Strong Gender-Specific Additive Effects of the *NYD-SP18* and *FTO* Variants on BMI Values

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Summary

The role of the *FTO* gene in obesity development is well established in populations around the world. The *NYD-SP18* variant has been suggested to have a similar effect on BMI, but the role of this gene in determining BMI has not yet been verified. The objective of our study was to confirm the association between *NYD-SP18* rs6971019 SNP and BMI in the Slavic population and to analyze i) the gender-specific effects of *NYD-SP18* on BMI and ii) the simultaneous effect of *FTO* rs17817449 and *NYD-SP18* on BMI. We analyzed a sample of a large adult population based on the post-MONICA study (1,191 males and 1,368 females). Individuals were analyzed three times over 9 years. *NYD-SP18* rs6971019 SNP is related to BMI in males (2000/1 GG 28.3±3.7 kg/m^2 vs. +A 27.5±3.7 kg/m^2 $P<0.0005$; in other examinations $P<0.05$ and <0.005), but not in females (all P values over 0.48 in all three examinations). Further analysis revealed the significant additive effect (but not the interaction) of *FTO* and *NYD-SP18* SNPs on BMI in males (all $P<0.01$). These results suggest that association between *NYD-SP18* rs6971019 SNP and BMI may be restricted to males. Furthermore, variants within *NYD-SP18* and *FTO* genes revealed a significant additive effect on BMI values in males.

Key words

FTO • *NYD-SP18* • BMI • Polymorphism

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Introduction

Obesity is one of the serious non-communicable diseases, which further increases the risk of cardiovascular diseases, cancer and diabetes development. In the most affected populations, about 60 % of individuals are overweight or obese and prevalence of obesity is significantly increasing even in children (Pastucha *et al.* 2013, Vrablik *et al.* 2014). Generally, overweight and obesity result from a positive energy balance, caused by the combination of low physical activity and high-energy intake. However, "non-traditional" factors like sleeping deficits, side effects of commonly prescribed drugs (mainly insulin, hypertensives, hormonal contraceptives) or social factors can significantly contribute and should not be omitted (Hubacek 2009, Adamkova *et al.* 2009, McAllister *et al.* 2009).

Body weight/body mass index (BMI) is also significantly influenced by genetic factors. Twin studies have estimated that genetic factors can be responsible for even about 60 % of body mass index (BMI) variability. However, despite intensive efforts in recent decades, BMI heritability is still poorly understood and remains hidden within the context of concrete genes and variants.

Using the genome-wide analysis approach, two genes (among others), with largely unknown mechanisms by which they may influence the risk of obesity development, have been detected – *FTO* and *NYD-SP18*.

The association between BMI and *FTO* ("fat mass and obesity-associated gene") was discovered by

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many groups simultaneously (Dina *et al.* 2007, Frayling *et al*. 2007, Scuteri *et al*. 2007). FTO has a potential to demethylate DNA (Gerken *et al.* 2007) and function as a possible transcriptional cofactors (Wu *et al.* 2010). To date, the general consensus is that *FTO* variants within the first intron are the strongest determinants of BMI among different ethnic groups around the world (reviewed by Cheung and Yeo 2010, Dlouha and Hubacek 2014). The association between rs17817449 SNP and BMI has also been confirmed in the Czech population (Hubacek *et al.* 2009, Dušátková *et al.* 2013). However, studies that have focused on the functional association between *FTO* and BMI reveal no clear results. Thus, it is not completely clear whether *FTO* affects BMI through its effect on physical activity, basal metabolism or energy intake (Berentzen *et al.* 2008, Cecil *et al.* 2008, Haupt *et al.* 2009, Hubacek *et al.* 2011, Liu *et al*. 2010, Harbron *et al*. 2014).

