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

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6 **Transcription Factor AP-2B associated with obesity**

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29 **ABSTRACT**

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

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

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

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

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

## 54 INTRODUCTION

55 Prevalence of overweight, obesity and abdominal obesity has increased worldwide (1–3).

56 Obesity was previously considered to be only a disorder of energy imbalance, but now we  
57 know that its pathogenesis is more complex involving an interaction between genetic,  
58 environmental, physiological, behavioural, social, and economic factors (4).

59 The development of obesity has a large genetic component, and heritability estimates of  
60 BMI around 80% have been reported, while a large variety of genes appears to play a role  
61 (5–7). We have previously demonstrated that the intron 2 variable number tandem repeat  
62 (VNTR) polymorphism of the transcription factor AP2B gene (*TFAP2B*) was associated with  
63 abdominal obesity and insulin resistance among 15-year old subjects. Homozygotes for the  
64 5-repeat allele had higher levels of fasting insulin, Homeostasis Model Assessment (HOMA)  
65 estimates and subscapular skinfold thickness, as compared to the carriers of the 4-repeat  
66 allele (8). These associations were however present only in male subjects. Recent large-scale  
67 studies have reinforced the implication of *TFAP2B* in BMI and obesity. A meta-analysis of  
68 genome-wide association studies (GWAS) in individuals of European and non-European  
69 descent and MetaboChip studies, with a total of 339 224 individuals, identified 97 loci  
70 including *TFAP2B* as associated with BMI (9). A meta-analysis of GWAS in children (aged 2–  
71 10 years) produced similar results: It included 20 studies (n = 35 668) in the discovery phase  
72 and 13 studies (n = 11 873) in the replication phase; 15 loci, including *TFAP2B*, reached  
73 genome-wide significance and were thus reliably associated with childhood BMI (10). These  
74 data make *TFAP2B* a highly interesting candidate gene for overall obesity as well as  
75 abdominal obesity and insulin resistance that has its effect already manifested in early  
76 childhood.

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

86

## 87 **SUBJECTS AND METHODS**

### 88 **Study sample**

89 The sample was originally formed for the European Youth Heart Study in 1998/1999 and was  
90 later incorporated into the Estonian Children Personality Behavior and Health Study  
91 (ECPBHS). The study procedure and the selection of the original sample has been described  
92 in detail elsewhere (14). In brief, ECPBHS is a longitudinal cohort study with a population  
93 representative sample of participants, all of European descent, with school as the sampling  
94 unit. All schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56)  
95 were included into the sampling and 25 schools were selected. All children from grades 3  
96 (aged 9 years; n = 583) and grades 9 (aged 15 years; n = 593) were invited to participate (14).  
97 Follow-up studies for the younger birth cohort have been taken place in ages 15 years (n =  
98 483), 18 years (n = 454) and 25 years (n = 441) and for the older birth cohort in ages 18 years  
99 (n = 417 + additional 62), 25 years (n = 541) and 33 years (n = 504) (ref. 15).

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

108 The average age of the subjects was 15.2 (SD = 0.6) years (n = 1022; 54.7% female), 17.8 (SD  
109 = 0.6) years (n = 796; 56.3% female), 24.8 (SD = 0.6) years (n = 832; 54.7% female) and 33.0  
110 (SD = 0.8) years (n = 470; 55.3% female). Written informed consent was obtained from the  
111 participants and, in case of minors, also from their parents. Permission for the study was  
112 obtained from the Ethics Review Committee on Human Research of the University of Tartu.  
113 The study was conducted in accordance with the Declaration of Helsinki.

