Common genetic variation in obesity, lipid transfer genes and risk of Metabolic Syndrome: Results from IDEFICS/I.Family study and meta-analysis

Rajini Nagrani1✉, Ronja Foraita1, Francesco Gianfagna2,3, Licia Iacoviello4, Staffan Marild5, Nathalie Michels6, Dénes Molnár7, Luis Moreno8, Paola Russo9, Toomas Veidebaum10, Wolfgang Ahrens1,11 & Manuela Marron1

As the prevalence of metabolic syndrome (MetS) in children and young adults is increasing, a better understanding of genetics that underlie MetS will provide critical insights into the origin of the disease. We examined associations of common genetic variants and repeated MetS score from early childhood to adolescence in a pan-European, prospective IDEFICS/I.Family cohort study with baseline survey and follow-up examinations after two and six years. We tested associations in 3067 children using a linear mixed model and confirmed the results with meta-analysis of identified SNPs. With a stringent Bonferroni adjustment for multiple comparisons we obtained significant associations ($p < 1.4 \times 10^{-4}$) for 5 SNPs, which were in high LD ($r^2 > 0.85$) in the 16q12.2 non-coding intronic chromosomal region of FTO gene with strongest association observed for rs8050136 ($\beta = 0.31, p_{Wald} = 1.52 \times 10^{-5}$).

We also observed a strong association of rs708272 in CETP with increased HDL ($p = 5.63 \times 10^{-40}$) and decreased tRG ($p = 9.60 \times 10^{-5}$) levels. These findings along with meta-analysis advance etiologic understanding of childhood MetS, highlighting that genetic predisposition to MetS is largely driven by genes of obesity and lipid metabolism. Inclusion of the associated genetic variants in polygenic scores for MetS may prove to be fundamental for identifying children and subsequently adults of the high-risk group to allow earlier targeted interventions.

A collection of risk factors, including central obesity, insulin resistance, dyslipidemia, and hypertension, describes metabolic syndrome (MetS). Additionally, MetS is a known precursor in cardiovascular disease development1. MetS has become a major public health concern globally due to its increasing prevalence and association with various chronic diseases2. MetS etiology is quite complex, involving a strong interplay between multiple genetic, environmental and lifestyle-related factors. In European ancestry, the heritability of the MetS was estimated to be between 13–30%3,4. The early prognosis of MetS is therefore extremely valuable for early detection of individuals at high genetic risk of developing the disease later in life and for encouraging change in lifestyle to reduce risk. While numerous single nucleotide polymorphisms (SNPs) associated with individual metabolic components and diseases have been reported in genome-wide association studies (GWAS)5–8, the effect of these polymorphisms on the MetS network and related diseases is not well studied.

1Leibniz Institute for Prevention Research and Epidemiology – BIPS, Bremen, Germany. 2Mediterranea Cardiocentro, Napoli, Italy. 3EPIMED Research Center, Department of Medicine and Surgery, University of Insubria, Varese, Italy. 4IRCIS Istituto Neurologico Mediterraneo Neurumedi, Pozzilli, Italy. 5Department of Paediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 6Department of Public Health and Primary Care, Ghent University, 9000, Ghent, Belgium. 7Department of Paediatrics, Medical School, University of Pécs, Pécs, Hungary. 8GENUD (Growth, Exercise, Nutrition, and Development) Research Group, University of Zaragoza, Zaragoza, Spain. 9Institute of Food Sciences, National Research Council, Avellino, Italy. 10National Institute for Health Development, Tallinn, Estonia. 11Institute of Statistics, Faculty of Mathematics and Computer Science, Bremen University, Bremen, Germany. ✉e-mail: rajni.nagrani@gmail.com
Further, of all MetS components, lipid levels seem under higher genetic determination. This has also been observed in the genetic association studies suggesting that genetic effects on lipid levels are more pronounced than for other traits. Most of the genetic association studies for MetS have been conducted in adult populations and are limited by the usage of one-point measurements. As the prevalence of MetS in children and young adults is increasing, a better understanding of the genetics that underlies MetS throughout childhood and adolescence will provide critical insights into the origin of the disease. We performed a longitudinal analysis using a repeated measurement design for the effect of genetic variants on a quantitative MetS score from early childhood to adolescence. We examined the association between 350 pre-selected variants and the MetS score derived from measured waist circumference (WC), high-density lipoprotein (HDL), homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides (TRG), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in a pan-European children cohort.

**Methodology**

**Study population.** The study population was enrolled in a pan-European, multi-center, prospective IDEFICS/I.Family cohort across three-time points. The IDEFICS baseline survey included a population-based sample of 16,229 children aged 2 to 9.9 years from eight European countries (Belgium, Cyprus, Estonia, Germany, Hungary, Italy, Spain, and Sweden) who were examined the first time in 2007/2008. Follow-up examinations were conducted after two (T1) and six (T3, I.Family study) years. In our longitudinal analysis using repeated measurement design, both baseline and follow-up data from the IDEFICS and I.Family study were included from all countries except Cyprus, for understanding the associations of genetic variants with MetS. In the IDEFICS/I.Family study, risk factors of lifestyle-related outcomes were investigated in young children and anthropometric and clinical examinations were conducted at each survey wave. Additionally, health characteristics and lifestyle behaviors were collected and biosamples were taken (Details in Supplementary methods). Parents gave written informed consent before study participation and children gave oral consent before the examinations. Ethical approval was obtained from the relevant local or national ethics committees by each of the study centers, namely from the Ethics Committee of the University Hospital Ghent (Belgium), the Tallinn Medical Research Ethics Committee of the National Institutes for Health Development (Estonia), the Ethics Committee of the University Bremen (Germany), the Scientific and Research Ethics Committee of the Medical Research Council Budapest (Hungary), the Ethics Committee of the Health Office Avellino (Italy), the Ethics Committee for Clinical Research of Aragon (Spain), and the Regional Ethical Review Board of Gothenburg (Sweden). We certify that all applicable institutional and governmental guidelines and regulations concerning the ethical use of human volunteers were followed during this research.

**MetS Score.** There are no universal definitions of MetS in children, we have, therefore, utilized a continuous MetS score as documented in a recent publication on the IDEFICS study. The MetS score was calculated summing age and sex-specific z-scores of WC, HOMA-IR, HDL, TRG, SBP, and DBP according to the following formula by Ahrens et al.: 

\[
\text{MetS score} = z_{WC} + \frac{z_{SBP} + z_{DBP}}{2} + \frac{z_{TRG} - z_{HDL}}{2} + z_{HOMA-IR}
\]

The components used to calculate the MetS score were based on the same risk factors used in the adult MetS definition. A higher score was associated with an unfavorable metabolic profile. A detailed description of the measurements of components of MetS has been published previously.

