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Association between Transcription Factor AP-2B genotype, obesity, insulin resistance and dietary intake in a longitudinal birth cohort study

Transcription Factor AP-2B associated with obesity

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ABSTRACT

BACKGROUND: The development of obesity has a large genetic component, and the gene encoding the transcription factor 2 beta (*TFAP2B*) has been identified as one of the responsible factors. We investigated the effect of *TFAP2B* intron 2 variable number tandem repeat (VNTR) genotype on obesity, insulin resistance and dietary intake from 15 to 33 years of age.

METHODS: The sample included both birth cohorts (originally n = 1176) of the longitudinal Estonian Children Personality Behaviour and Health Study. The association between *TFAP2B* genotype, and anthropometric measurements, glucose metabolism and dietary intake at ages 15, 18 and 25 years was assessed using the linear mixed-effects regression models. Differences in anthropometric measurements, biochemical measures, blood pressure and dietary intake between *TFAP2B* genotypes at different age, including data of the older cohort at age 33, were assessed by one-way ANOVA.

RESULTS: Male homozygotes for the *TFAP2B* 5-repeat allele had significantly higher body weight, body mass index, sum of 5 skinfolds, proportion of body fat, waist circumference, hip circumference, waist to hip ratio, waist to height ratio, fasting insulin and HOMA index. In female subjects, homozygotes for the *TFAP2B* 5-repeat allele had significantly larger increase in the rate of change per year in body weight, body mass index and hip circumference between years 15 and 25. By age 33 the findings were similar. A decrease in daily energy intake from adolescence to young adulthood was observed. In males, heterozygotes had significantly smaller decrease in the rate of change per year in daily energy intake.

CONCLUSIONS: The association of *TFAP2B* with the development of obesity and insulin resistance is present throughout adolescence to young adulthood in males. In females the

effect of *TFAP2B* on obesity appears later, in young adulthood. The *TFAP2B* effect is rather related to differences in metabolism than energy intake.

INTRODUCTION

Prevalence of overweight, obesity and abdominal obesity has increased worldwide (1–3). Obesity was previously considered to be only a disorder of energy imbalance, but now we know that its pathogenesis is more complex involving an interaction between genetic, environmental, physiological, behavioural, social, and economic factors (4).

The development of obesity has a large genetic component, and heritability estimates of BMI around 80% have been reported, while a large variety of genes appears to play a role (5–7). We have previously demonstrated that the intron 2 variable number tandem repeat (VNTR) polymorphism of the transcription factor AP2B gene (*TFAP2B*) was associated with abdominal obesity and insulin resistance among 15-year old subjects. Homozygotes for the 5-repeat allele had higher levels of fasting insulin, Homeostasis Model Assessment (HOMA) estimates and subscapular skinfold thickness, as compared to the carriers of the 4-repeat allele (8). These associations were however present only in male subjects. Recent large-scale studies have reinforced the implication of *TFAP2B* in BMI and obesity. A meta-analysis of genome-wide association studies (GWAS) in individuals of European and non-European descent and Metabochip studies, with a total of 339 224 individuals, identified 97 loci including *TFAP2B* as associated with BMI (9). A meta-analysis of GWAS in children (aged 2–10 years) produced similar results: It included 20 studies (n = 35 668) in the discovery phase and 13 studies (n = 11 873) in the replication phase; 15 loci, including *TFAP2B*, reached genome-wide significance and were thus reliably associated with childhood BMI (10). These data make *TFAP2B* a highly interesting candidate gene for overall obesity as well as abdominal obesity and insulin resistance that has its effect already manifested in early childhood.

Earlier studies have shown that polymorphisms in the first intron of *TFAP2B* affect the transcriptional activity of the gene (11). Overexpression of TFAP2B in 3T3-L1 adipocytes decreased the expression and secretion of adiponectin, by directly inhibiting adiponectin gene expression (12). Moreover, overexpression of TFAP2B causes adipocyte cell enlargement, stimulation of glucose transport activity, triglyceride accumulation and insulin resistance (13). However, it is not known, how the association of *TFAP2B* genotype with obesity and insulin resistance develops over time or which are the mediating factors. In this study we examined the longitudinal association between *TFAP2B* intron 2 VNTR genotype and obesity, abdominal obesity, insulin resistance and dietary intake in a birth cohort study.

