

## Association of Adenovirus 36 Infection With Obesity-Related Gene Variants in Adolescents

L. DUŠÁTKOVÁ<sup>1,2</sup>, H. ZAMRAZILOVÁ<sup>1</sup>, I. ALDHOON HAINEROVÁ<sup>1,3</sup>,  
R. L. ATKINSON<sup>4</sup>, B. SEDLÁČKOVÁ<sup>1,2</sup>, Z. P. LEE<sup>4</sup>, J. VČELÁK<sup>1</sup>, B. BENDLOVÁ<sup>1</sup>,  
M. KUNEŠOVÁ<sup>1</sup>, V. HAINER<sup>1</sup>

<sup>1</sup>Institute of Endocrinology, Prague, Czech Republic, <sup>2</sup>Faculty of Science, Charles University, Prague, Czech Republic, <sup>3</sup>Department of Pediatrics and Center for Research of Diabetes, Metabolism and Nutrition, Third Faculty of Medicine, Charles University, Prague, Czech Republic, <sup>4</sup>Virginia Obesity Research Institute, Richmond, VA, USA

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### Summary

Both, common gene variants and human adenovirus 36 (Adv36) are involved in the pathogenesis of obesity. The potential relationship between these two pathogenic factors has not yet been investigated. The aim of our study was to examine the association of obesity susceptibility loci with Adv36 status. Genotyping of ten gene variants (in/near *TMEM18*, *SH2B1*, *KCTD15*, *PCSK1*, *BDNF*, *SEC16B*, *MC4R*, *FTO*) and analysis of Adv36 antibodies was performed in 1,027 Czech adolescents aged 13.0-17.9 years. Variants of two genes (*PCSK1* and *BDNF*) were associated with Adv36 seropositivity. A higher prevalence of Adv36 antibody positivity was observed in obesity risk allele carriers of *PCSK1* rs6232, rs6235 and *BDNF* rs4923461 vs. non-carriers ( $\chi^2=6.59$ ,  $p=0.010$ ;  $\chi^2=7.56$ ,  $p=0.023$  and  $\chi^2=6.84$ ,  $p=0.033$ , respectively). The increased risk of Adv36 positivity was also found in *PCSK1* variants: rs6232 (OR=1.67, 95 % CI 1.11-2.49,  $p=0.016$ ) and rs6235 (OR=1.34, 95 % CI 1.08-1.67,  $p=0.010$ ). *PCSK1* rs6232 and *BDNF* rs925946 variants were closely associated with Adv36 status in boys and girls, respectively ( $\chi^2=5.09$ ,  $p=0.024$ ;  $\chi^2=7.29$ ,  $p=0.026$ ). Furthermore, *PCSK1* rs6235 risk allele was related to Adv36 seropositivity ( $\chi^2=6.85$ ,  $p=0.033$ ) in overweight/obese subgroup. In conclusion, our results suggest that obesity risk variants of *PCSK1* and *BDNF* genes may be related to Adv36 infection.

### Key words

Adolescence • Obesity • Genome-wide association • Single nucleotide polymorphism • Adenovirus 36

### Corresponding author

L. Dusatkova, Obesity Management Center, Institute of Endocrinology, Národní 8, 116 94 Prague 1, Czech Republic. Fax: +420 224 905 325. E-mail: ldusatkova@gmail.com

### Introduction

The prevalence of obesity in children and adolescents remains high despite numerous recent attempts to improve strategies in prevention and treatment (Ahluwalia *et al.* 2015, Ahrens *et al.* 2014). Novel insights into the multifactorial pathogenesis of obesity have, however, been made during the last decade. The genome-wide association study (GWAS) approach has identified a number of common variants related to body mass index (BMI) and obesity. Several associations have been replicated in various populations, including in children and adolescents (den Hoed *et al.* 2010, Nead *et al.* 2015). A total of 97 gene variants (56 novel) were described by the latest GWAS in 2015 (Locke *et al.* 2015). Variations have been mostly found in or near unknown genes whose function and impact on body weight regulation still need to be elucidated (Locke *et al.* 2015). The role of human adenovirus 36 (Adv36) in the pathogenesis of obesity was first documented in experimental animal models (Dhurandhar *et al.* 2000). Adv36 infection induces adipogenesis by promoting proliferation and differentiation of preadipocytes and by increasing lipid accumulation in adipocytes (Pasarica *et al.* 2008). An association of Adv36 with increased body

weight in humans has clearly been confirmed over the last decade (Aldhoon-Hainerova *et al.* 2014, Atkinson *et al.* 2005, Shang *et al.* 2014). An important role of genetic factors in susceptibility to various infectious diseases has been demonstrated by family studies and multiple GWAS (Chapman and Hill 2012). Additionally, an impaired immunity in obesity (Tanaka *et al.* 1993) leads to a greater susceptibility to different infections (Karlsson *et al.* 2010). If the pathogenesis of obesity is partly mediated by infectious agents we may speculate whether there is a relationship between obesity susceptibility gene loci and Adv36 infection.

