FTO and *INSIG2* Genotyping Combined with Metabolic and Anthropometric Phenotyping of Morbidly Obese Patients

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Key Words

Association · *FTO* · *INSIG2* · Metabolic and anthropometric phenotyping · Morbid obesity · SNPs

Abstract

Obesity is a major health problem worldwide. Associations of obesity with common variants of the fat mass- and obesity-associated gene (*FTO*) and insulin-induced gene 2 (*INSIG2*) have been reported in various studies. We aimed to further investigate the association of 2 single nucleotide polymorphisms (SNPs), rs9939609 in *FTO* and rs7566605 in *INSIG2*, with body mass index (BMI) and other anthropometric and metabolic parameters in subjects with morbid obesity (BMI ≥ 40). SNPs rs9939609 and rs7566605 were genotyped in 124 unrelated morbidly obese patients (mean BMI = 50, range 40.1–77.1) from Mainz, Germany, and in 253 normal controls without a history of morbid obesity. Metabolic and anthropometric parameters were analyzed in 109 of the 124 patients, and associations with the genotype data were examined. The high-risk AA genotype for *FTO* rs9939609 was observed in 32.3% of patients versus 15.8% of controls ($p = 0.0004$) and was associated with an increased obesity risk [odds ratio (OR) = 2.54, 95% confidence interval (CI) = 1.53–4.21]. The in-

termediate-risk AT genotype was found in patients and controls at similar frequencies (48.4 vs. 48.6%, OR = 0.99). The low-risk TT genotype for rs9939609 was found in 19.4% of patients (35.5% of controls; $p = 0.0013$) and was associated with a decreased risk for morbid obesity (OR = 0.43, CI = 0.26–0.73). In contrast, *INSIG2* rs7566605 showed no association with obesity in our patients. Evaluation of metabolic data indicated associations between the high-risk *FTO* genotype (rs9939609_AA) and increased levels of serum glutamic oxaloacetic transaminase (GOT) and between the high-risk *INSIG2* genotype (rs7566605_CC) and lower waist-to-hip ratio and lower hemoglobin A1c (HbA1c) levels. Our results confirm an association of the *FTO* SNP with extreme obesity. However, we found no association of the potential obesity risk allele of *INSIG2* in our sample and thus cannot confirm an association of the *INSIG2* CC genotype with obesity. We identified an association between the high-risk *FTO* genotype (rs9939609_AA) and higher GOT levels, which could possibly reflect the increased frequency of nonalcoholic steatohepatitis with obesity. We also detected associations of the high-risk *INSIG2* genotype (rs7566605_CC) with lower waist-to-hip ratios and lower HbA1c levels, which may indicate amelioration of impaired glucose tolerance and type 2 diabetes for patients with this genotype after bariatric surgery.

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Introduction

Morbid obesity has become alarming worldwide, and numerous comorbidities make obesity one of the leading causes of early death and disablement [World Health Organization, 2007]. Obesity is a multifactorial disease, and its etiology is not yet fully understood. Genetic and epigenetic factors play an important role in the pathogenesis of obesity. Genetic polymorphisms that have been detected by genome-wide association studies (GWAS) appear to contribute little to the current world wide 'obesity epidemic' [Walley et al., 2009]. Nevertheless, they have been extensively studied because the identification of obesity genes may help to better understand the etiopathogenesis of this complex disorder. In recent years, a number of single nucleotide polymorphisms (SNPs) have been linked to obesity, including rs9939609 in the first intron of the fat mass and obesity gene (*FTO*) and rs7566605 10 kb upstream of the transcription start site of the insulin-induced gene 2 (*INSIG2*). Adults who are homozygous for the A allele of the *FTO* SNP weighed ~3 kg more than individuals carrying the low-risk T allele [Frayling et al., 2007]. The *INSIG2* SNP was identified in a GWAS of the offspring cohort of the Framingham Heart Study [Herbert et al., 2006]. However, this association could be replicated in only 5 of 9 cohorts drawn from different populations [Lyon et al., 2007]. A recent meta-analysis of 27 studies on Caucasian adults suggested an association of the *INSIG2* CC genotype with extreme obesity, but not with obesity in general [Heid et al., 2009].

