GENETIC DETERMINANTS AND HEALTH CONSEQUENCES OF COMMON CHILDHOOD OBESITY

PhD Thesis

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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

e8 – exon 8

FFM – fat free mass

HOMA – homeostasis model assessment

IGR – impaired glucose regulation

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

OGTT – oral glucose tolerance test

OR – odds ratio

PPARG2 – peroxisome proliferator-activated receptor-γ2

RBMI – relative BMI

RBW – relative body weight

ROS – reactive oxygen species

RQ – respiratory quotient

SD – standard deviation

T2DM – type 2 diabetes mellitus

UCP – uncoupling protein

U-ACR – urinary albumin/creatinine ratio

U-BMCR – urinary β2-microglobulin/creatinine ratio

WAT – white adipose tissue

WBISI – whole body insulin sensitivity index

WHR – waist to hip ratio

INTRODUCTION

The dramatically increasing rate of pediatric obesity in populations throughout the world has triggered intensive research focusing on the development and consequences of excess body weight during the growing years. Obesity affects up to one third of the population in many industrialized countries; according to a survey conducted among school-aged children (10–12 years) in 2010, 22.6% of Hungarian girls and 27.7% of Hungarian boys were overweight or obese (1). It is well established that rapid globalization of the westernized lifestyle (general availability of high-calorie food and limited need for physical exercise) is fuelling the obesity epidemic, yet the relationship is not a simple one. Not all children are affected equally by our unhealthy lifestyles; while some carry gene variants rendering them particularly sensitive to the obesogenic environment, others are protected from its deleterious effects.

Adverse health consequences of obesity are numerous, affecting practically all parts of the human body and can already be present in early childhood. Joining the classical components of the obesity-related metabolic syndrome (derangements of carbohydrate and lipid metabolism, and hypertension) are a growing number of target organs with obesity-induced dysfunctions, including the liver (non-alcoholic fatty liver disease) and the kidney (microalbuminuria). Comorbidities of obesity – especially features of the metabolic syndrome – cluster in families and also do not affect all children equally; thus besides the set of genes responsible for susceptibility to common paediatric obesity there also seems to be a partly overlapping set of genes that determine the progression and health consequences of the obese state.

1. Genetic basis of common childhood obesity

Common obesity is a typical multifactorial disorder with a strong genetic component. According to family and twin studies genetic influences on body size and body composition are substantial and by some estimates 40–70% of the interindividual variation in obesity-related phenotypes is heritable (2,3). The genetic background for the susceptibility to common childhood obesity seems to result from the possession of risk alleles at many genes involved in the regulation of energy balance (4). These obesogenic gene variants have small effect sizes, but the risk alleles for obesity are quite common in populations, and may act synergistically in response to obesogenic environmental conditions (5). 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 (6).

Candidate gene association studies are one of the main genetic epidemiological approaches to identify gene loci for common obesity and related traits. Candidate gene studies are hypothesis-driven and rely on the current understanding of the biology and pathophysiology that underlies the susceptibility to obesity. Candidate genes are identified based on their presumed biological roles in the development of obesity and can be tested for association with obesity by examining whether or not variations in body weight are accompanied by increased frequencies of certain alleles of these genes.

Obesity results from a long-term imbalance between caloric intake and energy expenditure, resulting in the storing of energy as fat, primarily in adipose tissue. Energy expenditure in humans can be divided into (1) the energy needs of physical activity; (2) the basal metabolism (BMR – basic metabolic rate) measured under resting conditions and required for cell and organ survival; and (3) adaptive thermogenesis which confers the ability to adapt to prolonged exposure to cold (non-shivering thermogenesis) or increase energy expenditure after feeding (diet-induced thermogenesis). All components show considerable variations between subjects and have genetic determinants. In children, lower energy expenditure rather than increased energy intake has been reported to predict weight gain (7). Thus, genes concerned with energy metabolism and storage constitute one of the major groups 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-γ2 (PPARG2) with obesity and related traits (8) 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.

1.1 β_3-adrenoreceptor gene – Trp64Arg polymorphism

The β_3-adrenoreceptor (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 (9). Thus, the ADRB3 plays an important role in regulating metabolic rate and lipid metabolism and low ADRB3 activity could promote obesity through several pathways. A missense mutation in codon 64 of the gene for ADRB3 results in a tryptophan substitution by arginine in the first intracellular loop of the receptor protein. The Trp64Arg polymorphism of the ADRB3 gene was associated with

decreased lipolytic sensitivity (10) and is one of the most firmly established obesity susceptibility gene polymorphisms (11,12). A higher prevalence of the Arg64 allele among obese subjects has been described in adult (10,13) and paediatric (14) populations.

1.2. Uncoupling protein-1 gene – –3826 A/G polymorphism

Uncoupling proteins (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 (15). UCP-1 (also called "thermogenin") is expressed mainly in brown adipose tissue (BAT) where it is the key component of cold-induced non-shivering thermogenesis as well as contributing to diet-induced thermogenesis (16). BAT differs from white adipose tissue (WAT) in that it has large numbers of mitochondria containing UCP-1, and instead of storing fat it serves to oxidize lipids to produce heat and to rid the body of excess fat. BAT and UCP-1 have an established role in the nutritional homeostasis of rodents; in humans however, the importance of BAT thermogenesis in weight regulation has been strongly debated, since in large mammals such as humans, BAT is mainly active in infancy after which it atrophies; thus no discernible brown fat depots are present in adults. Brown fat cells remain, however, dispersed amongst the white adipose tissue so that substantial amounts of brown adipocytes expressing UCP-1 exist throughout life in intraperitoneal and deep-cervical/axillary adipose depots that are generally classified as white and may represent up to 1-2% of body weight even in adults (17,18). It has been hypothesized, that BAT (i.e. the "brown compartment" of adipose tissue) might be responsible for 1–2% of the energy expenditure in humans, and that defects in BAT function of this magnitude might lead to weight gain of 1–2 kg/year (18). Decreased expression of UCP-1 was indeed detected in WAT of obese as compared to lean adults (19). Furthermore, BAT retains capacity of expansion in adults and certain physiological and pathological stimuli (chronic cold exposure, adrenergic signals [pheochromocytoma], alcoholism, malignancies) can reactivate dormant brown adipocytes or lead to the recruitment of fully differentiated BAT from preadipocyte precursor cells in adult humans (20). A group of scientists recently reported that the transition of WAT to BAT (a phenomenon called WAT browning) is the precursor to cachexia, a wasting syndrome characterized by excessive weight loss of patients. (21). Thus, UCP-1 activity in BAT is thought to contribute to facultative energy expenditure and the development of obesity in humans (19). The first discovered genetic polymorphism of UCP-1 was an A to G point mutation in the promoter region of the gene at position –3826 (22). The G variant of the polymorphism was associated with reduced mRNA expression (23); and supporting the hypothesis of a role for UCP-1 and BAT in the body weight regulation of humans this functional polymorphism of UCP-1 was associated with a higher

frequency among obese adults (24), higher body mass index (BMI) (25, 26) higher capacity of weight gain (27) and resistance to low calorie diet (28, 29).

1.3 Uncoupling protein-2 gene – –866 G/A and exon 8 45 base pair deletion/insertion polymorphisms; uncoupling protein-3 gene – –55 C/T polymorphism

In 1997, two UCP-1 homologues have been discovered. UCP-2 is widely distributed in humans, with predominant expression seen in WAT, but also considerable amounts found in skeletal muscle, liver, heart, kidney, pancreas, brain and cells of the immune system, while UCP-3 expression is restricted mainly to skeletal muscle and brown adipocytes (15). UCP-2 and UCP-3 have 59% and 57% identity with UCP-1, and 73% identity with each other (30). 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, and thus serve as regulators of energy homeostasis. Although it has been demonstrated that these UCP-1 homologues are also able to reduce the mitochondrial membrane potential and thereby control or influence the efficiency of oxidative phosphorylation (31), they are not as thermogenic as UCP-1 (32), and almost two decades of intense research has shown by now, that their main function is not body weight or body temperature regulation. Despite the intense interest in defining the functions of UCP-2 and UCP-3 their precise physiological and biochemical roles are still uncertain. Several hypothesized functions have been described, among others, UCP-2 has been proposed to play a role in cellular energy balance, regulation of ATP/ADP ratio, protection from obesity, regulation of insulin secretion and action, type-2 diabetes mellitus (T2DM), lipid metabolism and storage, fatty acid oxidation and transport, regulation of reactive oxygen species (ROS) production, thermoregulatory response to infection, thermogenesis in certain tissues (brain), and neuroprotection (33–40). UCP-3 is also implicated in the modulation of ROS production and is presently thought to be primarily involved in the regulation of fuel partitioning (39,40). Whatever the main physiological roles of UCP-2 and UCP-3 may be, by virtue of their uncoupling activity these proteins can influence the efficiency of energy coupling in mitochondria as a secondary effect of their main function and thus play an important role in human energy homeostasis and in the mechanisms underlying the variability in human energy expenditure and affecting obesity risk (31,40,41). Supporting this notion, linkage was demonstrated between genetic markers in the vicinity of the chromosomal region containing the UCP-2/UCP-3 gene cluster (11q13) and resting metabolic rate in humans (41) and gene expression of both UCP-1 homologues have been positively related to resting metabolic rate in adults (42,43). Furthermore, the UCP-2/UCP-3 gene cluster was identified as a genetic locus for childhood and adolescent obesity in a genome scan conducted in

German families (44). UCP-3 is an especially attractive candidate gene for obesity since skeletal muscle is a major contributor to whole body thermogenesis and BMR in humans after infancy which could, in part, be due to proton leaks caused by UCP-3.

Functional variants of the UCP-2 gene include the –866 G/A polymorphism in the promoter region, with the A allele related to enhanced transcriptional activity (45) and lower BMI and risk of obesity (45, 46); and the 45 bp deletion/insertion (del/ins) polymorphism in the 3' untranslated region of exon 8 with the ins variant associated with reduced mRNA stability (45) and a greater risk of developing obesity (47–49).

In the UCP-3 promoter region a C to T substitution 55 bp upstream of the transcription starting site was identified (–55 C/T) and the T variant displayed association with enhanced transcriptional activity (50) and lower BMI and risk of obesity (46, 51, 52).

1.4 Peroxisome proliferator-activated receptor-γ2 gene – Pro12Ala polymorphism

Peroxisome proliferator-activated receptor-γ2 (PPARG2) is an adipocyte-specific nuclear hormone receptor that regulates fat cell differentiation, lipid metabolism and insulin sensitivity. Since the volume of lipid that an individual adipocyte can accumulate is fixed, significant expansion of adipose tissue mass requires de novo differentiation of adipocytes from precursor cells. As a lipid-activated transcription factor PPARG2 represents a potential molecular link between cellular or systemic lipid metabolism and adipocyte differentiation, thereby its genetic variations can modify fat accumulation and obesity risk (53).

The Pro12Ala missense mutation is a common coding gene variant located in the 5' region specific for PPARG2 and was associated with reduced DNA binding and transactivation in vitro (54). Data are conflicting as to whether the Pro12Ala mutation is associated with obesity (55,56) or with a lower BMI (53,54). Considering that PPARG2 promotes lipid storage, it is somewhat surprising that the majority of data point to the Ala12 allele – which reportedly renders the receptor less active – to be associated with human obesity: two large meta-analyses have concluded that the Ala12 allele is associated with increased BMI among obese adults (57,58). Furthermore, a higher frequency of the Ala12 allele was detected among obese men and women compared with controls (56,59) and among Spanish children and adolescents presence of the mutation was associated with significantly increased risk of obesity (60). It has also been proposed, that the consequences of the Pro12Ala polymorphism could be divergent in the lean and obese states: a study of Danish males suggested, that the Ala12 allele could be associated with lower BMI among lean subjects and higher BMI among the obese (61).

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

The basic metabolic rate is highly variable in humans, but sources of this variability remain to be identified. Reduced BMR and increased respiratory quotient (RQ) (implying a higher ratio of carbohydrate to fat oxidation) are known risk factors for weight gain (62,63) and are in part genetically determined (64). The development of childhood obesity – although perhaps to a lesser extent compared with adulthood obesity – is strongly influenced by environmental factors, which are difficult to precisely measure and control for. Intermediary traits related to obesity like BMR and RQ are less likely than BMI to be affected by extrinsic factors.

Obesity is associated with several metabolic complications and cardiovascular risk factors attributed to obesity-driven insulin resistance such as hyperinsulinemia, impaired glucose tolerance (IGT)/type 2 diabetes mellitus (T2DM), increased triglyceride/decreased HDL-cholesterol level, increased blood pressure, and non-alcoholic fatty liver disease (NAFLD); and also with the clustering of these, the metabolic syndrome. Genetic predisposition is hypothesized to be a key factor in the susceptibility of obese individuals to metabolic disorders. 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 and adolescence (65). According to recent estimates from the European Union (66), one third of obese children have hyperinsulinemia, 8.4% suffer from IGT and 0.5% from T2DM, about 20% have dyslipidemia, 20% have hypertension, and 28% show evidence of hepatic steatosis. The majority of obese children are affected by one or more features of the metabolic syndrome (66).

Besides being potential risk factors for the development of obesity, energy expenditure gene polymorphisms are also implicated in the polygenic background of common obesity-related traits and metabolic complications (8).

2.1 β_3-adrenoreceptor gene – Trp64Arg polymorphism

Low ADRB3 activity may cause decreased thermogenesis in BAT and retention of lipids in white fat cells. In humans, the latter may be especially important, since ADRB3 activity is much more prominent in visceral fat (67) and thus ADRB3 dysfunction may lead to visceral adiposity, a key component of the metabolic syndrome. In accordance with these, the Trp64Arg ADRB3 polymorphism was previously associated with lower BMR (68), a higher capacity of obese subjects to gain weight (69), and features of the metabolic syndrome including visceral adiposity, insulin resistance, T2DM, dyslipidemia and raised blood pressure among adults (70–72). In studies of

Japanese children the variant Arg allele was associated higher degree of adiposity and lower HDL-cholesterol level among obese boys (73) and with the presence of the metabolic syndrome among obese children (74). Two other studies conducted among Japanese and Chinese schoolchildren found no significant differences in serum lipid, glucose and insulin levels or blood pressure between the different Trp64Arg genotype groups (14, 75), but the mutation did affect the success of dietary intervention among obese Chinese children (75).

2.2 Uncoupling protein-1 gene – –3826 A/G polymorphism

Reduced BAT thermogenesis has been etiologically related to the development of insulin resistance and diabetes in animal models of obesity (18). Since brown adipocytes in humans are present only in „internal" sites of adipose depots and absent from subcutaneous adipose tissue, defects of BAT-type thermogenesis might have a role in central fat distribution (18). Indeed, low amount of BAT has been associated with abdominal obesity in adult males (76), potentially implying UCP-1 in the development of the features of the metabolic syndrome.

Among obese adults the UCP-1 –3826 A/G polymorphism was related to reduced BMR (77) and an increased tendency to gain weight (78). The G variant was associated with T2DM (26), insulin resistance (79), dyslipidemia (80) and higher blood pressure (81) among adults. Among children the G allele was associated with diminished diet-induced thermogenesis (82).

2.3 Uncoupling protein-2 gene – –866 G/A and exon 8 45 base pair del/ins polymorphisms; uncoupling protein-3 gene – –55 C/T polymorphism

UCP-2 and UCP-3 have been implicated in several mechanisms that underlie or influence the development of obesity-related traits and features of the metabolic syndrome – including body fat distribution, lipid metabolism, insulin secretion and action, fuel substrate partitioning, cellular energy metabolism, and ROS production – making variations in these genes strong candidates for obesity-associated conditions.

By lowering the intracellular ATP concentration UCP-2 expressed in pancreatic beta-cells has been demonstrated to be a negative regulator of glucose-stimulated insulin secretion (36) and the –866 G/A polymorphism was repeatedly (although not consequently) related to disturbances of glucose metabolism and altered risk of T2DM. Thus, the G allele – while being conductive for obesity – was shown to afford relative protection against T2DM (83), while the A allele was associated with lower glucose-stimulated insulin secretion (84). In other studies, however, the G allele was more frequent among subjects with T2DM (85,86). Furthermore, Pima Indian carriers of the A allele were shown to have an increased BMR (87). In Japanese adults, the A allele was significantly more frequent among hypertensives (88). In paediatric studies, the A allele was associated with increased

resting energy expenditure and increased carbohydrate oxidation among obese European children (89). In a Spanish study of school-aged children, the G allele was associated with obesity and insulin resistance risk while the A allele was associated with lower risk of hyperinsulinemia and insulin resistance among the obese (90). The A allele has also been linked to markers of reduced cardiovascular risk among children and adolescents (91).

The UCP-2 exon 8 45 bp del/ins polymorphism was associated with metabolic rate during sleep and over 24 hours among Pima Indians (35) and related to increased BMI and abdominal obesity among Danish men (92). In a smaller group of children, association was demonstrated between the ins allele and greater skinfold thicknesses and body fat mass, although no relationship with resting energy expenditure could be demonstrated (48). The UCP-2 del/ins polymorphism was also indicated as a determinant of insulin resistance in obese children (90).

The – 55 C/T UCP-3 polymorphism was implicated in several phenotypes associated with obesity and the metabolic syndrome, including central fat distribution (93), insulin resistance (90), and T2DM and dyslipidemia (94). Among children significant association was observed between the – 55 C/T polymorphism and serum insulin levels, as well as waist circumference (46).

2.4 Peroxisome proliferator-activated receptor-γ2 gene – Pro12Ala polymorphism

PPARG2 acts as a master regulator of fat cell function by regulating differentiation of new adipocytes, and by inducing expression of genes promoting fatty acid uptake, triglyceride synthesis and insulin sensitivity; it is therefore an excellent candidate gene for influencing susceptibility to the features of the metabolic syndrome. In humans, loss-of-function mutations of PPARG2 confer an extreme phenotype of partial lipodystrophy, early-onset severe insulin resistance, T2DM, dyslipidemia, hypertension, and hepatic steatosis (95); while gain-of-function mutations leading to the permanent activation of PPARG2 were found in four unrelated subjects with severe obesity (96). Since the activity of PPARG2 differs between subcutaneous and intraabdominal adipose tissue, it has been suggested that PPARG2 regulates body fat distribution, thereby affecting insulin resistance (59).

Observations regarding the associations between the Pro12Ala mutation and obesity-related metabolic complications are controversial. Thus, the Pro12 allele has been associated with insulin resistance (58) and T2DM (54), and the Ala12 allele related to higher insulin sensitivity (59), protection against the onset of T2DM (53), a lower risk of hypertriglyceridemia (49), higher HDL-cholesterol levels (54), and a decreased risk of the metabolic syndrome (97). However, in other studies the Ala12 allele was linked to higher risk of combined hyperlipidemia (98), central obesity and higher cholesterol levels (55), multiple traits associated with the insulin resistance syndrome,

including higher glucose levels and blood pressure (99), and an increased risk of the metabolic syndrome (100).

It must be emphasized, that the above cited observations are only examples selected from the large number of papers that can be found in the literature on the relationship between the candidate gene polymorphisms of our interest and obesity, with focus on the positive findings and data pertaining to children. Despite the numerous studies investigating the allelic associations of these polymorphisms with obesity propensity and obesity-related traits results concerning their impacts on these are controversial, and thus their role and significance in obesity are still under debate. Besides the studies with positive results cited above many other investigations have been published; some providing further support for the link between the polymorphisms and obesity but others demonstrating no or even an opposite association (some examples: 8,10,101–111). Since the limited success of the candidate gene approach can mainly be ascribed to small sample sizes ($n<1000$) that are insufficiently powered to identify modest effects that are expected for common obesity (11), it is generally agreed upon, that more studies with larger populations of subjects are needed to clarify the controversial results that are presently available on the associations of obesity candidate genes with the risk and consequences of obesity.

