INTRODUCTION

Forkhead box (Fox) proteins are a large family of factors that share a highly homologous Forkhead DNA-binding domain but diverge in the remaining sequences. These factors have been implicated in differentiation and developmental processes in both mice and humans. In particular, the three members of the Foxa subfamily, Foxa1, 2 and 3, which are most closely related to the archetype Drosophila Fork HEAD protein, have been shown to transcriptionally control early development, organogenesis and metabolism in mice. Through a genetic screen to assess the role of each Foxhead factor family member in adipocyte differentiation, we demonstrated that Foxa3 is the only Forkhead protein positively affecting adipocyte differentiation through regulation of the nuclear receptor peroxisome proliferator-activated receptors γ (PPARγ), in cooperation with the transcription factors CCAAT-enhancer-binding proteins. In addition, we showed that mice with Foxa3 ablation have decreased adipose tissue expansion in response to high-fat diet regimens and during aging, suggesting a new critical role of this factor in fat tissue biology.

Foxa3 functions through two transcriptional activation domains located at the amino- and carboxy-termini, in addition to the centrally located Forkhead DNA-binding motif, through which it binds to the upstream regulatory elements present in the promoters of target genes. Given the newly established importance of Foxa3 in fat metabolism in mice and the absence of data on potential associations of genetic variants of FOXA3 with human metabolic outcomes, we sequenced FOXA3 in 392 lean, overweight and obese subjects, assessed the potential correlation between genotype and phenotype by functionally analyzing two novel FOXA3 missense mutations and tested the association of the common single-nucleotide polymorphisms (SNPs) identified with a series of metabolic parameters. Through these analyses we identified 14 novel FOXA3 variants, demonstrated that the missense mutations c.185C>T (p.Ser62Leu) and c.731C>T (p.Ala244Val) have increased adipogenic function in vitro and that the FOXA3 polymorphism rs28666870 is associated with increased appendicular muscle mass.

MATERIALS AND METHODS

Subjects

We performed a cross-sectional study of 392 lean and obese children, adolescents and young adults selected from several NIH-NICHD (National Institutes of Health-National Institute of Child Health and Human Development) cohorts based on the availability of dual-energy X-ray absorptiometry data, magnetic resonance imaging scans and DNA samples (described in details in the following NIH clinical trials: NCT00001195, NCT007958108, NCT00631644, NCT00001522, NCT00001723 and NCT00005669 at clinicaltrials.gov). Non-Hispanic black (NHB) and non-Hispanic white (NHW) in the...
DNI-NICHD group were defined by the subject having all four grandparents of the same race. Ethnicity was ascertained by self-identification. All the clinical protocols were approved by the Institutional Review Board of the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Each child and adolescent provided written assent and parents gave written consent for their participation.

Body composition measurements
All participants underwent a screening visit by a pediatric endocrinologist or nurse practitioner. Blood samples were obtained for genomic DNA isolation.12 Weight and height were measured in the post-absorptive state.13 Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated from post-absorptive serum insulin and glucose concentrations.1,14 Total body fat and lean mass were determined by dual-energy X-ray absorptiometry.15 Magnetic resonance imaging was performed 3 days before cells were harvested for RNA analysis.

Sequencing of human FOXA3 gene
The entire FOXA3 gene (Exons 1 and 2) and 65 base pairs of 5'-UTR and 66 base pairs of the intron were sequenced (Polymeric DNA Technologies, Alameda, CA, USA) using PCR amplification (ABI) in 392 subjects of the NIH-NICHD group. Bidirectional sequencing was performed (ABI, Grand Island, NY, USA) and variants were identified by comparison to reference sequence number NM_004497. The primers used for sequencing were the following: FOXA3-1: forward (F) 5′-GGTGTCGCCTGCTATAA-3′ and reverse (R) 5′-AGGCCCTCCCATCAG-3′; FOXA3-2: F: 5′-GGTCTCTAGCAGATAAGGTA-3′ and R: 5′-TTCCTCAAGTGGCTCATCAG-3′; FOXA3-3: F: 5′-CTGGGGCCCACTT-3′ and R: 5′-CTTCTCTCCCTACGTTGA-3′; FOXA3-4: F: 5′-CCTCTATAACCCGGA-3′ and R: 5′-AGACCCACCACCATG-3′.