**Genotyping and quality control of SNP data.** Genomic DNA was extracted either from saliva or blood samples. Genotyping was conducted in two batches on 3492 children using the UK Biobank Axiom 196–Array from Affymetrix (Santa Clara, USA). We applied extensive quality control metrics to the data following the recommendations of Weale M, based on which we excluded the following: SNPS with a call rate of less than 97.5%, failure to meet Hardy-Weinberg equilibrium at a p-value of less than $10^{-4}$, a minor allele frequency (MAF) of less than 0.5% (batch 1) and 0.08% (batch 2), samples with a call rate of less than 98% (batch 1) and 96% (batch 2), poor intensity, sex mismatch, anomalous high heterozygosity (cut-off of 3 standard deviations (SD) from mean), cryptic relatedness, no phenotypic information or as population outliers with any of a sample’s standardized principal component (PC) loading exceeds the interval mean ±3 SD. We did quality control filtering using Affymetrix calling software APT and the R packages genABEL and SNPRelate. A sample of 3067 children remained for further analyses. Genome-wide imputation was carried out using the Minimac3 v2.0.1 software and reference haplotypes from unrelated individuals from the 1000 Genomes Project phase III v5.

To address the issue of population stratification, we performed a detailed genetic relatedness analysis using the SNPRelate v1.10.2 R package, where the eigenvectors or PCs are sorted in decreasing order of the corresponding eigenvalues. The first eigenvector (PC1) has the most variation in the data on the genetic matrix (SNP by sample); the second eigenvector (PC2) has the second-most, and so on. To account for relatedness in our sample, we calculated the genetic relatedness matrix (GRM) from the genotype data using the program EMMAX v20120210. The GRM matrix along with relatedness further adjusts for population stratification.

**Selection of candidate SNPs.** A custom panel of SNPs were selected for analysis in this study using the following three strategies: (a) SNPs significantly associated in previous GWAS studies (p < 5 × 10^{-8}) with MetS were identified using NHGRI-EBI GWAS Catalog and PubMed search (n = 29); (b) All SNP from candidate studies which were significantly associated (p < 0.05) with MetS were included using SNP curator platform (n = 193); (c) genes associated with MetS (using DisGenet browser) and involved in lipid metabolism pathway (CTDbase)
The characteristics of study participants were presented as means (± SD) for continuous variables and as frequencies (percentages) for categorical variables. Associations between SNPs and repeated MetS score values of non-independent individuals were analyzed using the Wald \( t \)-test with one degree of freedom applied on linear mixed models (LMM), using the R package GMMAT\(^{30} \) adjusting for age, sex, country of residence and the top five PCs as fixed effects, and using a kinship matrix to define the covariance structure of the random effect included in the model.

To account for multiple testing, we corrected the statistical significance level to \( \alpha = 0.05/350 = 1.4 \times 10^{-4} \) by the Bonferroni correction and false discovery rate (FDR) method for the 350 hypothesis tests. For further analysis, we presented results for only those SNPs that survived the FDR correction.

We stratified association models by sex, controlling for age, country of residence, first five PCs and kinship matrix. Additionally, we performed conditional analyses on the FTO locus rs8050136 as a covariate. To identify the driving factor in the association of SNPs and MetS, we recalculated the LMM with each of the MetS components: WC, HOMA-IR, HDL, TRG, FTO, SBP, and DBP. Throughout, we used \( \lambda \) to report LD between pairs of SNPs. Quantile-quantile (Q-Q) plots and the genomic inflation factor (\( \lambda \)) were used to evaluate control of type I error. LocusZoom\(^{31} \) was used to plot regions harboring significant signals (\( p < 1.4 \times 10^{-4} \)) to visualize LD patterns. Statistical analyses were performed using R 3.5.3 and Stata 15. All statistical tests were two-sided.
Table 1. Study characteristics at baseline. BMI = body mass index, DBP = diastolic blood pressure, HDL = high density lipoprotein, HOMA-IR = homeostasis model assessment of insulin resistance, SBP = systolic blood pressure, SD = standard deviation, TRG = triglycerides, WC = waist circumference. N stated in case of missingness.

| Characteristics               | Mean (±SD)/n (%)N = 3067 |
|-------------------------------|---------------------------|
| Girls                         | 1535 (50.05)              |
| No. of children               |                           |
| \(T_0\)                       | 2987 (35.05)              |
| \(T_1\)                       | 2907 (34.12)              |
| \(T_3\)                       | 2627 (30.83)              |
| New children enrolled at \(T_1\) | 80 (2.61)                |
| Age (years)                   | 6.20 (±1.77)              |
| Study Region                  |                           |
| Italy                         | 644 (21.00)               |
| Estonia                       | 299 (9.75)                |
| Belgium                       | 214 (6.98)                |
| Sweden                        | 434 (14.15)               |
| Germany                       | 634 (20.67)               |
| Hungary                       | 461 (15.03)               |
| Spain                         | 381 (12.42)               |
| BMI categories by Cole \textit{et al}, 2012 |          |
| Thinness grade 1–3            | 305 (9.94)                |
| Normal weight                 | 2162 (70.49)              |
| Overweight/obese              | 600 (19.56)               |
| SBP (mmHg), n = 2965          | 100.44 (±9.07)            |
| DBP (mmHg), n = 2966          | 63.26 (±6.39)             |
| WC (cm), n = 3010             | 54.44 (±7.03)             |
| HOMA-IR, n = 1946             | 0.92 (±0.74)              |
| TRG (mg/dl), n = 2636         | 57.62 (±25.94)            |
| HDL (mg/dl), n = 2640         | 52.51 (±14.28)            |
| Metabolic Syndrome Score, n = 1845 | 0.21 (±2.65)         |
| Relatedness                   |                           |
| 1st degree (sharing ≥ 50% DNA)| 141 (4.59)                |
| 2nd degree (sharing < 50 to ≥ 25% DNA) | 188 (6.12)            |
| Distant relation (sharing < 25 to ≥ 1% DNA) | 2728 (88.94)        |

Table 2. Association of markers with longitudinal Metabolic Syndrome score in children of IDEFICS/I.Family study. \(\beta\) = estimated coefficient, Chr = chromosome, EAF = effect allele frequency, FDR = false discovery rate, SNP = single nucleotide polymorphism, SE = standard error. The effect allele is the allele corresponding to the calculated risk. Adjusted for age, sex, country of residence, first five principal components as fixed effects and kinship matrix to define the covariance structure of the random effect. SNPs significant after Bonferroni correction are marked in bold. *imputed SNPs.
To identify potential causal genes explaining the observed genetic associations with MetS, we searched for existing expression quantitative trait loci (eQTL) SNPs in the eQTL dataset GTEx V8. We estimated the associations between the identified lead SNP and transcript expression levels for genes within a +/− 1 Mb cis window around the transcription start site or a trans-gene.

