

Evidence of an influence of a polymorphism near the *INSIG2* on weight loss during a lifestyle intervention in obese children and adolescents

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ABSTRACT

Objective: Homozygotes for the C-allele of the single nucleotide polymorphism rs7566605 (SNP), which is located ~10kb upstream of the insulin-induced gene 2 (*INSIG2*), showed a slightly increased risk to become obese. The aim of this study was to analyze whether children homozygous for the C-allele lose less weight in an intervention than children with the GG- or GC-genotype.

Methods: We genotyped rs7566605 in 293 obese children (mean age 10.8y, 45% male, mean BMI 28.1 kg/m²) who presented for a one-year intervention. The reduction of SDS-BMI was compared based on an intention-to-treat analysis between the children with different genotypes. Blood pressure, triglyceride, total-, HDL- and LDL-cholesterol, insulin, and glucose concentrations were measured before and after intervention.

Results: After 1 year, obese children with the CC-genotype had reduced their SDS-BMI to a lower extent than obese children with GC- or GG- genotypes (recessive model $p=0.007$). There was no evidence for an association of rs7566605 with the cardiovascular risk factor profile (nominal $p>0.1$).

Conclusions: CC-homozygotes at SNP rs7566605 in the vicinity of *INSIG2* lost less weight in this lifestyle intervention. This finding further implicates this polymorphism in weight regulation.

KEYWORDS. obesity, children, *INSIG2*, intervention, cardiovascular risk factors

Insulin-induced gene-1 (*INSIG1*) and its homolog *INSIG2* encode closely related proteins of the endoplasmic reticulum (ER) that block proteolytic activation of sterol regulatory element binding proteins and membrane-bound transcription factors that activate synthesis of cholesterol and fatty acids in animal cells. These proteins also restrict lipogenesis in mature adipocytes and block differentiation of preadipocytes [1,2,3]. Furthermore, *INSIG2* mediates both cholesterol and fatty acid metabolism in rodents [1,2,3,4]. It is regarded as a strong candidate susceptibility gene for total plasma cholesterol levels in mice [5].

Recently, a SNP located ~10kb upstream of *INSIG2* (rs7566605) was shown to be associated with an increased obesity risk for CC-homozygotes in a cross-sectional study of adults and children [6]. However, the influence of this SNP on obesity risk is uncertain: It has been genotyped in another 25,166 individuals of different ethnic backgrounds [7,8,9,10,11,12,13,14,15]. In 8,197 of these, an association with obesity could not be detected. However, a meta-analysis of case-controlled and family-based approaches comprising 16,969 individuals confirmed an association of rs7566605 and obesity [11]. Assuming that the C-allele of this polymorphism has a relevant impact on weight status in humans, one might expect that homozygous obese carriers have greater difficulties to reduce weight. However, the impact of this polymorphism on weight change in intervention programs has not been analyzed yet.

Therefore the primary aim of this study was to analyze whether the CC-genotype of the *INSIG2* polymorphism rs7566605 renders weight loss more difficult in obese children participating in an intervention. Moreover, we explored whether the CC-genotype is associated with cardiovascular risk factors in these children.

PATIENTS AND METHODS

The local ethics committees of the Universities of Witten/Herdecke and Duisburg-Essen approved this study. Written informed consent was obtained from all subjects and their parents.

We examined all 293 obese children aged 6 to 16 years (mean age 10.8±2.7 years, 45% male; 51% prepubertal, 30% pubertal, and 19% late/postpubertal; mean BMI 28.1±4.8 kg/m²; mean SDS-BMI 2.45±0.52) consecutively presenting to our outpatient obesity clinic in order to attend the one-year outpatient intervention program “Obeldicks”. None of the children were on any medication or suffered from endocrine disorders including type 2 diabetes mellitus, familial hyperlipidemia, or syndromal disorders. Blood pressure, triglycerides, HDL-, LDL- and total cholesterol, insulin, and glucose were determined as cardiovascular risk factors.

Obesity was defined by a BMI above the 97th percentile of German children according to the International Task Force of childhood obesity [16,17]. Because body mass index (BMI) is not normally distributed in childhood, we used the LMS method to calculate SDS-BMI as a measure for the degree of overweight. This method summarizes the data in terms of three smooth age specific curves termed L (lambda), M (mu), and S (sigma) [18]. The M and S curves correspond to the median and coefficient of BMI variation for German children at each age and gender, whereas the L curve allows for the substantial age dependent skewness in the distribution of BMI [17,18].

