Genetic determinants and health consequences of common childhood obesity

PhD Thesis

Katalin Csernus MD

Department of Paediatrics, Clinical Center, University of Pécs

Program leader: Dénes Molnár MD, PhD, DSc

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Abbreviations frequently used:

ADRB3 – β_3-adrenoreceptor

ALT – alanine aminotransferase

ANOVA – analysis of variance

AST – aspartate aminotransferase

BAT – brown adipose tissue

BMI – body mass index

BMR – basic metabolic rate

CI – confidence interval

del/ins – deletion/insertion

HOMA – homeostasis model assessment

IGR – impaired glucose regulation

NAFLD – non-alcoholic fatty liver disease

OGTT – oral glucose tolerance test

OR – odds ratio

PPARG2 – peroxisome proliferator-activated receptor-$\gamma 2$

RBMI – relative BMI

RBW – relative body weight

ROS – reactive oxygen species

RQ – respiratory quotient

SD – standard deviation

UCP – uncoupling protein

U-ACR – urinary albumin:creatinine ratio

U-BMCR – urinary $\beta 2$-microglobulin:creatinine ratio

WAT – white adipose tissue

WBISI – whole body insulin sensitivity index

INTRODUCTION

1. Genetic basis of common childhood obesity

Common obesity is a multifactorial disorder with a strong genetic component. The genetic background for common childhood obesity seems to result from the possession of risk alleles at many genes involved in the regulation of energy balance. These obesogenic gene variants have small effect sizes, but the risk alleles for obesity are quite common in populations. Because of the small effect sizes, data on large populations of children and numerous single gene polymorphisms are needed to identify or confirm associations with common paediatric obesity.

In children, lower energy expenditure rather than increased energy intake has been reported to predict weight gain. Thus, genes concerned with energy metabolism and storage constitute a major group of candidate obesity susceptibility genes. The replicated associations of common variants of genes encoding the β_3-adrenoreceptor (ADRB3), the mitochondrial uncoupling proteins (UCPs) 1,2 and 3, and the peroxisome proliferator-activated receptor-$\gamma2$ (PPARG2) with obesity and related traits has established these genes among the strongest candidates. Most of the relevant studies, however, have been conducted in adult subjects, and relatively little is known about how these genes influence the development of overweight in childhood.

ADRB3 is a fat-selective adrenoreceptor subtype expressed in brown adipose tissue (BAT) and mainly in visceral white adipose depots in humans. The adrenergic system has a key role in controlling energy expenditure, with ADRB3 as a principal receptor mediating catecholamine-stimulated thermogenesis in BAT and lipolysis in white fat cells. The Trp64Arg polymorphism of the ADRB3 gene was associated with decreased lipolytic sensitivity and a higher prevalence of the Arg64 allele among obese subjects has been described in adult and paediatric populations.

UCPs constitute a family of intramitochondrial transmembrane carriers that may uncouple the transport of protons across the inner mitochondrial membrane from ATP synthesis, thereby dissipating energy as heat and lowering metabolic efficiency. UCP-1 is expressed mainly BAT where it is the key component of cold-induced non-shivering thermogenesis as well as contributing to diet-induced thermogenesis. In humans, BAT is mainly active in infancy after which it atrophies; brown fat cells remain, however, dispersed amongst white adipose throughout life and may represent up to 2% of body weight in adults. The G variant of the UCP-1 –3826 A/G polymorphism was associated with reduced mRNA expression and a higher frequency among the obese.

UCP-2 is widely distributed in humans, while UCP-3 expression is restricted mainly to skeletal muscle and brown adipocytes. When they were discovered it was speculated that these proteins would have much the same function as UCP-1, but in tissues other than BAT, thus explaining the

mechanisms behind phenomena such as basal proton leak and non-shivering or diet-induced thermogenesis that can be demonstrated in the cells and tissues of adult humans lacking UCP-1. Despite the intense interest in defining the functions of UCP-2 and UCP-3, however, their precise physiological roles are still uncertain. UCP-2 has been proposed to play a role in cellular energy balance, regulation of insulin secretion and action, lipid metabolism and storage, and regulation of reactive oxygen species (ROS) production, while UCP-3 is implicated in the modulation of ROS production and the regulation of fuel partitioning. Whatever their main physiological roles may be, by virtue of their uncoupling activity these proteins can influence the efficiency of energy coupling in mitochondria and thus play an important role in human energy homeostasis and in the mechanisms underlying the variability in human energy expenditure. Functional variants of the UCP-2 gene include the –866 G/A polymorphism, with the A allele related to enhanced transcriptional activity and lower BMI and risk of obesity; and the exon 8 45 bp deletion/insertion (del/ins) polymorphism with the ins variant associated with reduced mRNA stability and a greater risk of developing obesity. The T variant of the –55 C/T UCP-3 polymorphism was related to enhanced transcriptional activity and lower BMI and risk of obesity.