Here, we investigated possible associations of BMI and other anthropometric and metabolic parameters with the well-established *FTO* SNP and the debatable *INSIG2* SNP in a sample of 124 morbidly obese patients that consulted a bariatric surgery clinic.

Patients and Methods

Patients and Controls

This study was approved by the ethics committee of the Medical Council of Rhineland-Palatinate, Germany, and included 124 unrelated morbidly obese patients [77 (62%) females, 47 (38%) males] from the bariatric surgery clinic of the University Medical Centre, Mainz, Germany. All patients were Caucasians from Southwestern Germany and were recruited before bariatric surgery. The mean age was 39.7 years, ranging between 19 and 66 years. Calibrated scales for weight and height were used to determine the BMI accurately. Two different SNPs, *FTO* rs9939609 and *INSIG2* rs7566605, were investigated. Metabolic and anthropometric parameters were analyzed in 109 of the 124 patients and were correlated with the *FTO* and *INSIG2* SNP genotypes. Because of the paucity of several genotypes, some of our data were analyzed according to the num-

bers of risk alleles in the patients. Forty-six patients displayed no or 1 risk allele (group A), 51 patients carried 2 risk alleles (group B) and 27 patients showed 3 or 4 risk alleles (group C).

Normal frequencies of the *FTO* and *INSIG2* genotypes were determined by molecular studies of 253 unrelated controls who had sought medical advice for reasons other than obesity; none of the controls had a medical history of morbid obesity. Controls were Caucasians from Southwestern Germany of either gender and were recruited at the Institute of Human Genetics, University Medical Centre, Mainz, Germany. All patients and controls provided written consent to the studies.

Anthropometric and Biochemical Analyses

Anthropometric and metabolic measurements included age, BMI, waist circumference, hip circumference, waist-to-hip ratio, head circumference, systolic and diastolic blood pressure, serum concentrations of glucose (mmol/l), hemoglobin A1c (HbA1c) (mmol/mol) and glutamic oxaloacetic transaminase (GOT) (U/I), sweet-eater status, educational status, employment status, and family status. All anthropometric measurements were performed using standardized procedures. Height (for determination of BMI) was determined using a calibrated stadiometer; weight was recorded in undressed state using a Seca 635 (Seca, Hamburg, Germany) digital scale for overweight or obese persons (capacity 300 kg, graduation weight 100 g, accuracy class 3). BMI was defined as the individual's body mass divided by the square of the height. Waist circumference, hip circumference and head circumference were measured in a standing position using a flexible tape of 300 cm scaled in millimeters. Maximum waist circumference was determined at the half distance between the lowest point of the rib cage and the pelvic ridge and maximum hip circumference at the height of the symphysis. Occipito-frontal circumference (head circumference) was measured according to standard procedures. All measurements were performed in duplicate with a precision of 1 mm and mean values were used.

Genetic Analyses

SNPs rs9939609 in the *FTO* gene and rs7566605 in the *INSIG2* gene were genotyped using TaqMan® Genotyping Assays C_30090620_10 (rs9939609) and C_29404113_20 (rs7566605) and an Applied Biosystems 7500 Real-Time Polymerase Chain Reaction System (Applied Biosystems, Foster City, Calif., USA). The Allelic Discrimination program (Applied Biosystems) was used for data analysis.

