INTRODUCTION

Obesity, defined as excessive accumulation of pathological adipose tissue, is a complex disorder whose pathogenesis involves both environmental and genetic factors (www.who.it). Obesity in children is one of the fundamental public health problems not only because of the increasing prevalence of this pathology but mainly because of its consequences in adult life, such as abnormal glucose metabolism (including type 2 diabetes mellitus), cardiovascular disease, hypertension and other conditions (1-3). The actual prevalence of obesity in children is unknown, although it is believed to have increased several times in the past few decades among Polish children (4). New anthropometric references have recently been published. They are based on a large population of children from all regions of Poland and adapted to the internationally accepted criteria for overweight and obesity based on body mass index standard deviation score (BMI SDS) (5). These authors were the only ones to have assessed the prevalence of overweight and obesity in a large group of Polish children based on the new references.

The phenomenon of excessive body mass increase in the general population of children and adults in the past few decades has been attributed to environmental changes, especially when superimposed on a predisposing genetic background. The fat mass and obesity-associated (FTO) gene has recently become one of the most extensively investigated genes associated with body mass. The aim of the study was to investigate the association of the FTO rs9939609 T>A single-nucleotide polymorphism with selected anthropometric and metabolic parameters and blood pressure in a large population of Polish children. A total of 968 children aged 4 to 18 years were included in the study. Genotyping was performed using a TaqMan assay. The rs9939609 marker was associated with standardised (standard deviation scores, SDSs) values of body mass, height, body mass index (BMI), waist circumference, hip circumference and arm circumference. When we compared normal-weight with obese children we observed a strong recessive predisposing effect of the A-allele (OR=2.11 95% CI: 1.50–2.99, p=2.23×10^{-6} for AA vs. TT+AT). Univariate analysis revealed associations of the FTO gene variants with the values of blood pressure, triglycerides, fasting glucose and HOMA insulin resistance index. After taking into account SDS BMI only the association with HOMA remained statistically significant. The FTO gene polymorphism may partially explain the predisposition to obesity in the population of Polish children. The potential effect on the remaining cardiovascular risk factors seems indirect and dependent on BMI changes. The polymorphism may be independently associated with insulin resistance.

Key words: blood pressure, body mass, cardiovascular risk factors, glucose level, fat mass and obesity-associated gene polymorphism, insulin resistance, obesity, overweight, triglycerides

Abbreviations: BMI - body mass index; BMI SDS - body mass index standard deviation score; BP - blood pressure; CI - confidence interval; FTO - fat mass and obesity-associated; HOMA - homeostatic model assessment; OR - odds ratio; SNP - single-nucleotide polymorphism

THE ASSOCIATION OF THE FTO RS9939609 POLYMORPHISM WITH OBESITY AND METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASES IN POLISH CHILDREN

1Department of Paediatrics, Endocrinology and Diabetes with a Cardiology Unit, Medical University of Bialystok, Bialystok, Poland; 2Department of Pathology, Medical University of Bialystok, Bialystok, Poland

W. LUCZYNSKI1, G. ZALEWSKI2, A. BOSSOWSKI1

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likely that many gene loci discovered in genome-wide association studies (GWAS) in adults also play a role in the pathogenesis of obesity in children (these genes include, among others: FTO, INSG2, MC4R, TMEM18, KCTD15), although the data available are still very scarce (7). It should also be borne in mind that a limitation of this field of knowledge is the small effect of the discovered genes and even their combinations explain only a small percentage of body mass index (BMI) variability (10). The fat mass and obesity-associated (FTO) gene has recently become one of the most extensively investigated genes associated with body mass (11). The presence of two A-alleles at the rs9939609 site of the FTO gene is associated with increased BMI by about 1 kg/m², increased body mass by 2.3 kg and 1.3-fold higher risk of overweight and obesity in both adults and children (11, 12). The role of the FTO gene product is unclear; all that is known at the moment is that it is expressed in the hypothalamus and its function is involved in the regulation of the appetite.