PPARG2 is an adipocyte-specific nuclear receptor that regulates fat cell differentiation, lipid metabolism and insulin sensitivity. As a lipid-activated transcription factor PPARG2 represents a potential molecular link between lipid metabolism and adipocyte differentiation, thereby its genetic variations can modify fat accumulation and obesity risk. The Pro12Ala mutation was associated with reduced DNA binding and transactivation in vitro. Data so far are conflicting as to whether this mutation is associated with obesity or with a lower BMI. Two meta-analyses have concluded that the Ala12 allele is associated with increased BMI and among Spanish children presence of the mutation was associated with increased risk of obesity.

2. Genetic determinants of obesity-related traits and metabolic complications of obesity in children

The basic metabolic rate (BMR) is highly variable in humans, but sources of this variability remain to be identified. Reduced BMR and increased respiratory quotient (RQ) are known risk factors for weight gain and are in part genetically determined.

Obesity is associated with several metabolic complications such as hyperinsulinemia, type 2 diabetes mellitus (T2DM), dyslipidaemia, high blood pressure, and non-alcoholic fatty liver disease; and also with the clustering of these, the metabolic syndrome. Although comorbidities of obesity usually present in adulthood, it is well known by now that obesity-related metabolic derangements and damage to different target organs of obesity can already accompany overweight during childhood. According to recent data from the European Union the majority of obese children are

affected by one or more features of the metabolic syndrome. Genetic predisposition is hypothesized to be a key factor in the susceptibility of obese individuals to metabolic disorders, and energy expenditure gene polymorphisms are implicated in the polygenic background of common obesity-related traits and metabolic complications. The above mentioned common polymorphisms of the ADRB3, UCP-1, UCP-2, UCP-3 and PPARG2 genes have reported to be associated with basic metabolic rate, respiratory quotient, diet-induced thermogenesis and tendency to gain weight, and also with features of the metabolic syndrome including visceral adiposity, insulin resistance, disturbances of carbohydrate and lipid metabolism, and raised blood pressure.

Most obesity candidate gene association studies are conducted in adults and there are relatively few data on the influence of energy expenditure gene polymorphisms on body weight regulation in children. Childhood onset common obesity is believed to be etiologically different from common adult obesity, as children are less influenced by prolonged exposure to environmental factors and comorbidities compared with adults; thus data from adult studies cannot be directly related to children. The manifestation of the effects of polymorphisms predisposing an individual to common multifactorial diseases depends on many factors including ethnicity, therefore it is important for studies to focus on populations with different genetic backgrounds and different environments.

3. Genetic influence on the hepatic complication of obesity: non-alcoholic fatty liver disease

Obesity-related hepatic injury includes a spectrum of conditions commonly referred to as non-alcoholic fatty liver disease (NAFLD), the hepatic component of the metabolic syndrome. Stages of NAFLD extend from simple steatosis to non-alcoholic steatohepatitis (NASH) and fibrosis, all of which can present as early as childhood. Diversity in the occurrence and phenotypic expression of NAFLD in obese subjects suggests a significant polygenic basis for the disorder, involving variations in single nucleotide polymorphisms. Theoretical considerations as well as experimental data indicate, that UCP-2 may have a role in the development of fatty liver disease. Hepatocytes induce UCP-2 mRNA and protein expression in obesity, leading to a substantially increased presence of UCP-2 in fatty livers. As to the possible protective or detrimental role of UCP-2 in human fatty liver disease, its concurrent effects of limiting ROS production but at the same time compromising cellular energy homeostasis by reducing ATP synthesis leave theoretical and experimental controversies yet to be resolved.

4. Renal effects of obesity: proteinuria indicating early renal dysfunction

There is increasing evidence that obesity may damage the kidney in otherwise healthy individuals. Enhanced excretion of albumin – indicating hyperfiltration and early renal damage – has been shown to be associated with adult obesity. Microalbuminuria, an indicator of disturbed glomerular

permeability, may also represent the renal expression of systematically increased transcapillary albumin leakiness reflecting general endothelial dysfunction and leading to vascular damage. Major epidemiological studies in adults have established microalbuminuria as an independent predictor of premature atherosclerosis and cardiovascular disease. Data concerning the presence of microalbuminuria in obese children and its association with cardiovascular risk factors were, however, lacking.