SUBJECTS AND METHODS

Study sample

The sample was originally formed for the European Youth Heart Study in 1998/1999 and was later incorporated into the Estonian Children Personality Behavior and Health Study (ECPBHS). The study procedure and the selection of the original sample has been described in detail elsewhere (14). In brief, ECPBHS is a longitudinal cohort study with a population representative sample of participants, all of European descent, with school as the sampling unit. All schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56) were included into the sampling and 25 schools were selected. All children from grades 3 (aged 9 years; n = 583) and grades 9 (aged 15 years; n = 593) were invited to participate (14). Follow-up studies for the younger birth cohort have been taken place in ages 15 years (n = 483), 18 years (n = 454) and 25 years (n = 441) and for the older birth cohort in ages 18 years (n = 417 + additional 62), 25 years (n = 541) and 33 years (n = 504) (ref. 15).

The sample of this analysis comprises of non-pregnant individuals with available complete data at age 15 years, 18 years and 25 years on anthropometric measurements, biochemical measures, dietary intake and *TFAP2B* intron 2 VNTR genotype (Supplementary Table 1). Data from the older birth cohort has by now been collected at age 33 years and is analyzed cross-sectionally. The study sample included 18 –21 pairs of siblings at each timepoint. To account for that, a separate analysis was done were one of the siblings was removed from the sample. The results did not differ significantly and thus both siblings were included in the final analysis.

The average age of the subjects was 15.2 (SD = 0.6) years (n = 1022; 54.7% female), 17.8 (SD = 0.6) years (n = 796; 56.3% female), 24.8 (SD = 0.6) years (n = 832; 54.7% female) and 33.0 (SD = 0.8) years (n = 470; 55.3% female). Written informed consent was obtained from the participants and, in case of minors, also from their parents. Permission for the study was obtained from the Ethics Review Committee on Human Research of the University of Tartu. The study was conducted in accordance with the Declaration of Helsinki.

Anthropometric measurements, blood pressure, biochemical measures and assessment of insulin resistance

Height and weight were measured by standardized procedures. BMI was calculated as weight / height squared (kg/m^2). Skinfold thickness was measured at the biceps, triceps, subscapular, suprailiac and medial calf areas on the left side of the body using a Harpenden caliper (Baty, West Sussex, England). Body fat percentage (BF%) was calculated using a formula by Durnin and Womersley (16,17). Waist circumference (WC) was taken between the lower rib margin and the iliac crest, at the end of gentle expiration and hip

circumference (HC) was measured over the buttocks, at the level of the great trochanter. All anthropometrical measurements were taken twice and a mean value was used.

Resting systolic (SBP) and diastolic blood pressure (DBP) was measured in a laboratory setting from the left arm with an automatic oscillometric method in a sitting position. Five consecutive measurements were made at 2 min intervals and the mean value was used in the analysis.

Venous blood samples were taken after an 8–12 h fast and analyzed in a certified clinical laboratory. Insulin resistance was estimated, using the HOMA index, which was calculated as fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5 (ref. 18).

Assessment of dietary intake

Dietary 24h (year 1998), 48h (years 2001, 2004, 2007) or 72h (years 2008, 2014) recall of food intake was used. The subjects were asked to complete a diet record at home during the day(s) before the study day. A face-to-face interview was performed on the study day. Data on portion size, that was not recorded in the food diary, was estimated using pictures of portion sizes (19). Where data on two or three days was available the mean consumption was calculated. Dietary intake was assessed from 1998–2004 using the Finnish Micro-Nutrica Nutritional Analysis program adapted to include Estonian foods, Estonian version 2.0 (Tallinn University of Technology, Food Processing Institute, Estonia) and from 2007–2014 using the NutriData food consumption database, versions 4.0–7.0 (National Institute for Health Development, Estonia). NutriData is an evidence-based food composition database, established by the National Institute for Health Development, and based on the Micronutrica

145 software. Over the years, the food list of Micronutrica has been updated with local food
146 data.