RESULTS AND DISCUSSION
We sequenced FOXA3 in 392 children, adolescents and young adults (NIH-NICHD group, Table 1) and identified 22 variants (Table 2), 14 of which had not been reported previously. The four common polymorphisms (http://www.1000genomes.org/) identified in the NIH-NICHD group were in Hardy–Weinberg equilibrium (data not shown). In addition, we detected three novel intronic variants and eight new missense mutations (Table 2), which were each present in heterozygosity only in one individual. The majority of the missense alterations were located in the amino- or carboxy-termini with three out of eight found within the last seven C-terminal amino acids. Sequence homology analysis indicated that the majority of the variants identified occurred at nucleotides highly conserved among species (Supplementary Table 1).

Given that Foxa3 potentiates adipocyte differentiation in vitro in 10T1/2 cells,7 we assessed the functionality of two FOXA3 mutants, c.185C>T and c.731C>T, in in vitro adipogenic assays. We chose to analyze the function of these two mutations because they were the only missense alterations that occurred as the sole genetic alteration in the FOXA3-coding sequence in the patients in which they were identified (Supplementary Table 2). Ectopic expression of FOXA3 c.185C>T and FOXA3 c.731C>T in 10T1/2 mesenchymal cells at levels comparable to those of FOXA3-WT (Figure 1a) was

Table 1. Characteristics of the 392 subjects analyzed

| Variable | Value | Range |
|----------|-------|-------|
| Sex | 44% Males, 56% females |
| Race | 55% NHW, 40% NHB, 5% Other |
| Age (years) | 12.2 ± 3.2 | 6.1 to 21.3 |
| Weight (kg) | 70.5 ± 30.8 | 17.0 to 146.1 |
| Height (cm) | 153.4 ± 16.6 | 108.3 to 186.4 |
| BMI (kg m⁻²) | 28.7 ± 9.2 | 14.4 to 53.5 |
| BMI-z score | 1.63 ± 1.14 | −2.52 to +3.18 |
| Body fat (%) | 35.1 ± 13.3 | 9.8 to 64.1 |
| Fat mass (kg) | 27.0 ± 17.1 | 3.2 to 83.6 |
| Lean mass (kg) | 42.1 ± 15.7 | 12.9 to 85.4 |
| SAT2-3 area (cm²) | 251.0 ± 238.2 | 11.0 to 3217 |
| VAT3-4 area (cm²) | 68.3 ± 59.2 | 3 to 598 |

Abbreviations: BMI, body mass index; BMI-z, body mass index standard deviation score; NHW, non-Hispanic white; NHB, non-Hispanic black; SAT, subcutaneous abdominal adipose tissue area in cm² from L2-3; VAT, visceral abdominal adipose tissue area in cm² from L3-4. Other race/ethnicities included: Asian, Hispanic, and mixed. Unadjusted means are reported with standard deviation of the means (±s.d.).

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GraphPad Prism. A Student’s t-test was used for comparison between two groups and differences between groups were considered significant with \( P < 0.05 \). FOXA3 association analyses in the NIH-NICHD group were performed using SPSS-version 18 software (IBM, Bethesda, MD, USA). Hardy–Weinberg equilibrium tests were performed on all variants. Associations were evaluated using Analysis of covariance (ANCOVA) for continuous variables and \( \chi^2 \) analyses for categorical variables. The \( P \) values were corrected for the four common variants found in the NIH-NICHD cohort and significant associations were called when \( P < 0.0125 \). Heterozygous and homozygous subjects were analyzed together, compared with wild-type subjects and NHW and NHB were analyzed separately, if a significant difference by race was found. Body mass index (BMI), fat mass, lean mass, appendicular muscle mass, trunk muscle mass, subcutaneous abdominal adipose tissue (SAT) and visceral abdominal adipose tissue (VAT) areas were log transformed before ANCOVA. Means and standard deviations were back transformed and adjusted for covariates. BMI was adjusted for sex, race and age. Fat, lean mass, appendicular lean mass and trunk lean mass were adjusted for sex, race, age and height squared. SAT2-3 and VAT3-4 areas were adjusted for sex, race, age and log fat mass. HOMA was adjusted for sex, race, age and fat mass. Given the relatively small number of subjects in the NIH-NICHD group, we used higher stringency in calling significant associations (\( P < 0.0125 \)) between SNPs and body composition measurements and adjusted for genotypes rather than for the traits studied.
Table 2. FOXA3 variants identified in 392 children, adolescents and young adults

