few with severe obesity in childhood; however, less progress has been made in establishing genetic influences on common early-onset obesity. We performed a North American, Australian and European collaborative meta-analysis of 14 studies consisting of 5,530 cases (≥95th percentile of body mass index (BMI)) and 8,318 controls (<50th percentile of BMI) of European ancestry. Taking forward the eight newly discovered signals yielding association with $P < 5 \times 10^{-6}$ in nine independent data sets (2,818 cases and 4,083 controls), we observed two loci that yielded genome-wide significant combined *P* values near *OLFM4* at 13q14 (rs9568856; $P = 1.82 \times 10^{-9}$; odds ratio (OR) = 1.22) and within *HOXB5* at 17q21 (rs9299; $P = 3.54 \times 10^{-9}$; OR = 1.14). Both loci continued to show association when two extreme childhood obesity cohorts were included (2,214 cases and 2,674 controls). These two loci also yielded directionally consistent associations in a previous meta-analysis of adult BMI[1].

Obesity is a major, increasingly prevalent health problem affecting modern societies. The problem is particularly severe for children in developed countries, where the prevalence of obesity is on the increase. Obesity present in adolescence is associated with increased overall mortality in later life[2]. Whereas the change in prevalence of obesity is likely to be explained by environmental changes over the last 30 years, there is also strong evidence for a genetic component to the risk for obesity. This is reflected in familial occurrences of childhood obesity, where the concordance of fat mass among monozygotic twins is reported to be higher than in dizygotic twins.

In the past 4 years, many genetic loci have been implicated in BMI and/or obesity phenotypes from the outcomes of genome-wide association studies (GWAS), primarily in adults. The first locus reliably found to harbor variation associated with adiposity, *FTO* (encoding fat mass and obesity associated)[3], has been shown to be associated with obesity in all sufficiently sized study groups. Subsequent larger studies have identified additional loci influencing BMI and/or obesity. The largest meta-analysis of adult BMI to date came from the Genetic Investigation of ANthropometric Traits (GIANT) Consortium, which

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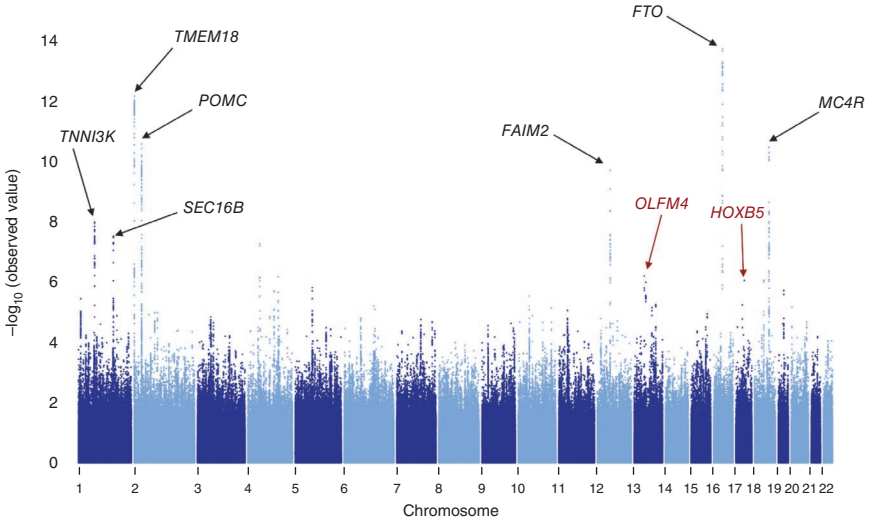

Figure 1 Manhattan plot of the results from meta-analysis of GWAS for childhood obesity in the discovery stage (5,530 cases and 8,318 controls), with each locus that achieved genome-wide significance ($P < 5 \times 10^{-8}$) indicated in black. The new loci uncovered in this study are indicated in red.

confirmed 14 known obesity susceptibility loci and identified 18 new loci associated with BMI in a study involving a total of 249,796 individuals[1]. However, these loci only account for a small fraction of the heritability that is known to contribute to obesity. There has been some work on extreme obesity in childhood (>99.5th percentile of BMI), but little progress has been made on less marked definitions of obesity that are more relevant to public health.

We reasoned that distilling the genetic component of this complex phenotype should be easier in children, where environmental exposure and impact have occurred for a relatively short period in their lifetimes. The relationship between BMI and body fat in children varies widely with age and with pubertal maturation. The Centers for Disease Control and Prevention defined overweight as at or above the 95th percentile of BMI for age[4]. By late adolescence, these percentiles approach those used for adult definitions, with the 95th percentile corresponding to ~30 kg/m^2 (ref. 5).