147

148 **Genotyping of *TFAP2B* variable number tandem repeat polymorphism**

149 Genotyping of *TFAP2B* intron 2 VNTR (a tetranucleotide repeat, 4–5 times) polymorphism
150 has been described in detail previously (8). Genotype frequencies (4/4 = 89, 4/5 = 407, 5/5 =
151 619) were in Hardy-Weinberg equilibrium.

152

153 **Statistical analysis**

154 All statistical analysis was performed with Stata software, version 13 (StataCorp LP, College
155 Station, Texas, USA). Significance level was set at 0.05.

156 The association between *TFAP2B* genotype and obesity, abdominal obesity, insulin
157 resistance and dietary intake was estimated from 15 to 25 years of age by using the linear
158 mixed-effects regression models with both random intercepts and random slopes. Linear
159 mixed-effects regression models take into account the correlations between repeated
160 measurements within each subject. Mixed models use all available observations and assume
161 that the missingness is independent of unobserved measurements, but dependent on the
162 observed measurements, and thus random (20). Models with 3-way interaction (time ×
163 TFAP2B × sex) were fitted to take into account differences between the sexes. Interaction
164 with sex was statistically significant and thus model with sex × TFAP2B and sex × time were
165 fitted. Thereafter, in the purpose of more clear presentation, separate models for male and
166 female subjects were fitted and presented. The measurements of obesity, abdominal

obesity, insulin resistance and dietary intake at baseline (at age 15 years) and at two follow-up points (ages 18 years and 25 years) were defined as the dependent variables. *TFAP2B* genotype (4/4, 4/5 or 5/5) was defined as the independent variable. Time was treated as a continuous variable. The goodness of fit of the statistical models was assessed using the likelihood-ratio test. In females, all the models included time × *TFAP2B* interactions. In males, time × *TFAP2B* interaction was not included in the final models for anthropometrical measurements and biomarkers, because the interaction was not statistically significant and the likelihood-ratio test did not show superiority of the more complicated models. Unstructured covariance structure and restricted maximum likelihood method was used. Heteroscedasticity was not detected based on graphical examination of standardized residual versus fitted values plot (not shown).

Continuous variables are presented as means and standard deviations and grouped by *TFAP2B* genotype and age. Differences in anthropometric measurements, metabolic biomarkers, blood pressure and dietary intake between *TFAP2B* genotypes in ages 15 years, 18 years, 25 years and 33 years were assessed by one-way ANOVA with the corrected significance level by Sidak method using the following equation $p^* = 1 - (1 - p)^3$ where p^* is compared with significance level 0.05.

RESULTS

Association between obesity and *TFAP2B* genotype

According to the linear mixed-effects regression model the interaction terms for sex × *TFAP2B* were significant ($p < 0.05$) for BMI and a trend ($0.05 \leq p < 0.10$) for body weight and BF% could be observed. The interaction terms for sex × time were significant for body

190 weight, BMI and BF% and a trend was observed for sum of 5 skinfolds (Supplementary Table
191 2).

192 Models for male subjects demonstrated that 5-repeat homozygotes of the *TFAP2B* had
193 significantly ($p < 0.05$) higher body weight, BMI, sum of 5 skinfolds and BF% compared to
194 heterozygotes (Table 1). The rate of change among male subjects in body weight was 1.94 kg
195 (95% CI 1.85, 2.03), in BMI 0.46 kg/m^2 (95% CI 0.43, 0.48), in sum of 5 skinfolds 2.37 mm
196 (95% CI 2.08, 2.66) and in BF% 0.20 % (95% CI 0.14, 0.25) per year (Figure 1A).

