Short Communication

Excessive fat accumulation is associated with the $TNF\alpha$ –308 G/A promoter polymorphism in women but not in men

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Abstract

Aims/hypothesis. Tumour necrosis factor alpha ($TNF\alpha$) is a candidate gene for the development of obesity, which in turn is a major risk factor for diabetes mellitus. The aim of our study was to investigate whether a previously known NcoI-sensitive polymorphism (–308 G/A) in the promoter region of the $TNF\alpha$ gene was related to body weight.

Methods. Genotyping was done in 239 male and 342 female non-diabetic subjects with a marked variation in body mass index (BMI).

Results. We found three genotypes; AA ($n = 13$), AG ($n = 158$) and GG ($n = 410$). When the material was divided according to sex, allele specific phenotypic differences were confined to women. The female subjects carrying the AA genotype were markedly more obese than both the AG and GG carriers (mean

BMI = 41.4 vs 32.3 and 31.7 kg/m², respectively, $p = 0.02$). The body fat content of female AA carriers was increased by one-third compared with AG/GG carriers ($p = 0.02$). We found no differences between genotypes with respect to waist-to-hip ratio, blood pressure or metabolic variables. Among obese female subjects (BMI > 27 kg/m²), we also found that the BMI and body fat content of AA carriers ($n = 7$) were also higher than for AG/GG carriers.

Conclusion/interpretation. The AA-variant at position –308 in the promoter region of the $TNF\alpha$ gene could be an important genetic factor behind excessive fat accumulation in women. [Diabetologia (2000) 43: 117–120]

Keywords Adipose tissue, BMI, obesity, polymorphism, $TNF\alpha$ gene.

Obesity is a major risk factor for non-insulin-dependent diabetes mellitus. Results of family studies suggest that hereditary factors are possibly of importance for the development of obesity [1]. Most of our knowledge about the genetic determination of obesity in man is derived from studies of candidate genes. Among these genes, tumour necrosis factor alpha ($TNF\alpha$) is an attractive candidate. The cytokine is

produced by adipocytes and the production is noticeably enhanced in obesity [2]. Genetic linkage between a marker near the $TNF\alpha$ locus and obesity has been reported [3]. This region is highly polymorphic and one polymorphism (G to A) at position –308 from the first transcribed nucleotide has been shown to influence the $TNF\alpha$ gene transcription in lymphocytes [4]. Recent data suggest that this polymorphism might be associated with obesity and insulin resistance. Using a small number of subjects ($n = 38$) it was found that carriers of the A allele had more body fat but similar BMI and also showed a lower insulin sensitivity index than GG carriers [5]. In another study it was reported that carriers of the A allele were more frequently obese than non-carriers [6]. In both studies, however, no information was given about the importance of sex or the effects of one or

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Abbreviations: DBP, Diastolic blood pressure; HDL, high density lipoprotein; SBP, systolic blood pressure; $TNF\alpha$, tumour necrosis factor alpha; WHR, waist-to-hip ratio.

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Table 1. Phenotypic and metabolic characteristics of male and female subjects grouped for a *NcoI* polymorphism at position -308 in the *TNF α* gene

	Men				Women			
	AA n = 5	AG n = 61	GG n = 173	p	AA n = 8	AG n = 97	GG n = 273	p
Age (years)	47 $\pm$ 3	44 $\pm$ 2	44 $\pm$ 1	0.85	42 $\pm$ 5	39 $\pm$ 1	39 $\pm$ 1	0.77
BMI (kg/m ²)	34 $\pm$ 4	32 $\pm$ 1	32 $\pm$	0.86	41 $\pm$ 3	32 $\pm$ 1	32 $\pm$	0.02
Body fat (%)	34 $\pm$ 7	31 $\pm$ 2	31 $\pm$ 1	0.84	56 $\pm$ 5	42 $\pm$ 2	41 $\pm$ 1	0.02
WHR	1.06 $\pm$ 0.03	1.01 $\pm$ 0.01	1.00 $\pm$ 0.05	0.12	0.92 $\pm$ 0.02	0.90 $\pm$ 0.09	0.91 $\pm$ 0.01	0.73
SBP (mmHg)	150 $\pm$ 5	138 $\pm$ 3	136 $\pm$ 1	0.25	128 $\pm$ 7	125 $\pm$ 2	124 $\pm$ 1	0.71
DBP (mmHg)	90 $\pm$ 5	85 $\pm$ 2	83 $\pm$ 1	0.45	82 $\pm$ 2	78 $\pm$ 1	77 $\pm$ 1	0.38
Insulin (pmol/l)	118 $\pm$ 32	106 $\pm$ 11	112 $\pm$ 6	0.61	110 $\pm$ 16	89 $\pm$ 7	81 $\pm$ 4	0.10
Glucose (mmol/l)	5.5 $\pm$ 0.5	4.9 $\pm$ 0.1	5.4 $\pm$ 0.1	0.12	5.2 $\pm$ 0.4	5.1 $\pm$ 0.1	5.0 $\pm$ 0.0	0.34
Cholesterol (mmol/l)	5.4 $\pm$ 1.0	5.5 $\pm$ 0.1	5.6 $\pm$ 0.1	0.89	5.6 $\pm$ 0.5	5.5 $\pm$ 0.1	5.3 $\pm$ 0.1	0.23
HDL-cholest. (mmol/l)	1.2 $\pm$ 0.2	1.1 $\pm$ 0.0	1.1 $\pm$ 0.0	0.65	1.2 $\pm$ 0.1	1.4 $\pm$ 0.0	1.4 $\pm$ 0.0	0.56
Triglycerides (mmol/l)	1.6 $\pm$ 0.5	1.9 $\pm$ 0.1	2.0 $\pm$ 0.1	0.67	1.8 $\pm$ 0.4	1.5 $\pm$ 0.1	1.5 $\pm$ 0	0.76