In an effort to systematically search for childhood obesity susceptibility loci, we performed a large-scale meta-analysis of 14 existing GWAS data sets for childhood obesity, totaling 5,530 cases (≥95th percentile of BMI reached before the age of 18 years, representing 5–30% of any given cohort) and 8,318 controls (relatively conservatively defined as <50th percentile of BMI consistently throughout all measures during childhood) of European ancestry (**Supplementary Table 1** and **Supplementary Note**).

In the meta-analysis of 2.7 million SNPs (directly genotyped or imputed), signals at seven discrete locations reached genome-wide significance at $P < 5.0 \times 10^{-8}$. All these loci have previously been identified through GWAS for adult BMI (*FTO*, *TMEM18*, *POMC*, *MC4R*, *FAIM2*, *TNNI3K* and *SEC16B*) and robustly reflect previous reports on individual pediatric cohorts[6,7]. *FTO* gave the strongest evidence for association, although *TNNI3K* and *POMC*, which were only detected in adult studies when samples came from hundreds of thousands of individuals, were also readily detected in our relatively small sample (**Fig. 1** and **Supplementary Tables 2** and **3**). Excluding the French and German studies from the meta-analysis, we did not observe association with variants previously reported by these groups as novel, where the defined childhood obesity was at a higher threshold[8], at the loci harboring *TNKS-MSRA* (rs17150703; $P = 0.22$) and *SDCCAG8* (rs12145833; $P = 0.57$).

We took forward all eight newly identified signals yielding association with $P < 5.0 \times 10^{-6}$ (**Table 1** and **Supplementary Table 4**). Heterogeneity analysis showed that the different distributions in each study did not affect the results (**Supplementary Table 4**). In addition, we applied a second genomic control correction to the overall discovery meta-analysis results in order to test for replication in multiple independent existing data sets, the majority of which were *in silico* analyses (**Supplementary Table 5**). In our replication effort, we initially tested these eight SNPs in nine study groups that had a comparable set of affected subjects with BMI distributed normally from the 95th percentile upward (2,818 cases and 4,083 controls). In combined analysis of the discovery and replication cohorts, we observed two loci that yielded consistent evidence of association near *OLFM4* (encoding olfactomedin 4) at 13q14 (rs9568856; $P_{combined} = 1.82 \times 10^{-9}$; OR = 1.22) and within the *HOXB5* gene (encoding homeobox B5) at 17q21 (rs9299; $P_{combined} = 3.54 \times 10^{-9}$; OR = 1.14) (**Table 1** and **Supplementary Figs. 1** and **2**).

Previous GWAS reports for extreme obesity case-control samples have both confirmed signals seen in samples with less extreme obesity or that are population based, such as *FTO*, as well as identified new signals that are distinct from those seen at the population level[8]. We reasoned, therefore, that further exploration in existing extreme obesity data sets (two cohorts totaling 2,214 cases (exclusively individuals who are approximately >4 s.d. above mean BMI, equating BMI > 99.5th percentile) and 2,674 controls) would offer further insight into how these signals operate, while acknowledging the phenotypic differences and limits of sample size. Indeed, both loci

Table 1 Two newly discovered loci associated with common early-onset obesity

	Locus	SNP	Allele 1/2	Nearest gene	Direction[a]	OR (95% CI)	*P* value
Discovery	13q14	rs9568856	A/G	*OLFM4*	+++++++++++−+	1.21 (1.12–1.30)	6.58×10^{-7}
	17q21	rs9299	T/C	*HOXB5*	++−++−++++++++	1.14 (1.08–1.21)	9.12×10^{-7}
Replication	13q14	rs9568856	A/G	*OLFM4*	+−++++−++	1.22 (1.09–1.38)	7.13×10^{-4}
	17q21	rs9299	T/C	*HOXB5*	++−+++++	1.14 (1.06–1.24)	0.00104
Combined	13q14	rs9568856	A/G	*OLFM4*		1.22 (1.14–1.29)	1.82×10^{-9}
	17q21	rs9299	T/C	*HOXB5*		1.14 (1.09–1.20)	3.54×10^{-9}

In the discovery stage (5,530 cases and 8,318 controls), these loci did not reach genome-wide significance but yielded $P < 5 \times 10^{-6}$. The outcome of the replication effort for these loci in nine comparable independent cohorts (totaling 2,818 cases and 4,083 controls) is shown, as is that of combined analysis, with signals at both loci reaching genome-wide significance in the latter analysis.