114

#### 115 **Anthropometric measurements, blood pressure, biochemical measures and assessment of** 116 **insulin resistance**

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

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

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

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

132

### 133 **Assessment of dietary intake**

134 Dietary 24h (year 1998), 48h (years 2001, 2004, 2007) or 72h (years 2008, 2014) recall of  
135 food intake was used. The subjects were asked to complete a diet record at home during the  
136 day(s) before the study day. A face-to-face interview was performed on the study day. Data  
137 on portion size, that was not recorded in the food diary, was estimated using pictures of  
138 portion sizes (19). Where data on two or three days was available the mean consumption  
139 was calculated. Dietary intake was assessed from 1998–2004 using the Finnish Micro-Nutrica  
140 Nutritional Analysis program adapted to include Estonian foods, Estonian version 2.0 (Tallinn  
141 University of Technology, Food Processing Institute, Estonia) and from 2007–2014 using the  
142 NutriData food consumption database, versions 4.0–7.0 (National Institute for Health  
143 Development, Estonia). NutriData is an evidence-based food composition database,  
144 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

167 obesity, insulin resistance and dietary intake at baseline (at age 15 years) and at two follow-  
168 up points (ages 18 years and 25 years) were defined as the dependent variables. *TFAP2B*  
169 genotype (4/4, 4/5 or 5/5) was defined as the independent variable. Time was treated as a  
170 continuous variable. The goodness of fit of the statistical models was assessed using the  
171 likelihood-ratio test. In females, all the models included time × *TFAP2B* interactions. In  
172 males, time × *TFAP2B* interaction was not included in the final models for anthropometrical  
173 measurements and biomarkers, because the interaction was not statistically significant and  
174 the likelihood-ratio test did not show superiority of the more complicated models.  
175 Unstructured covariance structure and restricted maximum likelihood method was used.  
176 Heteroscedasticity was not detected based on graphical examination of standardized  
177 residual versus fitted values plot (not shown).  
178 Continuous variables are presented as means and standard deviations and grouped by  
179 *TFAP2B* genotype and age. Differences in anthropometric measurements, metabolic  
180 biomarkers, blood pressure and dietary intake between *TFAP2B* genotypes in ages 15 years,  
181 18 years, 25 years and 33 years were assessed by one-way ANOVA with the corrected  
182 significance level by Sidak method using the following equation  $p^* = 1 - (1 - p)^3$  where  $p^*$  is  
183 compared with significance level 0.05.

184

## 185 **RESULTS**

### 186 **Association between obesity and *TFAP2B* genotype**

187 According to the linear mixed-effects regression model the interaction terms for sex ×  
188 *TFAP2B* were significant ( $p < 0.05$ ) for BMI and a trend ( $0.05 \leq p < 0.10$ ) for body weight and  
189 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<sup>2</sup> (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/m<sup>2</sup>; 95% CI 0.12, 1.39;  $p = 0.014$ ) and 18 years (by 0.95 kg/m<sup>2</sup>; 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/m<sup>2</sup>; 95% CI 0.97, 3.71;  $p < 0.001$ ) and 4-repeat homozygotes (by 2.90 kg/m<sup>2</sup>; 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

213 0.96, 7.83;  $p = 0.007$ ). Sum of 5 skinfolds was greater in male 5-repeat homozygotes at age  
214 18 years (by 9.72 mm; 95% CI 1.54, 17.89;  $p = 0.014$ ), compared to heterozygotes and at 33  
215 years compared to heterozygotes (by 13.23 mm; 95% CI 2.56, 23.91;  $p = 0.010$ ) and 4-repeat  
216 homozygotes (by 27.31 mm; 95% CI 7.13, 47.48;  $p = 0.004$ ). Similar trend was observed at  
217 age 15 years.

218 Among female subjects no statistically significant associations between weight, BMI, sum of  
219 5 skinfolds, BF% and *TFAP2B* genotype were identified by one-way ANOVA test, at any age  
220 (Supplementary Tables 3–6).

221

## 222 **Association between abdominal obesity and *TFAP2B* genotype**

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

226 According to the model, male 5-repeat homozygotes of the *TFAP2B* had significantly ( $p <$   
227 0.05) higher WC, HC, waist to hip ratio (WHR), waist to height ratio (WHtR) and subscapular  
228 skinfold thickness compared to heterozygotes (Table 1). The rate of change among male  
229 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  
230 0.005 units (95% CI 0.005, 0.006), in WHtR 0.007 units (95% CI 0.006, 0.007) and in  
231 subscapular skinfold thickness 0.94 mm (95% CI 0.86, 1.02) per year (Figure 2A).