197 In female subjects, the rate of change per year in body weight and BMI was significantly
198 larger in 5-repeat homozygotes compared to heterozygotes ($p < 0.05$ for interaction) and a
199 trend in sum of 5 skinfolds was observed (Tables 1–2, Figure 1B).

200 A one-way ANOVA test at ages 15, 18, 25 and 33 years revealed several associations
201 between weight, BMI, BF%, sum of 5 skinfolds and *TFAP2B* genotype in male subjects
202 (Supplementary Tables 3–6). At age 33 years, male 5-repeat homozygotes had greater body
203 weight compared to heterozygotes (by 6.78 kg; 95% CI 1.98, 11.58; $p = 0.002$) and 4-
204 repeat homozygotes (by 10.28 kg; 95% CI 1.20, 19.36; $p = 0.021$). Similar trend was observed
205 at age 18 years. BMI was higher in male 5-repeat homozygotes at age 15 years (by 0.75
206 kg/m2; 95% CI 0.12, 1.39; $p = 0.014$) and 18 years (by 0.95 kg/m2; 95% CI 0.03, 1.86; $p =$
207 0.042), compared to heterozygotes and at 33 years compared to heterozygotes (by 2.34
208 kg/m2; 95% CI 0.97, 3.71; $p < 0.001$) and 4-repeat homozygotes (by 2.90 kg/m2; 95% CI 0.30,
209 5.50; $p = 0.024$). Male homozygotes for the 5-repeat allele had higher BF% at age 15 years
210 (by 1.25 %; 95% CI 0.14, 2.36; $p = 0.022$) and 18 years (by 1.94 %; 95% CI 0.27, 3.60; $p =$
211 0.017), compared to heterozygotes and at 33 years compared to heterozygotes (by 2.23 %;
212 95% CI 0.41, 4.04; $p = 0.011$) and homozygotes for the 4-repeat allele (by 4.40 %; 95% CI

0.96, 7.83; p = 0.007). Sum of 5 skinfolds was greater in male 5-repeat homozygotes at age 18 years (by 9.72 mm; 95% CI 1.54, 17.89; p = 0.014), compared to heterozygotes and at 33 years compared to heterozygotes (by 13.23 mm; 95% CI 2.56, 23.91; p = 0.010) and 4-repeat homozygotes (by 27.31 mm; 95% CI 7.13, 47.48; p = 0.004). Similar trend was observed at age 15 years.

Among female subjects no statistically significant associations between weight, BMI, sum of 5 skinfolds, BF% and *TFAP2B* genotype were identified by one-way ANOVA test, at any age (Supplementary Tables 3–6).

Association between abdominal obesity and *TFAP2B* genotype

Interaction terms for sex × *TFAP2B* were significant ($p < 0.05$) for WC, WHR and WHtR and interaction terms for sex × time were significant ($p < 0.001$) for WC, HC, WHR, WHtR and subscapular skinfold thickness (Supplementary Table 2).

According to the model, male 5-repeat homozygotes of the *TFAP2B* had significantly ($p < 0.05$) higher WC, HC, waist to hip ratio (WHR), waist to height ratio (WHtR) and subscapular skinfold thickness compared to heterozygotes (Table 1). The rate of change among male subjects in WC was 1.43 cm (95% CI 1.36, 1.51), in HC 1.14 cm (95% CI 1.08, 1.20), in WHR 0.005 units (95% CI 0.005, 0.006), in WHtR 0.007 units (95% CI 0.006, 0.007) and in subscapular skinfold thickness 0.94 mm (95% CI 0.86, 1.02) per year (Figure 2A).

In HC the rate of change per year was greater ($p < 0.05$ for interaction) in female 5-repeat homozygotes compared to heterozygotes (Tables 1–2, Figure 2B).