It should also be emphasized, that most of the obesity candidate gene association studies are conducted in adult populations 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 and studies in pediatric populations are warranted. It has also been suggested, that alterations in genes that regulate energy metabolism would be more likely to contribute to childhood-onset rather than adult-onset obesity (46). Furthermore, 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, because presence or absence of association in one ethnicity or environment does not guarantee or exclude association in another.

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 (112–114). There is a growing concern about NAFLD, as it is emerging as the most common chronic liver condition in the developed world. 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 (115), but knowledge of the identity and effects of the genes and polymorphisms involved is currently limited (116).

Theoretical considerations as well as experimental data recently reviewed (117) indicate, that UCP-2 may have a role in the development of fatty liver disease. NAFLD begins with steatosis in the liver, which is generally accepted to be the result of insulin resistance and alterations in hepatic fat metabolism. In susceptible populations, and as the result of a “second hit” hepatic steatosis can progress to inflammation, or NASH. This course of NAFLD involves genetic determinants within several distinct processes linked to disease development and progression, including obesity, insulin resistance, lipid metabolism (synthesis, oxidation, transport and partitioning), cellular energy balance and oxidative stress (115), all of which are implicated as being regulated or influenced by the activity of UCP-2 (33–37). Experimental data implicating UCP-2 in the development and progression of NAFLD originate from research on both cell lines and animals. In normal liver, UCP-2 is expressed mainly in Kupffer cells, but not in hepatocytes; however hepatocytes induce UCP-2 mRNA and protein expression in obesity, leading to a substantially increased presence of UCP-2 in fatty livers (117,118). In liver biopsy specimens from young NAFLD patients, UCP-2 expression was correlated with the severity of inflammation and fibrosis (114). 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 (37) yet to be resolved. On the basis of these, the UCP-2 exon 8 del/ins polymorphism associated with altered UCP-2 activity (45) is a candidate gene polymorphism for the development/progression of or protection from obesity-associated NAFLD.

Definitive diagnosis of NAFLD requires liver biopsy, but the use of this invasive method is limited in large numbers of clinically healthy individuals, particularly children. Patients with NAFLD are

commonly characterized by elevated circulating concentrations of markers of liver injury, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, which are thus used extensively as surrogate markers for NAFLD (116). In obese children, an elevated ALT is a strong predictor of fatty liver disease (113) and associations between abnormal aminotransferase levels and histological and radiographic evidence of NAFLD have been repeatedly demonstrated both in adults and children (119–122).

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 urinary proteins – particularly that of albumin – indicating hyperfiltration and early renal damage has been shown to be associated with adult obesity (123–125). Furthermore, slightly increased urinary albumin excretion (microalbuminuria), an indicator of disturbed glomerular permeability, is not only a marker of renal damage, but may also represent the renal expression of systematically increased transcapillary albumin leakiness reflecting general endothelial dysfunction (126) and leading to vascular damage. Major epidemiological studies in adults have established microalbuminuria as an independent predictor of premature atherosclerosis and cardiovascular disease in both diabetics and non-diabetics (127–129). 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 (130). Prospective data regarding the association between markers of renal tubular dysfunction and obesity is limited and has been controversial (131–133), 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 polymorphism, the UCP-1 –3826 A/G polymorphism, the UCP-2 –866 G/A and exon 8 45 basepair del/ins polymorphisms, the UCP-3 –55 C/T polymorphism, and the PPARG2 Pro12Ala polymorphism among a large group of normal weight and overweight/obese Hungarian school-aged children, in order to provide basic, pediatric, nation-specific data on these polymorphisms and 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 energy expenditure gene polymorphisms on obesity-related traits and metabolic complications of common childhood obesity we looked for associations between the genetic variants and measures of obesity, BMR, blood pressure and parameters of lipid and carbohydrate metabolism 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 basepair 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 childhood obesity on urinary albumin and β2-microglobulin excretion as markers of renal glomerular and tubular dysfunction, respectively, we measured the urinary albumin/creatinine ratio (U-ACR) and urinary β2-microglobulin/creatinine ratio (U-BMCR) in clinically healthy obese and normal weight children. We also evaluated the relationship between the U-ACR and U-BMCR and multiple obesity-related cardiovascular risk factors among the obese children.

SUBJECTS

Most of the 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. Children were classified as overweight or obese according to international cut-off BMI values for overweight or obesity by sex and age (134). They underwent a detailed clinical examination aimed at determining the etiology and consequences of their obese state. Those with features to suggest rare metabolic or genetic conditions, endocrinological disorders, growth or renal problems or other forms of secondary obesity were excluded. As part of their examinations, the overweight/obese children underwent anthropometric measurements, indirect calorimetry, fasting blood sample collection, an oral glucose tolerance test (OGTT), and determination of blood pressure level.

1. Energy expenditure gene polymorphisms and risk of childhood obesity

Normal weight (control) children (n=637) aged 6 to 18 years, were recruited from elementary and middle schools of Pécs and surrounding cities. The overweight/obese group (n=709) included children aged 6 to 18 years, referred to our childhood obesity center for medical assessment and also those, who participated in the school recruitment programs but were above normal weight. In children recruited from the schools only basic physical examination and anthropometric measurements were performed, and random blood samples were collected for genetic analysis.

2. Energy expenditure gene polymorphisms and obesity-related traits

Overweight/obese children (n=528) aged 6 to 18 years, referred to our obesity center were included in this study.

3. UCP-2 exon 8 del/ins polymorphism and NAFLD

Overweight/obese children (n=252) aged 6 to 18 years, referred to our obesity center were included in this study. Children with features to suggest rare metabolic or genetic conditions, ongoing infections, or with a history of previous hepatitis or regular alcohol consumption, and also those taking medicine regularly were excluded.

4. Renal effects of obesity

During their clinical examination random morning spot urine samples were collected from 86 clinically healthy, obese children referred to our childhood obesity center. Random morning spot urine samples were also collected from 79 age-matched, healthy, normal weight children participating in a urinary screening program conducted among students of an elementary school.

Written informed consent was obtained from all parents of the children before enrollment in the studies. The studies were approved by the ethic review committee of the University of Pécs.

METHODS

1. Anthropometric measurements

Anthropometric measurements were carried out by the same investigators. Body weight in light clothing was determined to the nearest 0.5 kg by a standard beam scale, and height without shoes to the nearest 0.1 cm by a Holtain stadiometer. Relative body weight (RBW) and relative body mass index (RBMI) calculated as the ratio between actual body weight or BMI, and the ideal weight or BMI for age, gender and height were determined on the basis of Hungarian national standards (135,136), and are expressed as percentage.

Waist circumference was measured at midway between the inferior border of the rib cage and the superior border of the iliac crest, and hip circumference at the level of the greater trochanter, not including clothing. Skinfold thicknesses were measured on the left side of the body to the nearest 0.1 mm with a Holtain caliper and body fat was estimated using Slaughter et al. equations (137).

2. Clinical examinations

Overweight/obese children referred to our obesity center were examined for the presence of obesity-related metabolic and cardiovascular derangements. Blood samples were drawn after an overnight fast for measurement of fasting plasma glucose, serum insulin and lipid levels and a standard 2-hour OGTT was performed with administration of 1.75 g/kg (maximum 75 g) glucose and venous blood samples taken every 30 minutes for determination of plasma glucose and serum insulin concentrations.

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

hyperinsulinemia – fasting serum insulin >20 μU/mL (mean+2SD value of 100 control Hungarian children) and/or postload peak serum insulin during OGTT >150 μU/mL (138);

impaired glucose regulation (IGR) – fasting plasma glucose ≥5.6 mmol/L or 2-hour plasma glucose during OGTT ≥7.8 mmol/L (according to American Diabetes Association updated criteria [139]); except for the study on the renal effects of obesity, where the older criteria were used: fasting plasma glucose >6.1 mmol/L or 2-hour plasma glucose during OGTT >7.8 mmol/L (according to American Diabetes Association criteria [140]);

diabetes mellitus (DM) – fasting blood glucose ≥7.0 mmol/L or 2-hour blood glucose during OGTT ≥11.1 mmol/L (139);

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) concentration (criteria of the Hungarian Lipid Consensus Conference [141]);

hypercholesterolemia – total fasting cholesterol concentration > 5.2 mmol/L (141).

Insulin resistance/sensitivity was estimated with the homeostasis model assessment (HOMA) index (142) and the OGTT-derived whole body insulin sensitivity index (WBISI) using the Matsuda Formula (143).

Blood pressure was measured according to published recommendations for children at least three times in each subject on separate days by the same observer, using a mercury-gravity manometer with proper cuff size in standard conditions. We considered children to be hypertensive when the lowest blood pressure value of the three measurements was above the 95^{th} percentile for age and sex (144).

Basic metabolic rate (BMR) was measured by means of a Deltatrac metabolic cart (Datex Instrumentarium Corp., Helsinki, Finland), using the ventilated hood technique. After achieving steady state (which took typically 5–10 min) the BMR was measured for 45 min. Oxygen consumption, carbon dioxide production, standardized for temperature, barometric pressure and humidity, were measured at 1 min intervals and averaged over the whole measurement period. Oxygen and carbon dioxide concentrations were analyzed by a differential paramagnetic sensor and an infrared carbon dioxide analyzer, respectively. Before each test the calorimeter was calibrated with a reference gas mixture (95% oxygen, 5% carbon dioxide). To assess the precision of the indirect calorimeter ethanol burning tests were carried out. The coefficient of variation on BMR of a 1 day and a 1 week interval was less than 3%. In girls the timing of the BMR measurements was synchronized to the menstrual cycle. BMR and respiratory quotient (RQ) were adjusted for age, gender and fat free body mass (FFM).

Serum aminotransferase levels were determined from blood samples drawn after an overnight fast. We defined elevated aminotransferase levels as those exceeding the upper limit of normal specified for the method used in our laboratory as follows: aspartate aminotransferase (AST) > 50 U/L, alanine aminotransferase (ALT) > 40 U/L. Children with aminotransferase elevations were retested 3–6 months after the first examination, and only those with persistently elevated aminotransferase levels were considered as having indication of liver injury, with results of the initial tests used for the analyses.

Routine urinalysis by dip stick test and microscopic examination was performed on all analyzed urine samples, and protein or hemoglobin positive samples or those showing erythrocytes, leukocytes or cylinders were excluded from further assessment. Since time and temperature dependent degradation of β2-microglobulin occurs at a urine pH<5.5 (145), results for β2-

microglobulin were considered only for urine samples with pH greater than 5.5 (obese n= 56, control n= 62). To compensate for the variations in the concentration of random urine samples, we determined the urinary creatinine concentration and used the urinary albumin:creatinine ratio (U-ACR) and urinary β2-microglobulin:creatinine ratio (U-B2MCR) as estimation of the urinary excretion of albumin and β2-microglobulin.

3. Laboratory analyses

3.1 Biochemical analyses

Plasma glucose and serum total cholesterol, HDL-cholesterol, triglyceride, ALT and AST concentrations were determined by enzymatic methods using a Merck Selectra chemistry analyzer and reagents from Reanal Ltd (Budapest, Hungary). Serum insulin was determined by radioimmunoassay with the use of a commercially available kit (Isotope Institute of the Hungarian Academy of Sciences, Budapest, Hungary). Urinary albumin concentration was determined by immunoturbidimetric method, β2-microglobulin concentration by an enzyme immunoassay kit (Abbott Laboratories, Ill., USA), and creatinine concentration by the Jaffe method.

3.2 Examination of the common polymorphisms of genes associated with energy expenditure

Genomic DNA was isolated from peripheral leukocytes with the use of a commercially available DNA isolation kit (Generation Capture Column Kit, Gentra Systems, Minneapolis, Minnesota, USA). Genotyping for the common polymorphisms of genes associated with energy expenditure were performed by polymerase chain reaction (PCR)/PCR-restriction fragment length polymorphism (RFLP) methods previously described, with only minor modifications, as follows: the Trp64Arg polymorphism of the ADRB3 gene was examined by the method of Sipiläinen et al. (68); the –3826 A/G polymorphism of the UCP-1 gene was examined by the method of Valve et al. (77); the exon 8 45 basepair del/ins polymorphism of the UCP-2 gene was examined by the method of Walder el al. (35); the –866 G/A polymorphism of UCP-2 was examined by the method of Schauble et al. (107); the –55 C/T polymorphism of the UCP-3 gene was examined by the method of Cassel et al. (93); and the Pro12Ala polymorphism of the PPARG2 gene was examined by the method of Yen et al. (146). Important aspects of the methods applied are summarized in Table 3.2.1.

Table 3.2.1. Important details of the PCR/PCR-RFLP methods used for the examination of common polymorphisms of energy expenditure genes

Polymorphism	Primers	RE	Distinction of the wild type and variant allele
ADRB3 Trp64Arg	F: 5'–CGC CCA ATA CCG CCA ACA C–3' R: 5'–CCA CCA GGA GTC CCA TCA CC–3' **product size:** 210 basepairs	BstNI	Trp allele: 97+61+31+15+6 bp Arg allele: 158+31+15+6 bp
UCP-1 –3826 A/G	F: 5'–CCA GTG GTG GCT AAT GAG AGA A–3' R: 5'–GCA CAA AGA AGA AGC AGC AGA GAG G–3' **product size:** 279 bp	Bcl I	A allele: 157+122 bp G allele: 279 bp
UCP-2 –866 G/A	F: 5'–TGA TG AAC GTC TTT GGG ACT–3' R: 5'–GAT GAG AAA AGG CGT CAG GA–3' **product size:** 201 bp	Bsh 1236I	G allele: 122 + 79 bp A allele: 201 bp
UCP-2 exon 8 45 bp del/ins	F: 5'–CAG TGA GGG AAG TGG GAG G–3' R: 5'–GGG GCA GGA CGA AGA TTC–3'		del allele: 457 bp ins allele: 502 bp
UCP-3 –55 C/T	F: 5'–GGA TAA GGT TTC AGG TCA GGC–3' R: 5'–AAG GGA TGA GGG AGG AGA AA–3' **product size:** 194 bp	BsuRI	C allele: 100+64+20+10 bp T allele: 100+84+10 bp
PPARG2 Pro12Ala	F: 5'–GCC AAT TCA AGC CCA GTC–3' R: 5'–GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G–3' **product size:** 270 bp	BstUI	Pro allele: 270 bp Ala allele: 227+43 bp

RE – restriction endonuclease; F – forward; R – reverse; ADRB3 – β_3-adrenoreceptor; UCP – uncoupling protein; del/ins – deletion/insertion; PPARG2 – peroxisome proliferator-activated receptor-γ2; bp – basepairs.

4. Statistical analysis

Descriptive statistics are given as mean ± standard deviation (SD) for normally distributed continuous variables, median [interquartile range] for not normally distributed variables, and number (percentage) for categorical variables.

1–2. Energy expenditure gene polymorphisms and risk of childhood obesity, obesity-related traits and metabolic complications of childhood obesity

Genotype and allele frequencies were calculated for each polymorphism, and chi-square test was used to assess the concordance of estimated genotype frequencies with Hardy-Weinberg proportions. The comparison of allele/genotype frequencies in cases (overweight or obese) versus controls (normal weight) 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. In univariate analyses each gene was evaluated separately. For multivariate analysis, all of the examined polymorphisms were entered into a multiple logistic regression model with normal weight or overweight/obese as the dependent variable, genotype and allele frequencies according to the polymorphisms as independent predictor variables. Adjustment variables were age, height, and gender. Interaction between the gene polymorphisms was tested with the multiple logistic regression model containing the allele frequencies.

Intergroup comparisons of continuous variables were performed with analysis of variance (ANOVA) or unpaired Students' t-test and of categorical variables with chi-square test. Corrections for multiple comparisons were performed with the Bonferroni method. Adjustment variables were age, gender and FFM (ANCOVA) as appropriate.

3. UCP-2 exon 8 del/ins polymorphism and obesity-related liver injury

Chi-square test or Fisher's exact test was used to analyze frequencies of liver enzyme elevations between the different genotype groups. For comparisons across genotypes, significance tests for linear trend were performed. To strengthen the use of aminotransferase elevations as markers of obesity-related hepatic damage, presence of an elevated ALT *or* AST, elevated ALT, and elevated ALT *and* AST level were all tested for indication of liver injury. 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.

4. Renal effects of obesity

Bivariate analyses included both parametric (Students' t-test) and non-parametric (Mann-Whitney U test) methods. Correlation between the U-ACR and U-BMCR and anthropometric and metabolic parameters of the obese children were evaluated by calculation of Spearman's correlation coefficients.

Statistical analyses were performed using the SPSS for Windows statistical software (version 11.5–13.0, SPSS Inc., Chicago, IL, USA). A two-sided P-value of less than 0.05 was considered significant.

RESULTS

1. Energy expenditure gene polymorphisms and risk of childhood obesity

The anthropometric characteristics of the normal weight, and overweight or obese children included in this study are reported in Table 1.1. The mean ages of the groups were similar, while their indices of obesity were significantly different. For subsequent analyses the overweight and obese children were grouped together.

Table 1.1. Anthropometric data of the normal weight and overweight or obese children

	Normal weight n=637 (253 males)	**Overweight n= 205 (87 males)**	**Obese n= 504 (287 males)**	**Overweight/obese n=709 (374 males)**
Age (y)	13.4 ± 2.3	13.6 ± 2.3	13.1 ± 2.6	13.2 ± 2.6
Weight (kg)	48.6 ± 11.2	64.8 ± 12.6**	81.3 ± 21.9***	76.5 ± 21.0*
Height (cm)	159.7 ± 11.8	160.3 ± 11.3	158.7 ± 13.8	159.1 ± 13.2
BMI (kg/m^2)	18.8 ± 2.4	25.0 ± 2.0**	31.7 ± 4.7***	29.7 ± 5.1*
RBW (%)	100.3 ± 10.6	133.8 ± 10.9**	171.8 ± 21.9***	160.8 ± 25.9*
RBMI (%)	96.5 ± 10.0	127.8 ± 8.0**	165.1 ± 19.8***	154.3 ± 24.2*
WHR	0.77 ± 0.05	0.82 ± 0.07**	0.87 ± 0.06***	0.85 ± 0.07*

Data are mean ± SD.

* $p<0.001$ vs. normal weight (unpaired Students' t-test).

** $p<0.001$ vs. normal weight (ANOVA, Bonferroni post hoc test).

*** $p<0.001$ vs. overweight (ANOVA, Bonferroni post hoc test).

BMI – body mass index; RBW – relative body weight; RBMI – relative BMI; WHR – waist to hip ratio.

The genotype distributions and minor allele frequencies for the examined gene polymorphisms among the overweight or obese and control children are presented in Table 1.2. The allelic distributions were in Hardy Weinberg equilibrium for all of the variants investigated.

Table 1.2. Genotype distributions and minor allele frequencies of the examined gene polymorphisms in overweight or obese (ov/obese) and control children.