β2-microglobulin is a low molecular weight protein that is freely filtered through the glomerular barrier, and is normally almost completely reabsorbed and catabolized by the renal proximal tubules. Increased urinary β2-microglobulin excretion is a sensitive marker of proximal tubular dysfunction. Prospective data regarding the association between markers of renal tubular dysfunction and obesity is limited and has been controversial, and data pertaining to children is scarce.

AIMS

1. Energy expenditure gene polymorphisms and the risk of childhood obesity

We determined the frequencies of the ADRB3 Trp64Arg, UCP-1 –3826 A/G, UCP-2 –866 G/A and exon 8 45 basepair del/ins, UCP-3 –55 C/T, and PPARG2 Pro12Ala polymorphisms among a large group of normal weight and overweight/obese Hungarian school-aged (6–18 years) children to investigate their effects on the risk of common childhood obesity.

2. Energy expenditure gene polymorphisms and obesity-related traits and metabolic complications of childhood obesity

To study the influences of the above gene polymorphisms on obesity-related traits and metabolic complications we looked for associations between the genetic variants and measures of obesity, BMR, features of the metabolic syndrome among obese children.

3. Association of the UCP-2 exon 8 del/ins polymorphism with obesity-related liver injury

To investigate the potential role of UCP-2 in the development of pediatric obesity-related liver injury we examined the relationship between the UCP-2 exon 8 45 bp del/ins polymorphism and aminotransferase elevations indicative of liver dysfunction among obese children.

4. Effect of childhood obesity on renal glomerular and tubular function

To examine the effect of obesity on urinary albumin and β2-microglobulin excretion as markers of glomerular and tubular dysfunction, we measured the urinary albumin/creatinine ratio (U-ACR) and urinary β2-microglobulin/creatinine ratio (U-BMCR) in healthy obese and normal weight children.

SUBJECTS AND METHODS

Children were classified as overweight or obese according to international cut-off BMI values for overweight or obesity by sex and age. Overweight/obese children included in the studies were referred to the childhood obesity center of the Department of Pediatrics, University of Pécs because of their excess body weight. Those with features to suggest rare metabolic or genetic conditions, or other forms of secondary obesity were excluded. Overweight/obese children underwent a detailed clinical examination including anthropometric measurements, indirect calorimetry, fasting blood sample collection, an oral glucose tolerance test (OGTT), and determination of blood pressure level. Normal weight (control) children aged 6 to 18 years, were recruited from elementary and middle schools of Pécs and surrounding cities.

Anthropometric measurements were carried out by the same investigators. Relative body weight (RBW) and relative body mass index (RBMI) were determined on the basis of Hungarian national standards. For overweight/obese children blood samples were drawn after an overnight fast and a standard 2-hour OGTT was performed with administration of 1.75 g/kg (maximum 75 g) glucose.

Definitions used for the obesity-related metabolic conditions were as follows:

hyperinsulinemia – fasting insulin >20 µU/mL and/or postload peak insulin during OGTT >150 µU/mL; ***impaired glucose regulation (IGR)*** – fasting glucose ≥5.6 mmol/L or 2-hour glucose during OGTT ≥7.8 mmol/L; except for the study on the renal effects of obesity, where the older criteria were used: fasting glucose >6.1 mmol/L or 2-hour glucose during OGTT >7.8 mmol/L; ***diabetes mellitus (DM)*** – fasting glucose ≥7.0 mmol/L or 2-hour glucose during OGTT ≥11.1 mmol/L; ***dyslipidemia*** – high fasting triglyceride (>1.1 mmol/L [<10 years]; >1.5 mmol/L [>10 years]) or low fasting HDL-cholesterol (< 0.9 mmol/L); ***hypercholesterolemia*** – total fasting cholesterol > 5.2 mmol/L. Insulin resistance was estimated with the homeostasis model assessment (HOMA) index and the whole body insulin sensitivity index (WBISI) using the Matsuda Formula.

Basic metabolic rate was measured by means of a Deltatrac metabolic cart (Datex Instrumentarium Corp., Helsinki, Finland). BMR and respiratory quotient (RQ) were adjusted for age, gender and fat free body mass (FFM).

Genomic DNA was isolated from peripheral leukocytes and genotyping for the polymorphisms were performed by PCR/PCR- RFLP methods previously described: ADRB3 Trp64Arg by the method of Sipiläinen et al.; UCP-1 –3826 A/G by the method of Valve et al.; UCP-2 exon 8 45 bp del/ins by the method of Walder el al.; UCP-2 –866 G/A by the method of Schauble et al.; UCP-3 – 55 C/T by the method of Cassel et al.; PPARG2 Pro12Ala polymorphism by the method of Yen et al.