In order to participate in the intervention program “Obeldicks”, the children had to prove their motivation by filling out a questionnaire concerning their eating and exercise habits and by attending exercise groups for overweight children regularly for at least 8 weeks [19,20].

The intervention program “Obeldicks” has been described in detail elsewhere [19,20,21]. Briefly, this outpatient

intervention program for obese children was based on physical exercise, nutrition education, and behavior therapy including the individual psychological care of the child and his or her family. The recommended diet was fat and sugar reduced as compared to the every-day nutrition of German children [20]: The diet contained 30E% fat, 15E% proteins, and 55E% carbohydrates including 5E% sugar.

Seventy-seven of the 293 obese children (26%) dropped out in the motivation phase preceding the intervention and 31 (11%) in the first 3 months of the intervention period. The drop-outs did not differ in respect of age, gender, SDS-BMI, cardiovascular risk factor profile, or genotype distribution from the children completing the intervention. The 31 children who dropped-out during the intervention period had the same mean SDS-BMI at last visit as compared to baseline. The reasons for dropping out were a perceived lack of success in 29 children and disciplinary dismissal in 2 children.

The pubic hair stage was determined according to Marshall and Tanner. The pubertal developmental stage was categorized at baseline into 3 groups based on pubic hair and genital stages (prepubertal: boys with pubic hair stage I and gonadal stage I, girls with pubic hair stage I and breast stage I, pubertal: boys with pubic hair stage >II or gonadal stage >II and girls with pubic hair stage >II or breast stage >II, late/postpubertal: girls with menarche and boys with change of voice).

The following variables as parameters of the cardiovascular risk factor profile were measured in the fasting state in serum using commercially available test kits: Triglycerides, HDL- and LDL-cholesterol, glucose, and insulin (Roche Diagnostics, Mannheim, Germany; Boehringer, Mannheim, Germany; Ortho Clinical Diagnostics, Neckargemuend, Germany, Abbott, Wiesbaden, Germany). Intra- and interassay variations of these variables were less than 5%. The children and their parents had been carefully instructed to fast for a period of at least 10 hours. Homeostasis

model assessment (HOMA) was used to detect the degree of insulin resistance [22]: resistance (HOMA) = (insulin [mU/l] x glucose [mmol/l])/22.5.

The blood pressure was measured using a validated protocol before and after intervention [23]. Systolic and diastolic blood pressure were measured at the right arm twice after a ten-minute rest in the supine position by using a calibrated sphygmomanometer and averaged.

The SNP rs7566605 in the vicinity of *INSIG2* was genotyped as described previously [6]. All PCR products were visualized on ethidium bromide-stained 2.5% agarose gels. Allele sizes were determined with a molecular weight standard (123 bp ladder, Gibco BRL, Karlsruhe, Germany). Positive controls for the variant alleles were run on each gel. For validity of the genotypes, allele determinations were rated independently by at least two experienced individuals. Discrepancies were resolved unambiguously either by reaching consensus or by re-typing. The genotype distribution did not depart from Hardy-Weinberg equilibrium ($p=0.48$).

Statistical analysis was performed using the statistic software package SPSS 12.0. Relationships between genotypes and the baseline parameters (SDS-BMI, cardiovascular risk factors total, LDL-, HDL-cholesterol, triglycerides, insulin, and HOMA-index) were analyzed by linear regression under allele-dose model. For these analyses, we log transformed right-skewed distributed factors. The obtained effect estimates were adjusted for the covariates gender, age, stage of puberty, and baseline SDS-BMI.

According to a recessive model, we compared changes of SDS-BMI in the GG- and CG-carriers versus the CC-carriers. The p-values resulted from Mann-Whitney test based on the intention-to-treat approach and from the SDS-BMI at the beginning and at the end of the one-year intervention. In addition, the allele-dose effect of the risk C-allele was assessed in 185 children who

finished the therapy, by linear regression adjusted for stage of puberty and baseline SDS-BMI.

The two-sided power calculation for difference in mean weight loss per standard deviation unit between the CC- and CG+GG-group was performed by using the statistical software S-PLUS 6.0. As the calculation assumed normal distribution of SDS-BMI-reduction of each genotype group, we provided the difference in mean weight loss per standard deviation unit only in children who completed the intervention.

All reported p-values were two-sided and nominal. A p-value <0.05 was considered as statistically nominally significant.

RESULTS

Twenty-one of the 293 obese children and adolescents (7%) were homozygous for the C-allele, 125 (43%) were heterozygous, and 147 (50%) were homozygous carriers of the wild type (G) allele. Age ($p=0.444$) and pubertal stage ($p=0.946$) did not differ between the genotypes.