ADRB3 Trp64Arg (n=1337)	**Control (n=634)**	**Ov/obese (n=703)**	**p***
Trp64Trp	547 (86.3 %)	598 (85.1%)	
Trp64Arg	85 (13.4 %)	102 (14.5%)	0.80
Arg64Arg	2 (0.3 %)	3 (0.4%)	
Arg allele frequency	*0.07*	*0.08*	*0.75*
UCP-1 –3826 A/G (n=1346)	**Control (n=637)**	**Ov/obese (n=709)**	**p***
A/A	347 (54.5 %)	365 (51.5 %)	
A/G	245 (38.5 %)	284 (40.1 %)	0.44
G/G	45 (7.1 %)	60 (8.5 %)	
G allele frequency	*0.26*	*0.28*	*0.20*
UCP-2 –866 G/A (n=1346)	**Control (n=637)**	**Ov/obese (n=709)**	**p***
G/G	234 (36.7 %)	302 (42.6 %)	
G/A	303 (47.6 %)	314 (44.3 %)	0.07
A/A	100 (15.7 %)	93 (13.1 %)	
A allele frequency	*0.39*	*0.35*	***0.024***
UCP-2 e8 del/ins (n=1346)	**Control (n=637)**	**Ov/obese (n=709)**	**p***
del/del	340 (53.4 %)	330 (46.5 %)	
del/ins	248 (38.9 %)	313 (44.1 %)	**0.041**
ins/ins	49 (7.7 %)	66 (9.3 %)	
ins allele frequency	*0.27*	*0.31*	***0.016***
UCP-3 –55 C/T (n=1245)	**Control (n=567)**	**Ov/obese (n=678)**	**p***
C/C	316 (55.7 %)	410 (60.5 %)	
C/T	213 (37.6 %)	229 (33.8 %)	0.24
T/T	38 (6.7 %)	39 (5.8 %)	
T allele frequency	*0.25*	*0.23*	*0.10*
PPARG2 Pro12Ala (n= 1313)	**Control (n=637)**	**Ov/obese (n=676)**	**p***
Pro/Pro	484 (76.0 %)	490 (72.5 %)	
Pro/Ala	144 (22.6 %)	177 (26.2 %)	0.32
Ala/Ala	9 (1.4 %)	9 (1.3 %)	
Ala allele frequency	*0.13*	*0.14*	*0.20*

Data are n (%).

* p value in chi-square test.

ADRB3 – β_3-adrenoreceptor; UCP – uncoupling protein; e8 – exon 8; PPARG2 – peroxisome proliferator-activated receptor-γ2.

The genotype distributions and allele frequencies for the Trp64Arg polymorphism of ADRB3, the –3826 A/G polymorphism of UCP-1, the –55 C/T polymorphism of UCP-3 and the Pro12Ala polymorphism of PPARG2 did not significantly differ between the study groups. There were significant differences, however between the cases and controls with regard to the genotype distributions and minor allele frequencies of the examined UCP-2 polymorphisms. 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 G/A and A/A genotypes were more prevalent among controls compared with the overweight/obese group, although chi-square analysis of the genotype distribution for this polymorphism did not show statistical significance. The variant 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), and the genotype distribution for this polymorphism also showed a significant difference with the del/ins and ins/ins genotypes more prevalent among the overweight/obese compared with normal weight controls.

Obesity risk associated with the examined gene polymorphisms was estimated with univariate and multivariate logistic regression analyses, adjusted for age, height and gender (Table 1.3). Univariate analyses showed that the UCP-2 –866 G/A polymorphism and the UCP-2 exon 8 del/ins polymorphism slightly, but significantly influenced the risk of overweight/obesity. The presence of the UCP-2 –866 A allele was associated with an odds ratio (OR) for obesity of 0.84 (95% confidence interval [CI]: 0.71–0.97; p=0.024), with homozygotes for the A allele having a reduced risk of overweight/obesity (OR: 0.72, 95% CI: 0.52–1.00; p=0.049). The OR for obesity related to the presence of the UCP-2 exon 8 ins allele was 1.23 (95% CI: 1.04–1.45; p=0.016), and heterozygotes for the ins allele had a higher risk for overweight/obesity (OR: 1.30, 1.04–1.63; p=0.022).

Table 1.3. Odds ratios for the isolated (univariate, i. e., each gene polymorphism considered separately) and combined (multivariate, including all gene polymorphisms) effects of allelic variants and genotypes of the examined gene polymorphisms on obesity risk

Gene polymorphism		Univariate			Multivariate		
		OR	**95% CI**	**p***	**OR**	**95% CI**	**p***
ADRB3	Trp/Trp	1			1		
Trp64Arg	Trp/Arg + Arg/Arg**	1.10	0.81–1.50	0.53	1.20	0.86–1.69	0.29
	Arg allele	*1.03*	*0.88–1.20*	*0.75*	*1.00*	*0.82–1.23*	*0.99*
	AA	1			1		
UCP-1	AG	1.10	0.88–1.38	0.40	1.09	0.86–1.39	0.47
–3826 A/G	GG	1.27	0.84–1.92	0.26	1.31	0.82–2.09	0.26
	G allele	*1.12*	*0.94–1.32*	*0.20*	*1.10*	*0.90–1.33*	*0.38*
	GG	1			1		
UCP-2	GA	0.80	0.64–1.01	0.06	0.69	0.52–0.92	**0.013**
–866 G/A	AA	0.72	0.52–1.00	**0.049**	0.50	0.32–0.79	**0.003**
	A allele	*0.84*	*0.71–0.97*	***0.024***	*0.69*	*0.55–0.86*	***0.001***
UCP-2	del/del	1			1		
exon 8	del/ins	1.30	1.04–1.63	**0.022**	1.66	1.24–2.23	**0.001**
del/ins	ins/ins	1.39	0.93–2.07	0.08	2.12	1.23–3.63	**0.006**
	ins allele	*1.23*	*1.04–1.45*	***0.016***	*1.51*	*1.20–1.91*	***0.001***
	CC	1			1		
UCP-3	CT	0.83	0.65–1.05	0.12	1.08	0.82–1.41	0.59
–55 C/T	TT	0.79	0.49–1.27	0.33	1.02	0.61–1.70	0.94
	T allele	*0.86*	*0.71–1.03*	*0.09*	*0.94*	*0.76–1.17*	*0.59*
	Pro/Pro	1			1		
PPARG2	Pro/Ala	1.21	0.94–1.56	0.13	1.17	0.89–1.53	0.27
Pro12Ala	Ala/Ala	0.99	0.39–2.51	0.98	0.97	0.33–2.81	0.95
	Ala allele	*1.16*	*0.93–1.45*	*0.20*	*1.13*	*0.88–1.46*	*0.35*

* Wald-$\chi 2$ statistics. P-values were adjusted on age, height, and gender.

** Grouped together because of the small number of Arg/Arg homozygotes.

OR – odds ratio; CI – confidence interval. Other abbreviations as in table 1.2.

Considering all gene polymorphisms as independent factors in one model (Table 1.3 multivariate) did not substantially modify results as compared to the univariate estimates; however, for both UCP-2 polymorphisms the ORs for overweight/obesity corresponding to the minor alleles and heterozygote and homozygote genotypes were more pronounced and the p-values decreased. Thus, 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. The effects of these polymorphisms were altogether low, since the multivariate logistic regression model adjusted for age, height and gender and containing all examined polymorphisms accounted for only about 3.7% percent of variance. The search for possible effects of interaction between the examined genetic polymorphisms on risk of obesity was carried out with the multivariate logistic regression model for allele frequencies including all possible interaction terms. There were no evidence of a significant interaction between the different allelic variants, other than that between the two UCP-2 gene polymorphisms known to be in linkage disequilibrium (correlation coefficient r^2=0.52; p<0.001). The anthropometric characteristics of the children according to genotypes of the gene polymorphisms found to significantly influence obesity risk are presented in Table 1.4. 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 relative body weight and relative BMI compared to G/G homozygotes. 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 relative body weight and relative BMI as compared to del/del homozygotes. There were no significant differences in indices of obesity between the groups of children with different genotypes according to the examined ADRB3, UCP-1, UCP-3 and PPARG2 polymorphisms (data not shown).

Table 1.4. Anthropometric characteristics of the children according to the examined UCP-2 genotypes

UCP-2 –866 G/A (n=1346)						
	G/G (n=536) (256 males)	**G/A (n=617)** (396 males)	**A/A (n=193)** (75 males)	**p***	**A carrier** (n=810)	**p*****
Age (y)	13.3±2.4	13.3±2.4	13.4±2.5	0.87	13.3±2.5	0.94
Weight (kg)	64.7±22.3	62.7±22.0	61.2±21.2	0.12	62.4±21.8	0.06
Height (cm)	159.5±12.6	159.5±12.7	159.0±12.0	0.90	159.3±12.5	0.87
BMI (kg/m^2)	25.1±6.9	24.3±6.8	23.8±6.8	**0.045**	24.2±6.76	**0.017**
RBW (%)	135.2±36.9	130.9±35.9	128.6±35.8**	**0.048**	130.3±35.9	**0.021**
RBMI (%)	129.8±34.6	125.8±34.6	122.9±33.4**	**0.028**	125.1±34.3	**0.015**
WHR	0.81±0.07	0.82±0.08	0.81±0.07	0.13	0.82±0,08	0.68
UCP-2 exon 8 del/ins (n=1346)						
	del/del (n= 670) (332 males)	**del/ins (n=561)** (255 males)	**ins/ins (n=115)** (40 males)	**p***	**ins carrier** (n=676)	**p*****
Age (y)	13.3±2.4	13.3±2.5	13.4±2.5	0.95	13.3±2.5	0.98
Weight (kg)	62.7±22.3	63.6±21.5	65.0±23.5	0.52	63.9±21.8	0.33
Height (cm)	159.7±12.8	159.4±12.5	158.0±11.4	0.39	159.1±12.4	0.41
BMI (kg/m^2)	24.2±6.8	24.7±6.7	25.7±7.6	0.07	24.9±6.8	0.07
RBW (%)	130.2±36.0	133.2±35.6	139.1±40.8**	**0.030**	134.2±36.5	**0.038**
RBMI (%)	124.7±34.4	128.4±33.8	132.9±37.8**	**0.048**	129.2±34.5	**0.037**
WHR	0.82±0.07	0.82±0.08	0.81±0.07	0.51	0.82±0.08	0.72

* One-way ANOVA.

** $p<0.05$ vs. G/G or del/del; Bonferroni post hoc test.

*** Carrier vs. non-carrier; Students' t-test.

Abbreviations as in table 1.1. and 1.2.

Discussion

In a large population of Hungarian school-aged children, we found an association between higher risk of overweight or 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.

Although the physiological role of UCP-2 is still a matter of debate, numerous studies support its effect on mitochondrial energy metabolism and obesity (8,38,39,147,148). Since UCP-2 is an uncoupler of mitochondrial oxidative phosphorylation, its decreased expression or function might reduce energy expenditure and thereby increase the propensity to store energy as fat. There is evidence, that UCP-2 expression levels correlate with BMI in humans, with reduced expression in adipose tissue and skeletal muscle linked to obesity (149,150), and UCP-2 mRNA levels in adipose tissue were positively related to resting metabolic rate (43). The –866 G/A and the 45 basepair del/ins polymorphisms of UCP-2 have been reported to be functional variants. Esterbauer et al. (45) found the –866 A variant to be related with enhanced transcriptional activity both in vivo and in reporter gene constructs expressed in a human adipocyte cell line, and the exon 8 45 basepair ins variant to be associated with reduced mRNA stability. Thus, decreased UCP-2 message linked to the –866 G and the exon 8 ins variant could result in decreased energy expenditure and weight gain. Further data supporting this notion are contradictory. Le Fur et al. (89) reported that obese children with the −866 G/A and A/A genotype had a modest but significant increase of resting energy expenditure compared with the G/G genotype. Metabolic rate was also enhanced in Pima Indians carrying the –866 A allele, but also in those with the ins allele for the exon 8 del/ins polymorphism (106). Esterbauer et al. reported (45) that the –866 A allele was associated with a lower risk of obesity in middle-aged Caucasian subjects, but haplotype analysis of the two UCP-2 polymorphisms also showed that haplotypes including the -866 A and exon 8 ins alleles are more frequent in lean subjects. Other studies found no association between the UCP-2 –866 G/A polymorphism and risk of adulthood obesity (88,111). In a study of 193 obese and 170 control Spanish school-aged children, the individual polymorphisms of UCP-2 did not show linkage to obesity, but obesity was associated with a haplotype that included the –866 G and exon 8 del alleles (90). Schauble et al. could not demonstrate a significant influence of the –866 G/A polymorphism on the incidence of childhood-onset obesity among 277 extremely obese and 188 control children

and adolescents (107), although based on the slightly greater frequency of the G allele among the obese they concluded, that larger sample sizes could provide convincing evidence for a small effect of this polymorphism on body weight regulation in children. Among more than 700 Korean children, the A allele was associated with significantly decreased risk of overweight (46). Recently, a meta-analysis that included 12 studies with a total of 7390 cases and 9860 controls demonstrated a significant association between the UCP-2 –866 G allele and an increased risk of obesity among subjects of European descent (151) and another meta-analysis of data from 12 984 subjects also found the G allele to be related to obesity (152). A greater risk of developing obesity among individuals carrying the exon 8 45 basepair ins allele was found in Spanish and German adults (47,49), and an association of this polymorphism with BMI was observed in a variety of ethnic groups (148). Other studies, however, could not establish a relationship between the UCP-2 del/ins polymorphism and risk of adulthood obesity (108,109,153). In a smaller sample of children, the UCP-2 exon 8 ins variant was associated with childhood obesity and body composition (48). The two UCP-2 polymorphisms are in linkage disequilibrium (45,90) and can influence each other's effects, thus in some of the above mentioned studies the promoter polymorphism could have camouflaged the effect of the exon 8 del/ins variation. In our study, both univariate analysis examining the isolated and multivariate analysis examining the combined effects of the genetic variants identified the –866 G and the exon 8 ins variants to be risk alleles for obesity. Multivariate analysis suggested an interaction between the polymorphisms that did not attenuate, but enhanced their effects. Discrepancies between our results and the previous studies could be related to different haplotype distributions in the studied populations. We remain cautious in trying to interpret our results in the light of the physiology of UCP-2, since the precise role of this mitochondrial carrier protein remains to be defined.

The effect sizes (odds ratios) found for risk of obesity for the UCP-2 gene polymorphisms were moderate. In the multivariate model (adjusted for age, height and gender) identifying the UCP-2 –866 A variant as non-risk allele and the exon 8 ins variant as risk allele for obesity, the model accounted for only about 3.7% of the variance in normal weight or overweight/obese status. This observation is in accordance with the polygenic model of common obesity, and the small effect sizes expected for each one of the numerous susceptibility genes (6). Possession of the –866 A allele was associated with a 4.7% decrease in relative BMI compared with G/G wild type homozygotes, and possession of the exon 8 ins allele was related to a 4.5% increase in relative BMI compared with the del/del wild type homozygotes.

The ADRB3 Trp64Arg and UCP-1 –3826 A/G polymorphisms alone or in conjunction have been numerously associated with obesity, diabetes mellitus and related diseases although with contradictory results (8,16). Our analyses revealed no single or combined effects of these

polymorphisms on obesity risk. Although the Trp64Arg polymorphism has been shown to be associated with higher risk of obesity in Japanese children (14), a recent meta-analysis suggests, that its role in the development of common obesity might be confined to Asian populations (154). Likewise, UCP-1 was recently implicated in the regulation of body weight in healthy Japanese children (82), but data on large Caucasian populations could not prove a major role for the UCP-1 – 3826 A/G polymorphism in the development of obesity, nor its additive effect with the ADRB3 Trp64Arg genetic variation (104,105). The –55 T UCP-3 variant was associated with increased skeletal muscle expression of UCP-3 (50), and in large Caucasian populations the polymorphism was negatively correlated with BMI (51,155), and with lower risk of overweight among children (46). Controversial results suggesting an inverse relation of this polymorphism to BMI however, have also been published (90,156). The Ala12 allele of the PPARG2 was originally associated with reduced receptor activity and lower BMI (54), however in a large meta-analysis, obese Caucasian carriers of the Ala12 allele showed increased BMI (58). Among Spanish children the Pro12Ala polymorphism was associated with higher risk of obesity (60), however among German children and adolescents, the frequency of the polymorphism did not differ between lean and obese (157). We do not conclude that the above gene polymorphisms previously strongly indicated as factors in the genetic background of common obesity have no effect in Hungarian children. Possible explanations for the lack of associations to risk of overweight/obesity in our study population with regard to the examined polymorphisms of ADRB3, UCP-1, UCP-3 and PPARG2 may be related to ethnicity, interactions with other genes, uncontrolled environmental factors such as diet and physical activity, and also to the lack of sufficient statistical power. Since genetic variants with low minor allele frequencies especially require great sample sizes to identify association between the variant and obesity (6), our study may have been underpowered with regard to the ADRB3 and PPARG2 polymorphisms.

Observations and practical consequences

Our data provide evidence that common polymorphisms of the UCP-2 gene are genetic risk factors for overweight/obesity among Hungarian school-aged children.

Numerous studies have investigated the effects of obesity candidate genes associated with energy expenditure on the risk of developing overweight/obesity in adults, but only few studies have been conducted on large numbers of children. To the best of our knowledge, the present results provide the largest data on obesity candidate gene analysis among Hungarian children. Besides providing basic, nation-specific data on the investigated polymorphisms for the Hungarian pediatric population, we also have presented data valuable for future large-scale data integration (meta-

analysis), which is a key factor contributing to the success of candidate obesity gene studies to identify gene-disease associations with conclusive evidence.

Knowledge of the molecular background for childhood obesity is important from scientific, prognostic and prophylactic points of view. With the increasing prevalence of obesity, early detection of individuals at risk for becoming obese would be valuable in preventing childhood as well as adult obesity and obesity-related disorders. In the future our knowledge of the genetic factors that influence the development of obesity will also guide therapeutic decisions.

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

The anthropometric characteristics and metabolic parameters of this study population are reported in Table 2.1. The minor allele frequencies and genotype distributions for the polymorphisms studied are described in Table 2.2. The allelic distributions were in Hardy-Weinberg equilibrium for all of the variants investigated.

Carriers of the ADRB3 Arg64 allele (n=81) had a significantly higher relative body weight and relative BMI than the wild type homozygotes (Table 2.3.). 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).

Table 2.1. Anthropometric characteristics and metabolic parameters of the children included in the study

Anthropometric and metabolic parameters*	**n=528 (297 boys)**
Age (y)	13.2 ± 2.6
Weight (kg)	78.7 ± 20.8
Height (cm)	159.1 ± 13.8
BMI (kg/m^2)	30.6 ± 4.6
Relative body weight (%)	167.9 ± 28.9
Relative BMI (%)	158.8 ± 21.2
Waist-to-hip ratio	0.87 ± 0.06
Fasting glucose (mmol/L)	4.8 ± 0.7
OGTT 2-hour glucose (mmol/L)	6.7 ± 1.4
Fasting insulin (µU/mL)	29.7 ± 23.6
OGTT 2-hour insulin (µU/mL)	163.2 ± 107.0
Impaired glucose regulation	116 (22.0)
Diabetes mellitus	30 (5.7)
Hyperinsulinemia	339 (64.2)
HOMA	6.4 ± 5.3
WBISI	38.5 ± 23.5
Total cholesterol (mmol/L)	4.4 ± 0.9
Triglycerides (mmol/L)	1.4 ± 0.7
HDL-cholesterol (mmol/L)	1.2 ± 0.3
Dyslipidemia	199 (37.8)
Hypercholesterolemia	89 (16.9)
Systolic/diastolic blood pressure (mmHg)	125.3 ±11.7/72.2 ± 8.9
Basic metabolic rate (kcal)†	1993.6 ± 424.3
Respiratory quotient†	0.81 ± 0.07

Data are n (percentile) or mean ± SD.

*Definitions used for the obesity-related metabolic conditions are described under methods.

† adjusted for age, gender, and fat free body mass.

BMI – body mass index; OGTT – oral glucose tolerance test; HOMA – homeostasis model assessment index; WBISI: whole body insulin sensitivity index.

Table 2.2. Minor allele frequencies and genotype distributions for the examined polymorphisms

Polymorphism	Minor allele frequency	Wild type homozygote	Heterozygote	Variant homozygote
ADRB3 Trp64Arg (n= 522)	0.08	441 (84.5%)	79 (15.1%)	2 (0.4%)
UCP-1 –3826 A/G (n=528)	0.29	270 (51.1%)	214 (40.5%)	44 (8.3%)
UCP-2 –866 G/A (n=528)	0.34	230 (43.6%)	234 (44.3%)	64 (12.1%)
UCP-2 e8 del/ins (n=528)	0.32	244 (46.2%)	233 (44.1%)	51 (9.7%)
UCP-3 –55 C/T (n=515)	0.23	306 (59.4%)	178 (34.6%)	31 (6.0%)
PPARG2 Pro12Ala (n=508)	0.14	368 (74.2%)	122 (24.6%)	6 (1.2%)

ADRB3 – β_3-adrenoreceptor; UCP – uncoupling protein; e8 del/ins – exon 8 deletion/insertion; PPARG2 – peroxisome proliferator-activated receptor-γ2.