In male subjects several associations between WC, HC, WHR, WHtR, subscapular skinfold thickness and *TFAP2B* genotype were revealed by one-way ANOVA test at ages 15, 18, 25 and 33 years (Supplementary Tables 3–6). Homozygotes for the 5-repeat allele had higher WC at age 15 years (by 1.37 cm; 95% CI 0.04, 2.70; p = 0.041) and 18 years (by 2.78 cm; 95% CI 0.70, 4.87; p = 0.004) compared to heterozygotes and at 33 years compared to heterozygotes (by 5.82 cm; 95% CI 2.29, 9.36; p < 0.001) and 4-repeat homozygotes (by 6.80 cm; 95% CI 0.11, 13.49; p = 0.045). HC was higher in male 5-repeat homozygotes at age 18 years (by 2.10 cm; 95% CI 0.20, 4.01; p = 0.025) and 33 years (by 2.56 cm; 95% CI 0.05, 5.08; p = 0.44), compared to heterozygotes. Homozygotes for the 5 repeat allele had higher WHtR at age 15 years (by 0.009 units; 95% CI 0.001, 0.016; p = 0.012), 18 years (by 0.015 units; 95% CI 0.004, 0.027; p = 0.006) and 33 years (by 0.035 units; 95% CI 0.015, 0.055; p < 0.001), compared to heterozygotes. Subscapular skinfold thickness was greater at age 15 years (by 0.89 mm; 95% CI 0.01, 1.76; p = 0.046) and 18 years (by 2.02 mm; 95% CI 0.17, 3.87; p = 0.027) in male 5-repeat homozygotes compared to heterozygotes and at 33 years compared to heterozygotes (by 4.31 mm; 95% CI 0.81, 7.82; p = 0.010) and 4-repeat homozygotes (by 8.60 mm; 95% CI 1.96, 15.23; 0.006). Male 5-repeat homozygotes had higher WHR at age 18 years (by 0.010 units; 95% CI 0.0003, 0.0202; p = 0.041) and 33 years (by 0.033 units; 95% CI 0.012, 0.055; p = 0.001), compared to heterozygotes.

Female 5-repeat homozygotes had lower WHR at age 18 years (by 0.02 units; 95% CI 0.0004, 0.0407; p = 0.045) compared to 4-repeat homozygotes. In females, no other statistically significant associations between WC, HC, WHtR, subscapular skinfold thickness and *TFAP2B* genotype were identified at any age (Supplementary Tables 3–6).

Association between biochemical measures and *TFAP2B* genotype

In models with sex × time interaction, the interaction terms were significant ($p < 0.001$) for fasting insulin, cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides (Supplementary Table 2).

Male 5-repeat homozygotes of the *TFAP2B* had significantly ($p < 0.05$) higher fasting insulin levels and HOMA index compared to heterozygotes. Fasting glucose, cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels did not differ between genotypes (Table 1). The rate of change among male subjects in fasting insulin was -0.31 (95% CI -0.39, -0.24) and in HOMA was -0.07 (95% CI -0.09, -0.05) per year (Figure 3A).

In female subjects, fasting insulin, fasting glucose, HOMA index, cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride levels did not differ between genotypes (Table 1–2, Figure 3B).

At ages 15 and 33 years several associations were revealed in male subjects by one-way ANOVA test between fasting insulin, HOMA, HDL-cholesterol and *TFAP2B* genotype. Compared to heterozygotes, 5-repeat homozygotes had higher fasting insulin levels (by 2.22 mU/L; 95% CI 0.60, 3.83; $p = 0.003$) and HOMA (by 0.57 units; 95% CI 0.15, 1.00; $p = 0.004$) at age 15 years. At age 33 years HDL-cholesterol levels were lower in male 5-repeat homozygotes (by 0.16 mmol/L; 95% CI 0.04, 0.29; $p = 0.007$), compared to heterozygotes.

Among male subjects no other significant associations between cholesterol, LDL-cholesterol, triglycerides, glucose and *TFAP2B* genotype were identified by one-way ANOVA at any age (Supplementary Tables 3–6).