The comparison of allele/genotype frequencies was performed with a simple chi-square analysis. Association between risk of obesity and the different polymorphisms was estimated using univariate and multivariate logistic regression. For continuous parameters, distributional assumptions were verified, and parametric (unpaired Students' t-test, ANOVA, Pearson's correlation) and non-parametric (Mann-Whitney U-test, Kruskall-Wallis test, Spearman's correlation) methods were used as appropriate, for intergroup comparisons and tests of correlation. Categorical variables were examined by chi-square test or Fisher's exact test. For comparisons across genotypes, significance tests for linear trend were performed. Corrections for multiple comparisons were performed with the Bonferroni method.

RESULTS AND CONCLUSIONS

1. Energy expenditure gene polymorphisms and risk of childhood obesity

Results: The study included 1 346 (709 overweight/obese and 637 normal weight) children aged 6–18 years. The genotype distributions and allele frequencies for the ADRB3 Trp64Arg, UCP-1 –3826 A/G, UCP-3 –55 C/T, and PPARG2 Pro12Ala polymorphisms did not significantly differ between the overweight/obese and normal weight children. The variant A allele of the –866 G/A UCP-2 polymorphism was significantly more frequent among the normal weight children compared with the overweight/obese (0.39 vs. 0.35; p=0.024), and the ins allele of the UCP-2 exon 8 del/ins polymorphism was significantly more common among the overweight/obese children compared with controls (0.31 vs. 0.27; p=0.016). Univariate and multivariate logistic regression analyses showed that the UCP-2 –866 G/A polymorphism and the UCP-2 exon 8 del/ins polymorphism significantly influenced the risk of overweight/obesity. In multivariate analyses, the UCP-2 –866 A variant was associated with an OR for obesity of 0.69 (95% CI: 0.55–0.86; p=0.001), with both heterozygotes and homozygotes for the A allele showing significantly lower risk for overweight/obesity: OR=0.69 (95% CI: 0.52–0.92; p=0.013) and OR=0.50 (95% CI: 0.32–0.79; p=0.003), respectively, compared with G/G homozygotes. The ins allele for the UCP-2 del/ins polymorphism was associated with an OR for obesity of 1.51 (95% CI: 1.20–1.91; p=0.001), and heterozygosity and homozygosity for the ins allele were associated with significantly higher risk of overweight/obesity: OR=1.66 (95% CI: 1.24–2.23; p=0.001) and OR=2.12 (95% CI: 1.23–3.63; p=0.006), respectively, compared with the del/del genotype.

Measures of obesity decreased progressively across the three genotype groups G/G, G/A and A/A for the –866 G/A UCP-2 polymorphism, with the A allele carriers and A/A homozygotes showing a significantly lower RBW (130.3±35.9 and 128.6±35.8, respectively) and RBMI (125.1±34.3 and 122.9±33.4, respectively) compared to G/G homozygotes (RBW: 135.2±36.9, RBMI: 129.8±34.6; p<0.05) Likewise, indices of obesity showed a progressive increase across the del/del, del/ins and ins/ins UCP-2 genotypes, with the ins allele carriers and ins/ins homozygotes having a significantly higher RBW (134.2±36.5 and 139.1±40.8) and RBMI (129.2±34.5 and 132.9±37.8) as compared to del/del homozygotes (RBW: 130.2±36.0, RBMI: 124.7±34.4; p<0.05).

Conclusions: In a large population of Hungarian school-aged children, we found an association between higher risk of overweight/obesity and the ins allele of the UCP-2 exon 8 del/ins polymorphism, as well as lower risk of overweight/obesity and the A allele of the UCP-2 –866 G/A polymorphism. These observations suggest that genomic variations in or nearby the UCP-2 gene may influence the susceptibility to common pediatric obesity in the Hungarian population. On the other hand, our data did not reveal any evidence for a significant impact of the examined polymorphisms of the ADRB3, UCP-1, UCP-3 and PPARG2 genes on childhood obesity incidence.

2. Effects of energy expenditure gene polymorphisms on obesity-related traits and metabolic complications of obesity in children

Results: This study included 528 overweight/obese children aged 6–18 years, who underwent a detailed clinical examination aimed at determining the etiology and consequences of their obese state. Carriers of the ADRB3 Arg64 allele (n=81) had a significantly higher RBW (175.4 ± 27.1) and RBMI (164.0 ± 21.1) than the wild type homozygotes (166.4 ± 29.1 and 157.5 ± 21.0; p=0.01). There were no statistically significant differences in metabolic parameters between the ADRB3 genotype groups, although the two homozygote Arg allele carriers had a considerably lower mean adjusted BMR (1853.5 kcal) than the rest of the population (1991.3 kcal).