Table 2.3. Anthropometric parameters according to the presence of the ADRB3 Trp64Arg polymorphism

Parameters	Trp64Trp (n=441)	Trp64Arg/Arg64Arg* (n=81)	p
Weight (kg)	78.1 ± 20.5	82.2 ± 22.2	ns
Height (cm)	159.0 ± 13.6	160.4 ± 14.6	ns
BMI (kg/m^2)	30.2 ± 4.5	31.4 ± 4.7	ns
Relative body weight (%)	166.4 ± 29.1	175.4 ± 27.1	0.01
Relative BMI (%)	157.5 ± 21.0	164.0 ± 21.1	0.01
Waist-to-hip ratio	0.86 ± 0.06	0.87 ± 0.06	ns

Data are mean ± SD.

*Grouped together because of the small number of Arg/Arg homozygotes

BMI – body mass index; ns – not significant.

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, dyslipidemia and a lower adjusted BMR and higher RQ (Table 2.4.). Adjusting the insulin levels and related indices for fat free body mass did not significantly change the results.

Table 2.4. 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**
Weight (kg)	77.5±21.3	79.5±19.9	81.1±22.3	ns	79.8±20.3	ns
Height (cm)	159.4±14.2	159.2±13.7	157.17±12.8	ns	158.9±13.5	ns
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 glu (mmol/L)†	4.8±0.7	4.7±0.7	4.8±0.9	ns	4.8±0.8	ns
2h glu (mmol/L)†	6.6±1.4	6.7±1.5	6.6±1.4	ns	6.7±1.4	ns
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
IGR	51 (20.9)	53 (22.7)	12 (23.5)	ns	65 (22.9)	ns
DM	18 (7.4)	10 (4.3)	2 (3.9)	ns	12 (4.2)	ns
Hyperinsulinemia	133 (54.5)[1]	162 (69.5)[2]	44 (86.3)[3]	<0.001	206 (72.5)	<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
Chol (mmol/L)†	4.4±0.8	4.5±0.9	4.5±1.0	ns	4.5±0.9	ns
TG (mmol/L)†	1.4±0.6	1.5±0.7	1.6±0.8	0.05	1.5±0.7	0.04
HDL-chol (mmol/L)†	1.2±0.3	1.2±0.3	1.1±0.3	ns	1.2±0.3	ns
Dyslipidemia	76 (31.3)[1]	96 (41.2)	27 (59.2)[3]	0.005	123 (43.3)	0.005
Hyperchol	34 (14.0)	42 (18.0)	13 (25.5)	ns	55 (19.4)	ns
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 or number (percentage).

§ Definitions used for the obesity-related metabolic conditions are described under methods.

† adjusted for age and gender; ‡ adjusted for age, gender and fat free mass.

* for comparison of the three genotype groups; ANOVA for continuous variables and chi-squared test for categorical variables; across all genotypes.

** del/del vs. ins allele carriers; unpaired Students' t-test for continuous variables and chi-square test for categorical variables.

[1] $p < 0.05$ for del/del vs. del/ins (Bonferroni post hoc test or chi-squared test)

[2] p< 0.05 for del/ins vs. ins/ins (Bonferroni post hoc test or chi-squared test)

[3] p< 0.05 for del/del vs. ins/ins (Bonferroni post hoc test or chi-squared test)

RBW – relative body weight; RBMI – relative BMI; F – fasting; 2h – 2-hour (OGTT); glu – glucose; ins – insulin; IGR – impaired glucose regulation; DM – diabetes mellitus; chol – cholesterol; TG – triglycerides; hyperchol – hypercholesterolemia; BMR – basic metabolic rate; RQ – respiratory quotient; ns – not significant. Other abbreviations as in Table 2.1.

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 relative body weight (168.7±33.8) and relative BMI (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.

Discussion

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.

The Trp64Arg ADRB3 variant was one of the first polymorphisms for which association with obesity was reported, but although since the first reports in 1995 more than 100 studies have been published on the relation between this polymorphism and obesity-related traits, results are still inconsistent, and there are relatively few data on children. In pediatric studies, the Arg64 allele was associated with obesity, adiposity, features of the metabolic syndrome and effectiveness of dietary

intervention is Asian populations (14,73–75), but in studies of Caucasian children no associations with obesity-related traits were found (60,103,158). Recently, a large meta-analysis that combined data of 44 833 adults suggested, that the polymorphisms' effect on the risk of obesity may be confined to the East Asian populations (154). The minor allele frequency of the Trp64Arg ADRB3 polymorphism is highly heterogeneous among different populations and has been found to be about twice as high in Asian (0.18) than in Caucasian populations (0.075) (154), making it more difficult to demonstrate its effect in studies of Caucasian subjects. The minor allele frequency of the polymorphism in our study group was similar to that found among other European children (158), and lower than that reported among Asian children (14), and our study group was considerably larger than those of previous studies of Caucasian children (60,103). Our results are in concert with previous observations among young healthy Danes suggesting that the Trp64Arg ADRB3 mutation is associated with greater weight gain during childhood and adolescence (71). In our previous study including 295 obese children we also reported the Trp64Arg ADRB3 polymorphism to be associated with increased adiposity (159), however in the above analysis of a larger population of school-aged children (n=1337) the Arg64 allelic frequency was similar between the normal weight and obese subjects. These observations together suggest that the effect of this gene variant may be confined to the severity and not the risk of obesity. The underlying mechanism may involve changes induced by obesity (obesity-conditioned effect), for example down-regulation of the ADRB3 gene in enlarged adipose cells (160), which together with an already impaired receptor function could promote further obesity, establishing a vicious cycle.

The two homozygote carriers of the Trp64Arg polymorphism in our study population had a considerably lower BMR than the rest of the population. Other investigators have reported that influences of the Trp64Arg polymorphism on BMR and metabolic complications of obesity were only apparent in homozygote carriers of the Arg64 allele (13,71,161) as well, but because of the small number of homozygous subjects identified this observation must be interpreted with caution and needs further investigation.

We could not demonstrate any significant influence of the Trp64Arg mutation on blood pressure or metabolic parameters in our group of obese children. Results concerning an independent effect of this polymorphism on the development of the features of the metabolic syndrome are contradictory in adults and our results are in concert with previous studies of European children (103). Connection of the Trp64Arg mutation with features of the metabolic syndrome was demonstrated in Asian study participants (73,74), perhaps because of the higher frequency of the polymorphism in Asian populations, which may have caused our study to be underpowered in this respect.

The regulation and function of UCP-2 seems to be tissue-specific, and among others, UCP-2 has been proposed to play a role in cellular energy balance, obesity, BMR, fuel substrate partitioning,

insulin secretion and action, and lipid metabolism and storage (33,35,36,41,162). The UCP-2 exon 8 45 basepair ins variant was previously found to be associated with reduced mRNA stability (45) resulting in decreased UCP-2 message which could result in decreased energy expenditure and weight gain. In accordance with this hypothesis of an obesogenic effect for the UCP-2 exon 8 del/ins polymorphism, carriers of the ins allele had a lower adjusted BMR and higher degree of overweight in our group of obese children. BMR accounts for 60–80% of daily energy expenditure, has genetic determinants and reduced BMR is a known risk factor for weight gain (63). In the above analysis of 1346 normal weight and obese school-aged children the exon 8 ins allele was also associated with higher risk of obesity. In agreement with our findings an association of this polymorphism with BMI was observed in a variety of ethnic groups (148). In a smaller sample of children, the ins variant was associated with childhood obesity and body composition (48), however in that study no effect on BMR was found. In another pediatric study lack of an association between the ins allele and childhood obesity or BMR was demonstrated (163); however that study included a considerably smaller number of children than ours. In addition to an obesogenic effect, our data also show an association between the UCP-2 ins allele and increased insulin levels, decreased insulin sensitivity, higher triglyceride levels and a higher prevalence of dyslipidemia. These metabolic derangements are common consequences of obesity, however adjusting the insulin levels for fat free body mass did not change our results, suggesting a direct influence of the UCP-2 polymorphism on these metabolic parameters. Pathogenesis of overweight-associated metabolic conditions involves a variety of genetic determinants, and UCP-2 with a still debated biological function has been implicated in the processes presumed to underlie the development of the features of the metabolic syndrome (33,162). The higher insulin levels in carriers of the ins allele is also in accordance with previous studies demonstrating, that UCP-2 expressed in islet beta-cells influences glucose-stimulated insulin secretion in a way, that inhibition of UCP-2 promotes insulin secretion (36). The relationship between functional polymorphisms of the UCP-2 gene associated with decreased UCP-2 activity and insulin levels is however, also contradictory, and in a previous study the ins allele was associated with a lower risk of high fasting insulin levels in a group of obese children (90). The mildly increased triglyceride levels and higher frequency of dyslipidemia among children carrying the ins allele might be the consequence of the higher degree of obesity associated with the ins variant, or support a previously suggested role for the UCP-2 gene in the regulation of fat metabolism directly or indirectly via effects on insulin secretion (162). The higher ratio of carbohydrate to fat oxidation as evidenced by a higher RQ in carriers of the UCP-2 ins allele is also in accordance with the previously suggested role of UCP-2 as a regulator of intracellular fuel partitioning, with lower UCP-2 activity adversely affecting the availability of fatty acids for

oxidation (162). Increased RQ and low fat oxidation are also known to be risk factors for weight gain and development of obesity (62).

UCP-3 is predominantly expressed in human skeletal muscle where its expression was negatively correlated with BMI and positively with metabolic rate during sleep (42). In our group of obese children the –55 T/T genotype was associated with significantly lower adjusted BMR, than either the C/T or C/C genotypes. If UCP-3 does increase metabolic rate in humans, then this observation is contradictory to the initial report of the T allele being related with increased skeletal muscle expression of UCP-3 among 18 male, non-diabetic Pima Indians (50), and also with the studies associating the T variant with lower BMI among Caucasian adults (51,155), and with lower risk of overweight in Korean children (46). Studies demonstrating an inverse relation of the –55 C/T polymorphism to risk of obesity and related traits and also those reporting lack of association, however, have also been published (90,93,110,151,156), leaving the pathophysiological role of this UCP-3 variant uncertain. We cannot support the effect of the –55 C/T polymorphism on BMR we detected with consistent genotype-associated differences in degree of obesity. Although children with the T/T genotype had a higher relative body weight and BMI than those with the C/T genotype this difference did not reach statistical significance and measures of obesity were similar between the C/C and T/T genotypes. This, however, does not rule out a possible effect of the UCP-3 –55 C/T polymorphism on BMR, since intermediary phenotypes such as BMR or RQ are less likely than BMI to be influenced by extrinsic factors unrelated to obesity and may therefore provide more statistical power. Possible explanations for the great variability of results regarding the existence or direction of the impact of the UCP-3 –55 C/T polymorphism on human obesity and metabolic rate includes different effects of the polymorphism in different populations due to genetic or environmental interactions, and also the possibility that it is not a functional variant and the contrasting effects detected at the –55 C/T polymorphism reflect varying degrees of linkage disequilibrium with functional variants in the nearby genomic regions. UCP-2 and UCP-3 are neighboring genes on chromosome 11q13 and this region was previously linked to resting metabolic rate in humans (41). The UCP-2 exon 8 del/ins and UCP-3 –55 C/T, and also the UCP-2 –866 G/A polymorphisms are in considerable linkage disequilibrium and have all been implicated in human BMR. We, however, did not find significant correlations between the UCP-2 and UCP-3 genotypes that we analyzed (data not shown), and most importantly the UCP-2 ins allele was not correlated with the UCP-3 –55 T allele which could have explained their similar effects on BMR. Thus we think that the influences of the UCP-2 del/ins and UCP-3 –55 C/T polymorphisms we observed are independent of each other and of the UCP-2 –866 G/A polymorphism, but we cannot exclude other possible causative variants in the UCP-2–UCP-3 cluster nearby.

No associations of the UCP-1 –3826 A/G, UCP-2 –866 G/A, or PPARG2 Pro12Ala polymorphisms with obesity-related traits were evident from the present data. Possible explanations for the lack of associations may be related to ethnicity, interactions with other genes, uncontrolled environmental factors such as diet and physical activity, and also to the lack of sufficient statistical power.

Observations and practical consequences

These data provide support 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 or the metabolic complications of pediatric obesity. Further studies in pediatric populations are needed to clarify the role of the UCP-3 –55 C/T polymorphism in childhood obesity.

The current epidemic of obesity represents a major public health concern given the strong association of adiposity with cardiovascular, metabolic, and other comorbidities. Understanding the genetic parameters that contribute to the development and progression of obesity and its complications may lead to earlier identification of subjects at risk of being affected by the harmful consequences of obesity, offering the opportunity to prevent or delay the onset of disease through appropriate intervention.

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

Characteristics of the children participating in this study are presented in Table 3.1. Of the features of the metabolic syndrome, IGR was detected in 25.0% (n=63), hyperinsulinemia in 74.6% (n=188), dyslipidemia in 33.7% (n=85), and hypercholesterolemia in 19.4% (n=49) of the overweight/obese children. 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. ALT levels exceeded AST levels in most cases.

Table 3.1 Clinical and biochemical characteristics of the study population and of the UCP-2 exon 8 del/ins polymorphism genotype groups

Variable§	**Total population n=252**	***UCP-2 e8 del/del* n=126 (50%)**	***UCP-2 e8 del/ins* n=103 (40.9%)**	***UCP-2 e8 ins/ins* n=23 (9.1%)**	**p ***
Age (y)	12.8±2.7	12.7±2.7	12.9±2.6	12.7±2.7	ns
Weight (kg)	80.2±21.6	80.1±22.4	79.8±20.3	82.2±21.6	ns
Height (cm)	158.9±12.9	158.1±12.3	159.8±13.0	160.0±16.0	ns
BMI (kg/m^2)	31.2±4.9	31.5±5.2	30.7±4.4	31.7±4.8	ns
RBW (%)	168.7±22.1	171.1±24.2	165.0±18.4	173.4±24.8	ns
WHR	0.87±0.06	0.88±0.06	0.87±0.05	0.85±0.07	ns
F glu (mmol/L)	5.0 [4.7–5.3]	5.0 [4.7–5.3]	5.1 [4.7–5.3]	5.0 [4.7–5.5]	ns
2h glu (mmol/L)	6.7 [5.9–7.4]	6.6 [5.8–7.4]	6.8 [5.9–7.4]	6.9 [6.0–7.2]	ns
F ins (µU/mL)	28 [20–40]	28 [20–38]	26 [20–39]	35 [27–51]**	0.05
2h ins (µU/mL)	132 [94–250]	124 [93–230]	134 [92–275]	251 [129–326]**	0.02
HOMA	6.1 [4.6–8.7]	6.1 [4.4–8.4]	5.9 [4.5–9.5]	7.6 [6.0–11.3]**	0.04
WBISI	27.9 [19.1–39.1]	28.7 [19.5–40.7]	28.6 [20.1–40.2]	20.6 [14.4–26.3]**	0.02
Chol (mmol/L)	4.4 [4.0–5.0]	4.4 [4.0–5.1]	4.5 [4.0–5.0]	4.0 [3.3–4.6]	ns
TG (mmol/L)	1.2 [0.9–1.7]	1.2 [0.9–1.7]	1.2 [0.8–1.7]	1.3 [1.0–1.5]	ns
HDL-chol (mmol/L)	1.1 [1.0–1.3]	1.2 [1.0–1.3]	1.1 [1.0–1.3]	1.1 [1.0–1.2]	ns
ALT (U/L)	34 [21–43]	37 [24–49]	28 [20–35]†	24 [17–34]†	0.003
AST (U/L)	32 [20–36]	35 [25–41]	27 [22–33]†	25 [20–29]†	0.011
Elevated ALT *or* AST	65 (25.8)	44 (34.9)	19 (18.4)†	2 (8.7)†	0.003
Elevated ALT	60 (23.8)	41 (32.5)	17 (16.5)†	2 (8.7)†	0.004
Elevated ALT *and* AST	18 (7.1%)	15 (11.9)	3 (2.9)‡	0 (0)	0.013

Data are mean ± SD, median [interquartile range] or number (percentage).

§ Definitions used for the obesity-related metabolic conditions are described under methods.

* for comparison of the three genotype groups; ANOVA or Kruskall-Wallis test for continuous variables, as appropriate; chi-squared test for categorical variables; across all genotypes.

** p<0.05 for *del/del* vs. *ins/ins* and *del/ins* vs. *ins/ins* (Mann-Whitney *U* test).

† p<0.05 for *del/del* vs. *del/ins* and *del/del* vs. *ins/ins* (Mann-Whitney *U* test or chi-square test/Fisher's exact test).

‡ p<0.05 for *del/del* vs. *del/ins* (chi-square test).

UCP-2 – uncoupling protein-2; e8 – exon; 8; del/ins – deletion/insertion; BMI – body mass index; RBW – relative body weight; WHR – waist to hip ratio; F – fasting; 2h – 2-hour (OGTT); glu – glucose; ins – insulin; HOMA – homeostasis model assessment; WBISI – whole body insulin sensitivity index; chol – cholesterol; TG – triglycerides; HDL – high-density lipoprotein; ALT – alanine aminotransferase; AST – aspartate aminotransferase; ns – not significant.

Of the study participants 148 (58.7%) were boys and the genders were well matched for age and BMI. Compared with the boys, girls had a significantly lower WHR (0.84±0.05 vs. 0.90±0.05; p<0.001), higher fasting insulin level (35 [27–46] vs. 27[19–36] µU/mL; p=0.03), higher 2-hour insulin level during OGTT (174 [110–282] vs. 120 [85–235] µU/mL; p=0.004), and tended to be more insulin resistant (HOMA: 7.0 [5.4–9.8] vs. 5.9 [4.2–8.4]; p=0.07; WBISI: 24.4 [18.0–34.1] vs. 33.1 [20.4–41.2]; p=0.05). 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 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 (Table 3.1., Figure 3.1.). 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). Children with the ins/ins genotype had significantly higher fasting and 2-hour insulin levels during OGTT, higher HOMA index and lower WBISI compared with the del/del and del/ins groups (Table 3.1.).