Values are mean $\pm$ standard error of mean. Data were analysed using analysis of variance (ANOVA) or Kruskal-Wallis non-parametric test (BMI, insulin, triglycerides). n = Number of subjects, WHR = waist-to-hip ratio

two A alleles for the phenotype [5, 6]. To further investigate the relation between the -308G/A *TNF α* polymorphism and body weight, we examined a large group of unrelated Swedish subjects with a marked interindividual variation in BMI (range = 18–60 kg/m²).

Subjects and methods

The study group consisted of adult men (n = 239) and women (n = 342) who were either healthy control subjects with varying BMI (no medical history, n = 464) or referred to the hospital for uncomplicated hernia, gallstones or gastric binding for uncomplicated obesity (n = 117). All subjects belonged to the same cohort of Scandinavian subjects living in communities nearby Huddinge University Hospital. Both parents of all subjects were born in Scandinavia. The subjects had no other known disorder and were not on any regular medication. None had undertaken a weight-reducing effort during the recent 6-month period. Extensive details of this study group have been published before [7]. Obesity was defined as BMI 27 kg/m². The obese group had 327 subjects. In the morning after an overnight fast, height, weight, waist-to-hip ratio (WHR) and blood pressure were determined and a venous blood sample obtained for preparation of DNA. Analyses of plasma insulin, glucose, triglycerides, cholesterol and high density lipoprotein (HDL)-cholesterol were also done. Percentage body fat was calculated and evaluated as described [7]. It was not possible to obtain all of the phenotypic and metabolic data for each subject. Genotyping for the -308G/A polymorphism was carried out using a PCR fragment amplified by the forward primer 5'-AGGCAATAGGTTTGTAGGGCCAT and the reverse primer 5'-TCCTCCCTGCTCCGATTCCG, followed by digestion with the restriction enzyme *NcoI* [8].

Results

We found three genotypes at position -308; AA, AG and GG. The distribution of genotypes was consistent with the population being in Hardy-Weinberg equilibrium

and was not statistically significantly influenced by sex. The genotype distribution among non-obese subjects was AA = 3, AG = 68 and GG = 183 subjects. The corresponding distribution among the obese subjects was 10, 90 and 227. The values between obese and non-obese did not differ statistically significantly although AA occurred among 3 % of the obese and 1 % of the non-obese. Corresponding values in women were 4 % and 0.5 % and in men 2 % and 2 %, respectively. The allele frequency did not differ statistically significantly between obese and non-obese subjects. This is in contrast to the findings of another study in which an increased frequency of the A allele in obese male subjects was reported [6]. A number of reasons beside sex could explain these divergent findings. For example, the subjects in that study were selected on the basis of coronary heart disease and a much higher BMI cut-off point for obesity was used.

Phenotypic and metabolic variables were distributed normally with the exception of BMI and insulin and triglyceride concentrations. Non-parametric statistics (Kruskal-Wallis) were therefore used to evaluate the BMI, insulin and triglyceride data. As seen in Table 1, there were no apparent differences between carriers of AG and GG in either the male or female subgroups. The mean values for BMI and percent body fat for these genotypes were almost identical. Among women, however, the carriers of AA were noticeably and statistically significantly more obese than the other two groups. The mean BMI of female AA carriers was about 10 kg/m² higher and their body fat content 1/3 larger than for both AG and GG carriers. Of the female AA carriers (75 %) had a BMI > 40 kg/m², in contrast to 25 % of the AG and 22 % of the GG carriers.

