Clinical Study

Rs9939609 Variant of the Fat Mass and Obesity-Associated Gene and Trunk Obesity in Adolescents

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A common T/A polymorphism (rs9939609) in the fat mass and obesity associated (FTO) gene was found associated with early-onset and severe obesity in both adults and children. However, recent observations failed to find associations of FTO with obesity. To investigate the genetic background of early obesity, we analysed the single nucleotide polymorphism (SNP) rs9939609 of FTO in 371 styrian adolescents towards degree of obesity, subcutaneous adipose tissue (SAT)-distribution determined by lipometry, early metabolic and preatherosclerotic symptoms. The percentage of AA homozygotes for the rs9939609 SNP of FTO was significantly increased in the obese adolescents. Compared to the TT wildtype, AA homozygotes showed significantly elevated values of SAT thickness at the trunk-located lipometer measure points neck and frontal chest, body weight, body mass index, waist, and hip circumference. No associations were found with carotis communis intima-media thickness, systolic, diastolic blood pressure, ultrasensitive C-reactive protein (US-CRP), homocystein, total cholesterol, triglycerides, HDL cholesterol, oxidized LDL, fasted glucose, insulin, HOMA-index, liver transaminases, uric acid, and adipokines like resistin, leptin, and adiponectin. Taken together, to the best of our knowledge we are the first to report that the rs9939609 FTO SNP is associated with trunk weighted obesity as early as in adolescence.

1. Introduction

Juvenile obesity usually leads to obesity in adulthood which causes life threatening sequelae such as diabetes, cardiovascular disease, hypertension, stroke, and cancer [1]. In foregoing studies, we detected an increased carotid intima-media thickness (IMT) paralleled by a subclinical inflammation in obese adolescents and we provided the first evidence that preatherosclerosis is associated with individual risk profiles characterised by subcutaneous adipose tissue (SAT) topography and altered biomarkers [2–4]. In this paper, we analysed the single nucleotide polymorphism (SNP) rs9939609 of the fat mass and obesity-associated (FTO) gene in obese styrian adolescents and in normal weight controls of the same age and gender distribution. FTO is a gene located in chromosome region 16q12.2. Recently, it was brought into connection with the central control of energy homeostasis and fat cell lipolysis [5, 6]. The single-nucleotide polymorphisms (SNPs) rs1421085, rs17817449, and rs9939609, of FTO were reported to be linked to body mass index (BMI) and obesity in both adults and children [7–9]. However, Li et al. failed to find associations of FTO with obesity [10]. To clarify this issue, we examined the so far best described FTO gene variant (i.e., SNP rs9939609) [6, 8] for correlations with grade of obesity, SAT distribution, and obesity-related metabolic and cardiovascular risk parameters in styrian adolescents.

2. Material and Methods

2.1. Subjects. Study participants (obese persons and normal weight, age- and sex-matched controls) were from the STYrian Juvenile OBesity Study (STYJOBS), which is
Table 1: Baseline characteristics of study participants (age range 5 to 20 years, n = 371).

| Variable          | Normal weight (mean + 1 SD) | Obese (n = 268) |
|-------------------|-----------------------------|-----------------|
| Individuals       | 103                         | 268             |
| Female/male       | 51/52                       | 146/122         |
| Age (years)       | 14.0 ± 3.1                  | 12.5 ± 3.1      |
| Body length (m)   | 1.6 ± 0.12                  | 1.6 ± 0.15      |
| Body weight (kg)  | 54.0 ± 13.6                 | 76.2 ± 26.0     |
| BMI (kg/m²)       | 20.0 ± 2.9                  | 30.1 ± 6.2      |
| BMI-SDS           | 0.3 ± 1.0                   | 6.0 ± 2.6       |

BMI: body mass index.
BMI-SDS: body mass index standard deviation score.

Table 2: Occurrence of the rs9939609 FTO gene polymorphism within the experimental groups.

| FTO rs9939609 genotypes | Normal weight adolescents (n = 103) | Obese adolescents (n = 268) | P value |
|-------------------------|------------------------------------|-----------------------------|---------|
| TT                      | 31 (30.1%)                         | 75 (27.9%)                  |         |
| TA                      | 56 (54.3%)                         | 118 (44.0%)                 |         |
| AA                      | 16 (15.5%)                         | 75 (27.9%)                  | 0.015 (AA versus TT&TA) |
| A allele frequency      | 0.427                              | 0.500                       | 0.75    |

designed to investigate early stages of atherosclerosis and metabolic disorders in obese juveniles. STYJOBS is registered at Clinical-Trials.gov (Identifier NCT00482924), where detailed information of the study is available. The inclusion criterion for the obese probands was BMI > 97th percentile if under 18 years of age, BMI > 30 kg/m² if over 18 years of age. Exclusion criteria were endocrine diseases (e.g., hypothyreosis), infectious or any other chronic diseases. Further, STYJOBS participants aged above 20 years were excluded in this study. Controls were healthy age-matched volunteers. All controls had to be normal weight (BMI under 18 years of age, BMI > 30 kg/m² if over 18 years of age) free of infectious, chronic, and endocrine diseases. 268 obese juveniles recruited from July 2003 to December 2006 (mean age 12.5 ± 3.1 (SD) years) and 103 normal weight healthy controls of similar age and gender distribution were investigated. The study was approved by the ethical committee of the Medical University of Graz. At the time of blood collection, the probands were fasting. Blood samples were immediately centrifuged at 3500 rpm at ambient temperature and stored at −80°C until analysis.