The association of the FTO gene polymorphism with obesity has been confirmed in several populations, although no data on Polish children are available (11). In addition, new anthropometric references for children and adolescents have been developed recently, so an assessment of the association of the FTO rs9939609 polymorphism with BMI could be useful in establishing new cardiovascular risk factors. Our aim, therefore, was to assess the impact of the FTO rs9939609 polymorphism on BMI, the diagnosis of overweight and obesity, blood pressure values and lipid profile in a large population of Polish children while utilising the updated Polish anthropometric references.

**MATERIAL AND METHODS**

**Subjects**

This study is part of the research project FTO-DIAB whose aim is to evaluate the impact of genetic background on the development of overweight, obesity and metabolic syndrome features in healthy and diabetic Polish children (a multicentre study, registered on ClinicalTrials.gov, NCT01279161). A total of 968 children (521 boys (53.8%) and 447 girls (46.1%)) aged 4 to 18 years (mean age: 14.01±3.24 years) were included in the study. Inclusion criteria were as follows: Polish origin/nationality and parents’ or legal guardians’ consent. Children with endocrine disorders (thyroid disorders, adrenal disorders, type 1 or 2 diabetes mellitus), eating disorders (including anorexia nervosa and bulimia), genetic, metabolic and other diseases that may affect body mass were excluded from the study. The children included in the study were not taking any medication. All the study subjects were patients of the Department of Paediatrics, Endocrinology and Diabetes with a Cardiology Unit, Medical University of Bialystok, Poland, between September 2009 and June 2011. The most common reasons for hospitalisation included: heart murmurs and arrhythmia, haemodynamically insignificant valvular heart disease, overweight/simple obesity, and hypertension. The project was approved by the Medical University of Bialystok Ethics Committee and in the case of each subject informed consent was obtained from the child’s parents or legal guardians.

**Anthropometric methods**

The examination included measurements of height (cm; using the Harpenden stadiometer in standing position), body mass (kg), waist circumference (cm), hip circumference (cm), arm circumference (cm) and blood pressure (mmHg) taken by appropriately trained members of the study group. Height, body mass and waist circumference were measured in light sportswear or underwear, with shoes off, after voiding. Each parameter was measured twice. The measurements of height, waist circumference, hip circumference and arm circumference were taken to an accuracy of 0.1 cm. If the first and second measurements of height and waist circumference differed by more than 0.5 cm and more than 3 cm, respectively, a third measurement was taken. Body mass was measured to an accuracy of 0.1 kg and if the first and second measurements were discordant, a third measurement was taken. BMI was calculated from the formula, the child was represented on percentile charts and the standard deviation scores (SDSs) were calculated for BMI (BMI SDS), waist circumference, hip circumference, body weight, and height (5, 13). Blood pressure was measured with an arm sphygmomanometer (automated UA-767 Plus, A&D) using cuffs matching the length and circumference of the child’s arm. The measurement was performed after a 5-minute rest in the sitting position, with the child’s back propped and with a support under the extremity on which the cuff was placed. If values obtained during the first measurement exceeded the norm (the 95th percentile for age, sex and height), blood pressure was measured three times on three different days. In addition, if the relevant data were available, we analysed the fasting lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triacylglycerols) and glucose metabolism parameters (glucose and insulin levels at baseline and at 2 hours in the oral glucose tolerance test [OGTT]). The OGTT was performed using glucose at the dose of 1.75 g/kg (up to a maximum of 75 g). The above parameters were determined with commercially available kits and the reference ranges were those adopted by the National Cholesterol Education Program (NCEP) (14): fasting plasma glucose: ≤5.5 mmol/L; 2-hour OGTT glucose: <7.7 mmol/L; fasting total cholesterol: borderline 4.4–5.1 mmol/L, abnormal ≥5.1 mmol/L; LDL-cholesterol: borderline 2.8–3.3 mmol/L, abnormal ≥3.3 mmol/L; HDL-cholesterol: borderline 0.9–1.1 mmol/L, abnormal <0.9 mmol/L; triacylglycerols: borderline 1.0–1.4 mmol/L, abnormal ≥1.4 mmol/L. The insulin resistance index (HOMA index) was calculated from the following formula: (fasting glucose×fasting insulin)/22.5 with normal values ≤3.0 (15). After obtaining the results, normal body mass was defined as BMI SDS values above -1.0 and below 1.0, overweight as values between 1.0 and <2.0, and obesity as values equal or higher than 2.0 (5). High normal blood pressure was diagnosed if the blood pressure values were equal to or exceeded the 90th percentile and were below the 95th percentile, and hypertension was diagnosed if systolic and diastolic blood pressure values exceeded the 95th percentile (16). Central obesity was defined as waist circumference exceeding the 90th percentile (13).