| Chromosome 19 position (hg19 reference) | HGVS nucleotide change | SNP Location | Amino acid change | Genotype frequencies (%) | BMI- z |
|----------------------------------------|------------------------|--------------|-----------------|--------------------------|-------|
|                                        |                        |              |                 | Wt (Wt/Wt) | Het (Wt/SNP) | Hom (SNP/SNP) | Wt (Wt/Wt) | Het (Wt/SNP) | Hom (SNP/SNP) |
| 46367703                               | c.-12 T>C              | rs11667582   | 5'-UTR No       | 71.2 25.7 3.1 | 1.67±1.14 1.52±1.15 1.83±1.19 |
| 46367812                               | c.69+29 A>G            | Novel Intron | No              | 99.5 0.5 0.0 | 1.64±1.14 1.64±0.43 – |
| 46367831                               | c.69+48C>C             | Novel Intron | No              | 99.7 0.3 0.0 | 1.64±1.14 0.61 – |
| 46367832                               | c.69+94G>T             | Novel Intron | No              | 99.7 0.3 0.0 | 1.63±1.14 2.00 – |
| 46375364                               | c.101T>C               | Novel/N-Sym  | Exon 2 p.Met34Thr | 99.7 0.3 0.0 | 1.63±1.14 1.17 – |
| 46375448                               | c.185C>T               | Novel/N-Sym  | Exon 2 p.Ser62Leu | 99.7 0.3 0.0 | 1.63±1.14 2.30 – |
| 46375462                               | c.199C>T               | Novel/N-Sym  | Exon 2 p.Pro67Ser | 99.7 0.3 0.0 | 1.64±1.14 0.19 – |
| 46375608                               | c.345C>T               | rs144459600  | Exon 2 No       | 98.2 1.8 0.0 | 1.63±1.15 1.98±0.80 – |
| 46375620                               | c.357G>A               | rs147847262  | Exon 2 No       | 99.7 0.3 0.0 | 1.63±1.14 1.98 – |
| 46375779                               | c.516C>T               | rs16980091   | Exon 2 No       | 78.8 19.9 1.3 | 1.65±1.16 1.57±1.09 1.81±1.21 |
| 46375974                               | c.711T>C               | Novel/Sym    | Exon 2 No       | 99.7 0.3 0.0 | 1.64±1.14 0.94 – |
| 46375994                               | c.731T>C               | Novel/Sym    | Exon 2 p.Ala244Val | 99.7 0.3 0.0 | 1.63±1.14 1.31 – |
| 46376006                               | c.743C>A               | Novel/Sym    | Exon 2 p.Thr248Asn | 99.7 0.3 0.0 | 1.63±1.14 0.79 – |
| 46376043                               | c.780T>C               | rs28666870   | Exon 2 No       | 97.7 2.3 0.0 | 1.62±1.14 2.61±0.21 – |
| 46376089                               | c.826C>G               | rs140760356  | Exon 2 p.Pro276Ala | 99.7 0.3 0.0 | 1.64±1.14 0.23 – |
| 46376217                               | c.954C>A               | rs3810127    | Exon 2 No       | 86.7 13.0 0.3 | 1.63±0.16 1.65±1.06 2.31 |
| 46376226                               | c.963C>T               | rs146135785  | Exon 2 No       | 99.5 0.5 0.0 | 1.63±1.14 1.81±1.34 – |
| 46376244                               | c.981C>T               | Novel/Sym    | Exon 2 No       | 99.7 0.3 0.0 | 1.63±1.14 2.51 – |
| 46376293                               | c.1030C>T              | Novel/Sym    | Exon 2 p.Arg344Cys | 99.7 0.3 0.0 | 1.63±1.14 2.56 – |
| 46376309                               | c.1046C>T              | Novel/Sym    | Exon 2 p.Ala349Val | 99.7 0.3 0.0 | 1.63±1.14 2.25 – |
| 46376312                               | c.1049G>T              | Novel/Sym    | Exon 2 p.Ser350Phe | 99.7 0.3 0.0 | 1.63±1.14 2.81 – |
| 46376397                               | c.*81_82insT           | Novel Intron | No              | 99.5 0.5 0.0 | 1.63±1.14 2.17±0.23 – |

Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; BMI-z, body mass index standard deviation score; Cys, cysteine; Het, heterozygous; Hom, homozygous; ins, insertion; Leu, leucine; Met, methionine; N-Syn, non-synonymous; Phe, phenylalanine; Pro, proline; Ser, serine; SNP, single-nucleotide polymorphism; Syn, synonymous; Thr, threonine; Val, valine; Wt, wild type.