2.2. Laboratory Analysis. Genomic DNA was isolated from peripheral lymphocytes by standard methods and stored at −20°C. FTO genotypes were determined by 5′-exonuclease assay (TaqMan, Applied Biosystems, Applera International Inc., Austria GmbH, Mahlerstrasse 13, A-1010 Vienna, Austria). Primer and probe sets were designed and manufactured using Applied Biosystems “Assay-by-Design” custom service (Applied Biosystems, Applera International Inc., Austria GmbH, Mahlerstrasse 13, A-1010 Vienna, Austria), and assays were performed according to the manufacturer’s instructions. End-point fluorescence was measured and fluorescence plate reader data were exported into an Excel format, depicted, and analyzed as a scatter plot.

Liver transaminases, creatinine, glucose and uric acid were measured by routine laboratory methods on a Hitachi 917 chemical analyzer, cholesterol and triglycerides by means of ECLIA (ElectroChemiLuminiscenceAssay) on an Elesys 2010 analyzer (Roche Diagnostics Mannheim, Germany), and plasma insulin by ELISA (Mercodia, Uppsala, Sweden). HOMA-IR (homeostatic model assessment-insulin resistance) was calculated as reported [11]. Lipoproteins were separated by a combined ultracentrifugation-precipitation method (β-quantification) and analysed as outlined elsewhere [12]. Total adiponectin and subfractions were determined by Adiponectin (Multimeric) Enzyme-Linked ImmunoSorbent Assay (47-ADPH-9755) from Alpco Diagnostics, leptin and resistin by ELISAs from Biovendor Laboratory Medicine, Inc. (Brno, Czech Republic), oxidized low dense lipoprotein (oxLDL) by Merckodia oxidized LDL Competitive ELISA, SE-754 50 Uppsala, Sweden, ultra sensitive-CRP with a particle-enhanced immunoturbidimetric assay (Tina-quant C-reactive protein latex ultrasensitive assay, Roche diagnostics), homocysteine by triple quadrupole mass spectrometry (Applied biosystems, API 2000 LC/MS/MS-system) using a 3.3 × 0.46 cm HPLC column (SUPELCO LC-CN).

2.3. Carotid Artery Ultrasound. The ultrasound protocol involved scanning of the bulbous near the common carotid artery (CCA) on both sides with a 12-to-5-MHz broadband linear transducer on an HDI 5000 (ATL, Bothell, Washington, DC, USA). The carotid IMT was assessed at the far wall as the distance between the interface of the lumen and intima and the interface between the media and adventitia. All diameters were measured during diastole to avoid image blurring due to systolic arterial wall motion and to minimize the influence of blood pressure [13].

2.4. Lipometry. Measurements of SAT thickness were performed by means of a patented optical device (EU Pat.Nr. 0516251) on 15 anatomically well-defined body sites distributed from neck to calf on the right and left side of all obese juveniles [14] and then averaged for both body sides.

2.5. Statistics. Statistical analysis was performed by SPSS version 14. Kolmogorov-Smirnov test was used to examine for normal distribution. Means were compared by a two-tailed unpaired sample t-test or by Mann-Whitney Test, depending on the distribution of the data. A value of P < .05 was considered statistically significant.

3. Results

The clinical characteristics of the study participants are summarized in Table 1. FTO genotypes did not deviate from
and in normal weight controls of the same age and gender. We analysed the single nucleotide polymorphism (SNP) rs9939609 of the FTO gene in obese Caucasian adolescents and in normal weight controls of the same age and gender distribution. The percentage of AA homozygotes for the rs9939609 SNP of FTO was significantly increased in the obese adolescents indicating a relevance of this SNP in early phases of obesity. The observation that the SAT thicknesses of the trunk-located lipometer measure points, neck and frontal chest, were significantly increased in homozygote rs9939609 carriers indicates for the first time a link between trunk-weighted SAT distribution and a specific genetic disposition.

On the other hand, no associations could be found between the investigated SNP and cardiovascular risk parameters like carotis communis IMT, systolic, diastolic blood pressure, conventional laboratory-, metabolic-, inflammatory biomarkers (e.g., Liver enzymes, fasted glucose, HOMA-index, homocystein, lipids, oxidized LDL, and US-CRP), and adipokines such as adiponectin, resistin, and leptin. This may be caused by the fact that these cardiovascular and metabolic risk parameters reflect a more common pathologic phenotype. Genetic risk constellations, possibly more important for future clinical endpoints, may be present in the background without correlation to these markers but related to trunk-weighted obesity. It will be interesting to follow up this cohort of adolescents for development of overt metabolic and atherosclerotic disease symptoms later in life and to study correlations of clinical end points with homozygote rs9939609 carriers.