Hardy-Weinberg Equilibrium

Tables 1 and 2 summarize the genotype frequencies of rs9939609 and rs7566605 and of the 9 possible genotype combinations (for both genes) in the 124 patients and 253 controls. The Hardy-Weinberg equilibrium for the A and T alleles of *FTO* rs9939609 and the G and C alleles of *INSIG2* rs7566605 was studied using the χ^2 goodness-of-fit test with estimated probabilities and one degree of freedom. Results indicated an excellent coincidence of observed and expected frequencies. Independence of both genes was tested and confirmed by Fisher's exact test of the 3 × 3-table of counts. Both polymorphisms were found to be well within the Hardy-Weinberg equilibrium (*FTO*, $p = 0.85$; *INSIG2*, $p = 0.75$). For a subset of the 9 possible genotype combinations, small imbalances were observed, but this was to be expected with the limited sample sizes ($n = 253$ controls for all genotypes). The observed imbalances did not reach statistical relevance (maximum $p = 0.1379$).

Table 1. Frequencies and odds ratios for the different *FTO* and *INSIG2* genotypes in patients and controls

Genotype	Genotype frequency		p value	Odds ratio for morbid obesity (95% CI)
	patients (n=124)	controls (n=253)		
<i>FTO</i>				
TT (0 risk alleles)	19.4% (24/124)	35.5% (90/253)	0.0013*	0.43 (0.26–0.73)
AT (1 risk allele)	48.4% (60/124)	48.6% (123/253)	1.0000	0.99 (0.64–1.52)
AA (2 risk alleles)	32.3% (40/124)	15.8% (40/253)	0.0004*	2.54 (1.53–4.21)
<i>INSIG2</i>				
GG (0 obesity-prone alleles)	45.2% (56/124)	45.1% (114/253)	1.0000	1.00 (0.65–1.55)
CG (1 obesity-prone allele)	43.6% (54/124)	43.5% (110/253)	1.0000	1.00 (0.65–1.55)
CC (2 obesity-prone alleles)	11.3% (14/124)	11.5% (29/253)	1.0000	0.98 (0.50–1.94)

* Statistically significant differences (Fisher's exact test).

Table 2. Observed frequencies of the 9 possible *FTO-INSIG2* genotype combinations in 124 morbidly obese patients and 253 controls and odds ratios for morbid obesity

<i>FTO-INSIG2</i> genotype combinations (number of <i>INSIG2</i> potential obesity-prone alleles)	Genotype frequency		p value	Odds ratio for morbid obesity (95% CI)
	patients (n=124)	controls (n=253)		
No <i>FTO</i> risk alleles				
TT-GG (0)	8.1% (10/124)	15.0% (38/253)	0.0699	0.50 (0.23–1.03)
TT-CG (1)	10.5% (13/124)	15.4% (39/253)	0.2076	0.64 (0.33–1.25)
TT-CC (2)	0.8% (1/124)	5.1% (13/253)	0.0416*	0.15 (0.02–1.16)
1 <i>FTO</i> risk allele				
AT-GG (0)	18.6% (23/124)	23.3% (59/253)	0.3525	0.75 (0.44–1.28)
AT-CG (1)	21.8% (27/124)	22.1% (56/253)	1.0000	0.98 (0.58–1.65)
AT-CC (2)	8.1% (10/124)	3.2% (8/253)	0.4245	2.69 (0.98–6.99)
2 <i>FTO</i> risk alleles				
AA-GG (0)	18.5% (23/124)	6.7% (17/253)	0.0012*	3.16 (1.62–6.17)
AA-CG (1)	11.3% (14/124)	5.9% (15/253)	0.9777	2.02 (0.94–4.33)
AA-CC (2)	2.4% (3/124)	3.1% (8/253)	1.0000	0.76 (0.20–2.91)

* Statistically significant differences (Fisher's exact test).

Statistical Analyses

Statistical analyses were performed with the SPSS 17.0 software. The prevalence of the genotypes in the 124 patients was compared to a normal Caucasian population (n = 253 subjects) using odds ratios (ORs) with 95% confidence intervals (CI). Data in tables 1 and 2 were examined with Fisher's exact test and one-way analysis of variance for multiple comparisons using Bonferroni correction. In table 3, Student's t test was used for numerical data analysis (age, BMI, waist circumference, hip circumference, waist-to-hip ratio, head circumference, systolic blood pressure, diastolic blood pressure, glucose, HbA1c, GOT) and the χ^2 test for categorical data analysis (sweet eater, education, employment, family status).