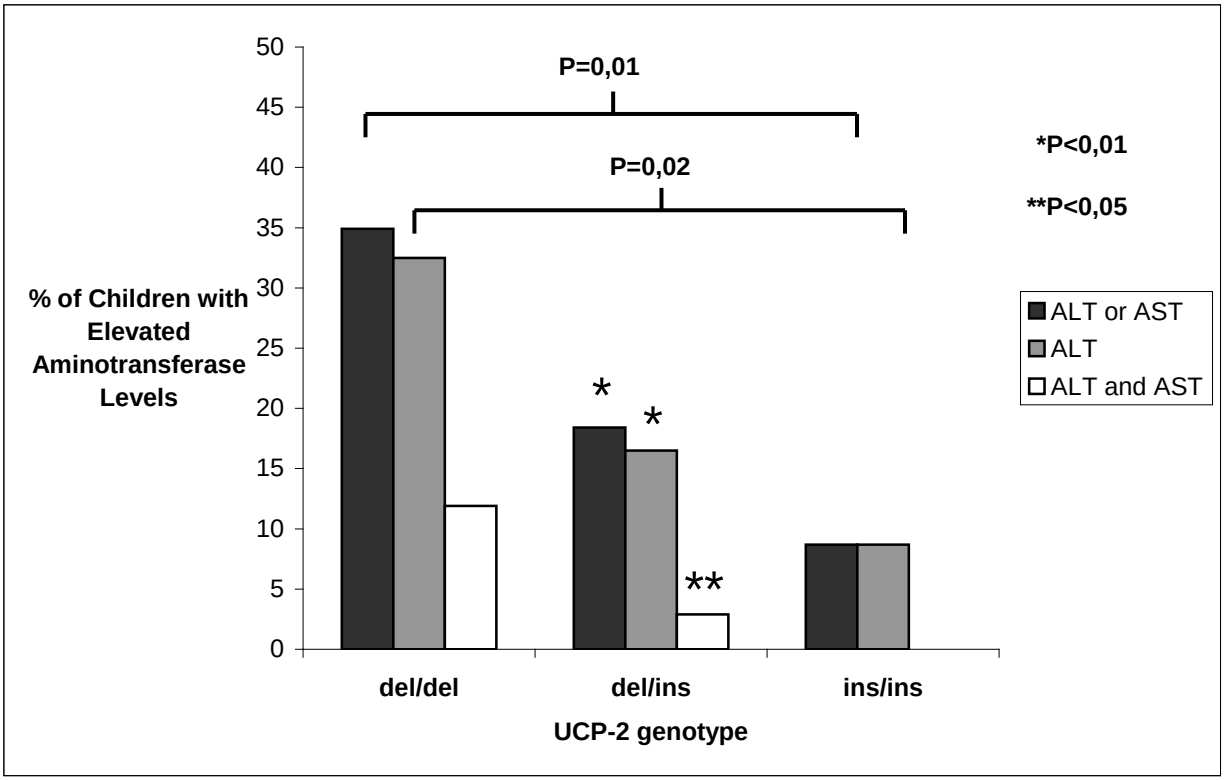

Figure 3.1 Percentage of children with elevated aminotransferase levels by genotype. Linear trends across genotypes for decreased prevalence of elevated liver function tests in heterozygote and homozygote carriers of the UCP-2 ins allele were significant with respect to all tested sets of hepatic enzymes: elevated ALT *or* AST: p=0.002; elevated ALT: p=0.003; elevated ALT *and* AST: p=0.012. Abbreviations as in Table 3.1.

Abnormal aminotransferase levels were generally associated with higher prevalence of or worse indices for features of the metabolic syndrome (Table 3.2.). In particular, children with an elevated liver function test were characterized by significantly higher BMI, relative body weight, and waist-to-hip ratio, fasting total cholesterol and triglyceride levels, and 2-hour blood glucose level during OGTT. Dyslipidemia, hypercholesterolemia and IGR were significantly more frequent among children with elevated aminotransferase levels, and hyperinsulinemia also tended to be more frequent. ALT positively correlated with body weight (r=0.16; p=0.01), 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).

Table 3.2. Clinical and biochemical characteristics according to normal or elevated aminotransferase (ALT *or* AST) levels

Variable§	Normal aminotransferase (n=186)	Elevated aminotransferase (n=66)	p value*
Age (y)	12.8±2.7	12.8±2.6	ns
Weight (kg)	78.7±21.1	84.5±22.8	0.06
Height (cm)	158.6±13.4	160.0±11.3	ns
BMI (kg/m^2)	30.7±4.6	32.5±5.4	0.01
Relative body weight (%)	166.5±20.7	174.9±24.6	0.02
Waist-to-hip ratio	0.86±0.1	0.9±0.06	<0.001
Fasting glucose (mmol/L)	5.0 [4.7–5.4]	5.0 [4.7–5.3]	ns
OGTT 2-hour glu (mmol/L)	6.6 [5.9–7.2]	7.2 [6.0–7.8]	0.04
Fasting insulin (µU/mL)	26 [19–38]	29 [20–46]	ns
OGTT 2-hour ins (µU/mL)	130 [90–240]	150 [101–291]	ns
HOMA	6.0 [4.4–8.5]	6.3 [4.6–10.2]	ns
WBISI	28.7 [20.1–40.9]	26.1 [17.9–36.4]	ns
IGR	40 (21.5)	23 (34.8)	0.03
Hyperinsulinemia	133 (71.5)	55 (83.3)	0.06
Total cholesterol (mmol/L)	4.3 [3.9–4.9]	4.7 [4.1–5.3]	0.05
Triglycerides (mmol/L)	1.2 [0.9–1.6]	1.5 [1.0–1.9]	0.005
HDL-cholesterol (mmol/L)	1.1 [1.0–1.3]	1.1 [0.9–1.3]	ns
Hypercholesterolemia	30 (16.1)	19 (28.8)	0.05
Dyslipidemia	53 (28.5)	32 (48.5)	0.006
ALT (U/L)	24 [19–30]	54 [47–70]	<0.001
AST (U/L)	25 [22–31]	44 [32–57]	<0.001

Data are mean ± SD, median [interquartile range] or number (percentage).

§ Definitions used for the obesity-related metabolic conditions are described under methods.

* for normal vs. elevated; Students' t-test or Mann-Whitney *U* test for continuous variables, as appropriate; chi-square test for categorical variables.

OGTT – oral glucose tolerance test; IGR – impaired glucose regulation. Other abbreviations as in Table 3.1.

Discussion

Previous works, as well as our findings demonstrate that obesity-related hepatic injury indicated by elevated serum aminotransferase levels is a frequent consequence of childhood obesity (120,164,165). Pathogenesis of overweight-associated conditions, such as NAFLD, involves a variety of genetic determinants, and UCP-2 with a still debated physiological role has been implicated in some of the mechanisms thought to underlie the development and progression of fatty liver disease, including insulin resistance, altered lipid metabolism, and ROS production (115,117).

The main finding of this study is, that 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.

As previously demonstrated, elevations in serum ALT usually occur in more severe cases of fatty liver (113,166), and ALT and AST were identified as significant predictors of hepatic inflammation and fibrosis in pediatric NAFLD (119,165). The elevated transaminase levels with the relatively high upper limit of normal we used (164,167,168) are therefore likely to have identified children with some degree of NASH.

Mitochondrial abnormalities involving the uncoupling of oxidation and phosphorylation have been proposed to play a role in the susceptibility to NASH (166). According to the „two hit" theory of the pathogenesis of NASH (169), accumulation of fat in the liver along with the compensatory responses of fat-laden hepatocytes increase hepatic vulnerability to secondary insults which cause necroinflammatory changes. Lipid-laden hepatocytes overexpress UCP-2 (170), presumably as part of an adaptive response to alleviate lipid toxicity by dissipating the mitochondrial membrane potential, and thereby enhancing the capacity for substrate oxidation and/or controlling ROS generation associated with increased substrate metabolism (34,118). Besides its potential advantages, however, UCP-2 upregulation proposes serious risks to cell viability, as increased uncoupling decreases the efficiency of energy production and the cell's capacity to respond to acute energy needs in conditions of stress (118,171). Only a subset of fatty livers develop necroinflammation, therefore, it is likely that the "second hit" involves environmental and genetic polymorphism. Lack of UCP-2 has recently been shown to reduce susceptibility of hepatocytes to acute injury (37). Decreased function associated with the ins allele of the exon 8 UCP-2 polymorphism may therefore protect fatty liver hepatocytes from the vulnerable state of compromised energy homeostasis associated with UCP-2 upregulation. Recently, a study aiming to identify clinical predictors distinguishing NASH from steatosis in young Koreans found, that UCP-

2 was expressed in all NASH liver biopsy specimens and was significantly related with the severity of inflammation and fibrosis (114).

NAFLD is commonly associated with insulin resistance and dyslipidemia and is now considered to be the hepatic manifestation of the metabolic syndrome (121). In agreement with previous findings, our data confirmed an association between elevated aminotransferase levels and degree of obesity, fat distribution, insulin resistance, and abnormalities of glucose and lipid metabolism associated with the metabolic syndrome (116,122,167,172). Like most published reports we found obesity-related liver injury to be more prevalent among boys, than girls (116).

Higher insulin levels in children with the UCP-2 ins/ins genotype is in accordance with previous studies demonstrating, that UCP-2 expressed in islet beta-cells influences glucose-stimulated insulin secretion in a way, that inhibition of UCP-2 promotes insulin secretion (36). Higher insulin levels in the girls, compared with the boys might be explained by their slightly older age (13.0±2.7 vs. 12.7±2.6 years; p=0.4), and thus more advanced stage of puberty, associated with the worsening of insulin resistance that is compensated for by an increase in insulin secretion (173). Interestingly, both children homozygous for the ins allele and our girl participants showed a lower prevalence of elevated liver function tests, compared with the other genotype groups, and the boys, respectively. Our cross sectional data do not allow to make an assumption of cause and effect relationship between hyperinsulinemia and lower prevalence of liver damage. Theoretically, hyperinsulinemia could protect against obesity-related liver injury in the setting of a different degree of peripheral and hepatic insulin resistance, with more preserved hepatic insulin sensitivity. Female gender has been associated with higher serum adiponectin levels, which correlated inversely with hepatic insulin resistance but not with peripheral insulin sensitivity (174); however adiponectin was not measured in our study.

Observations and practical consequences

These results support previous findings connecting the UCP-2 gene to obesity-related liver injury. The data suggest that a common polymorphism of the UCP-2 gene may contribute to the variation in the development and expression of obesity-associated hepatic damage in children.

Identification of candidate genes and polymorphisms that explain disease onset and progression of pediatric NAFLD can be useful in informing theories of disease pathogenesis and progression and ultimately improving management.

4. Renal effects of childhood obesity

The main characteristics of the children included in this study are presented in Table 4.1. The groups were well matched for age and sex, although the age range of the obese children was wider than that of the normal weight children.

Table 4.1. Characteristics of the study population

Characteristic	**Obese children (n=86)**	**Control children (n=79)**
Age (y)	12.9 [8.9–17.2]	13.5 [10.7–14.9]
Males (n)	49	44
Weight (kg)	80.6 [46.1–136.8]	51.0 [27.3–72.5]
Height (cm)	161.5 [122.8–184.4]	165.0 [142.3–189.5]
Body mass index (kg/m^2)	30.4 [24.5–43.2]	18.2 [13.2–23.9]
Relative body weight (%)	163 [125–231]	95 [73–119]

Data are median [range].

Obese children had a significantly higher urinary albumin:creatinine ratio (U-ACR) (median: 11.7 mg/g, interquartile range: 12.9 mg/g versus median: 9.0 mg/g, interquartile range: 5.1 mg/g; p=0.003) and urinary β2-microglobulin:creatinine ratio (U-BMCR) (median: 63.9 µg/g, interquartile range: 34.7 µg/g versus median: 34.6 µg/g, interquartile range: 44.1 µg/g; p<0.001) as compared to the normal weight children (Figure 4.1.).

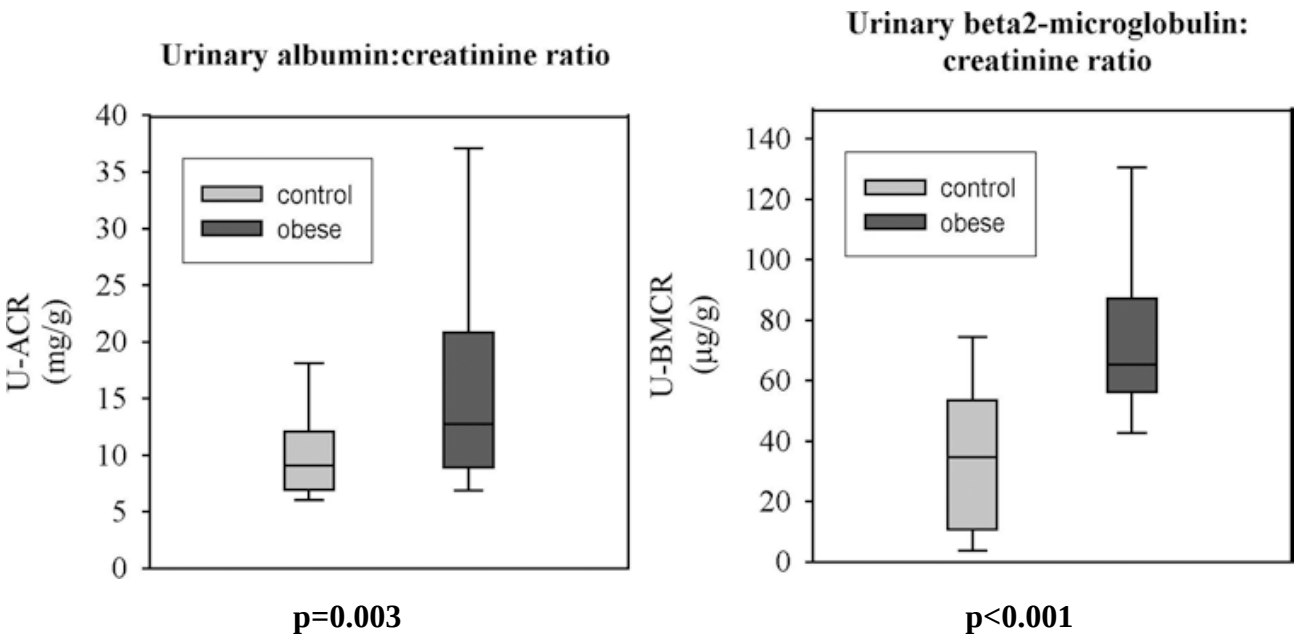

Figure 4.1. The urinary albumin:creatinine ratio (U-ACR) and urinary β2-microglobulin:creatinine ratio (U-BMCR) of the obese and control children (the median is depicted by the *line*, the interquartile range by the *box limits*, and the 10^{th}–90^{th} percentiles by the *error bars*).

Associations between the U-ACR and U-BMCR and the presence of obesity-related cardiovascular risk factors were investigated in the obese children by performing bivariate comparisons between the U-ACR and U-BMCR values of the obese children with or without a certain risk factor (Table 4.2). Among the obese children, 25 (29%) had fasting hyperinsulinemia, 56 (65%) had postprandial hyperinsulinemia, 20 (23%) had impaired glucose regulation (IGR), 45 (52%) had dyslipidemia, 17 (20%) had hypercholesterolemia, and 10 (12%) had hypertension. The presence of all tested cardiovascular risk factors was associated with a higher mean U-ACR, but the difference was significant only in the case of three factors. Thus, obese children with fasting hyperinsulinemia had a significantly higher U-ACR than obese children with normal fasting insulin and the same was true for obese children with or without IGR, and for the ones with or without hypercholesterolemia. The U-BMCR in the obese children was not significantly influenced by any of the cardiovascular risk factors studied.

Table 4.2 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* the cardiovascular risk factor		Obese children *with* the cardiovascular 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
IGR	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].

*The definitions used for the different risk factors are described under methods.

** Mann-Whitney *U* test.

IGR – impaired glucose regulation; ns: not significant.

The relationship between the degree of albuminuria and β2-microglobulinuria and the clustering of the cardiovascular risk factors that comprise the metabolic syndrome (hyperinsulinaemia [fasting or postprandial], IGR, dyslipidemia and hypertension) was further investigated. Obese children with no more than one of these traits had a significantly lower U-ACR, than those with two or more traits (median: 10.4 mg/g, interquartile range: 5.8 mg/g versus median: 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 both the obese and normal weight groups together, the U-ACR as well as the U-BMCR ratio 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 relative body weight ($r=0.23$; $p<0.005$ and $r=0.31$; $p<0.001$, respectively). When analyzing the normal weight and obese children separately, no correlations were found between the U-ACR or U-BMCR and these anthropometric measures. Among the obese children, no significant correlations were found between the U-ACR or U-BMCR and skinfold thicknesses or waist-to-hip ratio either.

Among the obese children, the U-ACR positively correlated with the fasting ($r=0.225$; $p< 0.05$) and 2-hour ($r=0.368$; $p<0.001$) plasma glucose concentrations measured during the OGTT. No correlations were found between the U-ACR of the obese children and other metabolic parameters

measured (fasting insulin, peak insulin, serum total cholesterol, triglyceride, HDL-cholesterol) or the systolic and diastolic blood pressure. Among the obese children, no significant correlations were found between the U-BMCR and any of the metabolic parameters or blood pressure values.

Discussion

Enhanced urinary albumin excretion is an indicator of systemic endothelial dysfunction (126), and an independent predictor of atherosclerosis and increased cardiovascular morbidity and mortality in the diabetic and general adult population (127–129). Previous studies have demonstrated that albuminuria is a continuous risk factor. Urinary albumin excretion levels relevant for cardiovascular risk have been shown to be substantially lower than the cutoff for the original definition of microalbuminuria as a marker of early diabetic nephropathy (128,129). A progressive graded relationship has been demonstrated between different degrees of albuminuria below the arbitrary threshold for defining microalbuminuria and cardiovascular events, extending to a U-ACR as low as 0.5 mg/mmol (4.4 mg/g). Therefore, reconsideration of the lower limit defining a “pathological” albuminuria had been suggested earlier, but no consensus has been reached on such a cut-off value so far. For this reason, we have not used a definition for microalbuminuria, but instead, have compared the U-ACR of obese and normal weight children.

Association between excessive albuminuria and common cardiovascular risk factors has been studied extensively in adults (124,125,175–177), with somewhat conflicting results. In the present study, increased albuminuria was observed in obese children and it was related to the presence of some features of the metabolic syndrome, but not with others. In particular, significant associations were found with the disorders of carbohydrate metabolism (hyperinsulinemia and IGR). This finding is in agreement with previous studies in adults demonstrating an association between microalbuminuria and insulin resistance (124,178) or increased blood glucose levels (179,180), and provides further evidence for the hypothesized central role of insulin resistance in the development of the metabolic syndrome and in the increased cardiovascular risk of subjects with excess urinary albumin excretion. The link between elevated insulin concentrations and the phenotypic traits of the metabolic syndrome are still only partially understood, mechanisms that might link hyperinsulinaemia to greater urinary albumin excretion include increased glomerular hemodynamic pressure (131,181) and endothelial dysfunction (126) that results in increased transcapillary leak of albumin.

Obesity is associated with atherogenic changes in lipoproteins and high lipid levels have previously been suggested to contribute to the obesity-associated pathological changes of the kidneys (181). Several studies have emphasized that increased total cholesterol may be associated with excessive albuminuria in some adult patient groups (131,182), and also with obesity-associated proteinuria and focal segmental glomerulosclerosis in children (183). Protective effect of dietary prevention of hypercholesterolemia in preventing obesity-linked renal disease has been demonstrated in animals (184). Our results have shown association between elevated serum cholesterol levels and enhanced albuminuria. The U-ACR was almost 2-fold increased in obese children with hypercholesterolemia compared to obese children with normal cholesterol levels, underscoring the importance of high cholesterol level in the development of obesity-related renal damage. We could not demonstrate a significant relationship between the U-ACR and serum triglyceride or HDL-cholesterol levels.

The synergistic effect of obesity and hypertension on renal function and albumin excretion in adults has been investigated by numerous studies, and the observations are controversial (125,131,132, 182). In our paediatric population, we could not demonstrate any significant influence of hypertension on the level of albuminuria in obese children, which is in accordance with the results of some of the adult studies (125) and might also be explained by the relatively mild degree of hypertension associated with childhood obesity, or the relatively few cases of obesity-associated hypertension we have identified among our obese patients.

We have found an association between excessive albuminuria and clustering of the traits of the metabolic syndrome in obese children. In 1998, the World Health Organization has designated microalbuminuria a feature of the metabolic syndrome (185), but this extension of the definition has raised debate, since results contradicting this relationship have also emerged (176,186). Our results, on the other hand, reinforce this association by demonstrating a link between enhanced albuminuria and presence of the features of the metabolic syndrome in children. Further longitudinal research is needed to evaluate the significance of the increased urinary albumin excretion of obese children in relation to the development of cardiovascular disease in adulthood.

