

Short Communication

***INSIG2* G-102A Promoter Variant Exhibits Context-Dependent Effect on HDL-Cholesterol Levels but Not on BMI in Caucasians**

(*INSIG2* / BMI / SNP / WHR / HDL cholesterol)

J. A. HUBÁČEK^{1,2,3}, P. SUCHÁNEK^{1,2}, V. LÁNSKÁ¹, J. PÍTHA^{1,2}, V. ADÁMKOVÁ^{1,3}

¹Institute for Clinical and Experimental Medicine, Prague, Czech Republic

²Centre for Cardiovascular Research, Prague, Czech Republic

³University of South Bohemia in České Budějovice, Faculty of Health and Social Studies, České Budějovice, Czech Republic

Abstract. The *INSIG2* (*INSIG2* is primarily involved in the regulation of fatty acid and cholesterol synthesis) gene is suggested to be obesity related. An *INSIG2* promoter variant, G-102A, has been detected and was demonstrated to be of potential functional significance. In two cohorts of middle-aged men, the association between this variant and BMI was suggested. We sought to replicate the association between the *INSIG2* G-102A variant and BMI in three large Slavonic Caucasian populations. Further, we analysed the possible effect of this variant on BMI changes in a short-time intervention study. One thousand ninety-nine males and 1368 females (population-based Czech MONICA three-year cohort), 908 females from the 3PMFs study, together with 94 overweight (BMI > 27 kg/m²) females who underwent nine weeks of dietary/exercise intervention were genotyped for the *INSIG2* G-102A variant using PCR-RFLP analysis. We could not detect any association between the *INSIG2* G-102A variant and BMI or WHR with or without adjusting for age and gender in any population. Neither the BMI change nor anthropometric and lipid parameter changes

were affected by the *INSIG2* G-102A gene variant in intervened overweight females. However, MONICA females (but not males) carrying the common GG genotype had higher plasma levels of HDL cholesterol (GG homozygotes 1.51 ± 0.36 mmol/l vs. A allele carriers 1.45 ± 0.33; P < 0.05) in both surveys. Our results indicated that the G-102A *INSIG2* polymorphism has no consistent effect on BMI in general populations, but could influence HDL cholesterol in females.

Introduction

The incidence of obesity is in excess of 15 % in almost all populations, but reaching about 30 % in the middle and east European countries as well as in Americans (MacDonald, 2007). Both external (primarily excess energy intake and lack of exercise but for example also sleeping habits, non-exercise activity thermogenesis and more stable inside room temperatures) (Hubáček, 2009) and genetic factors contribute to overweight development and it is estimated that the heritability of obesity is at least 30 % (Mutch and Clement, 2006).

Originally, a variant (rs7566605) within the insulin-induced gene 2 (*INSIG2*) was associated with increased risk of obesity in some but not all studies (Herbert et al., 2006; Lyon et al., 2007; Smith et al., 2007; Hubáček et al., 2010). Finally, a large meta-analysis has not detected an overall association of this polymorphism with obesity but suggests that heterogeneous effects from different study designs may mask an underlying association with extreme degrees of obesity (Heid et al., 2009). Functional polymorphism (G-102A) in the promoter of *INSIG2* gene was defined later and the possible relationship to the BMI was suggested after analysis of two cohorts of highly preselected men (50-year old men and mix of myocardial infarction survivors and age-matched controls) (Krapivner et al., 2008).

Received August 23, 2010. Accepted September 21, 2010.

This work was supported by projects Nos. 1M0510 (Ministry of Education, Youth and Sports of the Czech Republic), 00023001 and NS/10511-3 (Ministry of Health of the Czech Republic). Interventional part of the study was supported by project No. NS 10513-3/2009 (IGA, Ministry of Health of the Czech Republic).

Corresponding author: Jaroslav A. Hubáček, IKEM-DEM-LMG, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic. Phone: (+420) 261 363 367; Fax: (+420) 241 721 666; e-mail: jahb@ikem.cz

Abbreviations: 3PMFs – Prague Pre- and Post- Menopausal Females study, BMI – body mass index, HDL – high-density lipoprotein, *INSIG2* – insulin-induced gene 2, PCR – polymerase chain reaction, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, WHR – waist-hip ratio.

