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The common *-675 4G/5G* polymorphism in the plasminogen activator inhibitor –1 gene is strongly associated with obesity

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Abstract

Aims/hypothesis. Plasminogen activator inhibitor 1 (PAI-1) increases in several insulin-resistant conditions such as obesity. We tested the hypothesis that the *PAI-1* gene might be a candidate for obesity and Type II (non-insulin-dependent) diabetes mellitus.
Methods. We investigated the frequency of a common and functional *–675 4G/5G* promoter polymorphism in the *PAI-1* gene in 188 lean, 70 overweight (BMI 25–30 kg/m^2) and 247 obese otherwise healthy Scandinavian subjects.
Results. The genotypic ($p = 0.002$), or allelic ($p = 0.0004$) distribution differed markedly between the three groups. Homozygocity for *4G* was more common among obese people, whereas homozygocity for *5G* was more common among lean subjects. Heterozygocity was evenly distributed. The lean and overweight groups did not differ in frequency distribution. The relative risk for being obese in comparison to being lean for *4G/4G* was threefold higher ($p = 0.0003$). Also, carriers of the *4G* allele in the heterozygous or homozygous form were distributed differently between the three groups ($p = 0.006$). The *4G* carriers were more common among the obese than the lean group. The latter group did not differ from the overweight group. The relative risk of being obese in comparison with lean was twofold increased in *4G* carriers ($p = 0.0015$). Similar results were obtained in men and women.
Conclusion/interpretation. Thus, the common *–675 4G/5G* polymorphism in the *PAI-1* gene is strongly linked to obesity and a markedly increased risk for obesity is associated with the *4G* allele in its homozygous form. [Diabetologia (2002) 45:584–587]

Keywords Adipose tissue, body mass index, fat, humans, genes, obesity, overweight, polymorphism, plasmin activator inhibitor-1, single nucleotide polymorphism.

Obesity, which is a major risk factor for Type II (non-insulin-dependent) diabetes mellitus and other insulin-resistant conditions, is a rapidly growing health problem in the Westernised countries. The disease is multifactorial and probably caused by interactions between several environmental and genetic factors. Although a number of rare monogenic forms of morbid human obesity have been described recently, the genes involved in the common types of obesity remain to be established [1]. Several genetic pathways could be responsible for the development of obesity. The genes controlling adipose tissue function might be of particular importance because increased fat mass is the most prominent feature of the obese phenotype [2]. Adipose tissue has an important endocrine role as well as metabolic function. A number of proteins are secreted by fat cells and can regulate adipose tissue or peripheral organs in a paracrine and endocrine fashion [3]. Results of studies on these proteins, for example leptin, tumor necrosis factor α and interleukins for a relation between genetic polymorphism and obesity are conflicting [1, 2]. Another

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Abbreviations: PAI, Plasminogen activator inhibitor

protein that is secreted from fat cells is plasminogen activator inhibitor –1 (PAI-1). Adipose tissue produces large amounts of PAI-1 and circulating PAI-1 are higher in obesity and other insulin-resistant conditions [4–6]. Furthermore, studies of PAI-1 knockout mice shows an effect of PAI-1 on weight gain and adipose cellularity following high-fat dieting [7]. Furthermore, disruption of the *PAI-1* gene reduces the adiposity of the obese *ob/ob* mice [8]. This suggests that the *PAI-1* gene can control fat mass, although the mechanism of action is not yet known.

A functional polymorphism in the promoter region of the *PAI-1* gene (*-675 4G/5G*) affects the binding of nuclear proteins regulating the transcription of the gene [9, 10]. Because *PAI-1* is probably involved in atherosclerosis, this polymorphism has been intensively investigated in coronary artery disease [11]. In addition, the *4G/5G* polymorphism is associated with features of the insulin resistance (metabolic) syndrome in some, but not all, populations [12, 13]. However, the relation between obesity and the *–675 4G/5G PAI-1* polymorphism has, to the best of our knowledge, not been examined. We therefore genotyped consecutively recruited material of 505 healthy female and male subjects with a large individual variation in BMI for the *PAI-1* promoter polymorphism.

Subjects and methods

The subjects were recruited to an ongoing project to investigate candidate genes for obesity among the Swedish population. All subjects lived in the Stockholm area and both parents were born in Scandinavia. They were recruited by local advertisement or from the hospital's obesity clinic. They were included in a consecutive fashion. All subjects were healthy except for obesity and none was on regular medication. None was completely sedentary or involved in athletic performance. The 189 subjects were men and 316 women. Ages and BMI ranged between 18 and 77 years and between 18 and 56 kg/m^2, respectively. To obtain a better separation between phenotypes, the material was divided into three BMI groups, according to the World Health Organisation (WHO) definitions: lean (BMI < 25 kg/m^2), overweight (BMI 25–30 kg/m^2) and obese (BMI > 30 kg/m^2). The study was approved by the Huddinge hospital committee on ethics. It was explained in detail to each participant and the consent of all participants was obtained.