In-silico functional analysis. We examined the potential functional significance of the SNPs that reached the significance level using the combined annotation-dependent depletion (CADD) method proposed by Kircher and colleagues. CADD produces a single C score to measure the deleteriousness of a given variant, which will greatly improve in prioritizing the causal variants while conducting genetic analyses. We also extracted the RegulomeDB score to describe the regulatory potential of these SNPs.

Meta-analysis Crude ORs and 95% CIs in each study were estimated using a genetic additive model and evaluated for the strength of the associations between FTO variants and MetS risk. The study reported additive ORs were utilized when sufficient information on genotypic/allelic frequencies were not provided. Study-specific risk estimates were pooled by using random-effects meta-analyses and sensitivity analyses were performed using fixed-effect meta-analyses. To determine whether the genotypes in the control group deviated from Hardy-Weinberg Equilibrium (HWE) we used the R-package HardyWeinberg. Heterogeneity was assessed using the standard \( \chi^2 \) tests and \( I^2 \) statistic, where \( I^2 > 50\% \) indicated substantial heterogeneity. Evidence of publication bias was sought using the Egger regression test for funnel asymmetry in addition to visual inspection of the funnel plots. Two-sided \( P \) values < 0.05 were considered statistically significant.

Results After quality control and analytical exclusions, we performed longitudinal analyses with genotypic information on 350 SNPs and repeated measures on study calculated MetS Scores from 3067 children at 3-time points (Fig. 1). Boys and girls were equally present in the analysis with a mean age of 6.20 (±1.77). Almost 5% of study participants were first degree relatives (Table 1).

MetS score was not available for 314 study participants in any survey. In total, 2,753 children were utilized for the main analysis to test the association between pre-selected candidate SNPs and longitudinal MetS score; however, we made use of all children to test SNP effects on the components of the MetS score. Details of exclusions are shown in the appendix (Supplementary Table 1). A genomic control factor \( \lambda \) of 1.22 in the Q-Q plot of the association \( p \)-values suggested slight systematic inflation (Supplementary Fig. 1). The first five PCs explain only 1% of variance suggesting there may be no hidden pattern in the dataset (Supplementary Fig. 2).

Our results yielded significant associations for 13 SNPs with \( p \)-values corrected for FDR (Table 2). With a stringent Bonferroni adjustment for multiple comparisons, we obtained significant associations (\( p < 1.4 \times 10^{-4} \))
Table 3. Association of markers with longitudinal Metabolic Syndrome stratified by sex. ß = estimated coefficient, Chr = chromosome, EAF = effect allele frequency, FDR = false discovery rate, PVAL = p-value, SNP = single nucleotide polymorphism, SE = standard error. The effect allele is the allele corresponding to the calculated risk. Adjusted for age, sex, country of residence, first five principal components as fixed effects and kinship matrix to define the covariance structure of the random effect. The results here are presented for the markers that reached statistical significance after correction for FDR in the main analysis in Table 2.