The ins allele of the UCP-2 exon 8 del/ins polymorphism was associated with worse indices of obesity, as well as higher insulin levels during OGTT, insulin resistance, higher triglycerides, and a lower adjusted BMR and higher RQ (Table 1.).

Table 1. Anthropometric and metabolic parameters according to the UCP-2 exon 8 del/ins genotypes

Parameters	del/del (n=244)	del/ins (n=233)	ins/ins (n=51)	p*	ins carriers (n=284)	p**
BMI (kg/m^2)	29.9±4.5	30.9±4.4	32.3±5.2[3]	0.001	31.1±4.6	0.002
RBW (%)	164.1±29.7[1]	170.1±26.8	176.1±31.8[3]	0.008	171.2±27.8	0.005
RBMI (%)	154.8±21.1[1]	160.9±20.2	167.3±22.8[3]	<0.001	162.0±20.8	<0.001
F ins (µU/mL)†	26.7±17.6	30.1±19.3[2]	42.8±49.0[3]	<0.001	32.4±27.5	0.004
2h ins (µU/mL)†	147.0±108.5	167.1±102.2[2]	223.1±99.5[3]	<0.001	177.1±101.8	0.001
HOMA†	5.8±4.3	6.4±4.5[2]	9.2±10.3[3]	<0.001	6.9±6.0	0.01
WBISI†	41.1±22.4	38.2±24.9[2]	27.3±18.3[3]	0.001	36.3±24.2	0.02
TG (mmol/L)†	1.4±0.6	1.5±0.7	1.6±0.8	0.05	1.5±0.7	0.04
BMR (kcal)‡	2063.2±463.[1]	1937.0±390.7	1933.5±367.2	0.001	1936.3±382.5	0.001
RQ‡	0.800±0.07	0.813±0.07	0.824±0.08	ns	0.815±0.07	0.027

Data are mean ± SD. † adjusted for age and gender; ‡ adjusted for age, gender and fat free mass.

* for comparison of the three genotype groups; ** del/del vs. ins allele carriers

[1] p< 0.05 for del/del vs. del/ins; [2] p< 0.05 for del/ins vs. ins/ins; [3] p< 0.05 for del/del vs. ins/ins

RBW – relative body weight; RBMI – relative BMI; F – fasting; 2h – 2-hour (OGTT); ins – insulin; HOMA – homeostasis model assessment index; WBISI – whole body insulin sensitivity index; TG – triglycerides; BMR – basic metabolic rate; RQ – respiratory quotient; ns – not significant.

Children with the UCP-3 –55 T/T genotype had a significantly lower adjusted BMR (1808.1±295.8 kcal) than either those with the C/C (1991.7±431.5 kcal, p=0.04) or the C/T (2012.9±418.4; p=0.02) genotype. The T/T genotype group had a higher RBW (168.7±33.8) and RBMI (158.8±23.3) when compared with the C/T genotype group (164.1±27.1 and 155.3±18.9, respectively), but similar when compared to the C/C genotype (169.7±29.3 and 160.1±21.8, respectively), and the differences were not statistically significant. There were no meaningful differences in the other anthropometric and metabolic parameters according to the presence of the UCP-3 –55 C/T polymorphism.

The UCP-1 –3826 A/G, UCP-2 –866 G/A and PPARG2 Pro12Ala polymorphisms were not associated with significant differences in measures of obesity, adjusted metabolic rate, or obesity-related metabolic parameters in this study population.

Conclusions: In our group of overweight/obese, school-aged Hungarian children the Tpr64Arg polymorphism of the ADRB3 gene and the exon 8 45 basepair del/ins polymorphism of the UCP-2 gene were associated with severity of obesity. The UCP-2 exon 8 del/ins and the UCP-3 –55 C/T polymorphisms influenced BMR and the UCP-2 exon 8 ins allele was also associated with obesity-related derangements of carbohydrate and lipid metabolism. We could not demonstrate any effect of the UCP-1 –3826 A/G, the UCP-2 –866 G/A or the PPARG2 Pro12Ala polymorphisms on BMR, or the severity or metabolic complications of pediatric obesity in this study group.