Female 5-repeat homozygotes had higher triglyceride levels (by 0.16 mmol/L; 95% CI 0.02, 0.293; p = 0.18) at age 33 years, compared to heterozygotes. No other statistically significant associations between cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, insulin, HOMA and *TFAP2B* genotype were identified by one-way ANOVA test among females at any age (Supplementary Tables 3–6).

Association between blood pressure and *TFAP2B* genotype

The linear mixed-effects regression model and one-way ANOVA test failed to demonstrate a statistically significant difference in blood pressure between *TFAP2B* genotypes in male or female subjects at any age (Supplementary Tables 3–6).

Association between dietary intake and *TFAP2B* genotype

The linear mixed-effects regression model showed a significant (p = 0.023 for interaction) difference in the rate of change per year in daily energy intake (DEI) (MJ) (1 kcal = 0.0042 MJ) between male 5-repeat homozygotes of the *TFAP2B* and heterozygotes, the former having a larger decrease in the rate of change per year in DEI (0.15 [95% CI 0.08, 0.21] *versus* 0.03 [95% CI 0.04, 0.11]) (Figure 4A). In female subjects, DEI did not differ between genotypes (Figure 4B).

A difference in protein-, lipid- and carbohydrate intake in grams per kilogram of body weight (g/kg) or protein-, lipid- and carbohydrate intake as a percentage from DEI (E%) was not observed between *TFAP2B* genotype in male or female subjects.

One-way ANOVA test revealed associations between DEI, lipid and carbohydrate intake (g/kg) with *TFAP2B* genotype in male subjects at ages 25 and 33 years (Supplementary Tables 9–10), but not at age 15 and 18 years (Supplementary Tables 7–8). At age 25 years male heterozygotes had higher DEI compared to 5-repeat homozygotes (by 0.95 MJ/day; 95% CI 0.09, 1.81; p = 0.026) and 4-repeat homozygotes (by 1.68 MJ/day; 95% CI 0.02, 3.33; p = 0.046). Lipid intake was greater in male heterozygotes at age 25 years (by 0.17 g/kg; 95% CI 0.04, 0.30; 0.007) and 33 years (by 0.20 g/kg; 95% CI 0.03, 0.37; p = 0.014) compared to 5-repeat homozygotes. At 25 years (by 0.36 g/kg; 95% CI 0.02, 0.70; p = 0.034) and 33 years (by 0.43 g/kg; 95% CI 0.07, 0.79; p = 0.015) male heterozygotes had higher carbohydrate intake compared to 5-repeat homozygotes.

Protein intake (g/kg) and protein-, lipid- or carbohydrate intake (E%) did not associate with *TFAP2B* genotype in males at any age (Supplementary Tables 7–10).

In female 4-repeat homozygotes protein intake (E%) was greater at age 33 years compared to heterozygotes (by 2.29 %; 95% CI 0.01, 4.57; p = 0.049) and 5-repeat homozygotes (by 2.30 %; 95% CI 0.08, 4.51; p = 0.39).

Protein-, lipid- or carbohydrate intake (g/kg) and lipid- or carbohydrate intake (E%) did not associate with *TFAP2B* genotype in female subjects at any age (Supplementary Tables 7–10).

DISCUSSION

Various GWAS have identified several loci that are associated with measurements of obesity and abdominal obesity in children (10) and adults (9,21–24) or loci which can predict the development of obesity in adulthood (25). *TFAP2B* is among loci frequently associated with BMI variability (9,10,22,23), WC (9,21) and overweight (24) in GWAS.

A meta-analysis of 16 GWAS (n = 38 580) with data on WC and WHR selected 26 SNPs for follow-up, for which the evidence of association with WC and WHR was strong. Stage 2 follow-up studies in a maximum of 70 689 individuals identified a strong association between *TFAP2B* ($p = 1.9 \times 10^{-11}$) and WC (21). Speliotes et al. (2010) examined associations between BMI and ~2.8 million SNPs in up to 123 865 individuals, with targeted follow-up of 42 SNPs in up to 125 931 additional individuals. They confirmed 32 loci associated with BMI, including *TFAP2B* (22). Guo et al. (2013) identified three novel-, three previously established- and replicated five previously identified loci, including *TFAP2B*, associated with BMI in a meta-analysis of gene-centric association studies (n = 92 903) (ref. 23).