The *INSIG2* (OMIM acc N. 608660) gene encodes a protein which binds to the SCAP/SREBP protein complex and causes its retention in the endoplasmic reticulum. *INSIG2* has the potential to block synthesis of fatty acids (and cholesterol) (Yabe et al., 2002), and thus is a promising candidate gene for BMI determination. Also the chromosome region where *INSIG2* is located was identified as obesity-associated by linkage study in humans (Liu et al., 2004). Finally, functional analysis revealed a marked increase in the expression of *INSIG2* during the adipocyte differentiation (Krapivner et al., 2008).

We have analysed the putative association between the *INSIG2* G-102A variant and BMI and WHR in three Slavonic Caucasian populations. Further, the same parameters were correlated with this variant in females under intervention leading to body weight loss.

Material and Methods

Groups of 1191 males and 1368 females (aged 25–65 years) were selected according to the WHO protocol (*Multinational monitoring of trends and determinants in cardiovascular diseases: "MONICA Project"*. Manual of operations WHO/MNC 82.2, Nov. 1983). The individuals were recruited in 1997–1998 and re-invited in 2000–2001. Anthropometrical measurements (height, waist and hip) and lipid parameters (total and HDL cholesterol, triglycerides) were available in both surveys.

Further, 5 % representative random sample of the population consisting of 29,440 women aged 45–54 years living in the Prague-4 district (Prague Pre- and Post- Menopausal Females - 3PMFs study) was selected from the registers of health insurance companies. Out of 1,472 women, 908 agreed to be examined.

Finally, we have analysed 98 unrelated overweight Czech females. The selection criteria were BMI over 27 kg/m² (average BMI 32.3 ± 4.4 kg/m²) and age over 25 years (average age 37.9 ± 9.0 years) with no medication. During the nine weeks of intervention, all volunteers adopted diet changes consisting of a decrease of energy to the optimal, age-matched, level (max. 8000 kJ/day, achieved mean was 7200 kJ/day) and saturated fat intakes (max. 30 % of daily energetic intake) and simultaneously increases of physical activity (4 times/week one hour, 3 times with trainer in fitness centre and once outdoor activity – walking or cycling). These interventions were weekly and individually controlled by nutritionists and personal trainers (Dvořáková-Lorenzová et al., 2006; Suchanek et al., 2008). Written informed consent was obtained from all study participants and the ethics committee approved the design of the studies.

DNA was extracted using the salting out method (Miller et al., 1988). Oligonucleotides *INSIG-F* 5' ggt cag caa aca aca gca ga and *INSIG-R* 5' gta ccc cct acc gcc tct t and restriction enzyme Eco47I were used for *INSIG2* G-102A SNP genotyping.

Deviations from Hardy-Weinberg equilibrium were tested using the χ^2 test (<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>). Differences in BMI and WHR between the subgroups according to the *INSIG2* genotype were tested by ANOVA. For the analysis, AA homozygotes were pooled with GA heterozygotes.

Results and Discussion

Allelic frequencies at the G-102A SNP were not different between the analysed groups and were almost identical as in the so far analysed populations. Genotype frequencies in the pooled sample were in Hardy-Weinberg equilibrium and their frequencies were as follows – 84.2 % for GG, 15.1 % for GA and 0.7 % for AA carriers. There was no significant association between G-102A genotypes and BMI (GG homozygotes 28.0 ± 3.9 kg/m² vs. A allele carriers 27.7 ± 3.6 kg/m² for males; 27.2 ± 5.5 kg/m² vs. 27.7 ± 5.2 kg/m² for MONICA females and 26.0 ± 4.9 kg/m² vs. 26.1 ± 4.9 kg/m² for 3PMFs females) or WHR (GG homozygotes 0.93 ± 0.06 vs. A allele carriers 0.93 ± 0.07 for males; 0.82 ± 0.07 vs. 0.81 ± 0.07 for MONICA females and 0.84 ± 0.07 vs. 0.85 ± 0.07 in 3PMFs females) regardless of whether the analysis was performed with unadjusted or adjusted variables (adjustment for age). All results were insignificant in all five analyses performed (for the MONICA study, only the results from 2000/2001 are presented, both surveys were analysed), despite the fact that rare homozygotes showed slightly lower (non-significant) BMI values, similarly to the original finding.