**Genotyping**

0.5-ml samples of peripheral blood were collected as part of routine laboratory testing necessary for the diagnosis of the patients. The FTO rs9939609 polymorphism was determined in all the children with the allele discrimination method using the ABI 7900HT fast real-time PCR System with SDS 2.1 software (Applied Biosystems, Foster City, CA, USA). The A/T variant was investigated using validated commercially available probes contained in the TaqMan SNP genotyping assay (assay ID: C_30090620_10, Applied Biosystems) labelled with a reporter dye at the 5’ terminus (VIC and FAM, respectively). DNA material was isolated from whole venous blood using the TaqMan Sample-to-SNP Kit (Applied Biosystems) as per manufacturer’s instructions. The reaction was carried out in 5 µL 384-well plates. The mixture contained 1 µL of the DNA material, 2.5 µL of TaqMan GTXpress Master Mix (Applied Biosystems), 0.25 µL of TaqMan Genotyping Assay Mix 20x
(Applied Biosystems) and 1.25 µL of DNase-free water. We employed the following thermal cycling profile: initial denaturation at 95°C for 10 min; 40 cycles of denaturation at 92°C for 15 s; hybridisation/elongation at 60°C for 60 s. In order to control contamination in each reaction plate we used negative samples containing water rather than the DNA material. The rate of correct reactions was 99.3%.

Statistical analysis

The frequencies of alleles and genotypes in the study population were determined by counting the genotypes. The resulting distribution was analysed for compliance with the Hardy-Weinberg law (Pearson’s $\chi^2$ test). Non-normally distributed parameters were log-transformed to approximate a normal distribution curve before statistical analysis. Standard deviation scores (SDS/z-scores) of anthropometric parameters were compared between the genotypes using the Wilcoxon signed rank test or the Kruskal-Wallis test. Generalised linear models were applied for the other quantitative variables. The analysis of blood pressure values was adjusted for BMI SDS and height, and the analyses of the other continuous variables (e.g. lipid values) were adjusted for BMI SDS. Discreet variables were compared using Pearson’s $\chi^2$ test. Relative risk (RR) was estimated by calculation of odds ratio (OR) and 95% confidence interval (95% CI) using unconditional logistic regression analysis. The hypothesis whereby the polymorphisms were associated with patient-related parameters was tested assuming the existence of various relations between the alleles. Both co-dominant and recessive models were fitted. In order to determine which model best described the data the Akaike information criterion (AIC) was used to compare the fits of the models. Unless otherwise indicated, variables in the interval scale were presented as arithmetic means ± standard deviations or as medians with their interquartile ranges. A two-sided p of <0.05 determined the level of statistical significance. All the calculations were performed using R project version 2.11.1, 2010 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Results of anthropometric examinations and laboratory tests

Anthropometric and blood pressure measurements were available for all 968 children (100%). Normal values of BMI SDS were observed in 634 children (65.5%), values meeting the criterion for overweight in 135 children (13.9%) and obesity was demonstrated in 199 children (20.5%). Normal waist circumference was observed in 667 children (68.9%) and central obesity defined as waist circumference exceeding the 90th percentile was observed in 301 children (31.0%).

Normal systolic blood pressure values for age and sex was observed in 738 children (76.2%), borderline values in 106 children (10.9%) and values exceeding the 95th centile (indicating hypertension) in 124 children (12.8%). Normal diastolic blood pressure values were observed in 753 children (77.7%), borderline values in 92 children (9.5%) and values exceeding the 95th centile (indicating hypertension) in 123 children (12.7%).