Figure 1. (a) mRNA levels of FOXA3 in 10T1/2 cells ectopically expressing either vector (Ctrl), FOXA3 wild-type (FOXA3), FOXA3 c.185C>T (185) or FOXA3 c.731C>T (731). Data are presented as mean ± s.e.m. **P<0.01 compared with control group. (b) mRNA levels of markers of adipocyte differentiation in 10T1/2 cells ectopically expressing either vector (Ctrl), FOXA3 wild-type (FOXA3), FOXA3 c.185C>T (185) or FOXA3 c.731C>T (731). Data are presented as mean ± s.e.m. *P<0.05, **P<0.01 compared with FOXA3 wild-type (FOXA3).

Table 3. Analysis of the association of common variants with body mass index (BMI) and lean mass in NHB and NHB subjects present in the NIH-NICHD group. Demographics of the NHB cohort re-evaluated are shown in Supplementary Table 3. This analysis confirmed that variant rs28666870 was significantly associated with greater total lean body mass (P<0.035), increased BMI (P<0.032) and appendicular lean mass (P<0.044). No significant associations with body mass parameters were identified for the additional three common variants identified in the NIH-NICHD group, rs11667582, rs3810327 and rs16980091. We subsequently assessed the association between these four SNPs and HOMA-IR. As shown in Table 3, none of the four common variants associated with increased mRNA levels of classic adipocyte differentiation markers, such as PPARγ, adiponectin, perilipin and αP2, in comparison to FOXA3-WT-expressing cells (Figure 1b). These data suggest that these two missense mutations confer increased adipogenic capacity to FOXA3.

We next assessed whether the FOXA3 variants identified in the NIH-NICHD group were associated with metabolic parameters. This analysis revealed a significant association between the common variant rs28666870 and increased total lean body mass (Table 3). A significant association with race/ethnicity was also identified in subjects heterozygous for this variant (χ² P of 0.002; Table 3). To further confirm that the association identified between rs28666870 and lean mass was not confounded by race, we re-evaluated the association strictly within the subgroup of 158 NHB subjects present in the NIH-NICHD group. No significant associations with body mass parameters were identified for the additional three common variants identified in the NIH-NICHD group, rs11667582, rs3810327 and rs16980091. We subsequently assessed the association between these four SNPs and HOMA-IR. As shown in Table 3, none of the four common variants associated with increased mRNA levels of classic adipocyte differentiation markers, such as PPARγ, adiponectin, perilipin and αP2, in comparison to FOXA3-WT-expressing cells (Figure 1b). These data suggest that these two missense mutations confer increased adipogenic capacity to FOXA3.

![Figure 1](image-url)
Table 3. Associations of FOXA3 SNPs with race/ethnicity and metabolic parameters

| rs11667582 | Wt | Het & Hom | P |
|-----------|----|-----------|--|
| Race (%)  |    |           | 0.964 |
| NHW       | 55.2 | 53.1 | |
| NHB       | 39.8 | 41.6 | |
| Other     | 5.0 | 5.3 | |
| Sex, females (%) | 54.8 | 59.3 | 0.433 |
| Sex, males (%) | 45.2 | 40.7 | |
| BMI (kg/m²) | 27.5 ± 1.4 | 26.4 ± 1.3 | 0.205 |
| Fat mass (kg) | 21.3 ± 1.9 | 20.2 ± 1.9 | 0.556 |
| Lean mass (kg) | 39.3 ± 1.2 | 38.2 ± 1.2 | 0.128 |
| SAT-2, area (cm²) | 158.8 ± 1.4 | 155.6 ± 1.4 | 0.303 |
| VAT-2, area (cm²) | 50.3 ± 1.8 | 48.9 ± 1.8 | 0.625 |
| HOMA-IR | 3.45 ± 2.7 | 3.80 ± 2.7 | 0.295 |