In both MONICA surveys, significant association between the GG genotype and elevated HDL cholesterol was detected in females (GG homozygotes 1.51 ± 0.36 mmol/l vs. A allele carriers 1.45 ± 0.33; P < 0.05), but not in males (GG homozygotes 1.27 ± 0.33 mmol/l vs. A allele carriers 1.25 ± 0.31; P = 0.41). The same association, however, was not found in females from the 3PMFs study. The obtained difference could be explained by the fact that in contrast to the MONICA study, 3PMFs females are mostly post-menopausal.

In the interventional part of our study, there were 77 GG homozygotes, 15 GA heterozygotes and two AA homozygotes. This genotype distribution corresponds to the general population (P = 0.69). Their average weight loss was not significantly associated with *INSIG2* genotypes (Δ -6.00 ± 2.65 kg for GG homozygotes and Δ -5.96 ± 1.73 kg for A allele carriers).

In our study, we did not confirm the association between the *INSIG2* variant and BMI found in north-European males (Krapivner et al., 2008). Discrepancies between the investigated studies could not be explained by use of different ethnics. More likely, the different definitions of so far analysed populations could cause the different results. The primary finding in our study is a weak but significant and reproducible association between the *INSIG2* G-102A variant and plasma levels of HDL cholesterol in younger females but not in older fe-

males and males. This result needs to be confirmed in further studies.

Our results suggest that similarly to the primarily described rs7566605 variant, also the G-102A *INSIG2* SNP is likely to have a heterogenous effect on BMI even within the same ethnic groups, but could have a sex-specific effect on plasma HDL-cholesterol levels.