Data on lipid profile were available in 266 children. Total cholesterol values were normal, borderline and elevated in 157, 67 and 42 children (59.0%, 25.2% and 15.7% of the children with available values), respectively. HDL-cholesterol values were normal, borderline and decreased in 177, 67 and 22 children (66.5%, 25.2% and 8.2% of the children with available values), respectively. LDL-cholesterol values were normal, borderline and elevated in 182, 41 and 43 children (68.4%, 15.4% and 16.1% of the children with available values), respectively. Triacylglycerol values were normal, borderline and elevated in 148, 65 and 53 children (55.6%, 24.4% and 19.9% of the children with available values), respectively.

Fasting plasma glucose was determined in all the patients and did not exceed 7 mmol/l in any instance. The OGTT and the assessment of insulin levels were performed in 85 patients. The 2-hour OGTT glucose did not exceed 7.7 mmol/l in any of the patients. The HOMA index exceeded values considered indicative of insulin resistance in 25 patients (29.4% of the children in whom insulin levels were determined).

Genetic testing results

The frequency of the A-allele, the less frequent one in the study population, was 49% and the distribution of the AA, AT and TT genotypes was as follows: 243, 461 and 264 children (25.1%, 47.6% and 27.2%), respectively. The frequencies of genotypes were in line with the one determined on the basis of allele frequencies according to the Hardy-Weinberg law (p=0.14).

Association of the fat mass and obesity-associated gene polymorphism with anthropometric and metabolic parameters

The FTO gene polymorphism was associated with BMI SDS. In children with the T genotype, median BMI SDS was 0.21 (–0.52 to 1.46) and did not differ significantly from the value in heterozygotes (0.24 (–0.50 to 1.39), p=0.99). In children with the AA genotype, median BMI SDS was significantly higher (0.75 (–0.1 to 2.31), p=1.93 ×10^-4). These differences were particularly marked in boys (with medians of 0.17, 0.21 and 0.81 for TT, AT and AA, respectively; p=0.0002) compared to girls (with medians of 0.26, 0.26 and 0.69 for TT, AT and AA, respectively, p=0.005). As already mentioned, although BMI is a continuous variable, standard cutoff values are used for the diagnosis of overweight and obesity. As the BMI SDS values in the AT and TT groups were similar, in addition to the co-dominant model assuming that each genotype has an independent effect probability (TT homozygotes vs. AT heterozygotes vs. AA homozygotes) a recessive model was tested (TT homozygotes and AT heterozygotes vs. AA homozygotes). The frequencies of genotypes were different between normal-weight children and obese children (Table 1). When the recessive model of heredity was assumed, the AA genotype carriers were characterised by a higher risk of obesity (OR=2.11 (95% CI 1.5 to 2.99), p=2.23×10^-4). In the co-dominant model, the odds ratios were: 0.89 (95% CI 0.6 to 1.33), p=0.57, for AT heterozygotes and 1.97 (95% CI 1.29 to 3.00), p=0.002, for AA homozygotes. The AIC values for the recessive and co-dominant models were 902.54 and 904.21, respectively, indicating a better goodness of fit in the case of the recessive model. There were also differences in the distribution of genotypes between normal-weight children and overweight/obese children (Table 1). The FTO rs9939609 polymorphism was also associated with all the anthropometric parameters we assessed and, as was the case with BMI SDS, the recessive model was best fit. AA homozygotes had higher standardised body mass, waist circumference, arm circumference and height (Table 2).

AA homozygotes also had higher values of systolic and diastolic blood pressure, triacylglycerol levels, fasting insulin and HOMA index, although - with the exception of the latter - all these associations were attenuated after adjusting for BMI SDS and height (in the case of blood pressure) or BMI SDS (in the
we observed no significant associations with total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting glucose, insulin or 2-hour OGTT glucose.

**DISCUSSION**

The continuously increasing prevalence of overweight and obesity in children and adolescents necessitates the search for new effective diagnostic methods and treatments. Human genome studies led to the discovery of \( \text{FTO} \) gene involvement in the genetic predisposition for obesity (17). Some \( \text{FTO} \) variants are also associated with cardiovascular risk factors. Although several years have elapsed and many studies have been conducted since this discovery, we are without a doubt only beginning to elucidate the role of the \( \text{FTO} \) gene in the pathogenesis of obesity.