Children homozygous for the C-allele lost significantly (recessive model, $p=0.007$) less weight when compared to children heterozygous or homozygous for the wild type allele (figure 1). Even if only children with complete follow-up were considered, this observed association held up and the effect of the C-allele seemed to be additive (figure 1B; additive effect of the C-allele: -0.10 with standard error 0.036).

Linear regression with gender, age, and puberty as covariates demonstrated no significant relationship between genotypes of rs7566605 and cardiovascular risk factors as blood pressure, cholesterol levels, triglycerides, insulin, and HOMA (p -values >0.1, table 1).

DISCUSSION

This is the first study pertaining to the polymorphism rs7566605 near *INSIG2*, weight loss in an intervention program, and cardiovascular risk factors. The achieved reduction of overweight and the success rate

in our intervention program were comparable to previous reports of interventions for obese children [21,24,25,26,27]. The frequency of the CC genotype was 7% and therefore lower than the frequency of 14% in our previously reported independent sample of German families (mean BMI of the obese children: 31.8 kg/m² [6]). Assuming a true effect of the CC-genotype on obesity, this difference could have arisen from the fact that the children analyzed here were less obese. Also sampling variabilities may explain this difference.

Most importantly, homozygous carriers of the C-allele at rs7566605 lost significantly less weight in the intervention. This finding supports a recessive impact of this polymorphism on weight status. It is also possible that an interaction between *INSIG2* and environmental factors such as diet may influence the change of weight status [8]. With regard to the validity of this finding, the small effect size, and the moderate study sample, subsequent replication studies are indispensable. By the given CC-frequency of 7% and the sample size of 185 children completing the intervention, we retrospectively had 92% power to detect the observed difference of 0.97 in mean weight loss per standard deviation unit between the CC- and CG+GG-group at the alpha level 0.05. Furthermore, we have to keep in mind that it is unlikely that rs7566605, located 10 kb upstream of the transcriptional start site of the gene, is itself functional, but rather a variant elsewhere within the gene. Genotyping using several SNPs may therefore be more fruitful and give more reliable results [8].

We did not observe an effect of the C-allele on the cardiovascular risk factor profile. A potential effect of the polymorphism on the cardiovascular risk factor profile may be too moderate to be detectable in our 293 obese children. The effect on insulin resistance could be biased due to assessment by HOMA. HOMA is only an assessment of insulin resistance;

insulin-clamp studies are the gold standard to analyze insulin resistance [28].

In summary, children carrying the CC-genotype of SNP rs7566605 in the vicinity of the *INSIG2* gene lost less weight as compared to children with the GG- or GC-genotypes in an intervention. There was no evidence of an association of the C-allele with the cardiovascular risk factor profile in our obese children and adolescents.

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TABLE 1. Tests for association of rs7566605 in the vicinity of *INSIG2* with SDS-BMI and cardiovascular risk factor profile at baseline in 293 obese children

	homozygous GG	heterozygous CG	homozygous CC	p-value
number	147	125	21	-
SDS-BMI	2.46 (2.14-2.75)	2.36 (2.07-2.71)	2.40 (2.11-2.73)	0.124
Systolic BP [mmHg]	120 (111-131)	112 (111-125)	115 (111-126)	0.146*
diastolic BP [mmHg]	61 (57-71)	61 (55-75)	61 (55-71)	0.578*
total cholesterol [mg/dl]	170 (152-192)	167 (153-190)	167 (152-190)	0.291
LDL- cholesterol [mg/dl]	104 (84-124)	104 (89-125)	102 (85-124)	0.267*
HDL- cholesterol [mg/dl]	49 (43-58)	48 (51-54)	48 (42-56)	0.188
triglycerides [mg/dl]	98 (72-135)	106 (77-136)	102 (76-138)	0.642*
insulin [mIU/l]	16 (11-23)	15 (11-21)	16 (11-22)	0.636*
HOMA	3.3 (2.3-4.82)	3.2 (2.3-4.6)	3.3 (2.3-4.8)	0.785*

data as median and interquartile range, BP: blood pressure, ST: skinfold thickness, p-values derived from linear regression analyses under allele-dose model adjusted for gender, age, stage of puberty and baseline SDS-BMI, *not normally distributed variables were log transformed

FIGURE LEGEND

Figure 1. rs7566605 genotypes and change of weight status (SDS-BMI) compared to baseline in 293 children after participation in the one-year intervention based on an intention-to-treat analysis (Figure A) and in the 185 children completing the one-year intervention (Figure B) (data as median and interquartile range)

FIGURE 1

