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The visfatin (*PBEF1*) G-948T gene polymorphism is associated with increased high-density lipoprotein cholesterol in obese subjects

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Abstract

The newly discovered adipokine visfatin has been hypothesized to be related to obesity and insulin resistance. In this study, we investigate if the 2 single nucleotide polymorphisms rs4730153 and G-948T are associated with obesity and/or related traits and whether they influence the messenger RNA (mRNA) levels of *PBEF1* (originally the abbreviation for *pre–B-cell colony-enhancing factor 1*) in visceral and subcutaneous adipose tissue (VAT and SAT). We found that obese carriers of the *PBEF1* G-948T variant allele had significantly higher levels of high-density lipoprotein cholesterol (GG, 1.1 [0.97-1.3] mmol/L; GT + TT, 1.3 [1.0-1.5] mmol/L; $P = .02$). Other than that, neither rs4730153 nor G-948T had any major impact on any of the obesity-related phenotypes. There was no difference in mRNA expression between VAT and SAT (2.08 ± 0.17 and 2.09 ± 0.14, respectively; $P = .26$), but there was a nonsignificant trend toward higher *PBEF1* mRNA levels in the variant allele carriers concerning both VAT and SAT for both single nucleotide polymorphisms. A significant correlation was observed between body mass index and *PBEF1* mRNA expression in SAT ($R = 0.37$, $P = .03$) but not in VAT ($R = 0.26$, $P = .12$). In conclusion, *PBEF1* G-948T is associated with increased high-density lipoprotein cholesterol; but genetic variation in *PBEF1* does not seem to have a major impact on the development of obesity or on the expression of the gene.

1. Introduction

The messenger RNA (mRNA) expression of the adipokine visfatin, encoded by *PBEF1* (originally the abbreviation for *pre–B-cell colony-enhancing factor 1*), increases during the development of obesity; and its plasma level strongly correlates with the amount of visceral fat [1]. Visfatin mimics insulin by binding and activating the insulin receptor, and lowering plasma glucose; and it has been suggested that it is involved in the pathogenesis of obesity and insulin resistance, although the mechanism is still unknown. *PBEF1* is located on chromosome 7q22.2 and consists of 11 exons and 10 introns [2]. Visfatin is a 473–amino acid protein with a molecular mass of 52 kd [3]. It is found in plasma and mainly expressed in liver, skeletal muscle, and bone marrow [3], but also in the adipose tissue as was recently discovered [1]. Results from studies on *PBEF1* expression levels in visceral and subcutaneous adipose tissue (VAT and SAT) and the relation to visfatin plasma levels, obesity, and type 2 diabetes mellitus have been inconsistent [1,4-8]. It is possible that genetic variation in *PBEF1* contributes to these conflicting results. The 2 *PBEF1* single nucleotide polymorphisms (SNPs) rs4730153 and G-948T are representative for their linkage disequilibrium groups and have been associated with the ratio of VAT to SAT *PBEF1* mRNA expression [8]. The aim of this study was to investigate if these 2 SNPs are associated with obesity and/or related traits in a white Scandinavian case-control population and whether they influence the mRNA levels of *PBEF1* in VAT and SAT from obese subjects.

2. Materials and methods

2.1. Subjects

Obese (body mass index [BMI] >30 kg/m^2), nondiabetic, white, Scandinavian subjects (n = 235) from the obesity outpatient clinic at the University Hospital MAS in Malmö,

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Sweden, were randomly and individually matched for age and sex with nondiabetic, normal-weight subjects (n = 235) participating in the Botnia study [9]. Diabetes status was based on a previous diagnosis, ongoing hypoglycemic medication, and fasting plasma glucose according to the World Health Organization criteria. Clinical characteristics are shown in Table 1. All patients gave their written consent to participate in the study, and it was approved by the local ethics committee.

Abdominal VAT and SAT biopsies were obtained from 37 white, Swedish, nondiabetic, obese subjects undergoing bariatric surgery (4 men and 33 women; median age, 35 years [29.5-47.5]; BMI, 41.5 kg/m^2 [37.6-44.8]; waist circumference, 139 [132-148] and 113 [106-121] cm for men and women, respectively). Biopsies were immediately frozen in liquid nitrogen and stored at −80°C until further processed.

