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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OBJECTIVE—Homozygotes for the C-allele of the single nucleotide polymorphism (SNP) rs7566605, located ~10 kb upstream of insulin-induced gene 2 (*INSIG2*), showed a slightly increased risk of becoming 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.

RESEARCH DESIGN AND METHODS—We genotyped rs7566605 in 293 obese children (mean age 10.8 years, 45% male, mean BMI 28.1 kg/m^2) who presented for a 1-year intervention. The reduction of SD score (SDS) BMI was compared based on an intention-to-treat analysis between the children with different genotypes. Blood pressure, triglycerides, insulin and glucose concentrations, and total, HDL, and LDL cholesterol 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. ***Diabetes*** **57:623–626, 2008**

Insulin-induced gene-1 (*INSIG1*) and its homolog *INSIG2* encode closely related proteins of the endoplasmic reticulum 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 single nucleotide polymorphism (SNP) located ~10 kb 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 (7–15), in 8,197 of whom 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 in reducing weight. However, the impact of this polymorphism on weight change in intervention programs has not yet been analyzed.

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.

RESEARCH DESIGN 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 6–16 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/m^2; and mean SD score [SDS] BMI 2.45 ± 0.52) consecutively presenting to our outpatient obesity clinic in order to attend the 1-year outpatient intervention program "Obeldicks." None of the children were on any medication or suffered from endocrine disorders including type 2 diabetes, familial hyperlipidemia, or syndromal disorders. Blood pressure, triglycerides, insulin, glucose, and HDL, LDL, and total cholesterol 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 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 (λ), M (μ), and S (σ) (18). The M and S curves correspond to the median and coefficient of BMI variation for German children at each age and sex, whereas the L curve allows for the substantial age-dependent skewness in the distribution of BMI (17,18).

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 compared with the

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Received for publication 23 March 2007 and accepted in revised form 7 November 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 14 November 2007. DOI: 10.2337/db07-0408. Clinical trial reg. no. NCT00435734, clinicaltrials.gov.

HOMA, homeostasis model assessment; SDS, SD score; SNP, single nucleotide polymorphism.

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everyday nutrition of German children (20). The diet contained 30 energy (E)% fat, 15E% proteins, and 55E% carbohydrates including 5E% sugar.

Of the 293 obese children, 77 (26%) dropped out in the motivation phase preceding the intervention and 31 (11%) in the first 3 months of the intervention period. The dropouts did not differ with respect to age, sex, 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 the last visit compared with 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 three 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, 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; and Abbott, Wiesbaden, Germany, respectively). Intra- and interassay variations of these variables were <5%. The children and their parents had been carefully instructed to fast for a period of at least 10 h. Homeostasis model assessment (HOMA) was used to detect the degree of insulin resistance (22): resistance (HOMA) = (insulin [mU/l] × glucose [mmol/l])/22.5.

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 10-min rest in the supine position 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 retyping. 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, and HDL cholesterol; triglycerides; insulin; and HOMA index) were analyzed by linear regression under an allele-dose model. For these analyses, we log transformed right-skewed distributed factors. The obtained effect estimates were adjusted for the covariates sex, 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 a Mann-Whitney test based on the intention-to-treat approach and from the SDS BMI at the beginning and end of the 1-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 SD unit between the CC- and CG+GG-group was performed 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 SD unit only in children who completed the intervention.

All reported *P* values were two sided and nominal. $P < 0.05$ was considered statistically nominally significant.