In contrast, *NYD-SP18* (a protein with a largely unknown function except for its role in testes development *via* its influence on circulating testosterone levels) has been identified using fine mapping of the region on chromosome 7, widely known for its high LOD score for obesity. In an original study (the NHLBI Family Heart Study; replicated in the Framingham study), the effect of the *NYD-SP18* rs6971091 variant on BMI was even larger than the effect of the widely-analyzed 1st intron *FTO* variants (Wilk *et al.* 2008). However, the effect of *NYD-SP18* on BMI was never confirmed or disproved in later studies.

The aim of our study was to confirm the original finding of the association between *NYD-SP18* and BMI and to analyze the possible *FTO – NYD-SP18* interaction in determining BMI.

Materials and Methods

Analyzed subjects

We analyzed *NYD-SP18* SNP rs6971091 in a group of representatively and randomly selected adult individuals with the known *FTO* genotype, rs17817449 (1,191 males and 1,368 females, aged 25-64 years at the age of first examination; mean age of 49.0±10.7 years) (Hubacek *et al*. 2009, 2015). The individuals had participated in the post-MONICA study (Cifkova *et al*. 2010). The WHO MONICA Project protocol (Tunstall-Pedoe *et al*. 2003) was aligned to examine risk factors of cardiovascular disease development, including BMI and WHR. The subjects were examined in 9 Czech districts (Kromeriz, Chrudim, Cheb, Jindrichuv Hradec, Pardubice, Litomerice, Plzen, Prague East, and Benesov) in 1997/1998 and were completely re-examined in 2000/2001 (cohort from 2000/2001) and mostly (95.1 %) also in 2007/8. Written, informed consent was given by all individuals. The study was approved by the institute's Ethics Committee at the Institute for Clinical and Experimental Medicine, Prague and is in agreement with the Helsinki Declaration of 1975.

Genotyping

Genomic DNA was extracted from peripheral blood white cells using a standard salting-out method (Miller *et al.* 1988).

NYD-SP18 SNP rs6971091 was genotyped using the PCR-RFLP technique. All PCR chemicals were obtained from Fermentas International Inc., Burlington, Ontario, Canada and PCR reactions were performed on a PCR device – DYAD Disciple (MJ Research). Briefly, DNA was amplified in a total volume of 25 µl with the oligonucleotides, 5' aag gcc tta acc acc tgg ttc tgc and 5' cct tgg tca tta gct gaa tga gaa gct. The final PCR product (105 bp) was cut with 5 units of the restriction enzyme, HindIII (Fermentas International Inc., Burlington, Ontario, Canada) and restriction fragments were separated on 10 % polyacrylamide gel using the MADGE platform (Day *et al.* 1996). Restriction fragments, 26 bp and 79 bp, represented the minor A-allele, while the presence of the uncut product represented the major G-allele.

FTO SNP rs17817449 has already been analyzed in more detail, as described previously (Hubacek *et al.* 2008, 2009).

Statistical analysis

Deviations in Hardy-Weinberg equilibrium were tested using the following link: http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls.

ANOVA was used for statistical analyses. Males and females were analyzed separately. For individual gene analyses, we pooled minor AA homozygotes (7.2 %) and GA heterozygotes together because of the relative low frequency of the minor A-allele of rs6971019 homozygotes. A P value of 0.05 was defined as significant.

We analyzed the simultaneous effect of the *NYD-SP18* and *FTO* variants in three subgroups defined by the presence of different numbers of risky alleles

(G for *FTO* rs17817449 and G for *NYD-SP18* rs6971091) – allele combinations of three and more, exactly two, or less than two. For this analysis, only men with records of both genotypes and BMI at all three examinations were included (N=1,034).

Results

Basic characteristics of examined individuals at the 2000/2001 examination are presented in Table 1.

Table 1. Baseline characteristics for individuals analyzed in 2000/2001.