| Locus | Chr | SNP ID | Effect allele | Boys | Girls |
|-------|-----|--------|---------------|------|-------|
| FTO | 16q12.2 | rs8050136 | A | 0.42 | 0.33 (0.10) | 0.001 | 0.42 | 0.29 (0.10) | 0.004 |
| FTO | 16q12.2 | rs1121980 | A | 0.44 | 0.37 (0.10) | <0.001 | 0.45 | 0.25 (0.10) | 0.012 |
| FTO | 16q12.2 | rs1558902 | A | 0.43 | 0.32 (0.10) | 0.001 | 0.43 | 0.28 (0.10) | 0.005 |
| FTO | 16q12.2 | rs9939609 | A | 0.42 | 0.30 (0.10) | 0.002 | 0.42 | 0.30 (0.10) | 0.004 |
| FTO | 16q12.2 | rs1421085 | C | 0.43 | 0.32 (0.10) | 0.001 | 0.43 | 0.28 (0.10) | 0.006 |
| FTO | 16q12.2 | rs8057044 | A | 0.49 | 0.33 (0.10) | 0.001 | 0.49 | 0.21 (0.10) | 0.043 |
| CETP | 16q13 | rs708272 | A | 0.41 | −0.32 (0.10) | 0.002 | 0.41 | −0.18 (0.10) | 0.072 |
| FTO | 16q12.2 | rs8044769 | T | 0.46 | −0.24 (0.10) | 0.015 | 0.46 | −0.24 (0.10) | 0.014 |
| SGCGA3 | 15q13.2 | rs3764220 | G | 0.0004 | 7.13 (2.42) | 0.003 | 0.0004 | 3.74 (2.79) | 0.180 |
| FTO | 16q12.2 | rs17817288 | A | 0.48 | −0.29 (0.10) | 0.004 | 0.48 | −0.18 (0.10) | 0.074 |
| FTO | 16q12.2 | rs8047395 | G | 0.46 | −0.35 (0.10) | 0.001 | 0.47 | −0.13 (0.10) | 0.210 |
| ACACB | 12q24.11 | rs2075260 | G | 0.17 | −0.27 (0.13) | 0.045 | 0.18 | −0.33 (0.13) | 0.010 |
| GNPDA2 | 4p12 | rs10938397 | G | 0.39 | 0.27 (0.12) | 0.022 | 0.42 | 0.26 (0.11) | 0.025 |
for 5 SNPs, which were highly correlated in the 16q12.2 chromosomal region in the non-coding intronic region of the FTO gene. The SNPs located in FTO gene were in high LD ($r^2 > 0.87$), with the strongest association signal observed for rs8050136 ($P_{\text{wald}} = 1.52 \times 10^{-5}$) (Fig. 2). In LMMs conditioned on rs8050136, the risk of other variants in 16q12.2 was completely attenuated and non-significant (Supplementary Table 2). We could not replicate...
| Author                  | Sample Size | MetS cases (n) | Controls (n) | FTO variants | Criteria for MetS | Ethnicity/Study Location     | Population Type | Study Quality, NOS |
|------------------------|-------------|----------------|--------------|--------------|-------------------|-----------------------------|-----------------|-------------------|
| Ahmad, 2010            | 21674       | 4775           | 16899        | rs8050136    | modified NCEP ATP III | White women                | Health professionals from an RCT | 9                |
| Al-Attar, 2008         | 2121        | 474            | 1647         | rs9939609    | IDF, NCEP ATP III  | Canadians of multi-ethnic origin | General          | 7                |
| Armamento-Villareal, 2016 | 165      | 53             | 112          | rs8050136    | JIS               | Caucasians                 | Obese older adults               | 6                |
| Attaoua, 2009          | 119         | 34             | 85           | rs1421085    | NCEP ATP III      | Caucasians                 | Obese women                      | 7                |
| Attaoua, 2008          | 207         | 75             | 132          | rs1421085    | NCEP ATP III      | Caucasians                 | Patients of PCOS                   | 6                |
| Baik, 2012             | 4590        | 1487           | 3103         | rs9939609    | AHA/NHLBI         | Korean                      | General                       | 9                |
| Chedraui, 2016         | 192         | 103            | 89           | rs9939609    | AHA/NHLBI         | Ecuador                     | postmenopausal women                      | 9                |
| Cheung, 2011           | 1446        | 225            | 1221         | rs8050136    | JIS               | Hong Kong                   | General                       | 9                |
| Col, 2017              | 100         | 60             | 40           | rs9939609    | NCEP ATP III      | Caucasians in Turkey       | Obese adolescents                | 6                |
| Cruz, 2010             | 936         | 389            | 547          | rs9939609    | AHA/NHLBI         | Mexico                      | Blood donors without a family history of diabetes | 7                |
| de Lui, 2013           | 457         | 186            | 271          | rs9939609    | NCEP ATP III      | Caucasians                 | Obese females                   | 6                |
| Dusatkova, 2013        | 1443        | 111            | 1332         | rs9939609    | IDF               | Czech adolescents          | overweight, normal, overweight and obese adolescents | 9                |
| Elouej, 2016           | 685         | 340            | 345          | rs9939609, rs1421085 | IDF               | Tunisian                   | General                       | 9                |
| Fawwad, 2015           | 296         | 194            | 102          | rs9939609    | IDF, NCEP ATP III  | Pakistan                    | Patients of Type 2 diabetes       | 7                |
| Freathy (NBFC1966), 2008 | 4423        | 293            | 4130         | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Freathy (Oxford Biobank), 2008 | 1149    | 169            | 980          | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Freathy (Caerphilly), 2008 | 1046       | 216            | 830          | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Freathy (UKT2D GCC Controls), 2008 | 1858     | 299            | 1559         | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Freathy (BWHS), 2008   | 3191        | 1449           | 1742         | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Freathy (InChianti), 2008 | 888        | 250            | 638          | rs9939609    | NCEP ATP III      | European                    | General                       | 8                |
| Guçu-Geyik, 2016       | 1967        | 923            | 1044         | rs1421085, rs9939609 | NCEP ATP III | Turkish                    | General                       | 9                |
| Horta, 2011            | 1677        | 1096           | 581          | rs1121980, rs1421085, rs1558902, rs8050136, rs9939609 | study-specific | Japanese                   | Hospital based                | 5                |
| Hu, 2015               | 489         | 245            | 244          | rs1421085, rs9939609 | IDF               | Kazakh adults of Xinjiang, China | General                       | 9                |
| Khella, 2017           | 197         | 92             | 105          | rs9939609    | IDF               | Egyptian                   | Hospital based                  | 7                |
| Liem, 2010             | 1275        | 886            | 389          | rs9939609    | IDF               | Dutch                      | General                       | 9                |
| Liguori, 2014          | 1000        | 372            | 628          | rs1121980, rs1421085, rs9939609 | AHA/NHLBI | Italy                      | morbidly obese                   | 6                |
| Malgorzata, 2018       | 425         | 162            | 263          | rs9939609    | IDF               | Polish                     | General                       | 8                |
| Petkeviciene, 2016     | 1020        | 360            | 660          | rs9939609    | IDF               | Lithuanian                 | General                       | 9                |
| Phillips, 2012         | 1753        | 877            | 876          | rs9939609    | NCEP ATP III      | French                     | General                       | 9                |
| Ramos, 2015            | 199         | 49             | 150          | rs8050136, rs9939609 | JIS               | Caucasians                 | Patients of PCOS               | 6                |
| Ranjith, 2011          | 485         | 295            | 190          | rs9939609    | IDF, NCEP ATP III  | Asian Indian                | Patients of AMI                 | 7                |
| Reynolds, 2013         | 179         | 93             | 86           | rs9939609    | IDF               | Irish/British Caucasian    | Chronically treated patients with Schizophrenia | 6                |
| Rodrigues, 2015        | 146         | 114            | 32           | rs9939609    | AHA/NHLBI         | Multietnic                 | Bariatric surgery patients                  | 6                |
| Rotton, 2016           | 272         | 144            | 128          | rs9939609    | IDF               | Caucasian                  | Volunteers from primary health care centres | 6                |
| Sedlaghati-khayat, 2018 | 746        | 341            | 405          | rs1121980, rs1421085, rs1558902, rs8050136 | JIS               | Iran                      | General                       | 7                |
| Sikhayeva, 2017        | 697         | 208            | 489          | rs8050136, rs9939609 | NCEP ATP III | Ethnic Kazahhs              | Hospital-based                 | 9                |
| Sjogren, 2008          | 14996       | 3843           | 11153        | rs9939609    | study-specific     | Swedish                    | General                       | 8                |
| Słężak, 2018           | 191         | 100            | 91           | rs1421085, rs1558902, rs9939609 | NCEP ATP III | Poland                     | Not given                      | 5                |
| Steemburgho, 2012      | 236         | 192            | 44           | rs9939609    | JIS               | Brazil                     | Patients of Type 2 diabetes      | 7                |
| Tabara, 2009           | 2043        | 333            | 1710         | rs9939609    | modified NCEP ATP III | Japanese                   | General                       | 6                |
| Vankova, 2012          | 164         | 16             | 148          | rs9939609    | WHO               | Bulgarian                  | Centrally obese and normal volunteers | 5                |
| Wang, 2010             | 236         | 108            | 128          | rs1421085, rs8050136, rs9939609 | IDF               | Han Chinese                | Outpatients of endocrinology unit | 6                |
| Zhao, 2014             | 3477        | 431            | 3046         | rs9939609    | modified NCEP ATP III | Chinese                    | General                       | 9                |
previously reported GWAS SNPs of MetS conducted on adults in the present children cohort (Supplementary Table 3). The allele frequencies reported in this study were comparable to those reported for European samples (Supplementary Table 4).

Using data for additional covariates, we performed sex-specific analyses for SNPs that reached statistical significance (Table 3). The associations were stronger in boys compared to girls. We further went ahead to analyze the repeated measures of components of the MetS score as the outcome to understand which of the components drove the observed association. The variants in FTO were associated with higher SBP and larger WC whereas the variant A of rs708272 in CETP was strongly associated with decreased TRG levels and increased HDL levels (Supplementary Table 5).

A CADD-scaled C score of more than 10 for SNP rs8047395 (Supplementary Table 6) was observed in in-silico analyses. Similarly, a RegulomeDB score of four for three SNPs (rs8050136, rs1121980, and rs8044769; Supplementary Table 6) in the FTO gene was observed. Using existing eQTL datasets, we found that the rs8050136-A allele in muscle-skeletal tissue was associated with higher FTO gene expression based on the linear regression model.