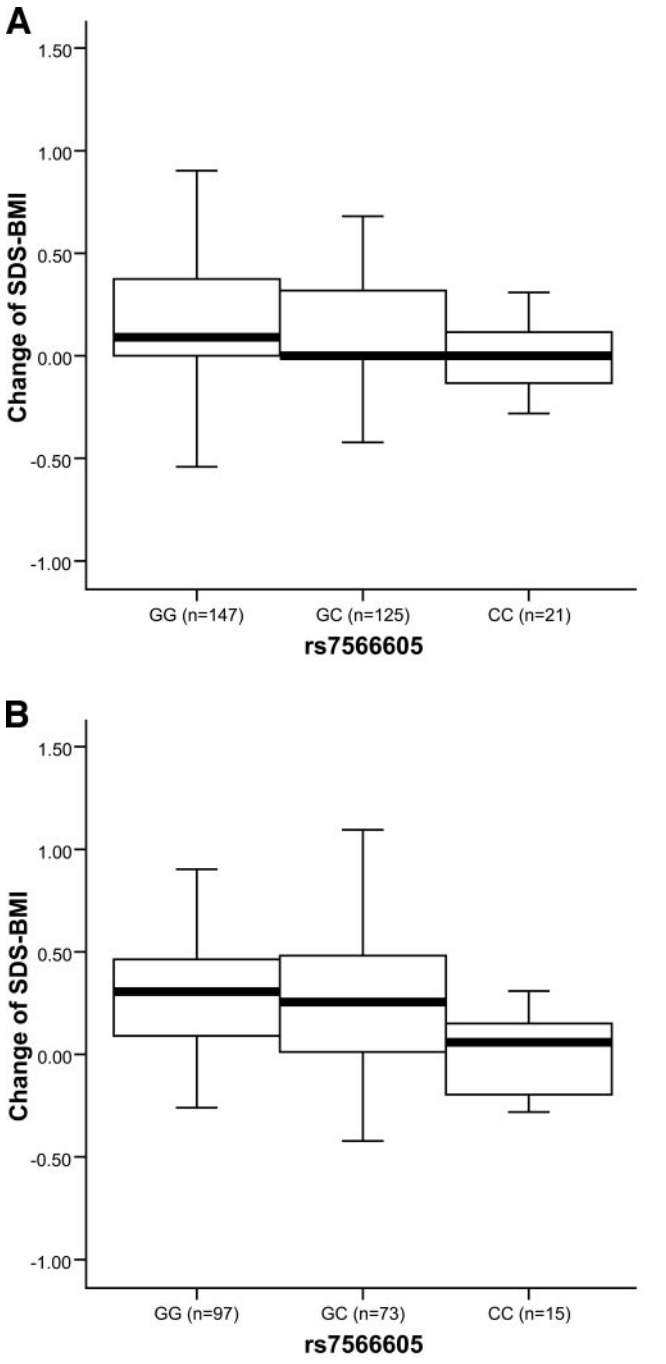

FIG. 1. rs7566605 genotypes and change of weight status (SDS-BMI) compared with baseline in 293 children after participation in the 1-year intervention based on an intention-to-treat analysis (*A*) and in the 185 children completing the 1-year intervention (*B*). Data are median and interquartile range.

RESULTS

Of the 293 obese children and adolescents, 21 (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 than children heterozygous or homozygous for the wild-type allele (Fig. 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 (Fig. 1*B*; additive effect of the C-allele: −0.10 with SE 0.036).

Linear regression with sex, age, and puberty as covariates demonstrated no significant relationship between genotypes of rs7566605 and cardiovascular risk factors such as blood pressure, cholesterol levels, triglycerides, insulin, and HOMA ($P > 0.1$, Table 1).

DISCUSSION

This is the first study pertaining to the polymorphism rs7566605 near *INSIG2*, weight loss in an intervention

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*
n	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
SBP (mmHg)	120 (111–131)	112 (111–125)	115 (111–126)	0.146*
DBP (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 are median (interquartile range). DBP, diastolic blood pressure; SBP, systolic blood pressure. *P* values derived from linear regression analyses under allele-dose model adjusted for sex, age, stage of puberty, and baseline SDS BMI. *Non-normally distributed variables were log transformed.

program, and cardiovascular risk factors. The achieved reduction of overweight and the success rate in our intervention program were comparable with previous reports of interventions for obese children (21,24–27). 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/m^2) (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. Given the 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 SD unit between the CC- and CG+GG-genotype group at the α level of 0.05. Furthermore, we must keep in mind that it is unlikely that rs7566605, located 10 kb upstream of the transcriptional start site of the gene, is itself functional; rather, it is 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 than 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 study's obese children and adolescents.

ACKNOWLEDGMENTS

This work was supported by grants from the German Ministry of Education and Research (Bundesministerium für Bildung und Forschung, National Genome Research Network, NGFN1 and 2).

We thank all probands and their families for their participation. The skillful technical assistance of J. Andrä Essen was highly appreciated.

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