2.2. Genotyping of PBEF1 SNPs

Deoxyribonucleic acid was extracted from blood samples using conventional methods. rs4730153, located in intron 6, and G-948T in the proximal promoter were genotyped using TaqMan allelic discrimination on ABI 7900HT (Applied Biosystems, Foster City, CA). For the rs4730153 SNP, we used Assay on Demand C_2673294_10 (Applied Biosystems). For the G-948T SNP, we used Assay by Design (Applied Biosystems) with the following primers: GCCCGTTGCCTTTTCCTT (forward) and GGTGGAATTCAGTCCTCACAGATAA (reverse), and probes: CCTAATTGAACCGAGTATT (VIC) and CCTAATTGAACAGAGTATT (FAM). Success rate for all genotyping was greater than 99%, and there was no deviation from Hardy-Weinberg equilibrium. Five percent of the samples were randomly selected and regenotyped with 100% overlap.

Table 1
Clinical characteristics of study subjects in case-control association

	Obese subjects	Control subjects	*P*
Sex (male/female)	235 (64/171)	235 (65/170)	.92
Age (y)	42.0 (33.0-52.0) (n = 235)	43.0 (34.4-53.0) (n = 235)	.53
BMI (kg/m^2)	40.2 (35.1-45.0) (n = 233)	22.5 (21.2-23.7) (n = 235)	<.0001
Waist (cm)	116.0 (106.0-128.0) (n = 180)	75.5 (70.3-81.5) (n = 220)	<.0001
Insulin (mU/L)	15.4 (11.3-21.9) (n = 77)	5.2 (3.9-8.0) (n = 165)	<.0001
Glucose (mmol/L)	5.5 (4.8-6.3) (n = 217)	4.8 (4.5-5.1) (n = 229)	<.0001
HOMA	3.3 (2.5-5.0) (n = 67)	1.1 (0.8-1.7) (n = 165)	<.0001
HDL (mmol/L)	1.2 (1.0-1.4) (n = 147)	1.5 (1.3-1.8) (n = 208)	<.0001
TG (mmol/L)	1.5 (1.0-2.2) (n = 219)	0.88 (0.70-1.2) (n = 209)	<.0001

Data are medians with interquartile range (25th-75th percentile). *P* values refer to the Wilcoxon rank sum test. HOMA indicates homeostasis model assessment index of insulin resistance; TG, triglycerides.

2.3. RNA extraction

Total RNA was extracted from the biopsies using a combination of TRI REAGENT (Sigma-Aldrich, St Louis, MO) and RNeasy Midi Kit (Qiagen, Hilden, Germany). Samples were stored at −80°C until used. Reverse transcriptase polymerase chain reaction (PCR) was performed using the QuantiTect Reverse Transcription Kit (Qiagen). Samples were analyzed with real-time PCR using the ABI 7900HT sequence detection system in 10-μL reaction volumes with 40 ng complementary DNA and Universal TaqMan 2× PCR MasterMix according to manufacturer's recommendations (Applied Biosystems). All samples were run in duplicates, and data were calculated using the standard curve method and expressed as a ratio to the endogenous control cyclophilin A (arbitrary units). The assays used in the study are 4326316E and Hs00237184_m1 for cyclophilin A and *PBEF1*, respectively (Applied Biosystems).

2.4. Statistical analyses

Allele frequencies were compared using Fisher exact test; and genotype frequencies, using χ^2 statistics. Clinical variables concerning the case-control material as well as differences in expression between genotype groups were log-transformed to obtain normal distribution, enabling adjustment (where appropriate) for age, sex, and BMI, and analyzed with analysis of variance. Wilcoxon signed rank test was used for paired comparisons; Mann-Whitney *U* test, for unpaired comparisons; and Spearman correlation, for estimating relationships between variables. A *P* value less than .05 was considered statistically significant. Data are presented as median with interquartile range (25th-75th percentile) in parentheses or as mean ± SEM. Statistical operations were performed using the Number Cruncher Statistical Systems program (NCSS, Kaysville, UT) apart from haplotype analysis, which was performed by Haploview software [10].

3. Results

3.1. Genetic variation in PBEF1

The 2 *PBEF1* SNPs rs4730153 and G-948T were genotyped in 235 obese subjects and 235 controls matched for sex and age (Table 1). There were no significant differences between cases and controls concerning allele or genotype frequencies for either SNP (Table 2). rs4730153 and G-948T were in linkage disequilibrium (D' = 0.99, r^2 = 0.28). There was no difference in haplotype frequency between cases and controls (data not shown).