**Meta-analysis.** We screened 193 records (Fig. 3) and identified 38 eligible studies39–77 for 5 FTO variants (8, 3, 32, 10 studies for rs8050136, rs1121980, rs9939609 and rs1421085, respectively) on 80856 participants with 22462 cases and 58394 controls (Table 4). Including the present study there were 29760, 6343, 5532, 59411 and 9908 participants for rs8050136, rs1121980, rs9939609 and rs1421085 respectively. The control populations of the included studies were in HWE. In addition to ours, only 4 studies were conducted on children or adolescents. A forest plot of association of FTO variants with MetS is provided in Fig. 4. The OR for MetS and rs8050136, rs1121980, rs9939609 and rs1421085 was 1.17 (95% CI: 1.09–1.26), 1.14 (95% CI: 1.00–1.31), 1.26 (95% CI: 1.11–1.43), 1.14 (95% CI: 1.09–1.19) and 1.21 (95% CI: 1.08–1.35) respectively. The degree of between-study heterogeneity was least with F = 20.3% (P = 0.263) for rs8050136 and highest for rs4121085 with F = 53.5% (P = 0.018). Sensitivity analyses that used fixed-effect meta-analysis (rather than random-effects meta-analysis as in the primary analysis) yielded similar OR as random effect meta-analysis (Supplementary Fig. 3). There was no evidence for publication bias, as indicated by funnel plot analyses and Egger test for asymmetry (Supplementary Fig. 4).

**Discussion**

Over the past decade, common genetic loci have been reported to be associated with MetS in different studies, mostly at a single time-point using a cross-sectional or a case-control approach.76,79. Our study took a step ahead in investigating 350 pre-selected loci for their longitudinal association with a continuous MetS score during the transition from childhood to adolescence in a pan-European cohort of children with a follow-up period of up to seven years. We observed a strong association between common genetic variants in the FTO and longitudinal MetS score after Bonferroni correction for multiple comparisons. We observed stronger associations in boys as compared to girls. The effect sizes observed in our study on children were much larger than those reported in adults further suggesting greater genetic predisposition and lower influence from environmental and behavioral factors in youth.

The FTO gene codes for a nuclear protein of the non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily, which is involved in post-translational modification, DNA repair, and fatty acid metabolism.80. FTO which is primarily expressed in the hypothalamus, plays a key role in energy homeostasis and regulation of food intake.81,82. FTO may thus play a role in metabolic regulation by altering gene expression in metabolically active tissues.83. While the exact mechanism remains to be unraveled, it has been shown that genetic variants within the FTO gene are linked functionally to another obesity-related gene called IRX3, which promotes browning of white adipocytes, maybe a connecting link between FTO variants and obesity-related disorders.76,84,85. Further, previous studies have observed that individuals homozygous for the risk alleles in FTO have impaired metabolic profile6–8. Similarly, our findings of the FTO association with MetS score may be related to its association with obesity,89,90, T2DM91 and/or lipid abnormalities.92,93. This is supported by the associations we observed between FTO variants and components of the MetS, particularly with WC and SBP. Various candidate gene studies have observed association between FTO variants and MetS in adults51,73,77,99 across different ethnicities.51,73,77,99. Our results confirm the association of FTO variants and MetS in children and adolescent populations via its implication in the regulation of body fatness.

Though the CETP variant did not survive conservative Bonferroni correction, we observed a strong association of rs708272 with increased HDL (β = 4.03, p = 5.63 × 10^{-6}) and decreased TRG (β = −2.43, p = 9.60 × 10^{-7}) levels. Consistent to our observations previous literature has shown that some variants in the CETP gene,
an essential protein of reverse cholesterol transport process are associated with decreased plasma CETP protein activity and protein levels, culminating in higher concentrations of HDL36,37 and reduced concentrations of TRG38. Similarly, meta-analyses have shown that carriers of the T allele, associated with lower CETP, have higher HDL concentrations than CC homozygotes39 and thereby showing an inverse association with MetS. Further, rs708272 of the CETP gene was moderately correlated ($r^2 = 0.47$, MAF = 0.41) with the GWAS-identified SNP rs1735390, a less common SNP (MAF = 0.30) which could not be detected in the present study given the moderate sample size. We observed a significant association of rs708272 with MetS score after adjusting for BMI z-scores (Supplementary Table 7), suggesting that the association may partly be driven by lipid metabolism in addition to obesity.

In-silico examinations of the possible functional significance of SNPs found in our sample suggested that the FTO gene had a CADD C score of over 10 for one SNP. Likewise, the RegulomeDB score of 4 in the FTO gene for three SNPs suggests that transcription factor binding could be impaired by these SNPs, thus indicating that one or more variants in the FTO gene are likely to have a functional effect. Analysis of the eQTL showed that the rs8050136-A allele may upregulate the level of FTO gene expression in the muscle-skeletal tissue. However, to establish the biological function of these variants of susceptibility, more functional work is needed.

To further assess whether the MetS score association results vary by sex, we performed stratified analysis. The associations remained significant for both boys and girls with slightly stronger associations observed in boys. This is obvious as MetS is more common in adult males as compared to adult females in Europeans and other high-income countries. A possible explanation could be due to the sex-modulated fat distribution interactions with the dynamics of cardiometabolic risk.

In recent years there has been no meta-analysis on the FTO variants and MetS94,100–102 therefore the present meta-analysis provides an updated overview of the risk associated with variants in 16q12.2 involving data from 38 studies on 80856 participants plus the present IDEFICS/L.Family study. Pooled estimates from the meta-analysis further confirmed our findings for rs8050136, rs1211980, rs1558902, rs9939609, rs1421085 and MetS risk. Again, most of the studies in the meta-analysis were conducted on adults which may not be an appropriate extrapolation to children, given its greater impact in children compared to adults103.

Strengths of our study include the design (samples derived from a well-phenotyped cohort of children), an accurate and highly standardized outcome measurement, and the ability to include several important covariates. To our knowledge, this is the first study to report common genetic variation conferring MetS risk with longitudinal analysis in children104. The study could have benefitted further by in-depth laboratory functional assays, but this was beyond the scope of this paper. We therefore conducted an in-silico functional analysis. Though the study was adequately powered to detect associations with common genetic variations, we couldn't replicate the previously identified GWAS SNPs conducted in adults, which could be for example attributable to absence of power to detect less common SNPs or SNPs with small effects, to differences in linkage disequilibrium, age group structure or the analytical methods across studies105. However, the greater impact of FTO variants in children as compared to adults is well known106,107, and therefore the association of the FTO variants in childhood MetS etiology, not observed by GWAS of the adult population, implies the involvement of different SNPs at different age groups.