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

234 In male subjects several associations between WC, HC, WHR, WHtR, subscapular skinfold  
235 thickness and *TFAP2B* genotype were revealed by one-way ANOVA test at ages 15, 18, 25  
236 and 33 years (Supplementary Tables 3–6). Homozygotes for the 5-repeat allele had higher  
237 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%  
238 CI 0.70, 4.87;  $p = 0.004$ ) compared to heterozygotes and at 33 years compared to  
239 heterozygotes (by 5.82 cm; 95% CI 2.29, 9.36;  $p < 0.001$ ) and 4-repeat homozygotes (by 6.80  
240 cm; 95% CI 0.11, 13.49;  $p = 0.045$ ). HC was higher in male 5-repeat homozygotes at age 18  
241 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;  
242  $p = 0.44$ ), compared to heterozygotes. Homozygotes for the 5 repeat allele had higher WHtR  
243 at age 15 years (by 0.009 units; 95% CI 0.001, 0.016;  $p = 0.012$ ), 18 years (by 0.015 units; 95%  
244 CI 0.004, 0.027;  $p = 0.006$ ) and 33 years (by 0.035 units; 95% CI 0.015, 0.055;  $p < 0.001$ ),  
245 compared to heterozygotes. Subscapular skinfold thickness was greater at age 15 years (by  
246 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;  $p =$   
247 0.027) in male 5-repeat homozygotes compared to heterozygotes and at 33 years compared  
248 to heterozygotes (by 4.31 mm; 95% CI 0.81, 7.82;  $p = 0.010$ ) and 4-repeat homozygotes (by  
249 8.60 mm; 95% CI 1.96, 15.23; 0.006). Male 5-repeat homozygotes had higher WHR at age 18  
250 years (by 0.010 units; 95% CI 0.0003, 0.0202;  $p = 0.041$ ) and 33 years (by 0.033 units; 95% CI  
251 0.012, 0.055;  $p = 0.001$ ), compared to heterozygotes.

252 Female 5-repeat homozygotes had lower WHR at age 18 years (by 0.02 units; 95% CI 0.0004,  
253 0.0407;  $p = 0.045$ ) compared to 4-repeat homozygotes. In females, no other statistically  
254 significant associations between WC, HC, WHtR, subscapular skinfold thickness and *TFAP2B*  
255 genotype were identified at any age (Supplementary Tables 3–6).

256

257 **Association between biochemical measures and *TFAP2B* genotype**

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

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

266 In female subjects, fasting insulin, fasting glucose, HOMA index, cholesterol, HDL-  
267 cholesterol, LDL-cholesterol and triglyceride levels did not differ between genotypes (Table  
268 1–2, Figure 3B).

269 At ages 15 and 33 years several associations were revealed in male subjects by one-way  
270 ANOVA test between fasting insulin, HOMA, HDL-cholesterol and *TFAP2B* genotype.

271 Compared to heterozygotes, 5-repeat homozygotes had higher fasting insulin levels (by  $2.22$   
272  $\text{mU/L}$ ; 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  
273 age 15 years. At age 33 years HDL-cholesterol levels were lower in male 5-repeat  
274 homozygotes (by  $0.16$   $\text{mmol/L}$ ; 95% CI  $0.04, 0.29$ ;  $p = 0.007$ ), compared to heterozygotes.

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

278 Female 5-repeat homozygotes had higher triglyceride levels (by 0.16 mmol/L; 95% CI 0.02,  
279 0.293;  $p = 0.18$ ) at age 33 years, compared to heterozygotes. No other statistically significant  
280 associations between cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, insulin, HOMA  
281 and *TFAP2B* genotype were identified by one-way ANOVA test among females at any age  
282 (Supplementary Tables 3–6).

283

#### 284 **Association between blood pressure and *TFAP2B* genotype**

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

288

#### 289 **Association between dietary intake and *TFAP2B* genotype**

290 The linear mixed-effects regression model showed a significant ( $p = 0.023$  for interaction)  
291 difference in the rate of change per year in daily energy intake (DEI) (MJ) (1 kcal = 0.0042 MJ)  
292 between male 5-repeat homozygotes of the *TFAP2B* and heterozygotes, the former having a  
293 larger decrease in the rate of change per year in DEI (0.15 [95% CI 0.08, 0.21] *versus* 0.03  
294 [95% CI 0.04, 0.11]) (Figure 4A). In female subjects, DEI did not differ between genotypes  
295 (Figure 4B).