Despite the efforts of multiple research facilities the prevalence of overweight and obesity in children has not been definitely assessed. Unified research tools continue to be lacking. The international percentile charts proposed by Cole et al. (18) seem the most universal, although the population studied by these authors did not include Polish children. Therefore, for the purpose of our study, we utilised the recently published Polish references taking into account cutoff values for the diagnosis of overweight and obesity in children in accordance with the international guidelines (the latest guidelines of the European Childhood Obesity Group (ECOG)) (5, 19). We believe that this is a form of a compromise between using Polish charts published nearly twenty years ago and references developed on the basis of examination of European children outside Poland. In our study population, 13.9% of the children were overweight and as many as 20.5% were obese. Using these same norms for children and adolescents in Poland between 2007 and 2009 the prevalence of overweight and obesity estimated by Kulaga et al. (5) differed relative to age and sex with the mean percentage of overweight or obese children being 18.7% for boys and 14.1% for girls. In the Polish province of Podlaskie, among children from randomly selected schools, overweight was observed in 11.3% and obesity in 5.4% of the children aged 7 to 10 years (20). The authors of this report point to a higher prevalence of overweight and obesity compared to that of malnutrition in children and conclude that there is a need for implementation of nutritional education already in the first years of primary-school education in order to curb the undesirable tendency towards the increasing prevalence of obesity in this age group. In a group of 3513 rural children from the south part of the Polish region of Podlasie, 11.5% of the girls and 6.5% of the boys were overweight and 3.0% of the girls and 1.8% of the boys were obese based on the criteria proposed by Cole et al. (21). Similarly, almost a third of our children met the

<table>
<thead>
<tr>
<th>Genetic model</th>
<th>Genotype</th>
<th>Normal n (%)</th>
<th>Obese n (%)</th>
<th>OR (95%CI)</th>
<th>P value†</th>
<th>Overweight+ obese n (%)</th>
<th>OR (95%CI)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codominant</td>
<td>TT</td>
<td>187 (30)</td>
<td>51 (26)</td>
<td>1</td>
<td>-</td>
<td>77 (23)</td>
<td>1.15</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>313 (49)</td>
<td>76 (38)</td>
<td>0.89</td>
<td>(0.6-1.33)</td>
<td>57 (44)</td>
<td>1.15</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>134 (21)</td>
<td>72 (36)</td>
<td>1.97</td>
<td>(1.29-3.00)</td>
<td>0.002</td>
<td>109 (33)</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>p*</td>
<td></td>
<td></td>
<td>8.66x10^-3</td>
<td></td>
<td></td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Recessive</td>
<td>AT+TT</td>
<td>500 (79)</td>
<td>127 (64)</td>
<td>1</td>
<td>-</td>
<td>222 (67)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>134 (21)</td>
<td>72 (36)</td>
<td>2.11</td>
<td>(1.5-2.99)</td>
<td>2.23x10^-6</td>
<td>109 (33)</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>p*</td>
<td></td>
<td></td>
<td>1.77x10^-5</td>
<td></td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

* Pearson \( \chi^2 \) p-value; †Univariate logistic regression p-value

**Table 2. Anthropometric parameters in the study population relative to the rs9939609 genotype of the \( \text{FTO} \) gene.**

<table>
<thead>
<tr>
<th>rs9939609 genotypes</th>
<th>Anthropometric parameters</th>
<th>Total (n=968)</th>
<th>TT+TA (n=725)</th>
<th>AA (n=243)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight-SDS</td>
<td>0.46</td>
<td>(0.39-1.81)</td>
<td>0.94</td>
<td>(0.26-1.58)</td>
</tr>
<tr>
<td></td>
<td>Height-SDS</td>
<td>0.3</td>
<td>(0.57-1.26)</td>
<td>0.23</td>
<td>(0.68-1.17)</td>
</tr>
<tr>
<td></td>
<td>BMI-SDS</td>
<td>0.36</td>
<td>(0.43-1.67)</td>
<td>0.22</td>
<td>(0.51-1.41)</td>
</tr>
<tr>
<td></td>
<td>Waist-SDS</td>
<td>0.52</td>
<td>(0.29-2.0)</td>
<td>0.42</td>
<td>(0.38-1.67)</td>
</tr>
<tr>
<td></td>
<td>Hip-SDS</td>
<td>0.54</td>
<td>(0.36-1.84)</td>
<td>0.11</td>
<td>(0.51-1.51)</td>
</tr>
<tr>
<td></td>
<td>Arm-SDS</td>
<td>0.68</td>
<td>(0.12-2.12)</td>
<td>0.44</td>
<td>(0.34-1.7)</td>
</tr>
</tbody>
</table>

Data are medians (interquartile range) *Wilcoxon test p-value with the assumption of the recessive model of inheritance, SDS – standard deviation score.

case of the other parameters (Table 3). We observed no significant associations with total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting glucose, insulin or 2-hour OGTT glucose.