In conclusion, the results from the present study along with the comprehensive meta-analysis advance etiologic understanding of childhood MetS, highlight that the genetic predisposition to MetS is largely driven by genes of obesity and lipid metabolism. Future work on functional characterization will further help in understanding the biological underpinnings underlying long-term MetS regulation. Our observation of distinct associations of variants of FTO and CETP for different component traits of MetS in children, suggests devising polygenic scores for MetS which may prove to be fundamental for identifying children and subsequently adults of the high-risk group to allow earlier targeted interventions.

Data availability

The authors declare that the data supporting the findings of this study are available within the article and its Supplementary Information files.

Received: 16 December 2019; Accepted: 7 April 2020;
Published online: 28 April 2020

References

1. Alberti, K. G. et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120, 1640–1645, https://doi.org/10.1161/circulationaha.109.192644 (2009).
2. Grundy, S. M. Metabolic syndrome pandemic. Arterioscler. Thromb. Vasc. Biol. 28, 629–636, https://doi.org/10.1161/atvbaha.107.151092 (2008).
3. Hennenman, P. et al. Genetic architecture of plasma adiponectin overlaps with the genetics of metabolic syndrome-related traits. Diabetes Care 33, 908–913, https://doi.org/10.2337/dc09-1385 (2010).
4. Monda, K. L. et al. The genetics of obesity and the metabolic syndrome. Endocr. Metab. Immune Disord. Drug. Targets 10, 86–108 (2010).
5. Lee, H. S., Kim, Y. & Park, T. New Common and Rare Variants Influencing Metabolic Syndrome and Its Individual Components in a Korean Population. Sci. Rep. 8, 5701, https://doi.org/10.1038/s41598-018-23074-2 (2018).
6. Jeong, S. W., Chung, M., Park, S. J., Cho, S. B. & Hong, K. W. Genome-wide association study of metabolic syndrome in koreans. Genomics Inf. 12, 187–194, https://doi.org/10.5808/GI.2014.12.4.187 (2014).
7. Zhu, Y. et al. Susceptibility loci for metabolic syndrome and metabolic components identified in Han Chinese: a multi-stage genome-wide association study. J. Cell Mol. Med. 21, 1106–1116, https://doi.org/10.1111/jcmm.13042 (2017).
8. Kristiansson, K. et al. Genome-wide screen for metabolic syndrome susceptibility Loci reveals strong lipid gene contribution but no evidence for common genetic basis for clustering of metabolic syndrome traits. Circ. Cardiovasc. Genet. 5, 242–249, https://doi.org/10.1161/CIRCGENETICS.111.961482 (2012).
9. McGarry, J. D. Banting Lecture 2001. Dysregulation Fat. Acid. Metab. Etiology Type 2 Diabetes 51, 7–18, https://doi.org/10.2337/ diabetes.51.1.7 (2002).

10. Kraja, A. T. et al. A bivariate genome-wide approach to metabolic syndrome: STAMPEED consortium. Diabetes 60, 1329–1339, https://doi.org/10.2337/db10-1111 (2011).

11. Aguilera, C. M., Olza, J. & Gil, A. Genetic susceptibility to obesity and metabolic syndrome in childhood. Nutr. Hosp. 28(Suppl 5), 44–55, https://doi.org/10.3039/nh.2013.28.sup5.s6917 (2013).

12. Penas-Steinhardt, A. et al. Association of common variants in JAK2 gene with reduced risk of metabolic syndrome and related disorders. BMC Med. Genet. 12, 166, https://doi.org/10.1186/1471-2350-12-166 (2011).

13. Hou, H. et al. Association between Six CETP Polymorphisms and Metabolic Syndrome in Uyghur Adults from Xinjiang, China. Int J Environ Res Public Health 14, https://doi.org/10.3390/ijerph14060653 (2017).

14. Calhuca-Pablo, J. A. et al. Polymorphisms in the LPL and CETP Genes and Haplotype in the ESR1 Gene Are Associated with Metabolic Syndrome in Women from Southwestern Mexico. Int. J. Mol. Sci. 16, 21539–21545, https://doi.org/10.3390/ijms160921539 (2015).

15. Al-Hamad, D. & Raman, V. Metabolic syndrome in children and adolescents. Transl. pediatrics 6, 397–407, https://doi.org/10.21037/tp.2017.10.02 (2017).

16. Ahrens, W. et al. Cohort Profile: The transition from childhood to adolescence in European children–how I.Family extends the IDEFICS cohort. Int. J. Epidemiol. 46, 1394–1395, https://doi.org/10.1093/ije/dyv317 (2017).

17. Ahrens, W. et al. The IDEFICS cohort: design, characteristics and participation in the baseline survey. Int. J. Obes. 35, S3, https://doi.org/10.1038/ijo.2011.30 (2011).

18. Ahrens, W. et al. Metabolic syndrome in young children: definitions and results of the IDEFICS study. Int. J. Obes. 38, 54–514, https://doi.org/10.1038/ijo.2014.130 (2014).

19. Weale, M. E. Quality control for genome-wide association studies. Methods Mol. Biol. 628, 341–372, https://doi.org/10.1007/978-1-60327-367-1_19 (2010).

20. Ziegler, A. Genome-wide association studies: quality control and population-based measures. Genet. Epidemiol. 33(Suppl 1), S45–S50, https://doi.org/10.1002/gepi.20472 (2009).

21. Aulchenko, Y. S., Ripke, S., Isaacs, A. & van Duijn, C. M. GenABEL: an R library for genome-wide association analysis. Bioinformatics 23, 1294–1296, https://doi.org/10.1093/bioinformatics/btm108 (2007).

22. Zheng, X. et al. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics 28, 3326–3328, https://doi.org/10.1093/bioinformatics/bts606 (2012).

23. MacArthur, J. et al. The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic acids Res. 45, D896–D901, https://doi.org/10.1093/nar/gkw1133 (2017).

24. Tawfik, N. S. & Spruit, M. R. The SNPcurator: literature mining of enriched SNP-disease associations. Database: J. Biol. databases curation 2018, bay020, https://doi.org/10.1093/database/bay020 (2018).

25. Piţero, J. et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic acids Res. 45, D833–D839, https://doi.org/10.1093/nar/gkw943 (2017).

26. Davis, A. P. et al. The Comparative Toxigenomics Database: update 2019. Nucleic Acids Res., https://doi.org/10.1093/nar/gky868 (2018).

27. Xu, Z. & Taylor, J. A. SNIPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. Nucleic acids Res. 37, W600–W605, https://doi.org/10.1093/nar/gkp290 (2009).