| rs16980091 | Wt | Het & Hom | P |
|-----------|----|-----------|--|
| Race (%)  |    |           | 0.011 |
| NHW       | 58.1 | 41.7 | |
| NHB       | 38.0 | 48.8 | |
| Other     | 3.9 | 9.5 | |
| Sex, females (%) | 55.2 | 59.5 | 0.379 |
| Sex, males (%) | 44.8 | 40.5 | |
| BMI (kg/m²) | 27.4 ± 1.3 | 26.4 ± 1.3 | 0.290 |
| Fat mass (kg) | 21.4 ± 1.9 | 19.1 ± 1.9 | 0.161 |
| Lean mass (kg) | 38.9 ± 1.2 | 39.2 ± 1.2 | 0.773 |
| SAT-2, area (cm²) | 157.4 ± 1.4 | 160.3 ± 1.4 | 0.896 |
| VAT-2, area (cm²) | 50.0 ± 1.8 | 49.8 ± 1.8 | 0.959 |
| HOMA-IR | 3.50 ± 2.7 | 3.73 ± 2.7 | 0.484 |

| rs28666870 | Wt | Het & Hom | P |
|-----------|----|-----------|--|
| Race (%)  |    |           | 0.002 |
| NHW       | 55.7 | 0.0 | |
| NHB       | 39.1 | 100.0 | |
| Other     | 5.2 | 0.0 | |
| Sex, females (%) | 56.3 | 50.0 | 0.731 |
| Sex, males (%) | 43.7 | 50.0 | |
| BMI (kg/m²) | 27.0 ± 1.4 | 35.8 ± 1.3 | 0.009 |
| Fat mass (kg) | 20.7 ± 1.9 | 30.5 ± 1.9 | 0.091 |
| Lean mass (kg) | 38.8 ± 1.2 | 45.3 ± 1.2 | 0.010 |
| Appendicular lean mass (kg) | 17.5 ± 1.2 | 20.9 ± 1.2 | 0.013 |
| Trunk lean mass (kg) | 18.1 ± 1.2 | 20.7 ± 1.2 | 0.045 |
| SAT-2, area (cm²) | 159.1 ± 1.4 | 154.5 ± 1.4 | 0.940 |
| VAT-2, area (cm²) | 50.0 ± 1.8 | 49.5 ± 1.8 | 0.973 |
| HOMA-IR | 3.58 ± 2.7 | 2.16 ± 2.7 | 0.144 |

| rs38103277 | Wt | Het & Hom | P |
|-----------|----|-----------|--|
| Race (%)  |    |           | 0.129 |
| NHW       | 53.8 | 59.6 | |
| NHB       | 41.8 | 30.8 | |
| Other     | 4.4 | 9.6 | |
| Sex, females (%) | 55.9 | 57.7 | 0.789 |
| Sex, males (%) | 44.1 | 42.3 | |
| BMI (kg/m²) | 27.1 ± 1.3 | 27.4 ± 1.3 | 0.797 |
| Fat mass (kg) | 20.6 ± 1.9 | 22.2 ± 1.9 | 0.460 |
| Lean mass (kg) | 39.0 ± 1.2 | 39.0 ± 1.2 | 0.960 |
| Appendicular lean mass (kg) | 157.0 ± 1.4 | 163.7 ± 1.4 | 0.381 |
| VAT-2, area (cm²) | 49.3 ± 1.7 | 54.4 ± 1.8 | 0.244 |
| HOMA-IR | 3.57 ± 2.7 | 3.44 ± 2.7 | 0.750 |

Abbreviations: BMI, body mass index; het, heterozygous for variant allele at one or both loci; HOMA-IR, homeostasis model assessment-estimated insulin resistance; hom/hom, homoyzygous for variant alleles; NHW, non-Hispanic white; NHB, non-Hispanic black; SAT, subcutaneous adipose tissue area in cm² from L2–3; VAT, visceral adipose tissue area in cm² from L1–2. VAT/VAT ratios were log transformed before ANCOVA. Adjusted means ± s.d. are reported. BMI was adjusted for sex, race and age. Fat and lean mass were adjusted for sex, race, age and height squared. SAT and VAT were adjusted for sex, race, age and log fat mass. HOMA-IR was adjusted for sex, race, age and log fat mass. P < 0.05 is considered significant and shown in bold.

