Short Communication

INSIG2 Promoter Variant, Obesity Markers and Lipid Parameters – No Association in a Large Slavonic Caucasian Population Sample

(INSIG / BMI / polymorphism)

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Abstract. Heritability studies have estimated the genetically attributable part of body mass index variance to be in the range of 30–70 %. Rs7566650 (G>C) single-nucleotide polymorphism (SNP) near the promoter of the *INSIG2* gene has been identified as associated with body mass index. The gene product of *INSIG2* is involved in regulation of fatty acid and cholesterol synthesis. In order to replicate this association we have analysed 2,559 unrelated individuals of Slavonic Caucasian origin from the population-based Czech MONICA 3-year cohort. Body mass index, waist-hip ratio and plasma lipids (total-cholesterol, HDL-cholesterol, triglycerides) were measured at two independent examinations within three years. We could not detect any association between the SNP rs7566605 and body mass index, waist-hip ratio or lipid parameters, both with or without adjusting for age and gender. Neither the body mass index change nor lipid changes were significantly affected by the *INSIG2* gene variant. Our results indicated that this *INSIG2* polymorphism has no significant effect on body mass index and plasma lipids in the Czech Slavonic population.

Introduction

The incidence of obesity is commonly in excess of 15 % (MacDonald, 2007), and obesity is one of the major health problems in most populations around the world and a large proportion of obese individuals die from overweight-related complications. Although the development of obesity/overweight is influenced by a variety of external factors (mostly, but not exclusively, by excess energy intake and lack of exercise) (Hubáček, 2009), it was estimated that the heritability of obesity is between 30 % and 70 % (Slawik and Beuschlein, 2006).

Genetic determination of obesity was intensively analysed in the last twenty years, but it is often difficult to confirm the results in more independent populations, as could be seen, for example, for one of the promising candidate genes – the gene for ghrelin (Korbonits et al., 2002; Ukkola et al., 2002; Hubáček et al., 2007).

Following a large genome-wide screening, a common genetic variant (G>C change, rs7566605) within the regulatory part of the *INSIG2* gene was described and independently associated with increased risk of obesity (Herbert et al., 2006). Some subsequent publications studying rs7566605 in probands from several ethnic groups did confirm the original association while others did not (Hall et al., 2006; Dina et al., 2007; Kumar et al., 2007; Loos et al., 2007; Lyon et al., 2007; Rosskopf et al., 2007). The inconsistent replication of the *INSIG2* variant's effect on body mass index (BMI) may be explained by several reasons, among them the possibility

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Abbreviations: BMI – body mass index, BSA – bovine serum albumin, HDL – high-density lipoprotein, *INSIG* – insulin-induced gene, PCR – polymerase chain reaction, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, SCAP/SREBP – SREBP cleavage activating protein/sterol regulatory elements binding protein, SNP – single-nucleotide polymorphism, TBE – Tris boric acid EDTA, WHR – waist-hip ratio.

that it may be apparent in certain ethnicities or that it may be confined to certain strata of the general population defined by other genetic or environmental factors.

The insulin-induced gene 2 (*INSIG2*) encodes a protein which together with INSIG1 inhibits the synthesis of fatty acids and cholesterol (Yabe et al., 2002). INSIG2 binds to the SCAP/SREBP protein complex and causes its retention in the endoplasmic reticulum. Despite the fact that the affinity of INSIG2 to the SCAP/SREBP protein complex is lower than that of INSIG1 (Yang et al., 2002), INSIG2 still has the potential to block cholesterol synthesis and synthesis of fatty acids. *INSIG2* is a promising candidate gene for BMI determination not only because it could have an effect on the fatty acid synthesis, but also because the chromosome region where *INSIG2* is located was identified as a factor in obesity by linkage studies (Deng et al., 2002; Liu et al., 2004).

We have analysed the putative association between the *INSIG2* rs7566605 variant and BMI, WHR and plasma lipids in a Slavonic Caucasian population sample.

Material and Methods

The 2,559 unrelated Caucasians (1,191 males and 1,368 females, aged 25–65 years) included in this study represent a 3-year cohort of the selected (in nine districts) 1% Czech population sample. The individuals were recruited in 1997–1998 and reinvited in 2000–2001 according to WHO (1983). The lipoprotein parameters were measured enzymatically by the WHO Regional Lipid Reference Centre, IKEM, Prague, in a Roche COBAS MIRA autoanalyzer (Hoffmann-LaRoche, Basel, Switzerland), using conventional enzymatic methods with reagents from Hoffmann-La Roche. Lipid parameters and anthropometric measurements (weight, height, waist and hip) are available for both years. BMI was calculated as the weight in kg divided by height squared in meters. Written informed consent was obtained from the study participants and the local ethics committee approved the design of the study.