3. Association of the UCP-2 exon 8 del/ins polymorphism with obesity-related liver injury

Results: The study population consisted of 252 overweight/obese children (age: 12.8±2.7 years). An elevated serum aminotransferase level was present in 25.8% (n=66) of the subjects. The liver enzyme elevations were usually mild to moderate, between 2 to 3 times the upper limit of the normal range. Of the study participants 58.7% (n=148) were boys and the genders were well matched for age and BMI. Compared with the girls, aminotransferase levels were significantly higher among the boys (ALT: 35 [22–47] vs. 24 [19–28] U/L; p<0.001, AST: 31 [25–40] vs. 25 [21–30] U/L; p<0.001), and elevated aminotransferase levels were significantly more frequent (elevated ALT *or* AST: 34.5% vs. 13.5%; p<0.001). The allelic frequency and genotype distribution of the UCP-2 gene exon 8 del/ins polymorphism did not differ between the genders.

Among the children with the different UCP-2 genotypes there was a progressive, statistically significant decrease in the proportion of subjects with aminotransferase elevations, as well as in mean aminotransferase levels in carriers of the ins allele in heterozygote or homozygote form (Figure 1.). Accordingly, prevalence of aminotransferase elevations was highest among children with the del/del genotype, lower among those with del/ins genotype, and the lowest among those with the ins/ins genotype, with respect to an elevated ALT *or* AST level (chi-square: 11.9, p=0.003), an elevated ALT level (chi-square: 11.2, p=0.004), or an elevated ALT *and* AST level (chi-square: 8.9, p=0.013). Linear trends across genotypes for lower prevalence of elevated liver function tests in heterozygote and homozygote carriers of the ins allele were significant with respect to all tested sets of hepatic enzymes (elevated ALT *or* AST: p=0.002, elevated ALT: p=0.003, elevated ALT *and* AST: p=0.012).
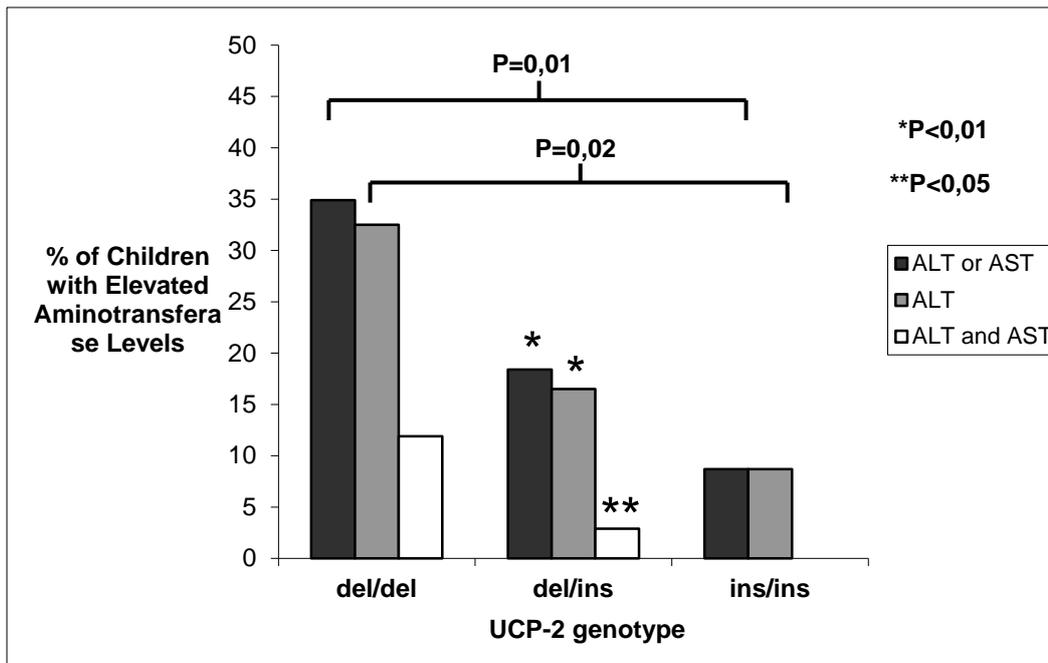
Figure 1 Percentage of children with elevated aminotransferase levels by genotype.