A subgroup analysis was also done in obese women (Table 2), combining AG and GG carriers since their phenotype appeared identical. Obese female

Table 2. Phenotypic characteristics of obese women

	AA subjects <i>n</i> = 7	AG/GG subjects <i>n</i> = 187	<i>p</i>
Age	45 $\pm$ 4	40 $\pm$ 1	0.21
BMI (kg/m ²)	44 $\pm$ 2	39 $\pm$ 0.5	< 0.05
Body fat (%)	60 $\pm$ 4	52 $\pm$ 1	0.03
WHR	0.93 $\pm$ 0.03	0.95 $\pm$ 0.01	0.46

Values are mean $\pm$ standard error of mean. Data were analysed using Student's *t* test or Mann-Whitney non-parametric test (BMI). *n* = Number of subjects, WHR = waist-to-hip ratio

AA-carriers were much more over-weight than the subjects with other allelic variants, as judged from the BMI and percent body fat values. Since only one female AA carrier was non-obese, we were unable to carry out investigations of this subgroup. The AA polymorphism had no statistically significant influence on BMI or body fat content in men (Table 1).

We also investigated men and women together. Male and female AG and GG carriers had similar BMI and percent body fat (data not shown). The BMI of AA carriers, however, was almost 7 kg/m² higher (mean $\pm$ SEM, BMI 38.6 $\pm$ 2.7 vs 32.1 $\pm$ 0.7 and 31.9 $\pm$ 0.5, respectively; *p* < 0.05 by Kruskal-Wallis). Also, the percent body fat was about 25 % higher in AA carriers (47.6 $\pm$ 5.1 vs 37.3 $\pm$ 1.2 and 36.9 $\pm$ 0.7, respectively; *p* = 0.04 by ANOVA) than in AG and GG carriers. No differences between the three genotypes were found with respect to either WHR, blood pressure or plasma concentrations of glucose, insulin, cholesterol, HDL-cholesterol or triglycerides, either when results for men and women were analysed together or separately (Table 1)

As differences in body fat between the three genotypes could feasibly be influenced by other factors, an analysis of covariance was done. Using percent body fat as the dependent variable, TNF α gene variant as factor and either age or insulin as covariates, we found no influence of either age or insulin on the results in any of the different subgroups (results not shown)

The data from subjects having no medical history (*n* = 464) were analysed separately and findings were essentially the same as for the whole cohort (results not shown).

Discussion

In the present study, we show that homozygous female, but not male, carriers of a functional [4] variation (G to A at position -308) in the promoter region of the TNF α gene are characterized by a considerable accumulation of fat tissue. This genotype occurs among 4 % of the obese female population (defined as BMI > 27 kg/m²) and might thus be a common genetic factor for obesity in this sex. Subjects with the

AA polymorphism did not have higher values of either WHR, blood glucose, lipids, insulin or blood pressure than did the other variants. This is not an unexpected finding, since female obesity is less frequently associated with abdominal fat accumulation and metabolic disturbances than is male obesity. An altered TNF α action on insulin receptor signalling in adipose tissue possibly only has local effects on fat cell function which may not result in systemic effects such as hyperinsulinaemia. Furthermore, we did not include diabetic subjects and therefore do not know how the polymorphism influences the risk profile in more complicated forms of obesity.

Although a minor, but not statistically significant, difference in age was found between carriers of the AA-variant compared with the GA/GG-carriers, it is unlikely that age had any major influence on the degree of obesity as judged by our analysis of covariance. Furthermore, the calculation of percent body fat is based on age, meaning that age is corrected for in this formula. It cannot be excluded, however, that age-related mechanisms such as female hormonal status could have influenced our study results.

It is not possible to determine the mode of inheritance in case control studies. Nevertheless, the observation that AG and GG carriers showed almost identical phenotypes possible suggests that the A allele is a recessive obesity trait. A previous study on the impact of this polymorphism on obesity and metabolic variables made no attempt to analyse homozygous and heterozygous subjects separately [5]. When we combined AA and AG carriers into one group, we found no statistically significant phenotypic differences compared with GG carriers (values not shown). The difference in results might be due to the small number of subjects examined in the previous study [5].

At present we can only speculate as to how TNF α might promote obesity. One way could be through interactions with insulin receptor signalling [2]. Another way could be through local effects in adipose tissue, for example by influencing lipolysis [9] or apoptosis [10] in adipocytes.

In summary, this study shows that a functional polymorphism in the promoter region of the TNF α gene is associated with female obesity when in a homozygous form. This genotype, being not uncommon among obese subjects, could be an important genetic factor behind excessive fat accumulation in women.

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