For both SNPs, minor allele carriers were compared with subjects homozygous for the major allele when

Table 2
Allele and genotype frequencies

		Obese subjects	Control subjects	*P*
rs4730153	G	271 (58.4%)	267 (57.5%)	.84
	A	193 (41.6%)	197 (42.5%)	
	GG	80 (34.5%)	68 (29.3%)	.17
	GA	111 (47.8%)	131 (56.5%)	
	AA	41 (17.7%)	33 (14.2%)	
G-948T	G	391 (83.9%)	378 (81.5%)	.34
	T	75 (16.1%)	86 (18.5%)	
	GG	165 (70.8%)	150 (64.7%)	.16
	GT	61 (26.2%)	78 (33.6%)	
	TT	7 (3.0%)	4 (1.7%)	

Data are number of obese subjects and controls (percentage) for each allele and genotype. *P* values refer to Fisher exact test and χ^2 statistics, respectively.

studying possible associations with clinical variables because of the small number of subjects homozygous for the minor allele. Obese carriers of the G-948T variant allele had significantly higher high-density lipoprotein (HDL) cholesterol (GG, 1.1 [0.97-1.3] mmol/L; GT/TT, 1.3 [1.0-1.5] mmol/L; $P = .02$, adjusted for sex, age, and BMI; Table 3). There were no differences concerning other relevant clinical variables for G-948T and none for rs4730153, in cases as well as controls (data not shown).

3.2. PBEF1 mRNA expression in VAT and SAT

PBEF1 mRNA levels were measured by real-time PCR in VAT and SAT biopsies from 37 obese subjects. The correlation between VAT and SAT *PBEF1* mRNA expression was high ($R = 0.62$, $P < .001$). Significant correlation was also observed between BMI and *PBEF1* mRNA expression in SAT ($R = 0.37$, $P = .03$) but not in VAT ($R = 0.26$, $P = .12$).

There was no difference between VAT and SAT *PBEF1* mRNA expression (2.08 ± 0.17 and 2.09 ± 0.14, respectively; $P = .26$). As each genotype was analyzed separately, a nonsignificant trend toward higher *PBEF1* mRNA levels in the variant allele carriers concerning both VAT and SAT for both SNPs was seen (Fig. 1). No significant difference was found when comparing wild-type with variant allele carriers concerning rs4730153 (VAT GG, 2.00 ± 0.32; GA/GG, 2.12 ± 0.25; $P = .85$; and SAT GG, 1.92 ± 0.15; GA/GG, 2.15 ± 0.16; $P = .54$). No correlations between VAT expression and relevant variables were found (data not shown).

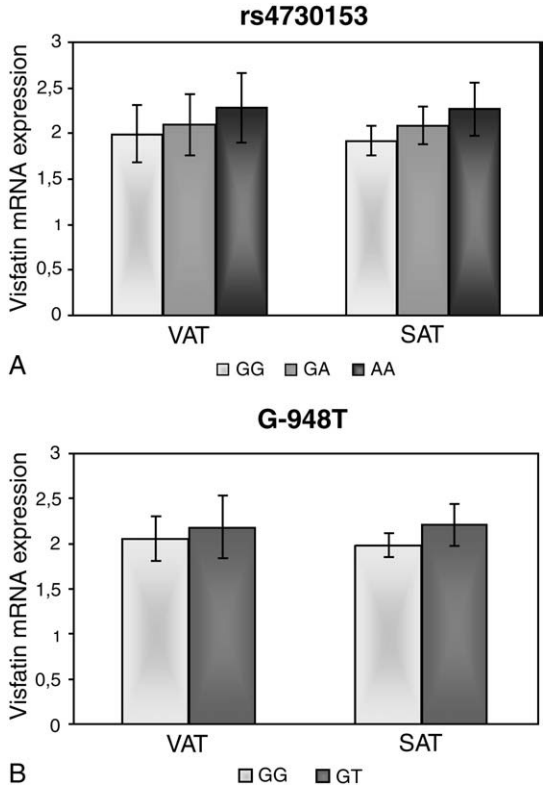

Fig. 1. Visfatin mRNA expression adjusted for the expression of the endogenous control (cyclophilin A) in VAT and SAT for each genotype separately. A, rs4730153: *P*(VAT) = .62 and *P*(SAT) = .60. B, G-948T: *P*(VAT) = .58 and *P*(SAT) = .46. There were no homozygous TT-genotype carriers concerning G-948T.