References

- Dvořáková-Lorenzová, A., Suchánek, P., Havel, P. J., Stávek, P., Karasová, L., Valenta, Z., Tintěra, J., Poledne, R. (2006) The decrease in C-reactive protein concentration after diet and physical activity induced weight reduction is associated with changes in plasma lipids, but not interleukin-6 or adiponectin. *Metabolism* **55**, 359-365.
- Heid, I. M., Huth, C., Loos, R. J., Kronenberg, F., Adamkova, V., Anand, S. S., Ardlie, K., Biebertmann, H., Bjerregaard, P., Boeing, H., Bouchard, C., Ciullo, M., Cooper, J. A., Corella, D., Dina, C., Engert, J. C., Fisher, E., Francès, F., Froguel, P., Hebebrand, J., Hegele, R. A., Hinney, A., Hoehe, M. R., Hu, F. B., Hubacek, J. A., Humphries, S. E., Hunt, S. C., Illig, T., Järvelin, M. R., Kaakinen, M., Kollerits, B., Krude, H., Kumar, J., Lange, L. A., Langer, B., Li, S., Luchner, A., Lyon, H. N., Meyre, D., Mohlke, K. L., Mooser, V., Nebel, A., Nguyen, T. T., Paulweber, B., Perusse, L., Qi, L., Rankinen, T., Rosskopf, D., Schreiber, S., Sengupta, S., Sorice, R., Suk, A., Thorleifsson, G., Thorsteinsdottir, U., Völzke, H., Vimalaswaran, K. S., Wareham, N. J., Waterworth, D., Yusuf, S., Lindgren, C., McCarthy, M. I., Lange, C., Hirschhorn, J. N., Laird, N., Wichmann, H. E. (2009) Meta-analysis of the *INSIG2* association with obesity including 74,345 individuals: does heterogeneity of estimates relate to study design? *PLoS Genet.* **5**, e1000694.
- Herbert, A., Gerry, N. P., McQueen, M. B., Heid, I. M., Pfeufer, A., Illig, T., Wichmann, H. E., Meitinger, T., Hunter, D., Hu, F. B., Colditz, G., Hinney, A., Hebebrand, J., Koberwitz, K., Zhu, X., Cooper, R., Ardlie, K., Lyon, H., Hirschhorn, J. N., Laird, N. M., Lenburg, M. E., Lange, C., Christman, M. F. (2006) A common genetic variant is associated with adult and childhood obesity. *Science* **312**, 279-283.
- Hubáček, J. A. (2009) Eat less and exercise more – is it really enough to knock down the obesity pandemic? *Physiol. Res.* **58 Suppl 1**, S1-S6.
- Hubáček, J. A., Kuthanová, L., Suchánek, P., Bohuslavová, R., Adámková, V., Lánská, V., Meitinger, T., Pfeufer, A. (2010) *INSIG2* promoter variant, obesity markers and lipid parameters – no association in a large Slavonic Caucasian population. *Folia Biol. (Praha)* **56**, 131-134.
- Krapivner, S., Popov, S., Chernogubova, E., Hellénus, M. L., Fisher, R. M., Hamsten, A., van't Hooft, F. M. (2008) Insulin-induced gene 2 involvement in human adipocyte metabolism and body weight regulation. *J. Clin. Endocrinol. Metab.* **93**, 1995-2001.
- Liu, Y. J., Xu, F. H., Shen, H., Liu, Y. Z., Deng, H. Y., Zhao, L. J., Huang, Q. Y., Dvornyk, V., Conway, T., Davies, K. M., Li, J. L., Recker, R. R., Deng, H. W. (2004) A follow-up linkage study for quantitative trait loci contributing to obesity-related phenotypes. *J. Clin. Endocrinol. Metab.* **89**, 875-882.
- Lyon, H. N., Emilsson, V., Hinney, A., Heid, I. M., Lasky-Su, J., Zhu, X., Thorleifsson, G., Gunnarsdottir, S., Walters, G. B., Thorsteinsdottir, U., Kong, A., Gulcher, J., Nguyen, T. T., Scherag, A., Pfeufer, A., Meitinger, T., Brönnner, G., Rief, W., Soto-Quiros, M. E., Avila, L., Klanderman, B., Raby, B. A., Silverman, E. K., Weiss, S. T., Laird, N., Ding, X., Groop, L., Tuomi, T., Isomaa, B., Bengtsson, K., Butler, J. L., Cooper, R. S., Fox, C. S., O'Donnell, C. J., Vollmert, C., Celedón, J. C., Wichmann, H. E., Hebebrand, J., Stefansson, K., Lange, C., Hirschhorn, J. N. (2007) The association of a SNP upstream of *INSIG2* with body mass index is reproduced in several but not all cohorts. *PLoS Genet.* **3**, e61.
- MacDonald, I. (2007) Viewpoint: obesity in Europe. *Circulation* **115**, f69-f71.
- Miller, S. A., Dykes, D. D., Polesky, H. F. (1988) A simple salting out procedure for DNA extraction from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.
- Mutch, D. M., Clement, K. (2006) Genetics of human obesity. *Best Pract. Res. Clin. Endocrinol. Metab.* **20**, 647-664.
- Smith, A. J., Cooper, J. A., Li, L. K., Humphries, S. E. (2007) *INSIG2* gene polymorphism is not associated with obesity in Caucasian, Afro-Caribbean and Indian subjects. *Int. J. Obes. (Lond)* **31**, 1753-1755.
- Suchanek, P., Lorencova, A., Poledne, R., Hubacek, J. A. (2008) Changes of plasma lipids during weight reduction in females depends on *APOA5* variants. *Ann. Nutr. Metab.* **53**, 104-108.
- Yabe, D., Brown, M. S., Goldstein, J. L. (2002) *Insig-2*, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. USA* **99**, 12753-12758.