**Table 1. Distribution of the investigated genotypes in normal-weight children, obese children and in a pooled group of overweight or obese children, along with relative odds ratios.**

**Table 3. Anthropometric parameters along with statistical significance of their differences between the genotype groups.**
criteria for central obesity. These data, however, seem overestimated and result from the fact that enrolment was restricted to children hospitalised at our Department only. Although there has been a slight increase in the prevalence of central obesity in Polish children in the past few years, the results reported by other authors are different from ours. For instance, the criteria were met by 3.9% and 6.4% of boys in 1983 and 2000, respectively, and by 2.4% and 3.1% of girls in 1983 and 2000, respectively (22). The differences were statistically significant. Despite the worrying forecasts some reports suggest a deceleration in the increasing prevalence of obesity in children and even stabilisation of this phenomenon (23). The waist circumference measurement is important indicator of central obesity, which probably plays a pathogenic role in metabolic consequences of the obesity (24).

Associations of many genes with body mass have been discovered in the recent years. We are aware of the fact that despite the spectacular discoveries in the field of the genomics of obesity the currently known polymorphisms explain only a small percentage of BMI variability and it is therefore very likely that significant discoveries are still ahead of us and that in the near future genetic variants will be discovered that will explain a larger part of the genetic predisposition to obesity. In a pioneering study, Timothy Frayling et al. (12) confirmed the association of the FTO gene with BMI variability.

### Table 3. Metabolic cardiovascular risk factors and blood pressure values relative to the rs9939609 genotype of the FTO gene.

<table>
<thead>
<tr>
<th>Cardiovascular risk factors (number of patients)</th>
<th>TT+TA</th>
<th>AA</th>
<th>P value</th>
<th>crude</th>
<th>adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L) (N=266)</td>
<td>4.23</td>
<td>4.37</td>
<td>0.19</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L) (N=266)</td>
<td>0.94</td>
<td>1.11</td>
<td>0.009</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L) (N=266)</td>
<td>1.31</td>
<td>1.22</td>
<td>0.07</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L) (N=266)</td>
<td>2.37</td>
<td>2.49</td>
<td>0.31</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (pmol/L) (N=85)</td>
<td>7.39</td>
<td>12.58</td>
<td>0.004</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>2-hour OGTT insulin (pmol/L) (N=85)</td>
<td>45.83</td>
<td>61.99</td>
<td>0.14</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/L) (N=85)</td>
<td>4.78</td>
<td>4.88</td>
<td>0.16</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>2-hour OGTT glucose (mmol/L) (N=85)</td>
<td>6.2</td>
<td>6.45</td>
<td>0.38</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>HOMA index (N=85)</td>
<td>2.63</td>
<td>3.9</td>
<td>0.001</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP mmHg (N=968)</td>
<td>114.7±15.89</td>
<td>120.4±16.6</td>
<td>2×10⁻⁵</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>DBP mmHg (N=968)</td>
<td>71.86±12.07</td>
<td>74.5±10.68</td>
<td>0.006</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± S.D. or geometric means (S.D.). *Adjusted for BMI-SDS, except for SBP and DBP which are adjusted for BMI-SDS and height, SBP – systolic blood pressure, DBP – diastolic blood pressure, OGTT- oral glucose tolerance test, HOMA - homoeostatic model assessment.