	Post-MONICA Males	Post-MONICA Females
N	1,191	1,368
Age (years)	49.2 ± 10.8	48.8 ± 10.6
BMI (kg/m^2)	28.2 ± 4.0	27.6 ± 5.5
WHR	0.929 ± 0.064	0.810 ± 0.072
Cholesterol (mmol/l)	5.75 ± 1.06	5.80 ± 1.15
Triglycerides (mmol/l)	1.98 ± 1.28	1.46 ± 0.85
HDL-cholesterol (mmol/l)	1.26 ± 0.33	1.50 ± 0.36
Diabetes (%)	6.0	4.4
Hypertension (%)	41.1	33.4
Smoking prevalence (%)	32.7	25.4

Call rates (calculated for individuals examined in 1998/9 and 2000/1, respectively) were 96.4 % for *FTO* and 97.8 % for *NYD-SP18* SNPs.

Distributions of individual genotypes were in Hardy-Weinberg equilibrium. Frequencies of the individual alleles and genotypes were similar to the frequencies observed in other Caucasian populations (for example Dina *et al.* 2007, Frayling *et al.* 2007, Scuteri *et al.* 2007, Dušátková *et al.* 2013, Wilk *et al*. 2008).

As described before in the Czech post-MONICA population study, we detected a significant association (P<0.02) between *FTO* genotypes and BMI in males in cases where a co-dominant model of analysis was used (Table 2). In females, the effect progressed in the same direction but the observed differences did not reach statistical significance (Hubacek *et al*. 2009), which suggests that age or menopausal status in females is a significant determining confounder of BMI through the *FTO* gene. Results for females were similar in all three examinations.

Furthermore, similar gender-specific results were also obtained in the case of the *NYD-SP18* polymorphism. We confirmed the findings from the original study (Wilk *et al.* 2008) in males only and detected higher BMI values in *NYD-SP18* GG homozygotes than in A-allele carriers (GG 28.3±3.7 kg/m^2 vs. +A 27.5±3.7 kg/m^2; P<0.0005). Similar significant differences were observed in both other examinations (P<0.05 and <0.005, respectively) (Table 2). In females, no effect of rs6971091 within the *NYD-SP18* gene on BMI values in any of the three examinations was observed (all P values over 0.48; results are not shown in detail).

Table 2. Genotype frequencies of analyzed individuals and the association between *FTO* rs17817449 and *NYD-SP18* rs6971019 polymorphisms and BMI values (kg/m^2) in the Czech post-MONICA study of 2000/2001.

rs17817449	**N**	**%**	**BMI**	**p**
Males				
GG	177	15.9	28.7 ± 3.7	
GT	572	51.4	28.3 ± 4.1	0.014
TT	364	32.7	27.8 ± 3.9	
Females				
GG	249	18.4	27.9 ± 5.7	
GT	652	48.1	27.5 ± 5.4	n.s.
TT	453	33.5	27.4 ± 5.0	
rs6971019	**N**	**%**	**BMI**	**p**
Males				
GG	630	53.6	28.3 ± 3.7	0.0005
+A	545	46.4	27.5 ± 3.6	
Females				
GG	696	52.4	27.4 ± 5.3	n.s.
+A	632	47.6	27.5 ± 5.4	

In males, we further analyzed whether the combination between the *FTO* rs17817449 and *NYD-SP18* rs6971091 polymorphisms had an influence on BMI or whether it had an additive effect, or whether these SNPs exhibited any interaction. Distinct combinations, based on the number of risky alleles of

FTO and *NYD-SP18* SNPs, revealed a significant effect on BMI in the additive model (all three P values were over 0.004; all three analyses remained significant after Bonferroni correction for multiple testing). However, no interaction (all three P values were over 0.56) between the analyzed SNPs was found (Table 3). Briefly, carriers of at least three risky alleles had the highest BMI values and carriers of at least three protective alleles had the lowest, with carriers of exactly two risky and two protective alleles exhibiting medium values (P<0.0001 in 2000/2001; for more details see Table 3). Adjustment for age did not change the differences significantly.

Table 3. Additive effects of the *FTO* rs17817449 and *NYD-SP18* rs6971019 polymorphisms on BMI (kg/m^2) in males from the 2000/2001 Czech post-MONICA study, according to subgroups with different numbers of risky alleles.