28. Wells, G. A. et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (2011).

29. Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Group, P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 6, e1000977, https://doi.org/10.1371/journal.pmed.1000977 (2009).

30. Chen, H. et al. Control for Population Structure and Relatedness for Binary Traits in Genetic Association Studies via Logistic Mixed Models. Am. J. Hum. Genet. 98, 653–666, https://doi.org/10.1016/j.ajhg.2016.02.012 (2016).

31. Pruim, R. J. et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 26, 2336–2337, https://doi.org/10.1093/bioinformatics/btq419 (2010).

32. Consortium, G. T. The Genotype-Tissue Expression (GTEx) project. Nat. Genet. 45, 580–585, https://doi.org/10.1038/ng.2653 (2013).

33. Kircher, M. et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. 46, 301–310, https://doi.org/10.1038/ng.2892 (2014).

34. Boyle, A. P. et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22, 1790–1797, https://doi.org/10.1101/137332.112 (2012).

35. Graffelman, J. Exploring Diabetic Genetic Markers: The HardyWeinberg Package. Journal of Statistical Software 60 (2015).

36. Higgins, J. P. T., Thompson, S. G., Deeks, J. J. & Altman, D. G. Measuring inconsistency in meta-analyses. Biochemical Biophysical Res. Commun. 337, 230–234, https://doi.org/10.1016/j.bbrc.2008.06.039 (2008).

37. Baik, I. & Shin, C. Interactions between the FTO rs9939609 polymorphism, body mass index, and lifestyle-related factors on metabolic syndrome risk. Nutr. Journal 19, 346–350, https://doi.org/10.1016/j.jclinepi.2007.11.010 (2007).

38. Egger, M., Smith, G. D., Schneider, M. & Minder, C. Bias in meta-analysis detected by a simple, graphical test. BMJ 325, 153, https://doi.org/10.1136/bmj.325.7398.160 (1998).

39. Hönig, D. et al. An overview of functional polymorphisms and HTRA1, the metabolic syndrome, and related disorders in healthy older women. J. Endocrinol. Invest. 39, 885–890, https://doi.org/10.1007/s40618-016-0443-7 (2016).

40. Col, N., Demircioğlu-Kılıç, B., Nacar, M. & Arar, M. Adolescent obesity and the role of the fat mass and obesity-associated gene polymorphism. Clin. Invest. Med. 40, E235–E242, https://doi.org/10.25011/cim.v40i6.29124 (2017).

41. de Luis, D. A. et al. Relation of the rs9939609 gene variant in FTO with metabolic syndrome in obese female patients. J. Diabetes Complications 27, 346–350, https://doi.org/10.1016/j.jdcompc.2013.02.003 (2013).

42. Costello, J. K., et al. Association of obesity susceptibility genes with metabolic syndrome and related traits in 1,443 Czech adolescents. Folia Biol. 59, 123–133 (2013).

43. Elouej, S. et al. Association of genetic variants in the FTO gene with metabolic syndrome: A case-control study in the Tunisian population. J. Diabetes Complications 30, 206–211, https://doi.org/10.1016/j.jdcompc.2015.11.013 (2016).
83. Mizuno, T. M Fat Mass and Obesity Associated (FTO) Gene and Hepatic Glucose and Lipid Metabolism. 
80. Gerken, T. 
73. Elouej, S. 
72. Cruz, M. 
70. Al-Attar, S. A. 
68. Wang, T. 
64. Slezak, R., Leszczynski, P., Warzecha, M., Laczmanski, L. & Misiak, B. Assessment of the FTO gene polymorphisms in male patients 
65. Steemburgo, T. 
66. Tabara, Y. 
67. Vankova, D., Radanova, M., Kiselova-Kaneva, Y., Madjova, V. & Ivanova, D. The fto rs9939609, adipoq rs1501299, rs822391, and 
63. Sjogren, M. 
61. Sedaghati-Khayat, B. 
62. Sikhayeva, N. 
58. Ranjith, N., Pegoraro, R. J. & Shanmugam, R. Obesity-associated genetic variants in young Asian Indians with the metabolic 
55. Petkeviciene, J. 
54. Malgorzata, S. 
52. Liem, E. T. 
49. Fawwad, A., Siddiqui, I. A., Zeeshan, N. F., Shahid, S. M. & Basit, A. Association of SNP rs9939609 in FTO gene with metabolic 
50. Freathy, R. M. 
47. Lee, N., Kim, J. S., Park, J. H., Shin, C. E. & Han, S. K. Association between single nucleotide polymorphisms in the FTO gene and 
48. Youn, S. Y., Shin, J. H., Chung, E. J., Lim, S. & Ahn, C. S. Association of the FTO gene with body weight and metabolic syndrome in 
46. S grease, S. A., Amin, A. I. & El-Messallam, H. O. The (FTO) gene polymorphism is associated with metabolic syndrome risk in Egyptian females: a case- control study. BMC Med. Genet. 18, 101, https://doi.org/10.1186/s12881-017-0461-0 (2017). 
45. Huy, Y. H. et al. Association between polymorphisms of fat mass and obesity-associated gene and metabolic syndrome in Kazakh adults of Xinjiang, China. Genet. Mol. Res. 14, 14597–14606, https://doi.org/10.4238/2015.November.18.23 (2015). 
44. Bella, M. S., Hamdy, N. M., Amin, A. I. & El-Messallam, H. O. The (FTO) gene polymorphism is associated with metabolic syndrome risk in Egyptian females: a case- control study. BMC Med. Genet. 18, 101, https://doi.org/10.1186/s12881-017-0461-0 (2017). 
43. Rotter, L. et al. Relationships between FTO rs9939609, MC4R rs17782313, and PPARGamma rs1801282 polymorphisms and the occurrence of selected metabolic and hormonal disorders in middle-aged and elderly men - a preliminary study. Clin. Inters. Aging 11, 53–59, https://doi.org/10.2147/CIA.S120253 (2016). 
42. Hasan, H. A., Abu-Oudeh, R. O., Muda, W., Mohamed, H. & Samsudin, A. R. Association of Vitamin D receptor gene polymorphisms with metabolic syndrome and its components among Arab adults from the United Arab Emirates. Diabetes Metab. Syndr. 11(Suppl 2), S531–S537, https://doi.org/10.1016/j.dsx.2017.03.047 (2017). 
41. Larder, R., Cheung, M. K., Tung, Y. C., Yeo, G. S. & Coll, A. P. Where to go with FTO? Trends Endocrinol. metabolism: TEM. 22, 1421–1425, https://doi.org/10.1016/j.tem.2011.09.001 (2011). 
40. Gerken, T. et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318, 1469–1472, https://doi.org/10.1126/science.1157110 (2007). 
39. Javieiro, B. M. et al. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. Genome Res. 20, 170–179, https://doi.org/10.1101/gr.100289.109 (2010). 
38. Campion, J., Milagro, F. I. & Martinez, I. A. Individuality and epigenetics in obesity. Obes. Rev. 10, 383–392, https://doi. 
37. Mizuno, T. M Fat Mass and Obesity Associated (FTO) Gene and Hepatic Glucose and Lipid Metabolism. Nutrients 10, 1600, https://doi.org/10.3390/nu10111600 (2018).
84. Smemo, S. et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 507, 371–375, https://doi.org/10.1038/nature13138 (2014).