Results

Table 1 summarizes the genotype frequencies of the analyzed *FTO* and *INSIG2* SNPs as well as the corresponding ORs. The low-risk *FTO* TT genotype was significantly less frequent (p = 0.0013) in patients (19.4%) than in controls (35.5%) and associated with a decreased risk for morbid obesity (OR = 0.43, CI = 0.26–0.73). The intermediate AT genotype was observed at similar frequencies in patients and controls (48.4 vs. 48.6%, OR = 0.99). The AA

Fig. 1. Odds ratios (95% confidence intervals) for morbid obesity of the different genotypes of *FTO* rs9939609 (a) and *INSIG2* rs7566605 (b). For numbers of patients, see table 1.

Fig. 2. Genotype frequencies in 124 morbidly obese patients and 253 controls. The obesity risk alleles are *FTO* A and *INSIG2* C, the numbers of subjects as shown in table 2. The asterisk indicates statistically significant differences.

obesity risk genotype was identified in 32.3% of the patients but in only 15.8% of the controls, indicating a markedly increased risk ($p = 0.0004$) for morbid obesity (OR = 2.54, CI = 1.53–4.21). Figure 1a illustrates the association of the *FTO* genotypes with morbid obesity. In contrast, not even a trend towards an association was observed for the *INSIG2* SNP genotypes (fig. 1b).

We also evaluated the frequencies of the different *FTO* and *INSIG2* genotype combinations in patients versus controls (fig. 2; table 2). Based on the limited sample sizes, $n = 124$ patients for all 9 possible combinations, we expected few if any significant differences as compared to the controls. Only 2 genotype combinations displayed significant p values: *FTO* TT-*INSIG2* CC ($p = 0.0416$; OR = 0.15) and *FTO* AA-*INSIG2* GG ($p = 0.0012$; OR = 3.16).

All patients in this study were morbidly obese (BMI ≥ 40), and 53 patients (42.7%) were super-obese (BMI ≥ 50). The mean BMI of the patients was 50.0 (range 40.1–77.2). No significant differences in BMI were observed between patients with the *FTO* AA, AT or TT genotypes and the *INSIG2* GG, CG or CC genotypes, respectively.

Table 3 summarizes correlations between SNP genotypes and anthropometric and metabolic parameters in 109 patients. Significant associations were observed, respectively, between the high-risk AA genotype for *FTO* rs9939609 and higher levels of serum GOT (also called aspartate aminotransferase) ($p = 0.026$) and between the high-risk CC genotype for *INSIG2* rs7566605 and lower waist-to-hip ratios ($p = 0.0342$) as well as lower HbA1c levels ($p = 0.0394$).

Discussion

Our results confirm the association between *FTO* SNP rs9939609 and obesity that was documented in previous studies (fig. 1a) [Dina et al., 2007; Chu et al., 2008]. In our patients, the AA genotype was even more frequent (32.3 vs. 21%) and the TT genotype less frequent (19.4 vs. 31%) than in the Pennsylvanian cohort of 707 morbidly obese subjects, whereas the AT genotype was observed at a similar frequency (48%) [Chu et al., 2008]. However, we consider these differences in frequencies as marginal because they likely reflect minor differences in age (mean age 38.7 vs. 45.9 years), gender (62 vs. 81% females), ethnicity (this study comprised only Caucasians), and/or eating habits between the samples. Hardy et al. [2010] reported that association of the *FTO* SNP with BMI and weight peaks at

Table 3. Anthropometric and metabolic parameters of 109 patients correlated with the high-risk *FTO* rs9939609 (AA) and *INSIG2* rs7566605 (CC) genotypes