Study protocol. The subjects came to the laboratory for determination of height, weight and waist-to-hip ratio (WHR). Thereafter, a venous blood sample was obtained for the extraction of genomic DNA exactly as described [14] and kept at –20 °C. Genotyping for the *4 G/5 G* polymorphism at position *–675* in the promoter region of the *PAI-1* gene was done exactly as described previously [14].

Statistical methods. Differences between groups for genotype and allele frequencies were assessed using the χ^2 (chi-square test). Odds ratios were determined by logistic regression analysis. Values are means ± SD and were compared by analysis of variance.

Table 1. Clinical data in lean, overweight and obese subjects according to genotype

Measure	Genotype			*p*
	4G/4G	*4G/5G*	*5G/5G*	
Men/women	31/71	88/154	70/91	NS
Age (years)				
Lean	40 ± 14	39 ± 10	38 ± 10	NS
Overweight	38 ± 11	42 ± 13	43 ± 15	NS
Obese	42 ± 1	40 ± 10	41 ± 10	NS
BMI (kg/m^2)				
Lean	22 ± 2	22 ± 2	22 ± 2	NS
Overweight	26 ± 1	27 ± 1	26 ± 1	NS
Obese	40 ± 5	39 ± 5	40 ± 5	NS
WHR (men)				
Lean	0.94 ± 0.05	0.90 ± 0.07	0.93 ± 0.05	NS
Overweight	1.03 ± 0.05	0.97 ± 0.06	0.97 ± 0.04	NS
Obese	1.04 ± 0.05	1.04 ± 0.05	1.03 ± 0.05	NS
WHR (women)				
Lean	0.87 ± 0.05	0.85 ± 0.07	0.84 ± 0.07	NS
Overweight	0.85 ± 0.08	0.90 ± 0.08	0.86 ± 0.06	NS
Obese	0.93 ± 0.07	0.94 ± 0.06	0.95 ± 0.06	NS

Values are means ± SD. Data with *4G/4G*, *4G/5G* and *5G/5G* subjects were compared using analysis of variance except for gender distribution when chi-square was used. NS = not significant

Results

In the whole material, 102 subjects were *4G* homozygotes, 161 were *5G* homozygotes and the remaining 242 subjects were *4 G/5 G* heterozygotes. This distribution is not different from the Hardy Weinberg equilibrium. The almost equal distribution of the *5G* and *4 G* alleles is in accordance with the reported genotype frequencies in other Caucasian populations [10–15]. There was no significant difference in genotype frequency between men and women (Table 1). Likewise, neither age, BMI nor WHR differed between genotypes in the different BMI groups (Table 1).

In the whole material, there was a clear difference ($p = 0.002$) in the genotype frequency between groups (Table 2); *4G/4G* was more common among obese and *5G/5G* more common among lean subjects. Heterozygous subjects were evenly distributed between groups. Separate analysis of lean versus overweight revealed no difference in distribution of genotypes ($p = 0.41$). When lean and obese homozygous were compared, the odds-ratio (mean and 95 %-CI) for being obese and having *4G/4G* was 2.8 (1.6–4.8, $p = 0.0003$). Data for men and women were also analysed (Table 2). The same type of results were obtained for either sex as for the whole cohort.

In order to further evaluate the association between the *4G* allele and obesity, subjects were grouped into carriers (*4G/4G* or *4G/5G*) or non-carriers (i. e. *5G/5G*) of this allele (Table 3). An obvious difference in the distribution of the *4G* carriers be-

Table 2. PAI-1 genotype frequencies in lean, overweight and obese subjects

	All subjects (n = 505)				Women (n = 316)				Men (n = 189)			
Genotype	Lean	Overweight	Obese	p	Lean	Overweight	Obese	p	Lean	Overweight	Obese	p
4G/4G	0.149	0.129	0.263		0.176	0.161	0.271		0.101	0.103	0.247	
4G/5G	0.452	0.543	0.482	0.002	0.462	0.677	0.470	0.035	0.435	0.436	0.506	0.014
5G/5G	0.399	0.329	0.255		0.361	0.161	0.259		0.464	0.462	0.257	

Values were compared by chi square analysis. p denotes frequency distribution of *4G/4G*, *4G/5G* and *5G/5G* among all subjects or among either women or men (i.e. 6 groups in each comparison)

Table 3. Distribution of carriers (*4G/4G* or *4G/5G*) and non-carriers of the *4G* allele of the *–675 4G/5G* polymorphism in the *PAI-1* gene in lean, overweight and obese subjects