[a]Plus and minus signs represent the direction of effect based on the reference allele.

emerging from the main replication stage continued to show association with the more extreme phenotype (*OLFM4*: rs4833407; $P_{overall} = 5.33 \times 10^{-9}$; OR = 1.18 and *HOXB5*: rs9299; $P_{overall} = 1.54 \times 10^{-8}$; OR = 1.13) (**Supplementary Table 6**).

As the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort leveraged BMI measures made before the age of 2 years as part of the definition of cases and controls, we performed sensitivity analyses limiting case and control definitions to children over 2 years of age (**Supplementary Tables 7–9**). In addition to no diminishment in the ORs for the *OLFM4* and *HOXB5* loci, we observed support for associations of rs4864201 at *BC041448* (UCSC annotation) and rs4833407 at *ALPK1* (**Supplementary Tables 8** and **9**).

Finally, we were interested to see whether our two main signals of interest at *OLFM4* and *HOXB5* were evident in the GIANT Consortium adult BMI meta-analysis results (n = 123,864 study subjects). Indeed, both loci yielded evidence of association in this quantitative setting ($P = 7.75 \times 10^{-5}$ and 0.015, respectively), with the same alleles being associated in the same direction in the two analyses. Overall, seven of the eight signals initially taken forward to the replication stage, with the exception of rs1290002, yielded consistent directionality, although not all were statistically significant (**Supplementary Table 10**).

Taken together, these data indicate that the genetic architecture of BMI and obesity overlap to a large extent in children as well as in adults. In addition to the previously reported loci, we have uncovered at least two new loci associated with obesity in early life. The adult BMI data available from the GIANT Consortium[1] show that the influence of these two loci is also detected in adulthood. Of note, in addition to *OLFM4* and *HOXB5*, the GIANT Consortium data support associations with three more of the eight loci initially taken forward to the replication stage, namely rs4864201 at *BC041448*, rs4833407 at *ALPK1* and rs2300095 at *MTOR-ANGPTL7*, despite these signals not formally replicating in the main defined overall pediatric setting, suggesting that these loci should be studied further to fully understand their role in the pathogenesis of obesity as a whole.

OLFM4, encoding olfactomedin 4, is the nearest gene to rs9568856 but is still approximately 500 kb from the associated signal; the corresponding gene product has never been directly implicated in obesity but has been extensively studied in the context of various cancers. The OLFM4 protein is a secreted glycoprotein that facilitates cell adhesion via lectins and cadherin on the cell surface. Although the function of OLFM4 is not well understood, there are several intriguing observations that link it to gut microflora and to the relationship between the gut microbiome and obesity risk. For example, the *OLFM4* gene product downregulates innate immunity to infection by the stomach bacterium *Helicobacter pylori*[9], with obese subjects having a higher occurrence of *H. pylori* infection than their lean counterparts[10,11]. Indeed, weight loss induced by obesity surgery eradicates *H. pylori*[12].

rs9299 is in the 3′ UTR of the *HOXB5* gene, encoding homeobox B5, within the HOXB gene cluster. *HOXB5* is spatially and temporarily regulated during gut development[13], and it has been suggested to have a role in obesity by a study observing upregulation of homeobox transcription factors after fat loss[14]. Therefore, it is possible that *OLFM4* and *HOXB5* may impact BMI via different aspects of gut function.

In summary, as a consequence of extensive North American, Australian and European collaborative genome-wide meta-analyses on children, we have uncovered two new obesity loci that have the strongest evidence for association with elevated adiposity in the first 18 years of life. Further functional characterization of these signals is required to elucidate the precise mechanisms behind these observations.

URLs. Public Health Research Consortium (PHRC), http://www.york.ac.uk/phrc/; Copenhagen Studies on Asthma in Childhood (COPSAC), http://www.copsac.com/; Danish Obesity Research Center (DanORC), http://www.danorc.dk/; Infancia y Medio Ambiente (INMA) Project, http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html; publically available GIANT Consortium data sets, http://www.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files; International HapMap Project, http://hapmap.ncbi.nlm.nih.gov/; METAL, http://www.sph.umich.edu/csg/abecasis/metal/index.html; Wellcome Trust Case Control Consortium, http://www.wtccc.org.uk/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

