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

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Abstract

Multiple genetic variants have been associated with adult obesity and a few with severe obesity in childhood; however, less progress has been made to establish genetic influences on common early-onset obesity. We performed a North American-Australian-European collaborative meta-analysis of fourteen 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 novel signals yielding association with $P < 5 \times 10^{-6}$ in to nine independent datasets ($n = 2,818$ cases and 4,083 controls) we observed two loci that yielded a genome wide significant combined P -value, namely near *OLFM4* on 13q14 (rs9568856; $P=1.82 \times 10^{-9}$; OR=1.22) and within *HOXB5* on 17q21 (rs9299; $P=3.54 \times 10^{-9}$; OR=1.14). Both loci continued to show association when including two extreme childhood obesity cohorts ($n = 2,214$ cases and 2,674 controls). Finally, these two loci yielded directionally consistent associations in the GIANT meta-analysis of adult BMI¹.

Obesity is the 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 life². 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 of obesity. This is reflected in familial occurrences of

⁷²A full list of members is provided in the Supplementary Note.

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childhood obesity, where concordance for fat mass among monozygotic twins is reported to be higher than in dizygotic twins.

In the past four years, many genetic loci have been implicated for body mass index (BMI) / obesity from the outcomes of genome-wide association studies (GWAS), primarily in adults. The first locus reliably found to harbor variation associated with adiposity, the fat mass- and obesity-associated gene (*FTO*)³, has been shown subsequently to be associated with obesity in all sufficiently sized study groups. Subsequent larger studies have revealed additional BMI/obesity genes. The largest meta-analysis of adult BMI to date came from the GIANT consortium, which confirmed fourteen known obesity susceptibility loci and revealed eighteen new loci associated with BMI in a study involving a total of 249,796 individuals¹. 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 more relevant to public health.

We reasoned that distillation of the genetic component in this complex phenotype should be easier in children, where environmental exposure and impact has been for a relatively short period of their lifetime. The relationship between BMI and body fat in children varies widely with age and with pubertal maturation. The Center for Disease Control and Prevention defined overweight as at or above the 95th percentile of BMI for age⁴. By late adolescence, these percentiles approach those used for adult definitions; the 95th percentile is then approximately 30 kg/m²⁵.

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

Following 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 been previously reported within 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 while *TNNI3K* and *POMC*, which were only detected in adult studies when using hundreds of thousands of participants, were readily detected in our relatively small sample size (Figure 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 they defined childhood obesity was at a higher threshold⁸, at the loci harboring *TNKS-MSRA* (rs17150703; $P = 0.22$) and *SDCCAG8* (rs12145833; $P = 0.57$).

We took forward all eight novel signals yielding association with $P < 5.0 \times 10^{-6}$ (Table 1 and Supplementary Table 4, the latter of which includes a heterogeneity analysis showing that the different distributions in each study did not affect our results; in addition Supplementary

Table 5 shows the results after applying a second genomic control correction to the overall discovery meta-analysis results) in order to test for replication in multiple independent existing datasets, the majority of which were *in silico* analyses. In our replication effort, we initially tested these eight SNPs in nine study groups that had a comparable set of affected subjects i.e. BMI distributed normally from the 95th percentile upwards (n = 2,818 cases and 4,083 controls). From this attempt we observed two loci that yielded consistent evidence of association when combined with the discovery cohort, namely near olfactomedin 4 (*OLFM4*) on 13q14 (rs9568856; $P_{combined} = 1.82 \times 10^{-9}$; OR = 1.22) and within the gene encoding homeobox B5 (*HOXB5*) on 17q21 (rs9299; $P_{combined} = 3.54 \times 10^{-9}$; OR = 1.14) (Table 1; see also the regional plots in Supplementary Figures 1 and 2 for the discovery meta-analysis data).

Previous GWAS reports for extreme obesity case-control samples have demonstrated both confirmation of signals seen in less extreme or population based samples, such as *FTO*, as well as novel signals that are distinct from those seen at the population level⁸. We reasoned therefore that further exploration in existing extreme datasets [two cohorts totaling 2,214 cases (exclusively individuals approximately >4 standard deviations above the mean, equating to BMI >99.5th percentile) and 2,674 controls] would offer further insight in to how these signals operate, acknowledging the phenotypic differences and limits of sample size. Indeed, both loci emerging from the main replication step continued to show association folding in this 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 ALSPAC cohort leveraged BMI measures made before the age of two years old as part of defining cases and controls, we ran sensitivity analyses limiting case and control definitions to children over two years of age (Supplementary Tables 7–9). In addition to no diminishment in the odds ratios for the *OLFM4* and *HOXB5* loci, we observed support for rs4864201 at *BC041448* and rs4833407 at *ALPK1* (Supplementary Tables 8 and 9).

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

Overall, these data indicate that the genetic architecture of BMI and obesity overlap to a large extent in children as well as 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 GIANT¹ reveals that the influence of these two loci is also detected in adulthood. Interestingly, in addition to *OLFM4* and *HOXB5*, GIANT also supports an association with three more of the eight loci initially taken forward in to the replication effort, namely rs4864201 at *BC041448*, rs4833407 at *ALPK1* and rs2300095 at *MTORANGPTL7* loci, despite these signals not formally replicating in the main defined

overall pediatric setting, suggesting that these loci should be followed up further to fully understand their role in the pathogenesis of obesity as a whole.