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

299 One-way ANOVA test revealed associations between DEI, lipid and carbohydrate intake  
300 (g/kg) with *TFAP2B* genotype in male subjects at ages 25 and 33 years (Supplementary  
301 Tables 9–10), but not at age 15 and 18 years (Supplementary Tables 7–8). At age 25 years  
302 male heterozygotes had higher DEI compared to 5-repeat homozygotes (by 0.95 MJ/day;  
303 95% CI 0.09, 1.81;  $p = 0.026$ ) and 4-repeat homozygotes (by 1.68 MJ/day; 95% CI 0.02, 3.33;  
304  $p = 0.046$ ). Lipid intake was greater in male heterozygotes at age 25 years (by 0.17 g/kg; 95%  
305 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-  
306 repeat homozygotes. At 25 years (by 0.36 g/kg; 95% CI 0.02, 0.70;  $p = 0.034$ ) and 33 years  
307 (by 0.43 g/kg; 95% CI 0.07, 0.79;  $p = 0.015$ ) male heterozygotes had higher carbohydrate  
308 intake compared to 5-repeat homozygotes.

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

311 In female 4-repeat homozygotes protein intake (E%) was greater at age 33 years compared  
312 to heterozygotes (by 2.29 %; 95% CI 0.01, 4.57;  $p = 0.049$ ) and 5-repeat homozygotes (by  
313 2.30 %; 95% CI 0.08, 4.51;  $p = 0.39$ ).

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

316 **DISCUSSION**

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

321 A meta-analysis of 16 GWAS (n = 38 580) with data on WC and WHR selected 26 SNPs for  
322 follow-up, for which the evidence of association with WC and WHR was strong. Stage 2  
323 follow-up studies in a maximum of 70 689 individuals identified a strong association  
324 between *TFAP2B* ( $p = 1.9 \times 10^{-11}$ ) and WC (21). Speliotes et al. (2010) examined associations  
325 between BMI and ~2.8 million SNPs in up to 123 865 individuals, with targeted follow-up of  
326 42 SNPs in up to 125 931 additional individuals. They confirmed 32 loci associated with BMI,  
327 including *TFAP2B* (22). Guo et al. (2013) identified three novel-, three previously established-  
328 and replicated five previously identified loci, including *TFAP2B*, associated with BMI in a  
329 meta-analysis of gene-centric association studies (n = 92 903) (ref. 23).

330 Both genetic and environmental factors have an effect on the variation of BMI. Although  
331 heritability estimates of BMI around 80% have been reported (5–7), it is still debated to  
332 which extent genes and shared environment contribute to food intake, physical activity and  
333 BMI variation. Twin studies have indicated the importance of shared environment in  
334 adolescence and young adulthood to fast food intake, sedentary lifestyle and obesity (26).  
335 The effect of environmental factors on BMI is greater in childhood, but when reaching  
336 adolescence and young adulthood, the effect of genetic factors increase (27,28). It has been  
337 suggested that the effect of *TFAP2B* on BMI variability may differ across the life course  
338 (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,

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

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

398 This study has some limitations that should be considered. Our study sample consists of  
399 individuals of European descent, which means the study results cannot be extrapolated to  
400 individuals of other ancestry. Although we demonstrate the effect of *TFAP2B* intron 2 VNTR  
401 polymorphism on measures of obesity and abdominal obesity is consistent in time, we  
402 cannot determine at what age the effect occurs. The sample size, to assess the prevalence of  
403 the main cardiovascular risk factors, was calculated using estimates of 0.80 for power and  
404 0.05 for variability. Regarding the results where no significant associations were found,  
405 because of the size of our sample and limited statistical power, we cannot be certain  
406 whether the associations are truly zero.

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

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

413

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421

#### 422 **CONFLICT OF INTEREST**

423 The authors declare no competing financial interests.

424

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

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536

537 **FIGURE LEGENDS**

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

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

543

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

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

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

551

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

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

557

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

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

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