Aside from the direct effects of obesity and associated metabolic disorders, glomerular proteinuria may also be a causative factor of tubulointerstitial dysfunction in obesity, since proteinuria has been shown to increase the turnover of tubular cells (187). On the other hand, infusion of albumin in proteinuric patients had no relevant effect on the tubular reabsorption of β2-microglobulin (188), and thus β2-microglobulin can be useful as a parameter to detect tubular injury and alterations in tubular handling of proteins in patients with glomerular proteinuria. Our finding of a significantly and greatly increased U-BMCR in obese children therefore indicates that there is also a tubular component to the renal dysfunction caused by childhood obesity. Unlike in the case of the level of albuminuria, we have not found any associations between the investigated cardiovascular risk

factors and the level of β2-microglobulinuria among the obese children, which indicates a different mechanism for obesity-linked glomerular and tubular dysfunction.

Observations and practical consequences

The onset of obesity-associated renal disease is insidious and asymptomatic, so early markers will be extremely useful in its prevention and treatment. 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. The significance of this in relation to later development of obesity-related cardiovascular and renal disease should be further investigated.

CONCLUSIONS

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

We provide data on the prevalence of six obesity candidate gene polymorphisms associated with energy expenditure among Hungarian obese and normal weight children. 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.

REFERENCES

1. Brug J, van Stralen MM, te Velde SJ, et al. Differences in weight status and energy-balance related behaviors among schoolchildren across Europe: The ENERGY-Project. PLoS ONE 2012;7: e34742.

2. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human obesity. Behav Genet 1997;27:325–51.

3. Wardle J, Carnell S, Haworth CM, Plomin R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr 2006;87:398–404.

4. Blakemore AI, Frougel P. Is obesity our genetic legacy? J Clin Endocrinol Metab 2008;93:S51–6.

5. Bouchard C. Childhood obesity: are genetic differences involved? Am J Clin Nutr 2009;89(S5):S1494–501.

6. Li S, Loos RJ. Progress in the genetics of common obesity: size matters. Curr Opin Lipidol 2008;19:113–21.

7. Griffiths M, Payne PR, Stunkard AJ, Rivers JP, Cox M. Metabolic rate and physical development in children at risk of obesity. Lancet 1990;336:76–8.

8. Rankinen T, Zuberi A, Chagnon YC, et al. The human obesity gene map: the 2005 update. Obesity (Silver Spring) 2006;14:529–644.

9. Emorine LJ, Marullo S, Briend-Sutren MM, et al. Molecular characterization of the human beta 3-adrenergic receptor. Science 1989;245:1118–21.

10. Hoffstedt J, Poirier O, Thörne A, et al. Polymorphism of the human beta3-adrenoreceptor gene forms a well-conserved haplotype that is associated with moderate obesity and altered receptor function. Diabetes 1999;48:203–5.

11. Loos RJ. Recent progress in the genetics of common obesity. Br J Clin Pharmacol 2009;68:811–29.

12. Fujisawa T, Ikegami H, Kawaguchi Y, Ogihara T. Meta-analysis of the association of Trp64Arg polymorphism of beta3-adrenergic receptor gene with body mass index. J Clin Endocrinol Metab 1998;83:2441–4.

13. Kadowaki H, Yasuda K, Iwamoto K, et al. A mutation in the β3-adrenergic receptor gene is associated with obesity and hyperinsulinaemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555–60.

14. Endo K, Yanagi H, Hirano C, et al. Association of Trp64Arg polymorphism of the beta3-adrenergic receptor gene and no association of Gln223Arg polymorphism of the leptin receptor gene in Japanese schoolchildren with obesity. Int J Obes Relat Metab Disord 2000;24:443–9.
15. Schrauwen P, Walder K, Ravussin E. Human uncoupling proteins and obesity. Obes Res 1999;7:97–105.
16. Jia JJ, Tian YB, Cao ZH, et al. The polymorphisms of UCP1 genes associated with fat metabolism, obesity and diabetes. Mol Biol Rep 2010;37:1513–22.
17. Krief S, Lonqvist F, Raimbault S, et al. Tissue distribution of β3-adrenergic receptor mRNA in man. J Clin Invest 1993;91:344–49.
18. Lean MEJ. Brown adipose tissue in humans. Proc Nutr Soc 1989;48:243–56.
19. Oberkofler H, Dallinger G, Liu YM, et al. Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. J Lipid Res 1997;38:2125–33.
20. Garruti G, Ricquier D. Analysis of uncoupling protein and its mRNA in adipose tissue deposits of adult humans. Int J Obes Relat Metab Disord 1992;16:383–90.
21. Petruzzelli M, Schweiger M, Schreiber R, et al. A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab 2014;20:433–47.
22. Cassard-Doulcier AM, Bouillaud F, Chagnon M, et al. The Bcl I polymorphism of the human uncoupling protein (UCP) gene is due to a point mutation in the 5'-flanking region. Int J Obes 1996;20:278–9.
23. Esterbauer H, Oberkofler H, Liu YM, et al. Uncoupling protein-1 mRNA expression in obese human subjects: the role of sequence variations at the uncoupling protein-1 gene locus. J Lipid Res 1998;39:834–4.
24. Ramis JM, González-Sánchez JL, Proenza AM, et al. The Arg64 allele of the beta 3-adrenoceptor gene but not the -3826G allele of the uncoupling protein 1 gene is associated with increased leptin levels in the Spanish population. Metabolism 2004;53:1411–6.
25. Hayakawa T, Nagai Y, Taniguchi M, et al. Phenotypic characterization of the beta3-adrenergic receptor mutation and the uncoupling protein 1 polymorphism in Japanese men. Metabolism 1999;48:636–40.
26. Heilbronn LK, Kind KL, Pancewicz E, et al. Association of –3826G variant in uncoupling protein-1 with increased BMI in overweight Australian women. Diabetologia 2000;43:242–4.
27. Oppert JM, Vohl MC, Chagnon M, et al. DNA polymorphism in the uncoupling protein (UCP) gene and human body fat. Int J Obes 1994;18:526–31.
28. Fumeron F**,** Durack-Bown I, Betoulle D, et al. Polymorphisms of uncoupling protein (UCP) and beta 3 adrenoreceptor genes in obese people submitted to a lowcalorie diet. Int J Obes Relat Metab Disord 1996;20:1051–4.

29. Fogelholm M, Valve R, Kukkonen-Harjula K, et al. Additive effects of the mutation in the beta3-aderenrgic receptor and uncoupling protein-1 genes on weight loss and weight maintenance in finnish women. J Clin Endocrinol Metab 1998;83:4246–50.
30. Fisler JS, Warden CH. Uncoupling proteins, dietary fat and the metabolic syndrome. Nutr Metab (Lond) 2006;3:38.
31. Brand MD, Affourtit C, Esteves TC, et al. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic Biol Med 2004;37:755–67.
32. Vidal-Puig AJ. Uncoupling expectations. Nat Genet 2000;26:387–8.
33. Ricquier D. Uncoupling protein-2 (UCP2): Molecular and genetic studies. Int J Obes 1999;23(S6):S38–42.
34. Uchino S, Yamaguchi Y, Furuhashi T, et al. Steatotic liver allografts up-regulate UCP-2 expression and suffer necrosis in rats. J Surg Res 2004;120:73–82.
35. Walder K, Norman RA, Hanson RL, et al. Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima Indians. Hum Mol Genet 1998;7:1431–5.
36. Chan CB, Kashemsant N. Regulation of insulin secretion by uncoupling protein. Biochem Soc Trans 2006;34:802–5.
37. Fülöp P, Derdák Z, Sheets A, et al. Lack of UCP2 reduces Fas-mediated liver injury in ob/ob mice and reveals importance of cell-specific UCP2 expression. Hepatology 2006;44:592–601.
38. Nagy TR, Blaylock ML, Garvey WT. Role of UCP2 and UCP3 in nutrition and obesity. Nutrition 2004;20:139–44.
39. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005;2:85–93.
40. Jia ZZ, Zhang X, Ge CR, Jois M. The polymorphisms of UCP2 and UCP3 genes associated with fat metabolism, obesity and diabetes. Obes Rev 2009;10:519–26.
41. Bouchard C, Perusse L, Chagnon YC, Warden C, Ricquier D. Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. Hum Mol Genet 1997;6:1887–998.
42. Schrauwen P, Xia J, Bogardus C, Pratley RE, Ravussin E. Skeletal muscle uncoupling protein 3 expression is a determinant of energy expenditure in Pima Indians. Diabetes 1999;48:146-9.
43. Barbe P, Millet L, Larrouy D, et al. Uncoupling protein-2 messenger ribonucleic acid expression during very-low-calorie diet in obese premenopausal women. J Clin Endocrinol Metab 1998;83:2450–3.
44. Saar K, Geller F, Ruschendorf F, et al. Genome scan for childhood and adolescent obesity in German families. Pediatrics 2003;111:321–7.

45. Esterbauer H, Schneitler C, Oberkofler H, et al. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. Nat Genet 2001;28:178–83.
46. Jun HS, Kim IK, Lee HJ, et al. Effects of UCP2 and UCP3 variants on the manifestation of overweight in Korean children. Obesity (Silver Spring) 2009;17:355–62.
47. Marti A, Corbalán MS, Forga L, Martinez-González MA, Martinez JA. Higher obesity risk associated with the exon-8 insertion of the UCP2 gene in a Spanish case-control study. Nutrition 2004;20:498–501.
48. Yanovski JA, Diament AL, Sovik KN, et al. Associations between uncoupling protein 2, body composition, and resting energy expenditure in lean and obese African American, white, and Asian children. Am J Clin Nutr 2000;71:1405–20.
49. Evans D, Wolf AM, Nellessen U, et al. Association between polymorphisms in candidate genes and morbid obesity. Int J Obes Relat Metab Disord 2001;25(S1):S19–21.
50. Schrauwen P, Xia J, Walder K, Snitker S, Ravussin E. A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians. Int J Obes Relat Metab Disord 1999;23:1242–5.
51. Liu YJ, Liu PY, Long J, et al. Linkage and association analyses of the UCP3 gene with obesity phenotypes in Caucasian families. Physiol Genomics 2005;22:197–203.
52. Alonso A, Martí A, Corbalán MS, et al. Association of UCP3 gene –55C>T polymorphism and obesity in a Spanish population. Ann Nutr Metab 2005;49:183–8.
53. Meirhaeghe A, Amouyel P. Impact of genetic variation of PPARgamma in humans. Mol Genet Metab 2004;83:93–102.
54. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284–7.
55. Meirhaeghe A, Fajas L, Helbecque N, et al. Impact of the peroxisome proliferator activated receptor gamma2 Pro12Ala polymorphism on adiposity, lipids and non-insulin-dependent diabetes mellitus. Int J Obes Relat Metab Disord 2000;24:195–9.
56. Li WD, Lee JH, Price RA. The peroxisome proliferator-activated receptor gamma 2 Pro12Ala mutation is associated with early onset extreme obesity and reduced fasting glucose. Mol Genet Metab 2000;70:159–61.
57. Masud S, Ye S. Effect of the peroxisome proliferator activated receptor-gamma gene Pro12Ala variant on body mass index: a meta-analysis. J Med Genet 2003;40:773–80.

58. Tonjes A, Scholz M, Loeffler M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. Diabetes Care 2006;29:2489–97.

59. González Sánchez JL, Serrano Ríós M, Pérez Fernández C et al. Effect of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ-2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. Eur J Endocrinol 2002;147:495–501.

60. Ochoa MC, Marti A, Azcona C, et al. Gene-gene interaction between PPAR gamma 2 and ADR beta 2 increases obesity risk in children and adolescents. Int J Obes Relat Metab Disord 2004;28(S3):S37–41.

61. Ek J, Urhammer SA, Sørensen TI, et al. Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-gamma2 (PPAR-gamma2): divergent modulating effects on body mass index in obese and lean Caucasian men. Diabetologia 1999;42:892–5.

62. Zurlo F, Lillioja S, Esposito-Del Puente A et al. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol 1990;259:E650–7.

63. Rasvussin E, Lillioja S, Knowler W, et al. Reduced rate of energy expenditure as a risk factor for body weight gain. N Engl J Med 1988;318:467–72.

64. Bogadus C, Lillioja S, Rasvussin E, at al. Familial dependence of the resting metabolic rate. New Engl J Med 1986;315:96–100.

65. Reilly JJ, Mefhren E, McDowell ZC, et al. Health consequences of obesity. Arch Dis Child 2003;88:748–52.

66. Lobstein T, Jackson-Leach R. Estimated burden of paediatric obesity and co-morbidities in Europe. Part 2. Numbers of children with indicators of obesity-related disease. Int J Paeditr Obes 2006;1:33–41.

67. Giacobino JP. Beta3-adrenoreceptor: an update. Eur J Endocrinol 1995;132:377–85.

68. Sipilainen R, Uusitupa M, Heikkinen S, Rissanen A, Laakso M. Polymorphism of the beta3-adrenergic receptor gene affects basal metabolic rates in obese Finns. Diabetes 1997;46:77–80.

69. Clement K, Vaisse C, Manning BS, et al. Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 1995;333:352–4.

70. Widen E, Letho M, Kanninen T, et al. Association of a polymorphism in the beta3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 1995;333:348–52.

71. Urhammer SA, Clausen JO, Hansen J, Pedersen O. Insulin sensitivity and body weight changes in young white carriers of the codon 64 amino acid polymorphism of the beta 3-adrenergic receptor gene. Diabetes 1996;45:1115–20.

72. Sakane N, Yoshida T, Umekawa T, et al. Beta 3-adrenergic-receptor polymorphism: a genetic marker for visceral fat obesity and the insulin resistance syndrome. Diabetologia 1997;40:200–4.
73. Arashiro R, Katsuren K, Fukuyama S, Ohta T. Effect of Trp64Arg mutation of the beta3-adrenergic receptor gene and C161T substitution of the peroxisome proliferator activated receptor gamma gene on obesity in Japanese children. Pediatr Int 2003;45:135–41.
74. Oquri K, Tachi T, Matsuoka T. Visceral fat accumulation and metabolic syndrome in children: the impact of Trp64Arg polymorphism of the beta3-adrenergic receptor gene. Acta Paediatr 2013;102:613–9.
75. Xinli W, Xiaomei T, Meihua P, Song L. Association of a mutation in the β3-adrenergic receptor gene with obesity and response to dietary intervention in Chinese children. Acta Paeditr 2001;90:1233–7.
76. Kortelainen ML. Association between cardiac pathology and fat distribution in an autopsy series of men without premortem evidence of cardiovascular disease. Int J Obes Relat Metab Disord 1996;20:245–52.
77. Valve R, Heikkinen S, Rissanen A, Laakso M, Uusitupa M. Synergistic effect of polymorphisms in uncoupling protein 1 and beta3-adrenergic receptor genes on basal metabolic rate in obese Finns. Diabetologia 1998;41:357–61.
78. Clement K, Ruiz J, Cassard-Doulcier AM, et al. Additive effect of A→G (-3826) variant of the uncoupling protein gene and the Trp64Arg mutation of the beta 3-adrenergic receptor gene on weight gain in morbid obesity. Int J Obes Relat Metab Disord 1996;20:1062–6.
79. Bracale R, Labruna G, Finelli C, et al. The absence of polymorphisms in ADRB3, UCP1, PPARγ, and ADIPOQ genes protects morbid obese patients toward insulin resistance. J Endocrinol Invest 2012;35:2–4.
80. Oh HH, Kim KS, Choi SM, Yang HS, Yoon Y. The effects of uncoupling protein-1 genotype on lipoprotein cholesterol level in Korean obese subjects. Metabolism 2004;53:1054–9.
81. Forga L, Corbalan M, Marti A, et al. Influence of the polymorphism –3826 A → G in the UCP1 gene on the components of metabolic syndrome. An Sist Sanit Navar 2003;26:231–6.
82. Nagai N, Sakane N, Ueno LM, Hamada T, Moritani T. The -3826A-->G variant of the uncoupling protein-1 gene diminishes postprandial thermogenesis after a high fat meal in healthy boys. J Clin Endocrinol Metab 2003;88:5661–7.
83. Krempler F, Esterbauer H, Weitgasser R, et al. A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans. Diabetes 2002;51:3331–5.

84. Sesti G, Cardellini M, Marini MA, et al. A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects. Diabetes 2003;52:1280–3.
85. Wang H, Chu WS, Lu T, et al. Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion. Am J Physiol Endocrinol Metab 2004;286:E1–7.
86. Bulotta A, Ludovico O, Coco A, et al. The common -866G/A polymorphism in the promoter region of the UCP-2 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy. J Clin Endocrinol Metab 2005;90:1176–80.
87. Dalgaard LT, Andersen G, Larsen LH, et al. Mutational analysis of the UCP2 core promoter and relationships of variants with obesity. Obes Res 2003;11:1420–7.
88. Ji Q, Ikegami H, Fujisawa T et al. A common polymorphism of uncoupling protein 2 gene is associated with hypertension. J Hypertens 2004;22:97–102.
89. Le Fur S, Le Stunff C, Dos Santos C, Bougneres P. The common -866 G/A polymorphism in the promoter of uncoupling protein 2 is associated with increased carbohydrate and decreased lipid oxidation in juvenile obesity. Diabetes 2004;53:235–9.
90. Ochoa MC, Santos JL, Azcona C, et al. Association between obesity and insulin resistance with UCP2-UCP3 gene variants in Spanish children and adolescents. Mol Genet Metab 2007;92:351–8.
91. Labayen I, Ortega FB, Sjöström M, et al. Association of common variants of UCP2 gene with low-grade inflammation in Swedish children and adolescents: the European Youth Heart Study. Pediatr Res 2009;66:350–4.
92. Kring SI, Larsen LH, Holst C, et al. Genotype-phenotype associations in obesity dependent on definition of the obesity phenotype. Obes Facts 2008;1:138–45.
93. Cassell PG, Saker PJ, Huxtable SJ, et al. Evidence that single nucleotide polymorphism in the uncoupling protein 3 (UCP3) gene influences fat distribution in women of European and Asian origin. Diabetologia 2000;43:1558–64.
94. Meirhaeage A, Amouyel P, Helbecque N, et al. An uncoupling protein 3 gene polymorphism associated with a lower risk of developing Type II diabetes and with atherogenic lipid profile in a French cohort. Diabetologia 2000;43:1424–8.
95. Gurnell M. Peroxisome prolifertor-activated receptor gamma and the regulation of adipocyte function: lessons from human genetic studies. Best Pract Res Clin Endocrinol Metab 2005;19:501–23.
96. Ristow M, Muller-Wieland D, Pfeiffer A, Krone W, Kahn CR. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. N Engl J Med 1998;339:953–9.

97. Frederiksen L, Brodbaek K, Fenger M, et al. Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome. J Clin Endocrinol Metab 2002;87:3989–92.
98. Swarbrick MM, Chapman CM, McQuillan BM, et al. A Pro12Ala polymorphism in the human peroxisome proliferator-activated receptor-gamma 2 is associated with combined hyperlipidaemia in obesity. Eur J Endocrinol 2001;144:277–82.
99. Hasstedt SJ, Ren Q, Teng K, et al. Effect of the peroxisome proliferator-activated receptor-γ2 Pro12Ala variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds. J Clin Endocrinol Metab 2001;86:536–41.
100. Meirhaeghe A, Cottel D, Amouyel P, Dallongeville J. Association between peroxisome proliferator-activated receptor γ haplotypes and the metabolic syndrome in French men and women. Diabetes 2005;54:3043–8.
101. Gagnon J**,** Mauriege P, Roy S, et al. The Trp64Arg mutation of the beta3-adrenergic receptor gene has no effect on obesity phenotypes in the Québec Family Study and Swedish obese subjects cohorts. JCI 1996;98:2086–93.
102. Buettner R, Schaffler A, Arndt H, et al. The Trp64Arg polymorphism of the beta3-adrenergic receptor gene is not associated with obesity or type 2 diabetes mellitus in a large population-based Caucasian cohort. J Clin Endocrinol Metab 1998;83:2892–7.
103. Zawodniak-Szalapska M, Stawerska R, Brzezianska E, et al. Association of Trp64Arg polymorphism of beta3-adrenergic receptor with insulin resistance in Polish children with obesity. J Pediatr Endocrinol Metab 2008;21:147–54**.**
104. Urhammer SA, Fridberg M, Sorensen TI, et al. Studies of genetic variability of the uncoupling protein 1 gene in Caucasian subjects with juvenile-onset obesity. J Clin Endocrinol Metab 1997;82:4069–74.
105. Schaffler A, Palitzsch KD, Watzlawek E, et al. Frequency and singificance of the A→G (-3826) polymorphism in the promoter of the gene for uncoupling protein-1 with regard to metabolic parameters and adipocyte transcription factor binding in a large population-based Caucasian cohort. Eur J Clin Invest 1999;29:770–9.
106. Kovacs P, Ma L, Hanson RL, et al. Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. Diabetologia 2005;48:2292–5.
107. Schauble N, Geller F, Siegfried W, et al. No evidence for involvement of the promoter polymorphism –866 G/A of the UCP2 gene in childhood-onset obesity in humans. Exp Clin Endocrinol Diabetes 2003;111:73–6.