DNA was isolated from frozen EDTA blood (Miller et al., 1988). The single-nucleotide polymorphism (SNP) rs7566605 was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Briefly, oppositely oriented oligonucleotides *INSIG-F* 5' AAT ACC CCA TCG GAA TTG AAA TCA TTG and *INSIG-R* 5' AAA CCA AGG GAA TCG AGA GCT AAG G were used. Total volume of 25 µl of PCR mixture consisted of 0.4 of each oligonucleotide, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1× PCR reaction buffer, 0.05 U of Taq-DNA polymerase and ~50 ng of genomic DNA. The amplification was performed in 96-well polycarbonate plates at PCR device DYAD (MJ Research, Watertown, MA) under the following conditions – initial denaturation at 96 °C for 3 min, followed by 34 cycles of denaturation at 95 °C for 15 s, annealing at 59.2 °C for 30 s and amplification at 72 °C for 30 s. The final extension was prolonged to 3 min. Ten µl of PCR product (183 bp) was cleaved by 5 U of restriction enzyme *Mbo*I (Fermentas, Vilnius, Lithuania) together with 1.5 µl of reaction buffer R + BSA and separated on 10% MADGE gel (Day and Humphries, 1994) in 0.5x TBE buffer. The minor allele C is characterized by fragments of 119 bp and 64 bp, while an uncut fragment of 183 bp represents the major G allele.

Statistical analysis was performed by ANOVA, triglycerides were analysed with and without log transformation.

Results and Discussion

The basic characteristics of the study population in 2002 are summarized in Table 1a. Allelic frequencies at the rs7566605 SNP were not different between males and females (Table 1b) and were similar to others in so far that the analysed populations were of the same ethnicity (Caucasians). Genotype frequencies were in Hardy Weinberg equilibrium and the frequencies of the individual alleles were 67.3 % for the G and 32.7 % for the C in the entire population.

There was no significant association between rs7566605 genotypes and BMI or WHR (Table 2), regardless of whether the analysis was performed with unadjusted or adjusted variables (adjustment for age) either with the dominant (carriers of the C allele vs. GG homozygotes; $P = 0.90$ for males and $P = 0.57$ for females), codominant (CC vs. GC vs. GG; $P = 0.27$ for males and $P = 0.64$ for females) or recessive model (carriers of the G allele vs CC homozygotes; $P = 0.53$ for males and $P = 0.67$ for females). No significant associations were observed in the entire population (adjustment for age and sex) as well as when the males and females were analysed separately.

Finally, no significant differences were observed in genotype frequencies in subgroups of individuals when divided according to their BMI into three groups (BMI < 25, 25 < BMI < 30 and BMI > 30).

For plasma cholesterol and triglycerides, only results using the means across both surveys are displayed (Table 2). All statistical analyses were also performed separately for the measurements obtained in the 1997/8 and 2000/1 surveys, but the results were not significant for either survey.

The association between the *INSIG2* variant and obesity, first reported by Herbert et al. (2006), was confirmed in some populations (Lyon et al., 2007) but not in all (Hall et al., 2006; Dina et al., 2007; Kumar et al., 2007; Loos et al., 2007). Overall in about 50 % of populations analysed, the association was confirmed. The discrepancies between studies could be explained by different definitions or by use of different ethnic groups. For example, in the Indian population described by Kumar et al. (2007), obesity is defined as BMI over 25, in contrast to Caucasians, where obesity has a usual cut-off point at BMI of 30. Also longitudinal data from unrelated individuals in the Framingham Heart Study Offspring cohort across different examination cycles de-

Table 1. Basic characteristics (1a) and genotype frequencies at the INSIG rs756660 SNP (1b) of the individuals involved in the study (mean ± SD). Age, diabetes and smoking prevalence are from 1997/8, other data represent means from 1997/8 and 2000/1 surveys.