Abnormal aminotransferase levels were generally associated with higher prevalence of or worse indices for features of the metabolic syndrome. Children with an elevated liver function test were characterized by significantly higher BMI (32.5 ± 5.4 vs. 30.7 ± 4.6; p=0.01), RBW (174.9 ± 24.6 vs. 166.5 ± 20.7; p=0.02) fasting cholesterol (4.7 [4.1–5.3] vs. 4.3 [3.9–4.9]; p=0.05) and triglyceride levels (1.5 [1.0–1.9] vs. 1.2 [0.9–1.6]; p=0.005), and 2-hour glucose level during OGTT (7.2 [6.0–7.8] vs 6.6 [5.9–7.2]; p=0.04) compared with those with normal transaminase levels. Dyslipidemia (48.5% vs 28.5%; p=0.006), hypercholesterolemia (28.8 vs 16.1%; p=0.05) and IGR (34.8 vs. 21.5%; p=0.03) were significantly more frequent among children with elevated aminotransferase levels. ALT positively correlated with BMI (r=0.23; p<0.001), waist-to-hip ratio (r=0.37; p<0.001), 2-hour glucose level during OGTT (rho=0.12; p=0.05), total cholesterol (rho=0.15, p=0.01), and triglycerides (rho=0.15, p=0.02), and AST with waist-to-hip ratio (r=0.40; p<0.001), 2-hour glucose level during OGTT (rho=0.14; p=0.03) and triglycerides (rho=0.16, p=0.01).

Conclusions: Our findings demonstrate that obesity-related hepatic injury indicated by elevated serum aminotransferase levels is a frequent consequence of childhood obesity. The ins allele of the exon 8 45 basepair del/ins polymorphism of the UCP-2 gene was associated with decreased prevalence of aminotransferase elevations among overweight/obese children, suggesting, that the ins allele might be a protective factor, or the del allele a vulnerability factor for the development of pediatric obesity-linked hepatic damage.

4. Renal effects of childhood obesity

Results: The study population included 86 obese (age 12.9 [8.9–17.2] years) and 79 normal weight (age 13.5 [10.7–14.9] years) children. Obese children had a significantly higher urinary albumin:creatinine ratio (U-ACR) (11.7 mg/g, interquartile range: 12.9 mg/g vs. 9.0 mg/g, interquartile range: 5.1 mg/g; p=0.003) and urinary β2-microglobulin:creatinine ratio (U-BMCR) (63.9 µg/g, interquartile range: 34.7 µg/g vs. 34.6 µg/g, interquartile range: 44.1 µg/g; p<0.001) as compared to the normal weight children (Figure 2).
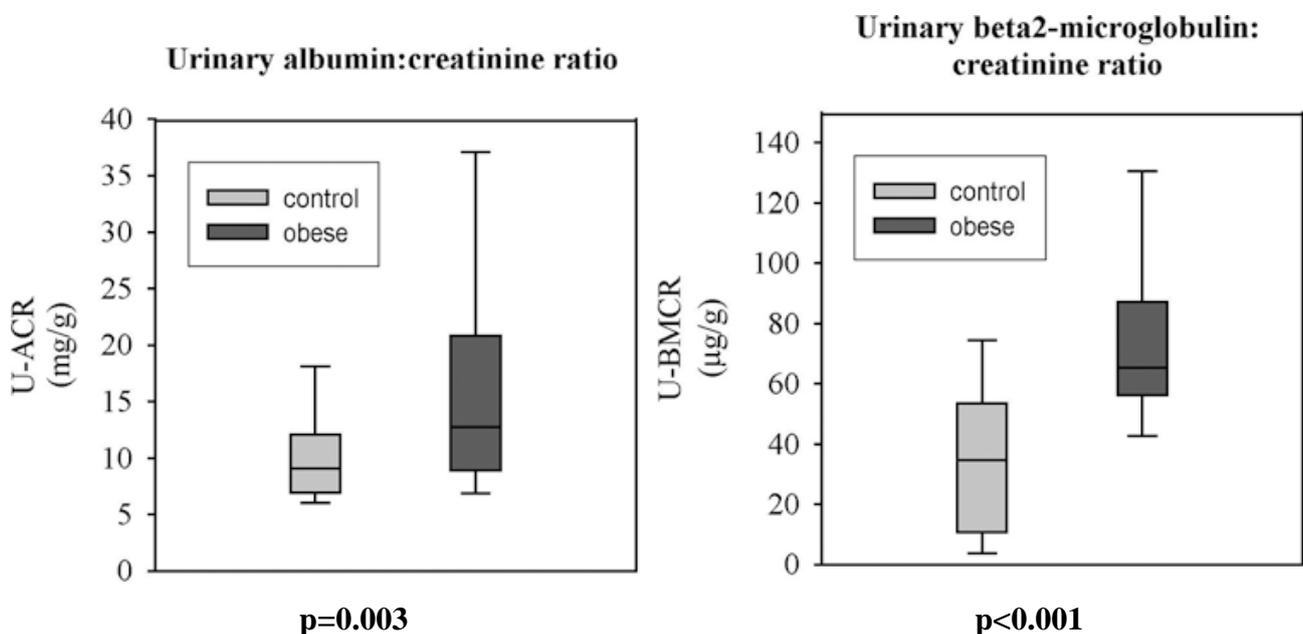
Figure 2. The urinary albumin:creatinine ratio (U-ACR) and urinary β2-microglobulin:creatinine ratio (U-BMCR) of the obese and control children (the median is depicted by the *line,* the interquartile range by the *box limits,* and the 10th–90th percentiles by the *error bars*).