108. Otabe S, Clement K, Rich N, et al. Mutation screening of the human UCP 2 gene in normoglycemic and NIDDM morbidly obese patients: lack of association between new UCP 2 polymorphisms and obesity in French Caucasians. Diabetes 1998;47:840–2.
109. Berentzen T, Dalgaard LT, Petersen L, Pedersen O, Sorense TI. Interactions between physical activity and variants of the genes encoding uncoupling proteins -2 and -3 in relation to body weight changes during a 10-y follow-up. Int J Obes (Lond) 2005;29:93–9.
110. Dalgaard LT, Sorensen TIA, Drivsholm T, et al. A prevalent polymorphism in the promoter of the UCP3 gene and its relationship to body mass index and long term body weight change in the Danish Population. J Clin Endocrinol Metab 2001;68:1398–402.
111. Mancini FP, Sabatino L, Colantuoni V, et al. Variants of uncoupling protein-2 gene and obesity: interaction with peroxisome proliferator-activated receptorgamma2. Clin Endocrinol 2003;59:817–22.
112. Moran JR, Ghishan FK, Halter SA, Greene HL. Steatohepatitis in obese children: a cause of chronic liver dysfunction. Am J Gastroenterol 1983;78:374–7.
113. Fishbein MH, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. J Pediatr Gastroenterol Nutr 2003;36:54–61.
114. Park JW, Jeong G, Kim SJ, Kim MK, Park SM. Predictors reflecting the pathological severity of non-alcoholic fatty liver disease: comprehensive study of clinical and immunohistochemical findings in younger Asian patients. J Gastroenterol Hepatol 2007;22:491–7.
115. Merriman RB, Aouizerat BE, Bass NM. Genetic influences in nonalcoholic fatty liver disease. J Clin Gastroenterol 2006;40(S1):S30–3.
116. Patton HM, Sirlin C, Behling C, et al. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. J Pediatr Gastroenterol Nutr 2006;43:413–27.
117. Baffy G. Uncoupling protein-2 and non-alcoholic fatty liver disease. Front Biosci 2005;10:2082–96.
118. Chavin KD, Yang S, Lin HZ, et al. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Biol Chem 1999;274:5692–700.
119. Schwimmer JB, Deutsch R, Rauch JB, et al. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. J Pediatr 2003;143:500–5.
120. Guzzaloni G, Grugni G, Minocci A, Moro B, Morabito F. Liver steatosis in juvenile obesity: correlations with lipid profile, hepatic biochemical parameters and glycemic and insulinemic responses to an oral glucose tolerance test. Int J Obes Relat Metab Disord 2000;24:772–6.

121. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–50.
122. Quirós-Tejeira RE, Rivera CA, Ziba TT, et al. Risk of nonalcoholic fatty liver disease in Hispanic youth with BMI ≥95th percentile. J Pediatr Gastroentrol Nutr 2007;44:228–36.
123. Lokkegaard N, Haupter I, Kristensen TB. Microalbuminuria in obesity. Scand J Urol Nephrol 1992;26:275–8.
124. Mykkanen L, Zaccaro DJ, Wagenknecht LE, et al. Microalbuminuria is associated with insulin resistance in nondiabetic subjects – the insulin resistance atherosclerosis study. Diabetes 1998;47:793–800.
125. Liese AD, Hense HW, Doring A, Stieber J, Keil U. Microalbuminuria, central adiposity and hypertension in the non-diabetic urban population of the MONICA Augsburg survey 1994/95. J Hum Hypertens 2001;15:799–804.
126. Jensen JS, Borch-Johnsen K, Jensen G, Feldt-Rasmussen B. Microalbuminuria reflects a generalized transvascular albumin leakiness in clinically healthy subjects. Clin Sci 1995;88:629–33.
127. Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. Lancet 1988;2:530–3.
128. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA 2001;286:421–6.
129. Hillege HL, Janssen WM, Bak AA, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Inter Med 2001;249:519–26.
130. Hong CY, Chia KS. Markers of diabetic nephropathy. J Diabetes Complications 1998;12:43–60.
131. Reisin E, Messerli FG, Ventura HO, Froflich ED. Renal hemodynamic studies in obesity hypertension. J Hypertens 1987;5:397–400.
132. Ribstein J, du Cailar G, Mimran A. Combined renal effects of overweight and hypertension. Hypertension 1995;26:610–5.
133. Hidaka S, Kaneko O, Shirai M, et al. Do obesity and non-insulin dependent diabetes mellitus aggravate exercise-induced microproteinuria? Clin Chim Acta 1998;275:115–26.
134. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. Brit Med J 2000;320:1240–3.
135. Eiben O, Pantó E. Body measurement in the Hungarian youth at the 1980s, based on the Hungarian National Growth Study. Antropol Közl 1987;31:49–68.

136. Joubert K, Darvay S, Gyenis Gy, et al. Az országos longitudinális Gyermeknövekedés-vizsgálat eredményei születéstől 18 éves korig. KSH Népességtudományi Kutató Intézetének kutatási jelentések 83. 2006.
137. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youths. Hum Biol 1988;60:709–23.
138. Tritos NA, Mantzoros CS. Syndromes of severe insulin resistance. J Clin Endocrinol Metab 1998;83:3025–30.
139. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care 2004;27:S5–10.
140. The Expert Committee on the diagnosis and classification of diabetes mellitus. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183–97.
141. Romics L, Szollar L, Zajkas G. Treatment of disturbances of fat metabolism associated with atherosclerosis. (in Hungarian with English summary) Orv Hetil 1993;134: 227–38.
142. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
143. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462–70.
144. Az Országos Csecsemő és Gyermekegészségügyi Intézet 35. sz. Módszertani Levél. Gyermekkori hypertonia. Gyógyszereink 1987;37:97–128.
145. Hong **C**Y, Chia KS. Markers of diabetic nephropathy. J Diabetes Complications 1998;12:43–60.
146. Yen CJ, Beamer BA, Negri C, et al. Molecular scanning of the human peroxisome proliferator activated receptor gamma (hPPAR gamma) gene in diabetic Caucasians: identification of a Pro12Ala PPAR gamma 2 missense mutation. Biochem Biophys Res Commun 1997;241:270–4.
147. Dahlman I, Arner P. Obesity and polymorphisms in genes regulating human adipose tissue. Int J Obes (Lond) 2007;31:1629–41.
148. Schonfeld-Warden NA, Warden CH. Physiological effects of variants in human uncoupling proteins: UCP2 influences body mass index. Biochem Soc Trans 2001;29:777–84.
149. Oberkofler H, Liu YM, Esterbauer H, et al. Uncoupling protein-2 gene: reduced mRNA expression in intraperitoneal adipose tissue of obese humans. Diabetologia 1998;41:940–6.
150. Nordfors L**,** Hoffstedt J, Nyberg B, et al. Reduced gene expression of UCP2 but not UCP3 in skeletal muscle of human obese subjects. Diabetologia 1998;41:935–9.

151. Qian L, Xu K, Xu X, et al. UCP2 –866G/A, Ala55Val and UCP3 –55C/T polymorphisms in association with obesity susceptibility – a meta-analysis study. PLoS One 2013;8:e58939.
152. Andersen G, Dalgaard LT, Justesen JM, et al. The frequent UCP2 -866G>A polymorphism protects against insulin resistance and is associated with obesity: a study of obesity and related metabolic traits among 17 636 Danes. Int J Obes (Lond) 2013;37:175–81.
153. Dalgaard LT, Sørensen TI, Andersen T, Hansen T, Pedersen O. An untranslated insertion variant in the uncoupling protein 2 gene is not related to body mass index and changes in body weight during a 26-year follow-up in Danish Caucasian men. Diabetologia 1999;42:1413–6.
154. Kurokawa N, Young EH, Oka Y, et al. The ADRB3 Trp64Arg variant and BMI: a meta-analysis of 44 833 individuals. Int J Obes (Lond) 2008;32:1240–9.
155. Halsall DJ, Luan J, Saker P, et al. Uncoupling protein 3 genetic variants in human obesity: the c-55t promoter polymorphism is negatively correlated with body mass index in a UK Caucasian population. Int J Obes Relat Metab Disord 2001;25:472–7.
156. Otabe S, Clement K, Dina C, et al. A genetic variation in the 5' flanking region of the UCP3 gene is associated with body mass index in humans in interaction with physical activity. Diabetologia 2000;43:245–9.
157. Hamann A, Münzberg H, Buttron P, et al. Missense variants in the human peroxisome proliferator-activated receptor-gamma2 gene in lean and obese subjects. Eur J Endocrinol 1999;141:90–2.
158. Hinney A, Lentes KU, Rosenkranz K, et al. β3-adrenergic-receptor allele distributions in children, adolescents and young adults with obesity, underweight and anorexia nervosa. Int J Obes 1997;21:224–30.
159. Erhardt É, Czakó M, Csernus K, Molnár D. Kosztolányi Gy. The frequency of Trp64Arg polymorphism of the beta3-adrenergic receptor gene in healthy and obese Hungarian children and its association with cardiovascular risk factors. Eur J Clin Nutr 2005;59:955–9.
160. Hollenga C, Brouwer F, Zaagsma J. Differences in functional cyclic AMP compartments mediating lipolysis by isoprenaline and BRL37344 in four adipocyte types. Eur J Pharmacol 1991;200:325–30.
161. Walston J, Silver K, Bougardus C, et al. Time of onset of non-insulin dependent diabetes mellitus and genetic variation in the beta3-adrenergic-receptor gene. N Eng J Med 1995;333:343–7.
162. Saleh MC, Wheeler MB, Chan CB. Uncoupling protein-2: evidence for its function as a metabolic regulator. Diabetologia 2002;45:174–87.
163. Tu N, Chen H, Winnikes U, et al. Structural organization and mutational analysis of the human uncoupling protein-2 (hUCP2) gene. Life Sci 1999;64:41–50.

164. Tazawa Y, Noguchi H, Nishinomiya F, Takada G. Serum alanine aminotransferase activity in obese children. Acta Paediatr 1997;86:238–41.
165. Franzese A, Vajro P, Argenziano A, et al. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. Dig Dis Sci 1997;42:1428–32.
166. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–92.
167. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab 2006;91:4287–94.
168. Kunde SS, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. Hepatology 2005;42:650–6.
169. Day CP, James OF. Steatohepatitis: a tale of two 'hits'? Gastroenterology 1998;114:842–5.
170. Kohjima M, Enjoji M, Higuchi N, et al. Re-evaluation of fatty acid metabolism-related gene expression in nonalcoholic fatty liver disease. Int J Mol Med 2007;20:351–8.
171. Rashid A, Wu TC, Huang CC, et al. Mitochondrial proteins that regulate apoptosis and necrosis are induced in mouse fatty liver. Hepatology 1999;29:1131–8.
172. Nadeau KJ, Klingensmith G, Zeitler P. Type 2 diabetes in children is frequently associated with elevated alanine aminotransferase. J Pediatr Gastroenterol Nutr 2005;41:94–8.
173. Caprio S, Plewe G, Diamond MP, et al. Increased insulin secretion in puberty: a compensatory response to reductions in insulin sensitivity. J Pediatr 1989;114:963–7.
174. Bugianesi E, Pagotto U, Manini R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005;90:3498–504.
175. Hoffmann IS, Jimenez E, Cubeddu LX. Urinary albumin excretion in lean, overweight and obese glucose tolerant individuals: its relationship with dyslipidaemia, hyperinsulinaemia and blood pressure. J Hum Hypertens 2001;15:407–12.
176. Jager A, Kostense PJ, Nijpels G, et al. Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: The Hoorn Study. Diabetologia 1998;41:694–700.
177. Palaniappan L, Carnethon M, Fortmann SP. Association between microalbuminuria and the metabolic syndrome: NHANES III. Am J Hypertens 2003;16:952–8.
178. Kuusisto J, Mykkanen L, Pyorala K, Laakso M. Hyperinsulinemic microalbuminuria: a new risk indicator for coronary heart disease. Circulation 1995;91:831–7.

179. Hiratsuka N, Shiba K, Nishida K, et al. Analysis of urinary albumin, transferrin, N-acetyl-beta-D-glucosaminidase and beta2-microglobulin in patients with impaired glucose tolerance. J Clin Lab Anal 1998;12:351–5.
180. Meigs JB, D'Agostino RB Sr, Nathan DM, Rifai N, Wilson PW. Longitudinal association of glycemia and microalbuminuria: the Framingham Offspring Study. Diabetes Care 2002;25:977–83.
181. Verani RR. Obesity-associated focal segmental glomerulosclerosis: pathological features of the lesion and relationship with cardiomegaly and hyperlipidaemia. Am J Kidney Dis 1992;20:629–34.
182. Bianchi S, Bigazzi R, Valtriani C, et al. Elevated serum insulin levels in patients with essential hypertension and microalbuminuria. Hypertension 1994;23:681–7.
183. Adelman RD, Restaino IG, Alon US, Blowey DL. Proteinuria and focal segmental glomerulosclerosis in severely obese adolescents. J Pediatr 2001;138:481–5.
184. Maddox DA, Alavi FK, Silbernick EM, Zawada ET. Protective effects of soy diet in preventing obesity-linked renal disease. Kidney Int 2002;61:96–104.
185. Groop L, Orho-Melander M. The dysmetabolic syndrome. J Intern Med 2001;250:105–20.
186. Zavaroni I, Bonini L, Gasparini P, et al. Dissociation between urinary albumin excretion and variables associated with insulin resistance in a healthy population. J Intern Med 1996;240:151–6.
187. Burton CJ, Harper SJ, Bailey E, et al. Turnover of human tubular cells exposed to proteins in vivo and in vitro. Kidney Int 2001;59:507–14.
188. Branten AJ, Wetzels JF. Influence of albumin infusion on the urinary excretion of beta2-microglobulin in patients with proteinuria. Nephron 1999;81:329–33.

PUBLICATIONS IN THE ISSUE OF THE THESIS

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.
2. Csernus K, Lanyi É, Erhardt É, Molnár D. Effect of childhood obesity and obesity-related cardiovascular risk factors on glomerular and tubular protein excretion. Eur J Pediatr 2005;164:44–9. **IF: 1.382**
3. Erhardt É, Czakó M, Csernus K, Molnár D, Kosztolányi Gy. The frequency of Trp64Arg polymorphism of the beta3-adrenergic receptor gene in healthy and obese Hungarian children and its association with cardiovascular risk factors. Eur J Clin Nutr 2005;59:955–9. **IF: 2.163**
4. Répásy J, Bokor Sz, Csernus K, Erhardt É, Molnár D. Béta3-adrenoreceptor gén Trp64Arg polimorfizmusának szerepe elhízott gyermekek energiaegyensúlyában. Gyermekgyógyászat 2006;4:425–31.
5. Bokor Sz, Csernus K, Erhardt É, Molnár D, Decsi T. Az uncoupling protein-2 gén –866 G/A polimorfizmusának szerepe az elhízás metabolikus szövődményeinek kialakulásában. Gyermekorvos Továbbképzés 2006;V/4:268–71.
6. Csernus K, Erhardt É, Molnár D. A túlsúlyos gyermekeknél előforduló emelkedett szérum aminotranszferáz-szintek összefüggése az uncoupling protein-2 egy gyakori polimorfizmusával. Gyermekgyógyászat 2010;61:22–8.
7. Csernus K, Pauler G, Erhardt E, Lanyi E, Molnar D. Uncoupling protein-2 gene polymorphisms are associated with obesity in Hungarian children. Acta Paediatr 2013;102:E200–4. **IF: 1.842**
8. Csernus K, Pauler G, Erhardt E, Lanyi E, Molnar D. Effects of energy expenditure gene polymorphisms on obesity-related traits in obese children. Obes Res Clin Pract 2015;9:133–40. **IF:0.697**

ABSTRACTS WHICH CAN BE CITED IN THE ISSUE OF THE THESIS

1. Csernus K, Erhardt É, Felszeghy E, Illyés I, Molnár D. The prevalence of disorders of carbohydrate metabolism in clinically healthy obese children. Int J Obes Relat Metab Disord 2003;27(S1):S78. **IF: 2.794**
2. Csernus K, Lanyi E, Erhardt E, Molnar D. Markers of renal glomerular and tubular dysfunction in childhood obesity. Int J Obes Relat Metab Disord 2003;27(S1):S4. **IF: 2.794**
3. Csernus K, Lányi É, Molnár D. Glomeruláris és tubuláris fehérjeürítés gyermekkori elhízásban. Obesit Hung 2003;4(S3):13.
4. Nagy E, Csernus K, Erhardt É, Molnár D. Elhízáshoz társuló zsírmáj gyermekkorban. Obes Hung 2003;4(S3):17.
5. Csernus K, Erhardt E, Lanyi E, Molnar D. Effect of childhood obesity on glomerular and tubular protein excretion. Int J Obes Relat Metab Disord 2004;28(S1):S42. **IF: 3.459**
6. Erhardt E, Csernus K, Czako M, Molnar D. Frequencies of single-nucleotide polymorphisms of some candidate genes playing role in thermogenesis in Hungarian children. Int J Obes Relat Metab Disord 2004;28(S1):S106. **IF: 3.459**
7. Csernus K, Erhardt É, Molnár D. Az energiafelhasználásban szerepet játszó gének polimorfizmusai gyermekkori elhízásban. Obes Hung 2004;5(2):52.
8. Nagy E, Csernus K, Erhardt É, Molnár D. Az elhízás és az uncoupling protein-2 exon 8 ins/del polimorfizmusának szerepe a gyermekkori zsírmáj kialakulásában. Obes Hung 2004; 5(S2):54.
9. Csernus K, Erhardt E, Molnar D. Non-alcoholic fatty liver disease in childhood obesity and role of uncoupling protein-2. Int J Obes Relat Metab Disord 2004;28(S3):S109. **IF: 3.459**
10. Csernus K, Lányi É, Erhardt É, Molnár D. A gyermekkori elhízás és metabolikus szindróma hatása a glomeruláris és tubuláris fehérjeürítésre. Gyermekgyógyászat 2004;55(S2):18.
11. Nagy E, Csernus K, Erhardt É, Molnár D. Elhízáshoz társuló zsírmáj gyermekkorban. Gyermekgyógyászat 2004;55(S2):73–4.
12. Erhardt E, Csernus K, Bokor S, Molnar D. Frequency and effect of Ala12 allele of PPARgamma on cardiovascular risk factors in Hungarian children. Int J Obes Relat Metab Disord 2005;29(S2):S146. **IF: 4.482**
13. Erhardt E, Csernus K, Molnar D. Examination of synergistic effects of some candidate genes playing role in thermogenesis. Obes Rev 2005;6(S1):S125.