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

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AUTHOR CONTRIBUTIONS

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Speliotes, E.K. *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat. Genet.* **42**, 937–948 (2010).
2. Must, A. Does overweight in childhood have an impact on adult health? *Nutr. Rev.* **61**, 139–142 (2003).
3. Frayling, T.M. *et al.* A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889–894 (2007).
4. Flegal, K.M., Wei, R. & Ogden, C. Weight-for-stature compared with body mass index–for-age growth charts for the United States from the Centers for Disease Control and Prevention. *Am. J. Clin. Nutr.* **75**, 761–766 (2002).
5. Daniels, S.R. *et al.* Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. *Circulation* **111**, 1999–2012 (2005).
6. Zhao, J. *et al.* The role of obesity-associated loci identified in genome-wide association studies in the determination of pediatric BMI. *Obesity (Silver Spring)* **17**, 2254–2257 (2009).
7. den Hoed, M. *et al.* Genetic susceptibility to obesity and related traits in childhood and adolescence: influence of loci identified by genome-wide association studies. *Diabetes* **59**, 2980–2988 (2010).
8. Scherag, A. *et al.* Two new loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and German study groups. *PLoS Genet.* **6**, e1000916 (2010).
9. Liu, W. *et al.* Olfactomedin 4 down-regulates innate immunity against *Helicobacter pylori* infection. *Proc. Natl. Acad. Sci. USA* **107**, 11056–11061 (2010).
10. Arslan, E., Atilgan, H. & Yavasoglu, I. The prevalence of *Helicobacter pylori* in obese subjects. *Eur. J. Intern. Med.* **20**, 695–697 (2009).
11. Abdullahi, M. *et al.* The eradication of *Helicobacter pylori* is affected by body mass index (BMI). *Obes. Surg.* **18**, 1450–1454 (2008).
12. Keren, D. *et al.* Sleeve gastrectomy leads to *Helicobacter pylori* eradication. *Obes. Surg.* **19**, 751–756 (2009).
13. Fu, M., Lui, V.C., Sham, M.H., Cheung, A.N. & Tam, P.K. *HOXB5* expression is spatially and temporarily regulated in human embryonic gut during neural crest cell colonization and differentiation of enteric neuroblasts. *Dev. Dyn.* **228**, 1–10 (2003).
14. Dankel, S.N. *et al.* Switch from stress response to homeobox transcription factors in adipose tissue after profound fat loss. *PLoS ONE* **5**, e11033 (2010).

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ONLINE METHODS

Research subjects. Descriptions of the individual cohorts are presented in the **Supplementary Note**. The discovery set for the meta-analysis consisted of 14 studies with BMI measured in childhood (age range from 2–18 years, except for ALSPAC, which also leveraged BMI data available from the first four clinical examinations before 2 years of age) and genome-wide genotype data available by the beginning of May 2010): the Avon Longitudinal Study of Parents and Children (ALSPAC; 976 cases and 1,244 controls); the Northern Finland 1966 Birth Cohort (NFBC1966; 700 cases and 521 controls); the British 1958 Birth Cohort–Type 1 Diabetes Genetics Consortium subset (B58C-T1DGC; 192 cases and 367 controls); the British 1958 Birth Cohort–Wellcome Trust Case Control Consortium Subset (B58C-WTCCC; 188 cases and 428 controls); the French Young Study (FRENCH YOUNG; 670 cases and 349 controls); the Lifestyle–Immune System–Allergy Study (LISA; 27 cases and 250 controls); the Western Australian Pregnancy Cohort Study (RAINE; 232 cases and 125 controls); the Children's Hospital of Philadelphia (CHOP; 1,445 cases and 2,802 controls); the Essen Obesity Study (ESSEN; 397 cases and 435 controls); the Helsinki Birth Cohort Study (HBCS; 261 cases and 403 controls); the Cardiovascular Risk in Young Finns Study (YF; 167 cases and 537 controls); the Copenhagen Study on Asthma in Childhood (COPSAC; 62 cases and 99 controls); the CM-GOYA Study (21 cases and 34 controls) and the Generation R Study (GENERATIONR; 192 cases and 724 controls).

The phenotypically comparable cohorts used for the replication effort were the Healthy Lifestyle in Europe by Nutrition in Adolescence Study (HELENA; 56 cases and 563 controls), the Young Hearts Studies (YH; 44 cases and 450 controls), the Lifestyle–Immune System–Allergy Study plus German Infant Study on the influence of Nutrition Intervention (LISA+GINI; 40 cases and 457 controls), the Children's Health Study (CHS; 311 cases and 330 controls), the Avon Longitudinal Study of Parents and Children (ALSPAC; 1,452 cases and 1,042 controls), the INfancia y Medio Ambiente (Environment and Childhood) Project (INMA; 55 cases and 213 controls), Project Viva (VIVA; 48 cases and 184 controls), the Prevention and Incidence of Asthma and Mite Allergy birth cohort study (PIAMA; 68 cases and 85 controls) and the Northern Finland 1986 Birth Cohort (NFBC1986; 744 cases and 759 controls). The two extreme obesity replication cohorts consisted of 705 German child-parent trios and the SCOOP-UK cohort (1,509 cases and 2,674 controls). Selected signals were further investigated in the GIANT Consortium[1] cohort, using the publically available GIANT Consortium data files (see URLs).