	<i>FTO</i> rs9939609			<i>INSIG2</i> rs7566605		
	genotype AA (n = 35)	genotype AT/TT (n = 74)	p value	genotype CC (n = 12)	genotype CG/GG (n = 97)	p value
Age, years	44.63±9.32	40.81±12.50	0.1113	39.58±11.52	42.57±11.19	0.6370
BMI	49.25±7.27	49.21±7.65	0.9787	51.28±6.86	48.97±7.57	0.3170
Waist circumference, cm	143.00±18.12	138.51±17.29	0.2662	135.00±13.09	140.45±18.02	0.2921
Hip circumference, cm	148.00±16.64	146.71±15.98	0.7912	151.00±13.00	145.92±16.46	0.3558
Waist-to-hip ratio	0.98±0.08	0.95±0.11	0.1332	0.90±0.09	0.97±0.10	0.0342*
Head circumference, cm	57.00±3.40	57.13±2.89	0.7913	56.00±2.31	57.29±3.13	0.3076
Systolic blood pressure, mm Hg	143.68±19.13	147.23±22.54	0.5485	152.00±32.07	142.86±26.07	0.3438
Diastolic blood pressure, mm Hg	92.37±11.50	92.76±10.77	0.8947	96.25±15.97	92.15±16.14	0.3242
Glucose, mmol/l	6.22±2.11	5.73±2.02	0.2978	6.33±1.64	5.83±2.10	0.4836
HbA1c, mmol/mol	6.60±1.42	6.46±1.37	0.6107	5.70±0.33	6.63±1.14	0.0394*
GOT, U/l	35.00±21.89	28.12±9.47	0.0260*	25.00±6.48	31.16±5.69	0.2230
Sweet eater ^a	11 (31.43)	32 (43.24)	0.2959	5 (41.67)	38 (39.19)	1.0000
No sweet eater ^b	24 (68.57)	42 (56.76)		7 (58.33)	59 (60.81)	
Education			0.9630			0.1990
No school-leaving certificate	3 (8.57)	9 (12.16)		1 (8.34)	6 (6.19)	
Graduation after 8 or 9 years ^c	14 (40.00)	23 (31.08)		4 (33.33)	35 (36.08)	
Graduation after 10 years ^d	13 (37.14)	31 (41.89)		3 (25.00)	43 (44.33)	
Graduation after 12 or 13 years ^e	4 (11.43)	9 (12.16)		4 (33.33)	10 (10.31)	
University degree	1 (2.86)	2 (2.70)	1.0000	0 (0)	3 (3.09)	0.5622
Employed ^f	21 (60.00)	42 (56.76)		8 (66.67)	55 (56.70)	
Not employed ^g	14 (40.00)	32 (43.24)		4 (33.33)	42 (43.30)	
Family status 1 ^h	22 (62.86)	45 (60.81)	0.8107	8 (66.67)	59 (60.82)	0.1175
Family status 0 ⁱ	13 (37.14)	29 (39.19)		4 (33.33)	38 (39.18)	

Values are means ± SD or numbers of patients (%). * Significant associations ($p < 0.05$). ^a More than 1 bar of chocolate or 1 liter of soft drinks or other sweet drinks per day; ^b not more than 1 bar of chocolate or 1 liter of soft drinks or other sweet drinks per day. ^c 8 or 9 years in the German school system (Volks- or Hauptschulabschluss); ^d 10 years in the German school system (Realschul-

abschluss); ^e 12 or 13 years in the German school system (Abitur or Fachabitur). ^f Earns a livelihood; ^g does not earn livelihood. ^h Single; ⁱ living in a partnership.

HbA1c = Glycosylated hemoglobin; GOT = glutamic oxaloacetic transaminase, alias aspartate transaminase.

the age of 20 years and weakens during adulthood. The association of the *FTO* AA genotype with increased BMI in Caucasians and other ethnicities has been confirmed in all sufficiently powered studies and could possibly be explained by an increased expression of *FTO* risk alleles [Fischer et al., 2009; Hinney et al., 2010]. We determined an OR of 2.54 (95% CI = 1.74–3.69) for morbid obesity with the *FTO* AA genotype, exceeding the 1.67-fold increased OR for obesity established by the meta-analysis of 38,759 individuals from 13 cohorts by Frayling et al. [2007].