1a

	Population	Males	Females
N	2559	1191	1368
Age (years)	49.0 ± 10.7	49.2 ± 10.8	48.8 ± 10.6
BMI (kg/m^2)	27.7 ± 4.8	28.0 ± 3.9	27.3 ± 5.4
WHR	0.865 ± 0.087	0.928 ± 0.061	0.810 ± 0.065
Cholesterol (mmol/l)	5.730 ± 1.050	5.750 ± 1.020	5.710 ± 1.090
Triglycerides (mmol/l)	1.730 ± 1.130	2.040 ± 1.370	1.460 ± 0.780
Diabetes (N/%)	132/5.2	72/6.0	60/4.4
Smoking prevalence (N/%)	737/28.8	389/32.7	348/25.4

1b

	Population		Males		Females	
	N	%	N	%	N	%
G/G	1114	45.1	509	44.6	605	45.7
G/C	1089	44.2	517	45.3	572	43.2
C/C	263	10.7	116	10.1	147	11.1
CR	96.2		95.9		96.8	
HWE	0.897		0.360		0.501	
MAF	0.328		0.328		0.327	

CR – call rate, HWE – Hardy-Weinberg equilibrium, MAF – minor allele frequency

Table 2. INSIG2 rs7566650 variant, BMI (kg/m^2), WHR and plasma levels of total cholesterol (mmol/l) and triglycerides (mmol/l) in the population. Both mean values and differences between parameters obtained in 1997/8 and 2000/1 are presented. All differences are non-significant.

Genotype	N	Mean BMI	Δ BMI	Mean WHR	Δ WHR	Mean TC	Δ TC	Mean TG	Δ TG
Entire population									
G/G	1114	27.6 ± 4.8	-0.54 ± 1.59	0.865 ± 0.086	-0.002 ± 0.060	5.72 ± 1.03	-0.16 ± 0.90	1.73 ± 1.00	-0.07 ± 1.00
G/C	1089	27.6 ± 4.7	-0.54 ± 1.70	0.866 ± 0.088	-0.002 ± 0.056	5.73 ± 1.08	-0.08 ± 0.89	1.74 ± 1.28	-0.07 ± 0.94
C/C	263	27.7 ± 4.9	-0.48 ± 1.61	0.862 ± 0.082	0.005 ± 0.060	5.75 ± 1.04	-0.09 ± 0.91	1.74 ± 1.01	-0.02 ± 0.88
P		0.75	0.55	0.67	0.86	0.78	0.38	0.88	0.73
Males									
G/G	509	27.9 ± 3.8	-0.52 ± 1.46	0.928 ± 0.060	-0.003 ± 0.055	5.75 ± 0.98	-0.05 ± 0.90	2.01 ± 1.14	-0.12 ± 1.27
G/C	517	28.1 ± 3.9	-0.44 ± 1.37	0.931 ± 0.060	-0.003 ± 0.055	5.73 ± 1.06	-0.00 ± 0.87	2.09 ± 1.60	-0.15 ± 1.18
C/C	116	28.0 ± 3.8	-0.52 ± 1.51	0.925 ± 0.061	-0.005 ± 0.065	5.83 ± 0.90	-0.05 ± 0.88	2.09 ± 1.18	-0.02 ± 1.10
P		0.59	0.67	0.61	0.95	0.68	0.64	0.63	0.56
Females									
G/G	605	27.3 ± 5.4	-0.56 ± 1.70	0.813 ± 0.067	-0.002 ± 0.064	5.69 ± 1.07	-0.25 ± 0.90	1.48 ± 0.79	-0.02 ± 0.69
G/C	572	27.1 ± 5.3	-0.62 ± 1.94	0.808 ± 0.067	0.000 ± 0.058	5.73 ± 1.09	-0.15 ± 0.90	1.42 ± 0.77	-0.01 ± 0.63
C/C	147	27.5 ± 5.7	-0.45 ± 1.70	0.816 ± 0.060	-0.005 ± 0.056	5.70 ± 1.13	-0.12 ± 0.93	1.46 ± 0.75	-0.01 ± 0.67
P		0.67	0.57	0.54	0.52	0.82	0.10	0.42	0.92

scribed time-associated variability in the effect size (Lyon et al., 2007). In a population-based cross-sectional study of Germans there was no association with BMI, but substratification revealed an elevated risk of obesity in already overweight individuals (Rosskopf et al., 2007).

In addition to the small effect size, the effect of the variant is likely to be heterogenous between different ethnic groups. Only a detailed analysis of the gene-gene and/or gene-environmental interactions could clarify the exact role of this variant in the pathogenesis of obesity. Additionally, fine mapping of the *INSIG2* gene region is necessary to detect the variants with potentially stronger effect on BMI across the different populations.

The *INSIG2* rs7566605 variant had no effect on BMI or WHR and was not causal for obesity development in a Slavonic Caucasian population sample. Further, we did not show any association between this variant and plasma levels of cholesterol and triglycerides.

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