The aim of our study was to investigate the association of ten previously reported gene variants associated with BMI and obesity with Adv36 seropositivity and their relation to gender and body weight in Czech adolescents.

## Methods

### Subjects

A study population consisted of 1,027 Czech adolescents aged 13.0-17.9 years (476 boys and 551 girls; of them 540 were normal weight and 487 overweight/obese) who were selected from the original Childhood Obesity Prevalence and Treatment project (Aldhoon-Hainerova *et al.* 2014). Only those with complete data on anthropometric parameters, genotypes and Adv36 antibody assessment were included in the present study. All participants and their parent(s) signed an informed consent before the initiation of study procedures. The study protocol was approved by the Ethical Committee of the Institute of Endocrinology in Prague and was in accordance with the Helsinki declaration II.

### Anthropometric assessment

Body height, body weight, BMI and its z-score were assessed as described previously (Aldhoon-Hainerova *et al.* 2014). Overweight and obesity was defined as BMI  $\geq$  90<sup>th</sup> percentile according to the Czech BMI references specified for sex and age (Kobzova *et al.* 2004).

### Genotyping

Ten single nucleotide polymorphisms (SNPs) – rs7561317 (transmembrane protein 18 gene, *TMEM18*), rs7498665 (SH2B adaptor protein 1 gene, *SH2B1*), rs29941 (potassium channel tetramerisation domain

containing 15 gene, *KCTD15*), rs6232 and rs6235 (proprotein convertase subtilisin/kexin type 1 gene, *PCSK1*), rs925946 and rs4923461 (brain-derived neurotrophic factor gene, *BDNF*), rs10913469 (SEC16 homolog B *S. cerevisiae* gene, *SEC16B*), rs17782313 (melanocortin 4 receptor gene, *MC4R*) and rs9939609 (fat mass and obesity associated gene, *FTO*) were studied. SNPs in both the *PCSK1* (rs6232, rs6235) and the *BDNF* (rs925946, rs4923461) were analyzed separately due to their incomplete linkage (Dusatkova *et al.* 2013). Genotyping was performed using the TaqMan SNP Genotyping Assays (Applied Biosystems, Waltham, MA, USA) on a Biomark (Fluidigm, South San Francisco, CA, USA) and LightCycler 480 (Roche, Basel, Switzerland) and described in detail by Dusatkova *et al.* (2013). In the present study all SNPs followed the Hardy-Weinberg equilibrium (evaluated by chi-square test,  $p > 0.05$ ).

### Adv36 antibody assessment

The detection of Adv36 antibodies from serum was done by a competitive enzyme-linked immunosorbent assay developed by Obetech, LLC (Richmond, VA, USA).

### Statistical analyses

Characteristics of Adv36 negative and Adv36 positive groups were compared using chi-squared test for descriptive variables and nonparametric Mann-Whitney test for quantitative variables (expressed as medians with lower and upper quartiles). Chi-squared tests and odds ratios (ORs) were calculated to determine associations of Adv36 seropositivity with genotypes and alleles, respectively. Analyses with genotypes were performed in the whole cohort as well as in subgroups stratified by gender and body weight. When the number of minor homozygotes was less than five, they were combined with heterozygotes for such a genotype. Statistical software NCSS 2004 (NCSS, LLC, Kaysville, UT, USA) was used. P-value (two-tailed)  $< 0.05$  was considered statistically significant.

## Results

Of the total 1,027 adolescents, 26.4 % presented with positive Adv36 antibodies. The basic characteristics of the cohort based on Adv36 antibody status are shown in Table 1. A significantly higher prevalence of Adv36 antibody positivity was found in girls as compared to

boys (Table 1). Genotype distributions and risk allele frequencies according to Adv36 status are presented in Table 2. Obesity risk allele carriers of *PCSK1* rs6232, *PCSK1* rs6235 and *BDNF* rs4923461 had significantly higher prevalence of Adv36 positivity than non-carriers (Table 2). Also the other *BDNF* variant rs925946 had a borderline association with Adv36 infection (Table 2). Furthermore, both minor alleles of *PCSK1* increased chances of having positive Adv36 antibodies (Table 2). Analyses taking into account gender revealed a higher prevalence of Adv36 positivity in males carrying risk *PCSK1* rs6232 ( $\chi^2=5.09$ ,  $p=0.024$ ) and in females carrying risk allele *BDNF* rs925946 ( $\chi^2=7.29$ ,  $p=0.026$ ). When stratified by body weight, the relation of *PCSK1* rs6235 with Adv36 was confirmed in the overweight/obese subgroup ( $\chi^2=6.85$ ,  $p=0.033$ ).