The gene encoding olfactomedin 4 (*OLFM4*) is the nearest gene to rs9568856 but is still approximately 500kb from the associated signal; the gene product has never been directly implicated in obesity but has been extensively studied in the context of various cancers. *OLFM4* 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 a relationship between the gut microbiome and obesity risk. For example, the *OLFM4* gene product down regulates innate immunity against infection by the stomach bacterium, *Helicobacter pylori*⁹, with obese subjects having a higher occurrence of *Helicobacter pylori* infection than lean counterparts^{10,11}; indeed, weight-loss induced by obesity surgery eradicates *Helicobacter pylori*¹².

rs9299 is in the 3' untranslated region of the gene encoding homeobox B5 (*HOXB5*) within a homeobox B cluster. *HOXB5* is spatially and temporarily regulated during gut development¹³, but its role in obesity has been suggested by a study observing up-regulation of homeobox transcription factors after fat loss¹⁴. Taken together, 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-European collaborative genome-wide meta-analyses on children, we have uncovered two novel obesity loci which have their strongest evidence for association with elevated adiposity in the first eighteen years of life. Further functional characterization of these signals is required to elucidate the precise mechanism behind these observations.

Supplementary Material

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British 1958 Birth Cohort (B58C)

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Helsinki Birth Cohort Study (HBCS)

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Copenhagen Study on Asthma in Childhood (COPSAC)

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CM-GOYA study (CM-GOYA)

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Generation R Study (GENERATIONR)

The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam. The generation and management of GWAS genotype data for the Generation R Study were done at the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, The Netherlands. We would like to thank Karol Estrada, Dr. Tobias A. Knoch, Anis Abuseiris, Luc V. de Zeeuw, and Rob de Graaf, for their help in creating GRIMP, BigGRID, MediGRID, and Services@MediGRID/D-Grid, (funded by the German Bundesministerium fuer Forschung und Technology; grants 01 AK 803 A-H, 01 IG 07015 G) for access to their grid computing resources. We thank Mila Jhamai, Manoushka Ganesh, Pascal Arp, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters for their help in creating, managing and QC of the GWAS database. Also, we thank Karol Estrada and Carolina Medina-Gomez for their support in creation and analysis of imputed data. The Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw 21000074). Vincent Jaddoe received an additional grant from the Netherlands Organization for Health Research and Development (ZonMw 90700303, 916.10159). Additional support was provided by a grant from the Dutch Kidney Foundation (C08.2251).

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SCOOP-UK (Severe Childhood Onset Obesity Project United Kingdom)

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Infancia y Medio Ambiente [Environment and Childhood] Project (INMA)

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Project Viva (VIVA)

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Prevention and incidence of asthma and mite allergy birth cohort study (PIAMA)

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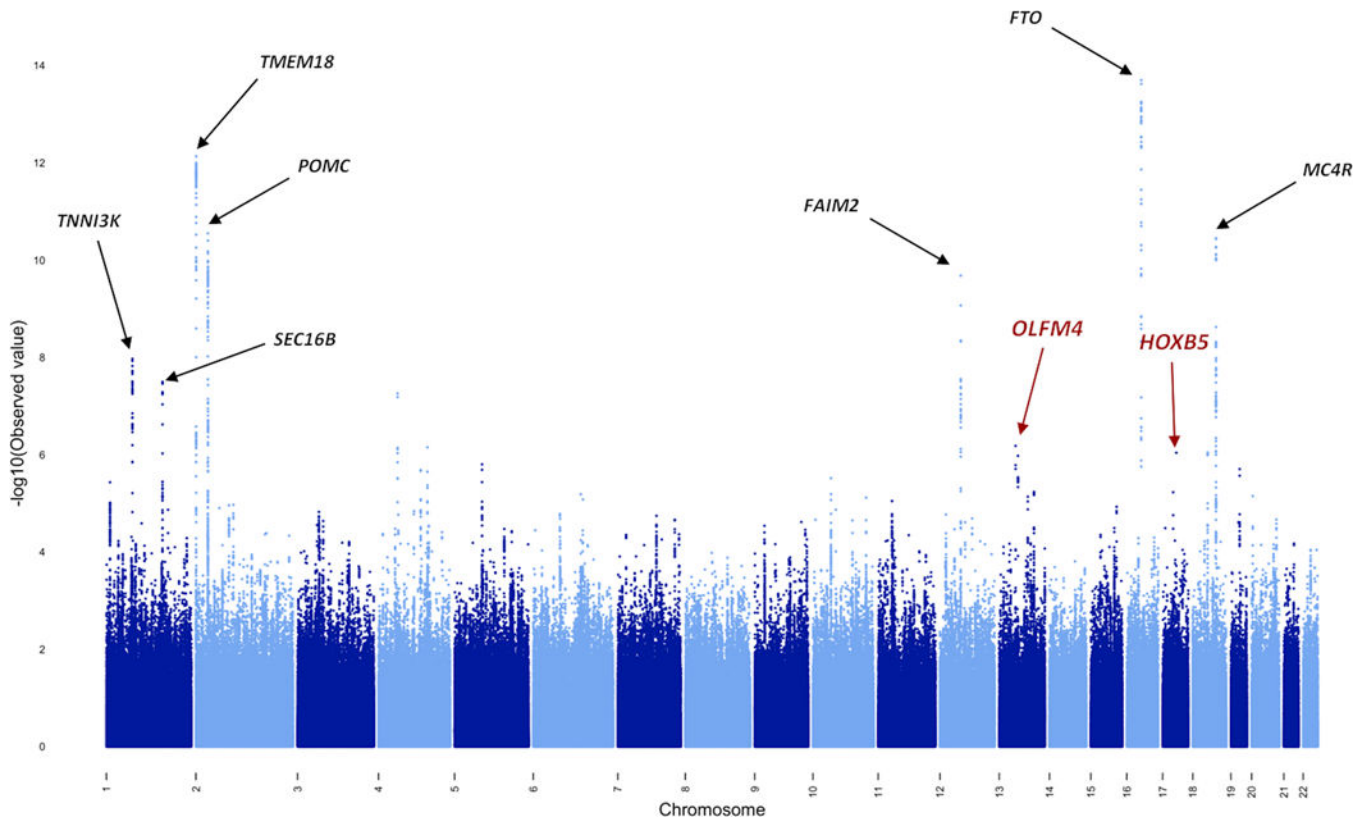