Genotype	Lean n = 188	Overweight n = 70	Obese n = 247	p
4G/4G or *4G/5G*	0.601	0.671	0.745	0.006
5G/5G	0.399	0.329	0.255	

Values are frequency and were compared by chi-square analysis. p in the table denotes comparison of all 6 groups. We also made a comparison of lean and obese subjects (4 groups). Then p was 0.002. Finally, lean and overweight were compared (4 groups). Then p was 0.30

tween groups was recorded in the whole material (p = 0.006). Carriers of the *4G* allele were more common among obese subjects and absence of the *4G* allele more common among lean subjects when the whole cohort was analysed. A separate analysis of lean compared with overweight subjects revealed no differences between groups as regards the distribution of carriers or non-carriers of the 4G allele (p = 0.39). The relative risk (measured as odds ratio with a 95%-CI) for being obese as compared to lean when carrying the *4G* allele was 1.9 (1.3–2.9, with a p value of 0.0015). There was also a difference in the distribution of *4G* carriers between BMI groups in women (p = 0.04) and men (p = 0.009).

The allele frequency was also determined. For *4G* it was 0.317, 0.126 and 0.558 in the lean, overweight and obese, respectively. The corresponding values for *5G* were 0.417, 0.149 and 0.434, respectively. This variation in allele frequency was highly significant (p = 0.0004) and remained so when the overweight group was removed from analysis (p = 0.0001). However, lean and overweight subjects did not differ in this respect (p = 0.60).

Discussion

This study found an association between a polymorphism in the *PAI-1* gene and obesity. We investigated a functional biallelic variation in the promoter region (*-675 4G/5G*), which is very common, the two alleles being almost equally distributed among the population. It is evident that the *4G* allele has a strong association to obesity. The risk of being obese was twofold increased in carriers of the *4G* allele, whereas the *5G* allele was more common among the lean. In homozygotes the risk of being obese instead of lean for those having *4G/4G* was threefold increased. There was, however, no apparent association between the *PAI-1* polymorphism and being overweight (BMI 25–27) kg/m^2. Also, heterozygocity had no significant link to any of the BMI groups. Furthermore, the polymorphism was associated to obesity in both sexes although the association tended to be strongest among men. It remains to be established whether this is a true gender difference.

Although the data suggest that the *PAI-1* gene is linked to obesity further studies are needed to clarify this role. We investigated healthy Scandinavian subjects. Other results might be obtained in subjects with co-morbidity or in other ethnic groups. Furthermore, the results need to be confirmed by family studies or investigations on population-based cohorts. It is also possible that our results simply reflect genetic variability at another locus in the vicinity of the *PAI-1* gene. Another *PAI-1* gene polymorphism near the present one has been reported (*A-844G*), although the two polymorphisms are in strong linkage disequilibrium [15–17]. In addition, it is not possible to say from the present results whether the polymorphism protects from obesity or promotes the disorder. For example, both BMI and WHR within the lean, overweight and obese groups were influenced by the polymorphism.

It is not known how *PAI-1* might promote (or protect) from the development of obesity although some mechanistic speculations could be offered. Besides its role in coagulation, *PAI-1* seems to be involved in cell migration and angiogenesis. Alterations in these effects might promote obesity, as discussed [18]. Furthermore, *PAI-1* binding to the extracellular matrix of adipose tissue could alter adipocyte function so that obesity is induced [5, 10, 18]. Inhibition of lipid second messengers in laboratory animals specifically inhibits angiogenesis as reviewed [19].

We previously reported no effect of the *4G/5G PAI-1* gene polymorphism on PAI-1 secretion from human subcutaneous adipose tissue [14], despite the fact that the secretion rate of PAI-1 from this tissue is markedly increased in obesity [20]. However, the

secretion of PAI-1 might not reflect the intracellular or tissue levels of the protein. As a matter of fact, PAI-1 mRNA expression in endothelial cells near pulmonary arterial trombi increased despite normal plasma PAI-1 amounts [21]. It is not known if the secretion rate of PAI-1 from adipose tissue is proportional to the adipocyte intracellular level of the protein. We measured adipose secretion and plasma PAI levels in some of the subjects; the data have been reported [14]. However, the number of subjects were too few (n = 244 for plasma PAI-1) to allow a reliable analysis of the interaction between PAI-1, genotyped BMI and gender; the two latter parameters strongly influence plasma PAI-1 amounts as discussed (6, 11, 14, 19).

In conclusion, a common and functional *– 675 4G/5G* polymorphism in the promoter gene region of the *PAI-1* gene is strongly associated with obesity and a markedly increased risk for this disorder is found in carriers of the *4G* allele.

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