**Table 1.** Characteristics of the study population according to the presence of Adv36 antibodies.

Characteristics	Adv36-	Adv36+	p
<b>All (n=1027)</b>	756 (73.6%)	271 (26.4%)	
<i>Boys/girls</i>	383/373	93/178	<0.001 <sup>a</sup>
<i>Age (years)</i>	15.7 (14.5; 16.8)	15.8 (14.7; 16.9)	0.445 <sup>b</sup>
<i>BMI z-score</i>	0.5 (-0.1; 2.6)	1.5 (0.1; 2.8)	0.027 <sup>b</sup>
<i>Overweight</i>	333 (44.1%)	154 (56.8%)	<0.001 <sup>a</sup>
<b>Boys (n=476)</b>	383 (80.5%)	93 (19.5%)	
<i>Age (years)</i>	15.6 (14.4; 16.7)	15.9 (15.1; 16.9)	0.134 <sup>b</sup>
<i>BMI z-score</i>	0.5 (0; 2.6)	1.3 (0.1; 2.7)	0.227 <sup>b</sup>
<i>Overweight</i>	171 (44.7%)	50 (53.8%)	0.114 <sup>a</sup>
<b>Girls (n=551)</b>	373 (67.7%)	178 (32.3%)	
<i>Age (years)</i>	15.9 (14.8; 16.9)	15.7 (14.6; 17)	0.560 <sup>b</sup>
<i>BMI z-score</i>	0.5 (-0.1; 2.7)	1.6 (0; 2.8)	0.082 <sup>b</sup>
<i>Overweight</i>	162 (43.4%)	104 (58.4%)	0.001 <sup>a</sup>

Quantitative data are described as medians (lower; upper quartiles). <sup>a</sup>p-values were derived from chi-squared tests, <sup>b</sup>from Mann-Whitney tests. Adv36-, adenovirus 36 antibody negative; Adv36+, adenovirus 36 antibody positive; BMI, body mass index.

## Discussion

Our association study of 1,027 Czech adolescents is the first to investigate the relationship between obesity susceptibility loci and Adv36 antibody status. As all identified GWAS loci are able to explain not more than 2.7 % of variation in BMI and their predictive value is low (Locke *et al.* 2015), an elucidation of these mostly unknown genes in pathophysiology of obesity could be

valuable. Of ten examined gene variants, variants of two genes showed some relation to the presence of Adv36 antibodies. The obesity risk alleles rs6232 and rs6235 of *PCSK1* and rs4923461 of *BDNF* were associated with a higher prevalence of Adv36 seropositivity in both genders regardless of body weight. The same tendency was observed for rs925946 of *BDNF* but only in girls. *PCSK1* encodes a protease from the subtilisin-like proprotein convertase family that process polypeptide hormones and neuropeptide precursors (Jansen *et al.* 1995). The rs6232 variant encodes a substitution N221D that reduces catalytic activity of the protease and leads to obesity, while the rs6235 (S690T) had no effect on catalytic activity (Benzinou *et al.* 2008). *BDNF*, a member of the nerve growth factor family essential for survival of striatal neurons in the brain, is involved in hypothalamic regulation of energy balance through the leptin-melanocortin signaling pathway (Nicholson *et al.* 2007). Both, rs4923461 and rs925946 are intergenic variants localized within the *BDNF* antisense non-coding transcript. It was shown that inhibition of this transcript upregulates *BDNF* mRNA (Modarresi *et al.* 2012), thus we could speculate that examined *BDNF* variants influence *BDNF* expression and consequently energy homeostasis. The minor allele of *PCSK1* rs6235 was significantly more prevalent in Adv36 positive overweight/obese subgroup, but not in normal weight counterparts. In obese subjects, particularly in those extremely obese, a more severe course of infectious diseases has been shown (Almond *et al.* 2013, Garcia *et al.* 2015). An association between obesity risk alleles and Adv36 seropositivity may partly be due to a greater susceptibility of obese individuals to infection. In addition, the combined effect of Adv36 infection with obesity risk genotype in the development of obesity should also be considered. An expression of *PCSK1* and *BDNF* is mainly situated to hypothalamic regions and mutations in these genes cause rare monogenic forms of early onset obesity (Hinney *et al.* 2014). The adipogenic effect of Adv36 studied in rats was also confirmed in the central nervous system (Pasarica *et al.* 2006). Moreover, Adv36 reduces leptin production (Vangipuram *et al.* 2007). Until now we may only speculate if these gene variants influence the susceptibility to Adv36 infection and consequently body weight. Nevertheless, we would point out the important role of genetic susceptibility as well as the role of Adv36 infection in body weight regulation. The major limitation of our study is the size of our cohort, which may not be sufficiently powered to detect low effects of common gene variants.