All cases and controls were of European ancestry. Cases were defined as having BMI >95th percentile at any point in childhood. Controls were defined as consistently having BMI <50th percentile throughout childhood for all measurements available for that individual. BMI percentiles were based on national standard growth curves, except in the HBCS and the NFBC1966, as pediatric measurements were made two decades ago, rendering contemporary curves not appropriate. HBCS and NFBC1966 generated their own reference curves. In addition, the density of data available longitudinally in the ALSPAC study gave rise to two differences in the definitions of cases and controls. First, this collection factored in data from subjects from the first four clinical examinations of childhood, thus using data from participants less than 2 years of age in the consideration of trait definition (sensitivity analyses considering the use of data from participants limited to being over the age of 2 years old are included; **Supplementary Tables 7–9**). Second, owing to the regularity of measures (11 measures available), controls in the ALSPAC sample were defined as those with BMI<50th percentile on at least 5 occasions. Known syndromic cases of obesity were excluded, as these individuals were likely to have a different underlying genetic architecture. Unless otherwise noted, all discovery sample analysis followed the same protocol and analysis plan.

Informed consent was obtained from all discovery and replication study participants (or parental consent, as appropriate), and study protocols were approved by the local ethics committees.

Statistical approaches. For the stage 1 meta-analysis of childhood obesity, we first performed statistical analyses within each discovery sample. Genotypes were obtained using high-density SNP arrays and then imputed for ~2.54 million HapMap Utah residents of Northern and Western European ancestry (CEU) SNPs (Phase 2, release 22). Before imputation, we excluded SNPs with a Hardy-Weinberg equilibrium P value (HWEP) $<1.0 \times 10^{-6}$, call rate of <95% or minor allele frequency (MAF) of <1%. After imputation, SNPs imputed with IMPUTE were excluded if proper info was <0.40, and SNPs imputed with MACH were excluded if r2hat was <0.30. SNPs were also excluded after imputation if the MAF was <1%. The association between each SNP and case-control status was assessed in each study sample using logistic regression of case-control status against genotype, assuming an additive model and taking into account genotype uncertainty. Imputed genotypes were only used where directly assayed genotypes were unavailable. Unless otherwise stated, all discovery analysis followed the same protocol.

For the meta-analysis of discovery samples, SNPs with a MAF of <1% and poorly imputed SNPs (proper_info of ≤0.4 (SNPTEST) or r^2 of ≤0.3 (MACH2QTL)) were filtered out before meta-analysis. Fixed-effects meta-analyses were conducted by two independent investigators. Meta-analysis was performed using the METAL software package. Genomic control[15] was applied to each cohort before meta-analysis. Meta-analysis was carried out using the inverse-variance method, and a fixed-effects model was assumed. SNPs available for less than half of the total expected sample were excluded. We used the Cochran Q test to assess evidence of between-study heterogeneity of effect sizes.

A total of 2.7 million SNPs were analyzed in the meta-analysis unfiltered for the number of cohorts in which they were available. Seven SNPs reached genome-wide significance, all of which were reported previously in the adult BMI GWAS[1,16]. Those loci that were below a P-value threshold of $<5 \times 10^{-6}$ in the discovery meta-analysis and were not identified with obesity-related traits before ($n = 8$) were considered for further analysis in additional samples.

For stage 2 follow-up studies, we used nine study samples representing a comparable data set (combined $n = 2,818$ cases and 4,083 controls) and two study samples representing an extreme obesity data set (combined $n = 2,214$ cases and 2,674 controls) to follow up the eight newly identified signals from the GWAS discovery meta-analysis (represented by index SNPs rs2300095, rs4833407, rs4864201, rs28636, rs1290002, rs9568856, rs9299 and rs17697518). If the index SNP was unavailable, the most closely correlated proxy SNP that was available was substituted. In four of the replication studies, the index SNPs were imputed from genome-wide genotype data.

We performed fixed-effects inverse-variance meta-analyses of the association results for the 8 lead signals in the 14 discovery samples and the 9 comparable replication samples. We subsequently did the same with the 14 discovery samples combined with the 2 extreme cohorts. Lastly, we combined all data sets for the final overall meta-analysis. Fixed-effects meta-analyses were conducted independently by two investigators, again using METAL.

15. Devlin, B. & Roeder, K. Genomic control for association studies. *Biometrics* **55**, 997–1004 (1999).
16. Willer, C.J. *et al.* Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat. Genet.* **41**, 25–34 (2009).

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