Both genetic and environmental factors have an effect on the variation of BMI. Although heritability estimates of BMI around 80% have been reported (5–7), it is still debated to which extent genes and shared environment contribute to food intake, physical activity and BMI variation. Twin studies have indicated the importance of shared environment in adolescence and young adulthood to fast food intake, sedentary lifestyle and obesity (26). The effect of environmental factors on BMI is greater in childhood, but when reaching adolescence and young adulthood, the effect of genetic factors increase (27,28). It has been suggested that the effect of *TFAP2B* on BMI variability may differ across the life course (29,30), but there is still little evidence on the longitudinal effect of obesity associated

339 genetic factors and the magnitude of difference over time. We investigated the effect of
340 *TFAP2B* intron 2 VNTR polymorphism on obesity and insulin resistance over a 10 year study
341 period from adolescence into young adulthood with a population representative sample of
342 participants, of European descent.
343 Our results show that *TFAP2B* intron 2 VNTR polymorphism is associated with
344 measurements of obesity and abdominal obesity from adolescence to young adulthood.
345 Furthermore, the *TFAP2B* genotype effect appeared earlier in males. Male homozygotes for
346 the *TFAP2B* 5-repeat allele had higher measures of obesity, abdominal obesity and insulin
347 resistance from 15 to 25 years of age. In female subjects, the rate of change per year in
348 measurements of obesity differed between *TFAP2B* genotypes, being larger in homozygotes
349 for the 5-repeat allele. We did not observe an association between *TFAP2B* genotype and
350 blood pressure. It would be interesting to see if and how *TFAP2B* genotype affects blood
351 pressure later in life.

352 The longitudinal effect of *TFAP2B* on BMI has only recently been reported by Graff et al.
353 (2017) in a nationally representative school-based cohort of US adolescents. The mean age
354 of subjects during Wave I was 15.9 years (11–20 years), and Wave IV 28.9 years (23–32
355 years). Results showed a positive association between six obesity loci, including *TFAP2B*, and
356 change in BMI over time, but only among subjects with European American ancestry. They
357 also found that two of the loci, *TFAP2B* and *MTCH2,* had different magnitudes of effect in
358 different ages, whereas *TFAP2B* had a stronger influence on BMI in young adulthood (greater
359 in those who were aged 21 years at Wave II compared to those who were 13 years), while
360 *MTCH2* had a stronger influence on BMI in young adolescents (greater in those who were
361 aged 13 years at Wave II versus those who were 21 years) (29).

362 The pathways through which *TFAP2B* influences the development of obesity and insulin
363 resistance are unclear. *TFAP2B* encodes a transcription factor expressed in neural crest cells,
364 regulating cell survival, promoting cell proliferation and suppressing differentiation (31). It is
365 likely that *TFAP2B* affects both the CNS and adipocyte function. We have previously shown
366 that a polymorphic region in the human transcription factor AP-2beta gene is associated
367 with specific personality traits (32) and furthermore that TFAP2B levels in the raphe where
368 the serotonergic perikarya are located were strongly correlated with serotonin turnover in
369 the frontal cortex of rats (33). Central serotonergic neurotransmission is critically important
370 in the regulation of food intake, thus we next analyzed the differences in dietary intake
371 between *TFAP2B* genotypes. Our results demonstrate that in male subjects, heterozygotes
372 had significantly smaller decrease in the rate of change per year in DEI. Furthermore, DEI
373 differed significantly between genotypes at age 25 years, where male heterozygotes had
374 higher DEI and higher lipid- and carbohydrate intake per body weight. Male homozygotes for
375 the 5-repeat allele had higher body weight already in adolescence and young adulthood
376 which may lead them to regulate their body weight by reducing DEI. Our results indicate that
377 the effect of *TFAP2B* on obesity is not mediated by dietary intake and hence further research
378 should concentrate on other factors.