**Table 2.** Associations of ten gene variants with the presence of Adv36 antibodies.

Variant	Genotypes (n)			RAF (%)	$\chi^2$ p	OR (95 % CI) p
<b><i>TMEM18</i> rs7561317</b>	<b>AA</b>	<b>AG</b>	<b>GG</b>	<b>G</b>		
Adv36-	13	202	541	84.9	3.17	0.79 (0.61; 1.03)
Adv36+	7	85	179	81.7	0.205	0.095
<b><i>SH2B1</i> rs7498665</b>	<b>AA</b>	<b>AG</b>	<b>GG</b>	<b>G</b>		
Adv36-	254	375	127	41.6	3.74	1.11 (0.91; 1.35)
Adv36+	91	121	59	44.1	0.154	0.338
<b><i>KCTD15</i> rs29941</b>	<b>AA</b>	<b>AG</b>	<b>GG</b>	<b>G</b>		
Adv36-	72	339	345	68.1	1.17	0.94 (0.76; 1.15)
Adv36+	32	117	122	66.6	0.557	0.571
<b><i>PCSK1</i> rs6232</b>	<b>CC</b>	<b>CT</b>	<b>TT</b>	<b>C</b>		
Adv36-	2	65	689	4.6	6.59 <sup>a</sup>	1.67 (1.11; 2.49)
Adv36+	1	38	232	7.4	0.010	0.016
<b><i>PCSK1</i> rs6235</b>	<b>CC</b>	<b>CG</b>	<b>GG</b>	<b>G</b>		
Adv36-	427	291	38	24.3	7.56	1.34 (1.08; 1.67)
Adv36+	131	117	23	30.1	0.023	0.010
<b><i>BDNF</i> rs925946</b>	<b>GG</b>	<b>GT</b>	<b>TT</b>	<b>T</b>		
Adv36-	387	315	54	28.0	5.79	1.16 (0.93; 1.43)
Adv36+	135	104	32	31.0	0.055	0.202
<b><i>BDNF</i> rs4923461</b>	<b>AA</b>	<b>AG</b>	<b>GG</b>	<b>A</b>		
Adv36-	484	225	47	78.9	6.84	1.17 (0.91; 1.50)
Adv36+	176	89	6	81.4	0.033	0.247
<b><i>SEC16B</i> rs10913469</b>	<b>CC</b>	<b>CT</b>	<b>TT</b>	<b>C</b>		
Adv36-	24	233	499	18.6	0.21	1.05 (0.82; 1.35)
Adv36+	10	85	176	19.4	0.899	0.735
<b><i>MC4R</i> rs17782313</b>	<b>CC</b>	<b>CT</b>	<b>TT</b>	<b>C</b>		
Adv36-	63	295	398	27.8	0.74	0.99 (0.80; 1.23)
Adv36+	19	112	140	27.7	0.690	0.985
<b><i>FTO</i> rs9939609</b>	<b>AA</b>	<b>AT</b>	<b>TT</b>	<b>A</b>		
Adv36-	162	385	209	46.9	2.20	1.16 (0.95; 1.41)
Adv36+	68	138	65	50.6	0.333	0.157

Chi-squared tests ( $\chi^2$ ) were performed among genotypes, odds ratios (OR) between alleles. <sup>a</sup> Minor homozygotes and heterozygotes were combined. Adv36-, adenovirus 36 antibody negative; Adv36+, adenovirus 36 antibody positive; CI, confidence interval; RAF, risk allele frequency.

In conclusion, our results suggest that obesity susceptibility loci of *PCSK1* and *BDNF* genes may be related to the status of Adv36 antibodies and consequently to body weight. However, to validate our findings, further investigations in larger cohorts are required.

### Conflict of Interest

Dr. R. Atkinson is the owner and Dr. Z. Lee is Laboratory Director of Obetech, LLC, a company that provides assays for adenoviruses that produce obesity and has

several patents for diagnostic assays and vaccines in the area of virus-induced obesity. Dr. Atkinson is a Co-Editor of the International Journal of Obesity. L. Dušátková, H. Zamrazilová, I. Aldhoon Hainerová, B. Sedláčková, J. Včelák, B. Bendlová, M. Kunešová and V. Hainer declare no conflict of interest.

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