***FTO/NYD-SP18* genotypes**	**N**	**BMI**	**P<**
GG/GG and GG/GA and GT/GG	393	28.8 ± 4.1	
GG/AA and TT/GG and GT/GA	442	27.9 ± 3.9	0.0001
GT/AA and TT/GA and TT/AA	199	27.3 ± 3.9	

Discussion

Body weight is a polygenic trait, which means that many variants within dozens of genes can significantly affect final individual values (Day and Loos 2011). It is estimated that single negative alleles increase body weight to a maximum of about one kilogram (*FTO*, *NYD-SP18* genes). However, most of the risky alleles add about 200-300 g "only". Some studies have focused on gene score in the context of BMI association (Peterson *et al.* 2011, Domingue *et al*. 2014). However, knowledge is sparse in the following areas: simultaneous presence of more risky alleles; additive effects and interaction; the critical number of risky alleles that leads to obesity in all cases; and at what stage alleles appear.

In our study, we analyzed the simultaneous effect of the two major BMI determinants, namely the genes for FTO and NYD-SP18. Briefly, we confirmed that the simultaneous presence of the distinct alleles of these genes is associated with enhanced BMI values. Importantly, we detected that there is a significant gender-specific effect of these genes on BMI. We also found a significant association between the variants of these genes and BMI in males only.

Dozens of papers have confirmed the role of the *FTO* gene in genetic determination of obesity (Hubacek *et al.* 2008, Dušátková *et al*. 2013, Tönjes *et al*. 2010, Hakanen *et al*. 2009) and many studies have focused on similar topics, e.g. determination of body mass changes after life style interventions (Dlouha *et al*. 2011, Schum *et al*. 2012, Zlatohlavek *et al*. 2014, Reinehr *et al*. 2014). Finally, the *FTO* gene has also been associated with enhanced risk of cardiovascular disease (Hubacek *et al*. 2010, Doney *et al*. 2009), renal failure (Hubacek *et al*. 2012), type 2 diabetes (Scott *et al.* 2007), Alzheimer's disease (Reitz *et al*. 2012), suicide (Chojnicka *et al.* 2014) and even with infection diseases, such as tuberculosis (Feng *et al*. 2014).

In relative contrast to the well-established role of *FTO*, our study is the first to confirm the role of the *NYD-SP18* rs6971091 polymorphism in BMI determination, as suggested by Wilk *et al.* (2008). Furthermore, we have recently presented data suggesting that this gene could be a significant predictor of body composition changes in adult females after intensive ten-week life-style interventions, based on intensive physical activity and age-adjusted optimal energy intake (Suchanek *et al*. 2015). This finding is of interest mainly because BMI is not strongly affected by this variant at the female population level.

Interestingly, in both genes, details in relation to the mechanism of action are unclear. It has been suggested that *FTO* first intron variants may have effects on epigenetic changes (Almén *et al.* 2012), transcriptional activity (Wu *et al*. 2010), RNA modification (Berulava *et al*. 2013) and leukocyte telomere length (Dlouha *et al*. 2012), which, surprisingly, point to some very heterogeneous regulatory effects. The function of the *NYD-SP18* gene is largely unknown, although it has been suggested that it may influence testosterone production.

The major limitation of our study is that we did not have a confirmatory group of individuals at our disposal. However, our subjects were examined three times within the nine-year period, of which all analyses led to identical conclusions, thus lowering the risk of false-positive results.

We conclude that the *NYD-SP18* rs6971091 polymorphism could be a significant genetic determinant of BMI values in males, but not in females. Furthermore, we observed an additive effect on BMI between the *FTO* rs17817449 and *NYD-SP18* rs697109 genetic variants in males, which underlines the importance of the simultaneous context-dependent analyses of variants in multiple genes.

Conflict of Interest

There is no conflict of interest.

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