Associations between the U-ACR and U-BMCR and obesity-related cardiovascular risk factors were investigated by performing bivariate comparisons between the U-ACR and U-BMCR values of the obese children with or without a certain risk factor (Table 2.). The presence of all tested risk factors was associated with a higher mean U-ACR, and the difference was significant in the case of fasting hyperinsulinemia, IGR, and hypercholesterolemia. The U-BMCR in the obese children was not significantly influenced by any of the cardiovascular risk factors studied.

Table 2. The urinary albumin:creatinine ratios (U-ACR) of the obese children with or without certain cardiovascular risk factors

Obesity-related cardiovascular risk factor	Obese children *without* risk factor		Obese children *with* risk factor		p**
	n	U-ACR (mg/g)	n	U-ACR (mg/g)	
Fasting hyperinsulinemia	61	10.4 [10.7]	25	13.6 [15.0]	< 0.05
Postprandial hyperinsulinemia	30	10.4 [8.2]	56	13.2 [15.3]	Ns
Impaired glucose regulation	66	10.8 [10.9]	20	19.3 [18.0]	< 0.05
Dyslipidemia	41	11.5 [12.3]	45	13.6 [15.1]	ns
Hypercholesterolemia	69	10.6 [12.5]	17	18.2 [10.6]	< 0.05
Hypertension	76	11.5 [11.6]	10	22.7 [17.8]	ns

Values are median [interquartile range]. ** Mann-Whitney *U* test. ns: not significant.

Furthermore, obese children with no more than one of these risk factors had a significantly lower U-ACR, than those with two or more traits (10.4 mg/g, interquartile range: 5.8 mg/g vs. 15.3 mg/g, interquartile range: 14.9 mg/g; p<0.05). There were no differences in the U-BMCR between these groups.

When analyzing all participants, the U-ACR as well as the U-BMCR was positively correlated with body weight (r=0.16; p<0.05 and r=0.34; p<0.001, respectively), BMI (r=0.22; p<0.05 and r=0.23; p<0.05, respectively), and RBW (r= 0.23; p<0.005 and r=0.31; p<0.001, respectively).

Among the obese children, the U-ACR positively correlated with the fasting (r=0.225; p< 0.05) and 2-hour (r=0.368; p<0.001) glucose concentrations measured during the OGTT.

Conclusions: According to our results, clinically healthy obese children have a higher level of albuminuria and β2-microglobulinuria than normal weight children. The U-ACR in the obese children was associated with certain metabolic derangements linked to obesity, and also with the clustering of the features of the metabolic syndrome. Our findings suggest that increased levels of albuminuria and β2-microglobulinuria indicating early glomerular and tubular dysfunction, respectively, are features of childhood obesity.

SUMMARY OF NEW OBSERVATIONS

1. Energy expenditure gene polymorphisms and the risk of childhood obesity

We show evidence that the UCP-2 –866 G and exon 8 45 basepair ins alleles are genetic risk factors for overweight/obesity among Hungarian school-aged children.

2. Energy expenditure gene polymorphisms and obesity-related traits and metabolic complications of childhood obesity

We show evidence for the roles of the Trp64Arg ADRB3 polymorphism and the exon 8 45 basepair del/ins UCP-2 polymorphism as genetic determinants of the severity and/or metabolic complications of pediatric obesity in the Hungarian population. Our results regarding the influence of the UCP-3 –55 C/T polymorphism on childhood obesity are inconclusive.

3. Association of the UCP-2 exon 8 del/ins polymorphism with obesity-related liver injury

We provide data suggesting that the exon 8 del/ins polymorphism of the UCP-2 gene may play a role in the development of hepatic injury, as indicated by elevated aminotransferase levels in obese children.

4. Effect of childhood obesity on renal glomerular and tubular function

We show that clinically healthy obese children have a higher level of albuminuria and β2-microglobulinuria than normal weight children, indicating early renal glomerular and tubular dysfunction as a consequence of childhood obesity.

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Papers

1. Erhardt É, Czakó M, Csernus K, Molnár D, Kosztolányi Gy. A beta3-adrenoreceptor gén Trp64Arg polimorfizmus kapcsolata cardiovascularis kockázati tényezőkkel. Metabolizmus 2004;II/3:147–50.

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