85. Zou, Y. et al. IRX3 Promotes the Browning of White Adipocytes and Its Rare Variants are Associated with Human Obesity Risk. Elife 24, 64–75, https://doi.org/10.1101/500716.2017.09.010 (2017).

86. Basile, K. J., Johnson, M. E., Xia, Q. & Grant, S. F. A. Genetic susceptibility to type 2 diabetes and obesity: follow-up of findings from genome-wide association studies. Int. J. Endocrinol. 2014, 769671–769671, https://doi.org/10.1155/2014/769671 (2014).

87. Kilpeläinen, T. O. et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. Nat. Genet. 43, 753–760, https://doi.org/10.1038/ng.866 (2011).

88. Wang, Q. et al. Relationship between fat mass and obesity-associated gene expression and type 2 diabetes mellitus severity. Exp. Ther. Med. 15, 2917–2921, https://doi.org/10.3892/etm.2018.5752 (2018).

89. 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, https://doi.org/10.1126/science.1141634 (2007).

90. Lauria, F. et al. Prospective analysis of the association of a common variant of FTO (rs9939609) with adiposity in children: results of the IDEFICS study. PLoS one 7, e48876–e48876, https://doi.org/10.1371/journal.pone.0048876 (2012).

91. Hertel, J. K. et al. FTO, type 2 diabetes, and weight gain throughout adult life: a meta-analysis of 41,504 subjects from the Scandinavian HUNT, MDC, and MPP studies. Diabetes 60, 1637–1644, https://doi.org/10.2327/db-10-1340 (2011).

92. Zabena, C. et al. The FTO obesity gene. Genotyping and gene expression analysis in morbidly obese patients. Obes. Surg. 19, 87–95, https://doi.org/10.1007/s11695-008-9727-0 (2009).

93. Al-Attar, S. A. et al. Association between the FTO rs9939609 polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. Cardiovasc. Diabetol. 7, 5, https://doi.org/10.1186/1475-2840-7-5 (2008).

94. Povel, C. M., Boer, J. M., Reiling, E. & Feskens, E. J. Genetic variants and the metabolic syndrome: a systematic review. Obes. Rev. 12, 952–967, https://doi.org/10.1111/1467-789X.2011.00907.x (2011).

95. Frisdal, E. et al. Functional interaction between –629C/A, −971G/A and −1337C/T polymorphisms in the CETP gene is a major determinant of promoter activity and plasma CETP concentration in the REGRESS Study. Hum. Mol. Genet. 14, 2607–2618, https://doi.org/10.1093/hmg/ddi291 (2005).

96. Boekholdt, S. M. et al. The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. PLoS Medicine/Public Library Sci. 2, e1000075, https://doi.org/10.1371/journal.pmed.0010075 (2003).

97. Zou, Y. et al. Metabolism disrupting chemicals and metabolic disorders. Reprod. Toxicol. 68, 3–33, https://doi.org/10.1016/j.reprotox.2016.10.001 (2017).

98. Wang, H., Dong, S., Xu, H., Qian, J. & Yang, J. Genetic variants in FTO associated with metabolic syndrome: a meta- and gene-based analysis. Mol. Biol. Rep. 39, 5691–5698, https://doi.org/10.1007/s11033-011-1377-y (2012).

99. Wang, H., Dong, S., Xu, H., Qian, J. & Yang, J. Genetic variants in FTO associated with metabolic syndrome: a meta- and gene-based analysis. Mol. Biol. Rep. 39, 5691–5698, https://doi.org/10.1007/s11033-011-1377-y (2012).

100. Fall, T. et al. The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. PLoS Medicine/Public Library Sci. 2, e1001474, https://doi.org/10.1371/journal.pmed.1001474 (2013).

101. Zhou, D. et al. Common variant (rs9939609) in the FTO gene is associated with metabolic syndrome. Mol. Biol. Rep. 39, 6555–6561, https://doi.org/10.1007/s11033-012-1484-4 (2012).

102. Qi, L. et al. Fat mass-and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test. Diabetes 57, 3145–3151, https://doi.org/10.2337/db08-0066 (2008).

103. Kelishadi, R., Hovsepian, S. & Javanmard, S. H. A Systematic review of single nucleotide polymorphisms associated with metabolic syndrome in children and adolescents. children 29, 46 (2017).

104. Kraft, P., Zeggini, E. & Ioannidis, J. P. A. Replication in genome-wide association studies. Stat. Sci. 24, 561–573, https://doi.org/10.1214/09-STS290 (2009).

105. Kraus, W. E. et al. BMI loci and longitudinal BMI from adolescence to young adulthood in an ethnically diverse cohort. Int. J. Obes. 41(2005), 759–768, https://doi.org/10.1038/ijo.2016.233 (2017).

106. Hardy, R. et al. Life course variations in the associations between FTO and MC4R gene variants and body size. Hum. Mol. Genet. 19, 545–552, https://doi.org/10.1002/hmg.ddp504 (2010).

Acknowledgements
This work was done as part of the IDEFICS/I.Family studies. We gratefully acknowledge the financial support of the European Commission within the Sixth RTD Framework Programme Contract No. 016181 (FOOD), and the Seventh RTD Framework Programme Contract No. 266044. The publication of this article was partially funded by the Open Access Fund of the Leibniz Association. We thank the IDEFICS and I.Family children and their parents for taking the time to participate in this extensive examination program. The funding sources had no role in the design, conduct, and analysis of the study or in the decision to submit the manuscript for publication. We are grateful for the support provided by school boards, headmasters, teachers, school staff and communities, and for the effort of all study nurses and our data managers, especially Claudia Brünings-Kuppe, Willemje Hummel-Bartenschlager, and Sandra Israel-Georgii.

Author contributions
R.N. and R.F. contributed to the association analysis of the genotyping data. R.N. and M.M. contributed to the large-scale epidemiologic analysis. R.N., R.F. and M.M. drafted the paper. R.N., R.F., F.G., L.I., S.M., N.M., D.M., L.M., P.R., T.V., W.A. and M.M. provided critical comments on the paper, draft, and analysis. All authors read and approved the final paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-64031-2.

Correspondence and requests for materials should be addressed to R.N.

Reprints and permissions information is available at www.nature.com/reprints.
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020