379 Previously, the 8-repeat allele of intron 1 and the 4-repeat allele of intron 2, and also the 9-
380 repeat allele of intron 1 and 5-repeat allele of intron 2 were found to be in significant linkage
381 disequilibrium, and indeed they were linked to the same phenotype (8). Polymorphisms in
382 the first intron of *TFAP2B* affect the transcriptional activity of the gene, whereas individuals
383 with the 9-repeat allele have higher expression of TFAP2B in adipose tissue (11).
384 Overexpression of TFAP2B in adipocytes cause decreased expression and secretion of
385 adiponectin (12), adipocyte cell enlargement, stimulation of glucose transport activity,

triglyceride accumulation and insulin resistance (13). Furthermore, it is possible that *TFAP2B* plays a role in intrauterine growth. We have previously found that the sex of the newborn influences the association of maternal *TFAP2B* genotype and maternal leptin with the weight of the newborn (34). *TFAP2B* has also been associated with type 2 diabetes (35,36).

The reasons behind sex differences remain unclear. The effects of sex on food intake can be observed already in childhood, where boys are more prone to eat in the absence of hunger ($p = 0.006$) (ref. 37). Women are more likely to make better dietary choices consuming more fiber, fruits and avoiding high-fat foods (38). Metabolic differences between males and females are well established, but little is known about the neuroendocrine basis of these differences (39). Serotonergic neurotransmission, affected by *TFAP2B* (33), plays a part in satiation and food reward (40) and a sexual dimorphism can be observed in the serotonergic system (39,40).

This study has some limitations that should be considered. Our study sample consists of individuals of European descent, which means the study results cannot be extrapolated to individuals of other ancestry. Although we demonstrate the effect of *TFAP2B* intron 2 VNTR polymorphism on measures of obesity and abdominal obesity is consistent in time, we cannot determine at what age the effect occurs. The sample size, to assess the prevalence of the main cardiovascular risk factors, was calculated using estimates of 0.80 for power and 0.05 for variability. Regarding the results where no significant associations were found, because of the size of our sample and limited statistical power, we cannot be certain whether the associations are truly zero.

Overall, the results strongly support the notion that *TFAP2B* plays an important role in the development of obesity and abdominal obesity. We have also demonstrated that the effect

of *TFAP2B* intron 2 VNTR polymorphism on anthropometric measures and glucose metabolism differs between male and female subjects. In males the *TFAP2B* genotype effect remains consistent from 15 to 25 years of age, but in females the rate of change differs in time between genotypes.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

Supplementary information is available at International Journal of Obesity's website.

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FIGURE LEGENDS

Figure 1. Association between *TFAP2B* intron 2 VNTR genotype and body weight, body mass index (BMI) and body fat percentage (BF%) from 15 to 25 years of age in male (graph A) and female (graph B) subjects.

*P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/5 and 5/5 genotypes

Figure 2. Association between *TFAP2B* intron 2 VNTR genotype and waist circumference, waist-hip ratio (WHR) and waist-height ratio (WHtR) from 15 to 25 years of age in male (graph A) and female (graph B) subjects.

*P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/5 and 5/5 genotypes

#P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/4 and 5/5 genotypes

Figure 3. Association between *TFAP2B* intron 2 VNTR genotype and fasting glucose, fasting insulin and HOMA index, from 15 to 25 years of age in male (graph A) and female (graph B) subjects.

*P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/5 and 5/5 genotypes

Figure 4. Association between *TFAP2B* intron 2 VNTR genotype and daily energy intake, lipid intake per body weight and carbohydrate intake per body weight, from 15 to 25 years of age in male (graph A) and female (graph B) subjects.

¤P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/4 and 4/5 genotypes

*P<0.05 significant difference between the mean values of the *TFAP2B* intron 2 VNTR 4/5 and 5/5 genotypes