In contrast, there was not even a trend association of the *INSIG2* SNP with obesity in our sample. The ques-

tionable CC obesity risk genotype was found in 11.3% of patients and 11.5% of controls, and these frequencies differed neither from each other nor from the 10% frequency of the rs7566605 CC genotype reported in a normal population [Herbert et al., 2006]. Therefore, our findings further weaken the assumption that the *INSIG2* CC genotype is associated with higher BMIs. The association had been first reported by a GWAS for body weight that analyzed 694 individuals of 288 families from the Framingham Heart Study offspring cohort [Herbert et al., 2006]. Several replication studies were conducted, but results were inconsistent [Heid et al., 2009; Hinney et al., 2010]. A recent extensive meta-analysis of the *INSIG2* associa-

tion with obesity, which incorporated data from 27 cohorts and 66,000 Caucasian adults, showed no overall association of the rs7566605 polymorphism with obesity, but suggested an association with extreme degrees of obesity, e.g. BMI ≥ 37.5 [Heid et al., 2009]. However, even this association was not supported by our findings.

Chu et al. [2008] found the strongest association with BMI, when both genes were affected and proposed interactions between *FTO* and *INSIG2*. In another study, the *INSIG2* CC genotype was found negatively associated with weight loss in a lifestyle intervention program in overweight children and adolescents. Individuals with the CC genotype lost less weight than those with a CG or GG genotype [Reinehr et al., 2008]. For the *FTO* AA genotype, only a trend towards lower weight loss was found. The combination of *INSIG2* CC and *FTO* AA was associated with the lowest reduction of overweight and even with an increase in overweight, suggesting that the effects of *FTO* and *INSIG2* could possibly aggravate each other [Reinehr et al., 2009]. In our study of morbidly obese patients awaiting laparoscopic adjustable gastric banding procedure, we observed the strongest effects with the *FTO* risk alleles alone, smaller effects with combinations of *FTO* and *INSIG2* genotypes and no significant effects for *INSIG2* alone. Our results do not support a cumulative effect of *FTO* and *INSIG2* risk alleles and/or *FTO*-*INSIG2* gene interactions. However, this could possibly be due to the limited sample size of this study.

Other studies have shown that the FTO protein interferes with the energy balance system; it appears to function as a demethylase in the cell nucleus with the highest expression in brain and particularly in hypothalamic nuclei involved in regulating energy balance [Gerken et al., 2007]. In addition, mouse knockout studies demonstrated functional roles for FTO in energy homeostasis and whole body metabolism [Fischer et al., 2009]. Another mouse model carrying a dominant point mutation

(Ile367Phe) in *Fto* showed unchanged physical activity but reduced fat mass and increased energy expenditure [Church et al., 2009].

When correlating our genotype data with metabolic and anthropometric parameters, we observed associations of the high-risk AA genotype for *FTO* rs9939609 with increased levels of GOT. This finding may be relevant with regard to the increased incidence of nonalcoholic steatohepatitis in morbidly obese patients. Patients carrying the high-risk AA genotype may be more susceptible to nonalcoholic steatohepatitis. Another potentially interesting finding of this study is the association of the high-risk CC genotype for *INSIG2* rs7566605 with a lower waist-to-hip ratio and lower HbA1c levels. This finding could be relevant in view of the association between type 2 diabetes and obesity. We would speculate that morbidly obese patients with the rs7566605 CC genotype could have better chances of an ameliorating impaired glucose tolerance and type 2 diabetes after bariatric surgery.

A main strength of this study is the analysis of 2 genetic variants in a relatively homogeneous cohort of extremely obese probands. Limitations include the sample size, only 124 patients, making this study too underpowered to draw firm conclusions in general and in particular, for the combined/interactive allelic analysis. However, the study strongly confirms the association of the *FTO* SNP with extreme obesity and may contribute to further meta-analyses.

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