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alleles identified showed a significant association (P = 0.10) with HOMA-IR. On the basis of the demonstration that FOXA3 is a novel transcriptional regulator of adipocyte differentiation and fat tissue expansion,7,8 we took a candidate gene approach to identify possible novel, naturally occurring, mutations in FOXA3, assessed their adipogenic capacity and tested the potential association of the variants identified with metabolic traits in human subjects. Our analysis revealed eight novel missense mutations in the NIH-NICHD group analyzed and demonstrated that two pyrimidine transitions C to T resulting in non-synonymous alterations (p.Ser62Leu and p.Ala244Val) conferred increased adipogenic activity to FOXA3 in vitro. Detailed mechanistic studies will further define whether phosphorylation at the serine residue 62 may alter FOXA3 nuclear localization and decrease its function, as demonstrated for other Forkhead factors,16 or whether posttranslational modifications alter FOXA3 interactions with components of the transcriptional machinery. Interestingly, the proadipogenic missense mutation c.185C>T (p.Ser62Leu) was identified in an obese subject (BMI-z score of 2.3) and c.731C>T (p.Ala244Val) was identified in an overweight individual (BMI-z of 1.3), suggesting a potential correlation between genotype and phenotype. In light of the role of FOXA3 in adipose tissue expansion in mice,7,8 our data suggest that the two novel FOXA3 missense alterations identified in this study may have an effect on the fat storage capacity of the adipocytes, possibly leading to increased fat tissue expansion. Our analysis also revealed an association of the FOXA3 variant rs28666870 with increased lean mass in the NIH-NICHD group analyzed. Given that the majority of lean mass measured by dual-energy X-ray absorptiometry (DEXA) scan is represented by muscle mass, we determined whether the variant rs28666870 was associated with appendicular or trunk muscle mass. Despite an association of rs28666870 with increased appendicular muscle mass, no significant association was found between this variant and increased insulin sensitivity. It is possible that this polymorphism may influence the development of insulin resistance during the course of aging and that its protective effects may not yet be evident, given that the NIH-NICHD cohort is mainly constituted by young subjects. Interestingly, the HOMA-IR value of 2.16 ± 2.7 (mean ± s.d.) for the heterozygous subjects carrying the rs28666870 variant appeared to be below the cutoffs for insulin resistance reported by Kurtoglu17 in pre-pubertal and pubertal boys (2.67 and 5.22, respectively) and girls (pre 2.22 and pubertal, 3.82), whereas the HOMA-IR values for the subjects carrying the WT allele (3.58 ± 2.7, mean ± s.d.) were higher, suggesting a possible protection from the development of insulin resistance among the subjects carrying the rs28666870 variant.

Our finding that the variant rs28666870 is significantly associated with increased lean mass, may suggest a role for FOXA3 in controlling the differentiation of mesenchymal tissues toward different lineages. This hypothesis is in line with our recent demonstration that Foxa3 plays a role in mouse tissues of mesenchymal origin, such as fat.7,8 Given that the synonymous variant rs28666870 is predicted by ESEfinder (http://rulai.cshl.edu/tools/ESF) to alter an exon splicing enhancer site for the binding of SR splicing regulators; it is plausible that rs28666870 could give rise to a new spliced isoform of FOXA3, potentially affecting muscle mass. Additional studies employing genome editing and functional assays will determine whether a novel FOXA3 isoform arises from the variant rs28666870 with transcriptional activity in adipose and/or in muscle cell lineages. In addition, further studies in larger cohorts will determine the presence of associations between metabolic parameters and body composition with FOXA3 variants.

In conclusion, our analysis identified 14 novel FOXA3 variants, demonstrated a novel association between one FOXA3 SNP and body mass parameters and showed increased adipogenic function of two missense mutations, each found in individuals with BMI-z...
scores greater than 1. These findings support a role for FOXA3 as a novel critical regulator of fat mass in humans. To our knowledge, this is the first study to report an association between FOXA3 variants and metabolic parameters, suggesting a potential role of FOXA3 DNA variants in regulation of body mass.

Supplementary information is available at the International Journal of Obesity’s website

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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