**Fig. 1.** Relationship between BMI and the FTO genotype. AA homozygotes show significantly higher values of BMI SDS values compared to AT heterozygotes or TT homozygotes (medians and percentiles). The difference between AT heterozygotes and TT homozygotes in BMI SDS is non-significant.
rs9939609 polymorphism with BMI, body mass, waist circumference, skinfold thickness and dual-emission X-ray absorptiometry (DEXA) measurement of fat mass in Caucasian adults and children. The strength of the influence of gene polymorphisms on phenotypic traits may vary in relation to sex, age and race. Our results suggest a more marked influence of FTO polymorphism on BMI in boys compared to girls. Initial reports on this topic also suggested differences in the influence of FTO on BMI in very obese children. In contrast to our findings, this association has been confirmed in girls but not in boys (25). In addition to data from Europe and the United States, a strong association of the A-allele with type 2 diabetes mellitus at the site we investigated has also been demonstrated in inhabitants of southern India (26). However, the effect on BMI was weaker than in the Europeans. Interestingly, the association with type 2 diabetes mellitus remained significant after elimination of the influence of BMI. Extremely interesting studies have been conducted in the Japanese population. According to some authors, FTO does not affect body mass or cardiovascular risk factors in the inhabitants of Japan (27). In meta-analyses, this effect is, however, statistically significant and concerns the risk of obesity and type 2 diabetes mellitus (28). Similarly, in the Chinese population, the effect of rs9939609 is significant and similarly strong as in Europe, and the frequency of the A-allele is much lower: 12.6% vs. 45.0% (49% in our study) (29).

Data on the impact of gene polymorphisms on cardiovascular risk factors in long-term observations are scarce and conflicting. Among the participants of the MRC National Survey of Health and Development the FTO variant was characterised by a variable effect on body mass depending on age and its strength increased in childhood, adolescence and adulthood until the age of 20 years and then gradually decreased (30). Slightly different results were obtained in the analyses based on the population followed up in the Bogalusa Heart Study (Louisiana), a study to assess the natural history of cardiovascular disease from childhood to maturity. In this group, the FTO rs9939609 polymorphism was associated with BMI in adulthood but not with birth weight or BMI in childhood (31). The participants of the Finnish study STRIP were observed from the age of 7 months to 15 years (32). The FTO polymorphism correlated with BMI from the age of 7 years (but not earlier), with systolic and diastolic blood pressure and with total cholesterol and LDL-cholesterol. No association of FTO with energy intake or physical activity at the age of 15 years has, however, been observed.

The impact on the rs9939609 variant of the FTO gene on BMI and features of overweight and obesity is unquestionable. The association of FTO with other cardiovascular risk factors is also being studied. The association with systolic blood pressure has been confirmed in the French Canadian founder population (33). These data were not, however, adjusted to BMI. In another study, which included 598 adolescents, an association of the FTO rs9939333 polymorphism with blood pressure values has also been observed (34). Interestingly enough, among obese adults with BMI ≥29 kg/m², carriers with the A-risk allele showed 2-fold higher risk of having high blood pressure trait compared with non-carriers even after taking BMI into account (35). On the other hand, as regards fat mass markers, of the several genes recently discovered only the rs17006633 polymorphism near the MC4R gene showed an effect on arm circumference as a marker of adipose tissue and this effect increased with age. No such relationship was observed in the remaining genes that were investigated, including FTO (36). We observed an association of FTO with HOMA index (an index of insulin resistance), which is consistent with the previously cited paper by Pausova et al. (33). Correlations of the FTO polymorphism with HOMA index were detected before puberty in obese girls but not boys (37). Another FTO polymorphism, namely rs1421085, is also associated with features of insulin resistance in women inhabiting southern France (38). Our results also suggest a correlation of the FTO genotype with certain lipid parameters. These associations, however, lose their significance after adjustment for BMI. This effect can also be influenced of low number of subjects studied. In another study, the A-allele was associated with lower values of HDL-cholesterol, higher values of triacylglycerols and a higher incidence of myocardial infarction (39). Furthermore, the presence of the AA genotype was associated with higher mortality in a long-term follow-up of Danish men irrespective of obesity (40). The TT genotype exerted a protective effect in this respect. The results of this observation should be confirmed in other populations. On the other hand, in a study of Chinese children, similarly to ours, the above correlations lost their significance when the data were adjusted for BMI (41).