14. Nagy E, Bokor Sz, Csernus K, Erhardt É, Molnár D. Szénhidrátanyagcsere-zavarok előfordulása kövér gyermekekben. Gyermekgyógyászat 2006;57(2):242.
15. Molnar D, Erhardt E, Csernus K, Bokor S. Metabolic syndrome and gene polymorphism. Int J Obes 2006;30(S2):S21. **IF: 4.055**
16. Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D. Energy expenditure and Trp64Arg polymorphism of the ß3 adrenoreceptor gene. Int J Obes 2006;30(S2):S22. **IF: 4.055**
17. Nagy E, Bokor S, Csernus K, Erhardt E, Molnar D. Relationship between the Pro12Ala polymorphism of the proliferator-activated receptor gamma gene and carbohydrate metabolism in obese children. Int J Obes 2007;31(S1):S20. **IF: 3.56**
18. Csernus K, Erhardt E, Molnar D. Frequencies of common polymorphisms of candidate obesity genes playing role in energy storage and expenditure in Hungarian children. Obes Rev 2007;8(S3):S41. **IF: 7.821**
19. Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D. Affect of gene polymorphisms on energy expenditure in obese children. Int J Pediatr Obes 2010;5(S1) **IF:2.654**
20. Csernus K, Pauler G, Erhardt E, Lanyi E, Molnar D. Common polymorphisms of the UCP-2 gene are associated with obesity in Hungarian children. Int J Obes Supp 2011;1:S10. **IF:4.691**

OTHER PUBLICATIONS AND ABSTRACTS

1. Erhardt É, Nyikos O, Csernus K, Molnár D. Szénhidrátanyagcsere-zavarok előfordulása és változása diéta hatására kövér gyermekekben. Gyermekgyógyászat 2003;54/4:415–22.
2. Erhardt E, Csernus K, Felszeghy E, Ilyes I, Molnar D. Impaired glucose tolerance and type 2 diabetes in obese children. Int J Obes Relat Metab Disord 2003;27(S2):S24. (abs) **IF: 2.794**
3. Lányi É, Csernus K, Erhardt É, Molnár D. Keringő aktív ghrelin szint változása orális glukózterhelés során elhízott gyermekekben. Obes Hung 2004;5(S2):55. (abs)
4. Lányi É, Csernus K, Erhardt É, Molnár D. Keringő aktív ghrelin szintjének változása orális glükózterhelés során kövér gyermekekben. Gyermekgyógyászat 2004;55(S2):54. (abs)
5. Bokor S, Csernus K, Erhardt E, Burus I, Molnar D, Decsi T. The –866A/A but not the –866G/A genoype of uncoupling protein 2 gene is associated with reduced dihomo-gamma-linolenic acid values in obese children. Paediatr Res 2005;58(2):362. (abs) **IF: 2.875**
6. Molnar D, Lanyi E, Csernus K, Erhardt E. Active ghrelin levels in childhood obesity. Paediatr Res 2005;58(2):398. (abs) **IF: 2.875**
7. Lanyi E, Csernus K, Erhardt E, Molnar D. Plasma levels of active form of ghrelin during an oral glucose tolerance test in obese children. Obes Rev 2005;6(S1):S123. (abs)
8. Bokor Sz, Csernus K, Erhardt É, Burus I, Marosvölgyi T, Molnár D, Decsi T. A béta-3 adrenoreceptor gén Trp64Arg polimorfizmusának összefüggése a zsírsavellátottsággal elhízott gyermekekben. Gyermekgyógyászat 2006;57:125-129.
9. Csernus K, Turai R, Ottóffy G, Kajtár P. Vállövi lágyrész terime érdekes esete. Gyermekgyógyászat 2006;57(2):210. (abs)
10. Nagy E, Bokor Sz, Csernus K, Erhardt É, Molnár D. Gyermekkori szénhidrátanyagcsere-zavarok előfordulása. Gyermekgyógyászat 2006;57(S1):61. (abs)
11. Répásy J, Bokor Sz, Csernus K, Erhardt É, Molnár D. Leptin-receptor gén Gln223Arg polimorfizmus szerepe elhízott gyermekek energia leadásában. Gyermekgyógyászat 2006;57(S1):38. (abs)
12. Lanyi E, Csernus K, Erhardt E, Toth K, Urban B, Lenard L, Molnar D. Plasma leves of acylated ghrelin during an oral glucose tolerance test in obese children. J Endocrinol Invest 2007;30:133–37. **IF: 2.021**
13. Bokor S, Csernus K, Erhardt E, Burus I, Molnar D, Decsi T. Association of n-6 long-chain polyunsaturated fatty acids to -866G/A genotypes of the human uncoupling protein 2 gene in obese children. Acta Paediatr 2007;96:1350–4. **IF: 1.411**

14. Repasy J, Bokor Sz, Csernus K, Erhardt E and Molnar D. Gln223Arg polymorphism of the leptin receptor gene and food-induced thermogenesis in obese boys. Obes Rev 2007;8 (S3):14. (abs) **IF: 7.821**.
15. Bokor S, Csernus K, Erhardt E, Molnar D. The Arg223Arg genotype of the leptin receptor gene is associated with obesity in Hungarian children. Int J Obes 2007;31(S1):12. (abs) **IF: 3.56**
16. Ottoffy G, Kajtar B, Kereskai L, Tornoczky T, Csernus K, Kajtar P. Vacuolated alveolar rhabdomyosarcoma cells mimicking FAB L3 lymphoblasts in bone marrow. Blood Rev 2007;21:(S1):S96–7. (abs) **IF: 5.756**
17. Vojcek Á, Csernus K, Kajtár P, Ottóffy G. Szöveti Plazminogén aktivátor (tPA) alkalmazása gyermekeknél centrális vénás kanül okklúziója esetén. Gyermekgyógyászat 2008;59:219–23.
18. Repasy J, Csernus K, Erhardt E, Molnar D. Polymorphism of the UCP1 gene and food-induced thermogenesis in obese boys. Obes Rev 2008;9(S2):19. (abs) **IF: 5.569**
19. Csernus K, Csábi Gy, Stankovics J. Adenovírus-fertőzés ritka manifesztációi csecsemőkorban: agyvelőgyulladás, májelégtelenség. Gyermekgyógyászat 2009;60(2):129. (abs)

LECTURES AND POSTERS IN THE ISSUE OF THE THESIS

1. Magyar Gyermekorvosok Társasága Éves Nagygyűlése, Pécs, 2001. június 15-16.
   Csernus K, Erhardt É, Czakó M, Molnár D, Kosztolányi Gy: Béta3-adrenoreceptor gén polimorfizmus előfordulása gyermekekben (poszter)

2. European Childhood Obesity Group 12th Workshop, Prague, Czech Republic, 2002. May 23-25.
   Csernus K, Erhardt E, Nyikos O, Molnar D: Prevalence of the disorders of carbohydrate metabolism in clinically healthy obese children
   Erhardt E, Czako M, Csernus K, Molnar D, Kosztolanyi G: The Trp64Arg polymorphism of the beta3-adrenergic receptor gene in normal and obese Hungarian children

3. A Magyar Elhízástudomány 10 éves Jubileumi Kongresszusa, Budapest, 2002. szept. 13-14.
   Csernus K, Erhardt É, Czakó M, Molnár D: Béta-3 adrenoreceptor gén polymorphismus normál és kövér magyar gyermekekben. Kapcsolata az insulin rezisztenciával
   Nyikos O, Csernus K, Erhardt É, Molnár D: Szénhidrátanyagcsere-zavarok előfordulása kövér gyermekekben.

4. 12th European Congress on Obesity, Helsinki, Finland, 2003. May 29–June 1.
   Csernus K, Erhardt E, Felszeghy E, Illyes I, Molnar D: The prevalence of disorders of carbohydrate metabolism in clinically healthy obese children (poster)

5. A Magyar Gyermekorvosok Társaságának 2003. évi Nagygyűlése, Szeged, 2003. június
   Erhardt É, Csernus K, Molnár D, Soltész Gy: Szénhidrát-anyagcserezavarok kövér gyermekekben

6. A Magyar Elhízástudományi Társaság 4. Kongresszusa, Budapest, 2003. szeptember 12-13.
   Csernus K, Lányi É, Molnár D: Glomeruláris és tubuláris fehérjeürítés gyermekkori elhízásban (poszter)
   Nagy E, Csernus K, Erhardt É, Molnár D: Elhízáshoz társuló zsírmáj gyermekkorban (poszter)

7. 3rd International Symposium on Obesity and Hypertension, Berlin, Germany, 2003. October 23-25.
   Csernus K, Lanyi E, Erhardt E, Molnar D: Markers of renal glomerular and tubular dysfunction in childhood obesity (poster)

8. Magyar Gyermekorvosok Társasága Ifjúsági Tagozat III. Találkozó és Szakmai Napok, Szeged, 2004. április 2-3.
   Csernus K, Lányi É, Erhardt É, Molnár D: Renális glomeruláris és tubuláris fehérjeürítés gyermekkori elhízásban

9. 13th European Congress on Obesity, Prague, Czech Republic, 2004. May 26–29.
   Csernus K, Erhardt E, Lanyi E, Molnar D: Effect of childhood obesity on glomerular and tubular protein excretion
   Erhardt E, Csernus K, Czako M, Molnar D: Frequencies of single-nucleotide polymorphisms of some candidate genes playing role in thermogenesis in Hungarian children (poster)

10. A Magyar Elhízástudományi Társaság V. Kongresszusa, Budapest, 2004. szeptember 10-11.
    Csernus K, Erhardt É, Molnár D: Az energiafelhasználásban szerepet játszó gének polimorfizmusai gyermekkori elhízásban
    Nagy E, Csernus K, Erhardt É, Molnár D: Az elhízás és az uncoupling protein-2 exon8 ins/del polimorfizmusának szerepe a gyermekkori zsírmáj kialakulásában

11. International Symposium „Childhood Obesity: From Basic Knowledge to Effective Prevention" 14th Workshop of the European Childhood Obesity Group, Zaragoza, Spain, 2004. September 23-25.
    Csernus K, Erhardt E, Molnar D: Non-alcoholic fatty liver disease in childhood obesity and role of uncoupling protein-2

12. Magyar Gyermekorvosok Társasága 2004. évi Nagygyűlése, Debrecen, 2004. október 7-9.
    Csernus K, Lányi É, Erhardt É, Molnár D: A gyermekkori elhízás és metabolikus szindróma hatása a glomeruláris és tubuláris fehérjeürítésre
    Nagy E, Csernus K, Erhardt É, Molnár D: Elhízáshoz társuló zsírmáj gyermekkorban

13. 14th European Congress on Obesity, Athens, Greece, 2005. May 29–June 1.
    Erhardt E, Csernus K, Molnar D: Examination of synergetic effects of some candidate genes playing role in thermogenesis (poster)

14. Magyar Gyermekorvosok Társasága Dél-Dunántúli Területi Szervezetének Tudományos Ülése, Siófok, 2005. szeptember 16–17.
    Bokor Sz, Csernus K, Erhardt É, Burus I, Molnár D, Decsi T: Egy genetikai sajátosságnak, az uncoupling protein 2 polimorfizmusának szerepe az elhízás metabolikus szövődményeinek kialakulásában

15. 15th European Childhood Obesity Group Workshop, Vienna, Austria, 2005. September 29–October 2.
    Erhardt E, Csernus K, Bokor S, Molnar D: Frequency and effect of Ala12 allele of PPARgamma on cardiovascular risk factors in Hungarian children

16. Fiatal Gyermekgyógyászok Konferenciája, Debrecen, 2006. április 7-8.
    Nagy E, Bokor Sz, Csernus K, Erhardt É, Molnár D: Szénhidrátanyagcsere-zavarok előfordulása kövér gyermekekben.

17. 16th Workshop of the European Childhood Obesity Group, Rzeszów, Poland, 2006. June 1–3.
    Molnar D, Erhardt E, Csernus K, Bokor S: Metabolic syndrome and gene polymorphism
    Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D: Energy expenditure and Trp64Arg polymorphism of the ß3 adrenoreceptor gene

18. Combating Obesity: Strategies for Prevention and Intervention, Erasmus Intensive Programme, Graz, Austria, 2007. February 3–17.
    Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D: Basal metabolic rate measurements in Hungarian obese children with Trp64Arg polymorphism of the β3 adrenergic receptor gene (poster)

19. European Congress on Obesity 2007, Budapest, 2007. March 22–25.
    Nagy E, Bokor S, Csernus K, Erhardt E, Molnar D: Relationship between the Pro12Ala polymorphism of the proliferator-activated receptor gamma gene and carbohydrate metabolism in obese children

20. Magyar Gyermekorvosok Társasága Országos Nagygyűlése, Székesfehérvár, 2007. május 24–26.

    Répásy J, Csernus K, Erhardt É, Molnár D: Alapanyagcsere vizsgálat UCP1 polimorfizmust hordozó elhízott gyermekekben

21. 17th Annual Meeting of the European Childhood Obesity Group, Athens, Greece, 2007. July 5–7.

    Csernus K, Erhardt E, Molnar D: Frequencies of common polymorphisms of candidate obesity genes playing role in energy storage and expenditure in Hungarian children (poster)

22. 19th Workshop of the European Childhood Obesity Group, Dublin, Ireland, 2009. September 17–19.

    Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D: Affect of gene-polymorphisms on energy expenditure in obese children

23. 21st Workshop of the European Childhood Obesity Group; 1st European Congress of Childhood Obesity, Pécs, 2011. September 8–10.

    Csernus K, Pauler G, Erhardt E, Lanyi E, Molnar D: Common polymorphisms of the UCP-2 gene are associated with obesity in Hungarian children

OTHER LECTURES AND POSTERS

1. Magyar Diabetes Társaság XVI. Kongresszusa, Debrecen, 2002. május 30.–június 2.
   Erhardt É, Csernus K, Molnár D, Soltész Gy: A 2-es típusú diabetes ritka, az IGT viszont gyakori tünetmentes, kövér gyermekekben

2. 13th European Childhood Obesity Group Workshop, Tenuta Moreno, Mesagne (BR) Italy, 2003. September 25–27.
   Erhardt E, Csernus K, Felszeghy E, Ilyes I, Molnar D: Impaired glucose tolerance and type 2 diabetes in obese children (poster)

3. A Magyar Elhízástudományi Társaság V. Kongresszusa, Budapest, 2004. szeptember 10-11.
   Lányi É, Csernus K, Erhardt É, Molnár D: Keringő aktív ghrelin szint változása orális glukózterhelés során elhízott gyermekekben
   Bokor Sz, Csernus K, Erhardt É, Decsi T, Molnár D: A béta-3-adrenoreceptor gén Trp64Arg polimorfizmusának zsírsavellátottsággal való összefüggése elhízott gyermekekben

4. Magyar Gyermekorvosok Társasága 2004. évi Nagygyűlése, Debrecen, 2004. október 7–9.
   Lányi É, Csernus K, Erhardt É, Molnár D: Keringő aktív ghrelin szint változása orális glukózterhelés során kövér gyermekekben

5. Magyar Gyermekorvosok Társasága Ifjúsági Szekció, Budapest, 2005. március 20-22.
   Csernus K, Rózsai B, Erhardt É, Kozári A, Sólyom J, Soltész Gy: Paralyticus ileus képében jelentkező hypokalaemia érdekes esete

6. 14th European Congress on Obesity, Athens, Greece, 2005. May 29–June 1.
   Lanyi E, Csernus K, Erhardt E, Molnar D. Plasma levels of active form of ghrelin during an oral glucose tolerance test in obese children (poster)

7. 46th Annual Meeting of the European Society for Pediatric Research, Siena, Italy, 2005. August 31–September 3.
   Bokor S, Csernus K, Erhardt E, Burus I, Molnar D, Decsi T: The –866A/A but not the 866G/A genotype of uncoupling protein 2 gene is associated with reduced dihomo-gamma-linolenic acid values in obese children
   Molnar D, Lanyi E, Csernus K, Erhardt E: Active ghrelin levels in childhood obesity

8. Magyar Gyermekorvosok Társasága – Magyar Gasztroenterológiai Társaság Kongresszusa, Eger, 2005. október 7-8.
   Bokor Sz, Csernus K, Erhardt É, Burus I, Molnár D, Decsi T: Az uncoupling protein 2 gén promoter régiójában lévő –866 A/A genotípus összefüggése a zsírsavellátottsággal elhízott gyermekekben

9. Fiatal Gyermekgyógyászok Konferenciája, Debrecen, 2006. április 7–8.
   Csernus K, Turai R, Ottóffy G, Kajtár P: Vállövi lágyrész terime érdekes esete

10. 16th Workshop of the European Childhood Obesity Group, Rzeszów, Poland, 2006. June 1–3.
    Bokor S, Erhardt E, Csernus K, Molnar D: Leptin receptor polymorphism in childhood obesity

11. European Society for Paediatric Gastroenterology, Hepatology and Nutrition, Dresden, Germany, 2006. June 7–10.
    Bokor S, Csernus K, Erhardt E, Molnar D, Decsi T: Fatty acid status in obese children stratified according to UCP2 and PPAR-gamma genetic polymorphisms

12. Magyar Gyermekorvosok Társasága 2006. évi Nagygyűlése, Siófok, 2006. október 6–7.
    Nagy E, Bokor Sz, Csernus K, Erhardt É, Molnár D: Gyermekkori szénhidrátanyagcsere-zavarok előfordulása (poszter)
    Répásy J, Bokor Sz, Csernus K, Erhardt É, Molnár D: Leptin-receptor-gén Gln223Arg polimorfizmus szerepe elhízott gyermekek energia leadásában (poszter)

13. European Congress on Obesity 2007, Budapest, 2007. March 22–25.
    Bokor S, Csernus K, Erhardt E, Molnar D: The Arg223Arg genotype of the leptin receptor gene is associated with obesity in Hungarian children (poster)

14. Fiatal Gyermekgyógyászok Konferenciája, Miskolc, 2007. március 23–25.
    Csernus K, Kajtár B, Kereskai L, Ottóffy G, Kajtár P: Leukémiát utánzó malignus csontvelő-infiltráció

15. 17th Annual Meeting of the European Childhood Obesity Group, Athens, Greece, 2007. July 5–7.

    Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D: Gln223Arg polymorphism of the leptin receptor gene and food-induced thermogenesis in obese boys

16. Combating Obesity: Strategies for Prevention and Intervention, Erasmus Intensive Programme, Grundlsee, Austria, 2008. February 24–March 8.

    Repasy J, Bokor S, Csernus K, Erhardt E, Molnar D: Gln223Arg polymorphism of the leptin receptor gene and food-induced thermogenesis in obese boys

17. Fiatal Gyermekgyógyászok Konferenciája, Pécs, 2008. március 28–30.

    Répásy J, Csernus K, Erhardt É, Molnár D: Energia-leadás UCP1 polimorfizmust hordozó elhízott fiú gyermekekben

18. 18th Annual Meeting of the European Childhood Obesity Group, Porto, Portugal, 2008. June 5–7.

    Repasy J, Csernus K, Erhardt E, Molnar D: Polymorphism of the UCP1 gene and food-induced thermogenesis in obese boys

19. 5-éme Congrés Annuel de la Nouvelle Société Francaise d'Athérosclérose, Biarritz, France, 2008. June 12–14.

    Bokor S, Meirhaeghe A, Erhardt É, Csernus K, Amouyel P, Molnár D, Dallongeville J: Impact of adipocytokine gene polymorphism on juvenile obesity

20. Pécsi Tudományegyetem Orvostudományi és Egészségtudományi Szakosztályának ülése, Pécs, 2009. március 16.

    Csernus K, Csábi Gy, Hollódy K, Stankovics J: Adenovírus-fertőzés ritka manifesztációi csecsemőkorban: agyvelőgyulladás, májelégtelenség

21. Fiatal Gyermekgyógyászok Konferenciája, Kőszeg, 2009. április 3–5.

    Csernus K, Csábi Gy, Stankovics J: Adenovírus-fertőzés ritka manifesztációi csecsemőkorban: agyvelőgyulladás, májelégtelenség.

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