Table 3
Clinical characteristics of obese subjects according to visfatin G-948T wild-type and variant allele carriers

	G/G	G/T and T/T	*P*
Sex (male/female)	48/117	15/53	N/A
Age (y)	43.0 (34.0-53.0) [165]	39.5 (32.0-50.8) [68]	.40
BMI (kg/m^2)	39.8 (35.5-44.9) [164]	40.5 (34.9-45.5) [67]	.93
Waist (cm)	117 (106-129) [131]	114 (103-124) [49]	.64
Insulin (mU/L)	16.1 (11.6-24.9) [57]	14.5 (9.7-19.7) [20]	.26
Glucose (mmol/L)	5.6 (4.8-6.4) [150]	5.4 (4.9-6.3) [65]	1.00
HOMA	3.5 (2.6-5.5) [47]	3.0 (2.4-4.2) [20]	.50
HDL (mmol/L)	1.12 (0.97-1.28) [101]	1.29 (1.01-1.53) [44]	.02
TG (mmol/L)	1.6 (1.0-2.2) [153]	1.4 (1.0-2.2) [64]	.97

Data are medians with interquartile range (25th-75th percentile). Values inside brackets represent the number of subjects. *P* values refer to analysis of variance adjusted for sex, age, and BMI (where appropriate).

4. Discussion

Because visfatin has insulin-mimicking effects and the expression of the gene has been reported to increase with obesity, variants in *PBEF1* may affect measures of obesity as well as insulin sensitivity. Here we found that obese carriers of the *PBEF1* G-948T variant allele had significantly higher levels of HDL cholesterol in a Scandinavian obesity case-control material. Other than that, neither rs4730153 nor G-948T had any major impact on any of the obesity-related phenotypes.

Several recent reports have shown a positive association between visfatin and HDL cholesterol in various populations [11-15]. These findings are likely a reflection of differences in insulin sensitivity between the subjects resulting in the typical dyslipidemia of insulin resistance and the metabolic

syndrome, that is, increased triglyceride levels and decreased HDL cholesterol. We did not see a corresponding decrease in triglycerides in our subjects; and unfortunately, the homeostasis model assessment index of insulin resistance (HOMA), as an estimate of insulin resistance, was only available for a minority of subjects. Variants in the promoter region of *PBEF1* have earlier been found to correlate with fasting plasma insulin (rs9770242, G-948T, and rs1319501) and fasting plasma glucose (rs9770242 and rs1319501) [16], indicating a possible association with insulin resistance. G-948T has also been reported to correlate with plasma insulin and 2-hour plasma glucose, as well as a borderline association with percentage of body fat [8]. Although we did not find any of these correlations, there are trends of lower mean insulin and glucose levels among the T-allele carriers in our study as well. Otherwise, we did not find any association between rs4730153 or G-948T and obesity, thereby confirming earlier observations [8,17]. With regard to the association between visfatin and HDL, there are negative studies as well [16-18], indicating the need for further research in this field.

In the original report on visfatin, *PBEF1* was primarily found in the visceral fat, where the expression was strongly correlated to visceral fat mass as well as visfatin plasma level [1]. Since this report, several studies on VAT and SAT *PBEF1* expression have shown conflicting results, some consistent with our finding of no difference between the depots [4,5] and others indicating a higher expression level in VAT [6] or in SAT [7,8]. The correlation we observed between BMI and *PBEF1* expression in SAT has earlier been observed in VAT [4,5,7]. It should be stressed that most of the participants in our biopsy cohort were female and that this may bias the results. However, there have been no previous reports on sex differences concerning *PBEF1* mRNA expression. Neither did we find any difference between men and women in this respect in our study (data not shown). It has been proposed that VAT *PBEF1* expression is elevated because of chronic inflammation, accomplished by macrophages accumulated in the tissue [19]. Viscerally obese subjects do as well have increased levels of the inflammatory markers C-reactive protein, interleukin-6, and tumor necrosis factor–α [11,20], which actually up-regulate the transcription of *PBEF1* [2]. Taken together, it is possible that *PBEF1* expression is differently regulated in the 2 depots, as well as in different regions of SAT. In subjects with a more even fat distribution, *PBEF1* mRNA expression might be higher in SAT than VAT, whereas the opposite is more likely to be expected in viscerally obese subjects.

In conclusion, *PBEF1* G-948T is associated with increased HDL cholesterol, likely as a reflection of insulin sensitivity; but genetic variation in *PBEF1* does not seem to have a major impact on the development of obesity or on the expression of the gene.

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