Several studies reported a strong link between the rs1421085, rs17817449, and rs9939609 SNPs of FTO and body mass index (BMI), even in children [7–9]. On the other

| Table 3: Associations of clinical parameters with the rs9939609 FTO gene polymorphism. |
|---------------------------------|----------------|----------------|----------------|
| Mean ± 1 SD                     | Wildtype       | Heterozygote   | Homozygote     |
| Body weight (kg)                | 65.9 ± 23      | 70.4 ± 26.4    | 74.2 ± 25**    |
| BMI (kg/m²)                     | 26.5 ± 7       | 27.7 ± 7.2     | 28.8 ± 7**     |
| BMI-SDS                         | 4.2 ± 3.5      | 4.2 ± 3.4      | 5.1 ± 3.5*     |
| Waist circumference (cm)        | 85.4 ± 16      | 88.1 ± 16.8    | 90.4 ± 13*     |
| Hip circumference (cm)          | 99.7 ± 16      | 102.9 ± 16.9   | 105.1 ± 16*    |
| SAT-T, neck (cm)                | 12.7 ± 6.5     | 13.9 ± 7       | 15.3 ± 5.8**   |
| SAT-T, front chest (cm)         | 16.7 ± 8.9     | 18.4 ± 8.8     | 20.4 ± 9.3**   |

*P < .05, **P < .01, ***P < .001, MannWhitney U-test (wildtype versus homozygote).

Table 4: Clinical parameters and the rs9939609 FTO gene polymorphism within all experimental groups.

| Mean ± 1 SD   | Obese adolescents | Normal weight adolescents |
|---------------|--------------------|---------------------------|
|               | Wildtype n = 75    | Heterozygote n = 118      | Homozygote n = 75 |
| Body weight (kg) | 72.1 ± 23.3        | 77.8 ± 27.7               | 77.9 ± 25.7      |
| BMI (kg/m²)     | 29.3 ± 6.1         | 30.3 ± 6.3                | 30.6 ± 6.1       |
| BMI-SDS         | 5.9 ± 2.6          | 6.0 ± 2.6                 | 6.2 ± 2.9        |
| Waist circumference (cm) | 89.9 ± 14.8 | 92.2 ± 15.8               | 92.7 ± 11.8      |
| Hip circumference (cm) | 102.8 ± 15.8    | 106.4 ± 16.5              | 106.7 ± 16.4     |
| SAT-T, neck (cm) | 14.3 ± 5.7         | 15.4 ± 6.4                | 15.6 ± 5.1       |
| SAT-T, front chest (cm) | 19.9 ± 7.4        | 20.8 ± 8.1                | 22.3 ± 9.4       |

*P < .05, **P < .01, ***P < .001, MannWhitney U-test (wildtype versus homozygote).

BMI: Body mass index.
BMI-SDS: body mass index standard deviation score.

the Hardy-Weinberg equilibrium. Compared to the normal weight controls, the percentage of carriers of the FTO AA genotype was significantly increased in the obese adolescents (Table 2). Heterozygous and wildtype genotypes were approximately equally distributed within the groups (Table 2).

Homozygote carriers showed significantly elevated values for body weight, body mass index (BMI), BMI standard deviation scores (BMI-SDS), and waist, hip circumferences, and the thickness of the SAT was significantly increased at the trunk-located lipometer measure points, neck and frontal chest (Table 3). Analysed separately in the obese and normal weight group, these variables showed a trend to higher levels in heterozygote and homozygote carriers; however no significant difference was achieved (Table 4). Further, no significant elevations were found with carotis IMT, systolic, diastolic blood pressure, conventional laboratory-, metabolic-, inflammatory biomarkers (e.g., Liver enzymes, fasted glucose, HOMA-index, homocystein, lipids, oxidized LDL, and US-CRP), and adipokines such as adiponectin, resistin, and leptin. This may be caused by the fact that these cardiovascular and metabolic risk parameters reflect a more common pathologic phenotype. Genetic risk constellations, possibly more important for future clinical endpoints, may be present in the background without correlation to these markers but related to trunk-weighted obesity. It will be interesting to follow up this cohort of adolescents for development of overt metabolic and atherosclerotic disease symptoms later in life and to study correlations of clinical end points with homozygote rs9939609 carriers.

4. Discussion

We analysed the single nucleotide polymorphism (SNP) rs9939609 of the FTO gene in obese Caucasian adolescents and in normal weight controls of the same age and gender...
hand, a recent study failed to find associations of FTO with obesity [10]. This may be influenced by the fact that these authors investigated Chinese and not Caucasian probands [10]. Our observations in juveniles are in accordance with the observations of adults by Frayling et al. [8].

Taken together, to the best of our knowledge, we are the first to report that homozygosity for the rs9939609 FTO SNP is critically associated with trunk-weighted obesity in obese adolescents.

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