As regards the role of the FTO gene, no data are available to sufficiently elucidate its mechanism of action. Cecil et al. suggest that children with the A-allele are characterised by higher energy intake, unchanged energy expenditure and, interestingly, higher physical activity compared to carriers of the T-alleles (42, 43). The weight of the consumed meals did not differ between the genetic groups. It is, therefore, suspected that the FTO gene product affects the regulation of food intake. However, no influence of the FTO rs9939609 polymorphism on nutritional behaviours were observed in 756 healthy pairs of adult twins (44). In another observational study conducted in children, this variant correlated with episodes of loss of control of eating and with intake of high-fat foods (45). The mechanism of action of FTO may be related to impaired feeling of satiety observed in carriers of the A-allele (46). Studies and discoveries of new peptides and genes also suggest a role of the central nervous system in the pathogenesis of obesity (47, 48). However, these are still speculations, not direct evidence of existing pathomechanisms.

There are very few data, unaccompanied by unequivocal results, on the influence of FTO variants on the therapeutic intervention in obese children. It seems that the presence of the A-allele leads to a lower weight loss or even to weight gain after lifestyle modification in this group of patients (49). Unexpected results were obtained in adults consuming the Mediterranean diet, where A-allele carriers were characterised by the lowest weight gain compared to T-allele carriers (50). The combination of undesirable genotypes of the FTO and INSIG2 genes (AA and CC, respectively) led to the lowest weight loss or even to weight gain during one year during which lifestyle modifications were being implemented (51). The German study Obeldicks, despite the presence of an obvious association of FTO with body mass and obesity, failed to demonstrate any influence of this polymorphism on weight reduction as a result of an intervention or any association with lipid parameters (52). The sample size was, however, lower than in our study. We hope that in the near future studies will be conducted to evaluate the influence of gene polymorphisms, including FTO polymorphisms, on the outcomes of lifestyle interventions in obese patients, including obese children.

Although in the majority of studies the influence of FTO on BMI was modest, its magnitude in women with polycystic ovary syndrome was 1.56 kg/m² vs. 0.46 kg/m² in women from the general population (53).

In the near future we are planning to investigate the impact of polymorphism of other genes (such as INSIG2, MC4R, other sites in the FTO gene, CD36 and others) on the development of cardiovascular risk factors. We might find that the effect of multiple genes affects BMI to a greater degree. Expectations associated with the results of genetic research, among both doctors and patients, are huge. There is theoretical evidence for the economic feasibility of therapeutic interventions in the general population aimed at reducing the epidemic of obesity in children (54). However, in order to implement them the
knowledge on groups at risk of this pathology is needed. Confirmation of the genetic risk of obesity might perhaps be a tool to reduce the stress associated with rejection of obese patients by the society and healthcare professionals.

Limitations of our study included the lack of assessment of the signs of puberty in children, participation of patients hospitalised rather than completely healthy children, high rate of obesity in the study population and the lack of all the details for all the participating children. The project was multicentric, cross-sectional and hospital-based but not prospective and population-based study. The study strenght is the enrolment of large number of polish children in the project. The results obtained in our study and in studies conducted by other authors to evaluate the influence of the genome on the development of overweight and obesity do not change the general recommendations to avoid obesity, including the recommendations related to healthy diet and physical activity. The data presented here may only indicate that some children will find it harder than others to achieve normal body mass in today’s world which promotes excessive energy intake and low physical activity. Elucidation of the way in which the FTO gene product affects fat mass will help us to understand the pathogenesis of obesity and perhaps to propose a new treatment. Evidence is therefore needed to support the functional significance of the polymorphisms discovered so far. Perhaps a genetic calculator of cardiovascular risk factors will be developed in the near future based on the studies investigating the polymorphisms of several or more genes.

Based on a study in a large group of patients we demonstrated for the first time in the literature the influence of the FTO rs9939609 polymorphism on the body weight and features of insulin resistance in Polish children. Our results may be the starting point for further studies investigating the genetic background of obesity in children and may, in the future, serve to develop an index of the genetic risk of obesity and its sequelae.

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Author’s address: Dr. Wlodzimierz Luczynski, Department of Pediatrics, Endocrinology with a Cardiology Unit, Medical University of Bialystok, 15-274 Bialystok, 17 Waszyngtona Street, Poland; E-mail: w.luczynski@wp.pl