Figure 1.

Manhattan Plot of the meta-analysis of childhood obesity GWAS runs in the discovery stage (5,530 cases and 8,318 controls), with each locus achieving genome wide significance ($P < 5 \times 10^{-8}$) indicated in black text. In addition, the novel loci uncovered in this study are indicated in red text.

Table 1
The two key novel loci established to be associated with common early-onset obesity

They did not reach genome wide significance but yielded $P < 5 \times 10^{-6}$ in the discovery stage (5,530 cases and 8,318 controls). The outcome of the replication effort of these loci taken forward in to nine comparable independent cohorts (n = 2,818 cases and 4,083 controls) is also indicated. Separate discovery and replication data plus combined data are shown, with the latter indicating genome wide significance in both instances.

Locus	SNP	Allele1/2	Nearest Gene	Direction	OR [95% C.I.]	P-value
Discovery	13q14	rs9568856	<i>OLFM4</i>	+++++	1.210 [1.123, 1.305]	6.58×10^{-7}
	17q21	rs9299	<i>HOXB5</i>	+++++	1.144 [1.084, 1.207]	9.12×10^{-7}
Replication	13q14	rs9568856	<i>OLFM4</i>	+++++	1.225 [1.089, 1.378]	7.13×10^{-4}
	17q21	rs9299	<i>HOXB5</i>	+++++	1.145 [1.056, 1.242]	0.00104
Combined	13q14	rs9568856	<i>OLFM4</i>	+++++	1.215 [1.140, 1.294]	1.82×10^{-9}
	17q21	rs9299	<i>HOXB5</i>	+++++	1.144 [1.094, 1.196]	3.54×10^{-9}

Discovery cohorts

1. Avon Longitudinal Study of Parents and Children (ALSPAC)
2. Northern Finland 1966 Birth Cohort (NFBC1966)
3. British 1958 Birth Cohort – Type 1 Diabetes Genetics Consortium subset (B58C-TIDGC)
4. British 1958 Birth Cohort – Wellcome Trust Case Control Consortium Subset (B58C-WTCCC)
5. French Young study (FRENCH YOUNG) PCA adjusted
6. Lifestyle Immune System Allergy Study (LISA)
7. Western Australian Pregnancy Cohort study (RAINE)
8. Children's Hospital of Philadelphia (CHOP) PCA adjusted
9. Essen Obesity Study (ESSEN) PCA adjusted
10. Helsinki Birth Cohort Study (HBCS)
11. Cardiovascular Risk in Young Finns Study (YF)
12. Copenhagen Study on Asthma in Childhood (COPSAC)
13. CM-GOYA study (CM-GOYA)
14. Generation R Study (GENERATIONR)

Comparable replication cohorts

1. Healthy Lifestyle in Europe by Nutrition in Adolescence study (HELENA)

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2. Young Hearts studies
3. Lifestyle – Immune System – Allergy Study plus German Infant Study on the influence of Nutrition Intervention LISA+GINI)
4. Children’s Health Study (CHS)
5. INfancia y Medio Ambiente [Environment and Childhood] Project (INMA) (2 proxies used - rs965013 and rs11099020 for rs4833407)
6. Project Viva (VIVA)
7. Prevention and incidence of asthma and mite allergy birth cohort study (PIAMA)
8. Northern Finland 1986 Birth Cohort (NFBC1986) (2 proxies used - rs10779751 for rs230095; rs4883723 for rs9568856)
9. Avon Longitudinal Study of Parents and Children ALSPAC)