Brief Genetics Report

Peroxisome Proliferator–Activated Receptor-γ Coactivator-1 Gene Locus

Associations With Obesity Indices in Middle-Aged Women

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Peroxisome proliferator–activated receptor-γ coactivator-1 (PPARGC1) is a transcriptional coactivator that has been implicated in the regulation of genes involved in energy metabolism. We studied associations of two polymorphisms identified in PPARGC1 transcripts with obesity indices in 591 middle-aged men and 467 middle-aged women of a cross-sectional Austrian population. Because neither polymorphic site was likely to be a functional site, we analyzed sex-specific associations of two loci haplotype combinations with obesity indices. Significant associations with BMI ($P = 0.006$), waist ($P = 0.01$) and hip circumference ($P = 0.03$), and total body fat ($P = 0.005$) and borderline significant associations with abdominal visceral and subcutaneous fat were observed in women but not men. In women, plasma triglycerides, HDL cholesterol, and glucose significantly differed by haplotype combinations, but these associations were not maintained after statistical consideration of BMI. The haplotype combination of the double-variant allele with the double–wild-type allele was associated with the lowest obesity indices, whereas homozygosity for the double-variant allele was not discriminatory among haplotype combinations. These studies suggest functional differences of PPARGC1 haplotypes in human energy metabolism and support a role of PPARGC1 in obesity. *Diabetes* 51:1281–1286, 2002

Peroxisome proliferator–activated receptor-γ coactivator-1 (PPARGC1) is a coactivator of PPAR-γ and other nuclear hormone receptors and plays an essential role in energy homeostasis. Studies in rodents and cell culture models showed that Pgc1 stimulates mitochondrial biogenesis and activates genes of the oxidative phosphorylation pathway and thermogenesis (1–4). Human PPARGC1 exhibits 94% amino acid identity with the mouse ortholog and was mapped to chromosome 4p15.1 (5). This chromosomal region has been associated with basal insulin levels in Pima Indians (6) and abdominal subcutaneous fat in the Quebec Family Study (7). Because of these associations and PPARGC1's role in energy metabolism in animal models, we tested the hypothesis that markers at the PPARGC1 gene locus are associated with obesity indices.

By sequencing eight cDNA alleles transcribed from muscle RNA, we identified four polymorphisms in PPARGC1 transcripts. Two polymorphisms, a G-to-A transition at position +1,564 in exon 8 (that predicted a glycine to serine amino acid substitution at position 482, and therefore referred to as *G482S*) and an A-to-G substitution at position +2,962 in the 3′-untranslated region (Genbank accession no. NM-013261) were selected because of their heterozygosity indices of 0.449 (95% CI 0.436–0.462) and 0.499 (0.498–0.501), respectively, to genotype 591 unrelated men and 467 unrelated women of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) (8). The two other polymorphisms, a silent G-to-A transition at +1,302 in exon 8 and an A-to-T transversion at +3,010 in exon 13 were not considered because heterozygosity indices were <0.10. Frequencies of the wild-type, heterozygous, and variant genotypes at the *G482S* site were 0.433 ($n = 457$), 0.453 ($n = 481$), and 0.114 ($n = 120$). The respective frequencies at the +2,962 A/G site were 0.264 ($n = 280$), 0.488 ($n = 516$), and 0.247 ($n = 262$). The wild-type and variant allele frequencies for the *G482S* polymorphism were 0.659 or 0.341. Respective frequencies for the +2,962 polymorphism were 0.509 and 0.492. Genotype frequencies at both polymorphic sites were in agreement with Hardy-Weinberg expectations (χ^2 for *G482S* or +2,962 A/G were 0.117 or 0.624, respectively). The two polymorphisms showed a highly significant standardized pairwise linkage disequilibrium (D/Dmax = +0.971, $P < 0.001$). Estimated haplotype frequencies were 0.503, 0.157, 0.006, and 0.335 for the double–wild-type allele (0,0), the allele with the variant nucleotide at +2,962 (0,1), the allele with the variant amino acid at *G482S* (1,0), and the double-variant allele

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PPAR, peroxisome proliferator–activated receptor; PPARGC1, PPAR-γ coactivator-1; SAPHIR, Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk.

(1,1), respectively. Sex-specific analyses revealed no significant differences in genotype, allele, or estimated haplotype frequencies.

Genotypes associated with each of the two polymorphic sites showed associations with obesity indices and metabolic parameters in women but not in men (online appendix, Tables 1 and 2). Interestingly, women heterozygous at either site displayed lower average values for BMI, waist circumference, and body fat and higher values for HDL cholesterol than women homozygous for the wild-type allele at the respective sites. Several considerations argued against either of the two polymorphisms being a major functional site. The amino acid exchange at *G482S* is conservative and does not create or eliminate protein motifs known to be functional. Furthermore, the variant amino acid is identical to the wild-type amino acid at this position in mice and rats. In addition, transient transfections of PAZ-6 cells showed that the magnitude of PPAR-γ–mediated transactivation of the uncoupled protein-1 promoter was identical for PPARGC1 constructs harboring the wild-type or variant amino acid at *G482S* (H.O., H.E., V.L., A.D. Strosberg, F.K., and W.P., unpublished observations). Moreover, the possibility that the +2,962 polymorphism in the 3′-untranslated region was a major determinant of mRNA expression appeared unlikely based on preliminary allele-specific expression studies in muscle and adipose tissues of obese subjects. The ratio of the wild-type to variant transcripts (with respect to the +2,962 site) showed wide variations in subjects carrying one variant or two wild-type amino acids at the *G482S* site (online appendix, Table 3).

We therefore analyzed associations with two loci haplotype combinations. Because three of the possible haplotype combinations, i.e., 1,0/0,0, 1,0/1,0, and 1,1/1,0 were each observed in less than five men or five women, these haplotype data are not shown. Haplotype combinations are unambiguous except for the double heterozygous genotype. Based on estimated haplotype frequencies, >98% of the double heterozygous individuals were predicted to carry a double–wild-type (0,0) and a double-variant allele (1,1), whereas <2% were predicted to carry alleles with one variant nucleotide each (0,1/1,0). In men, no significant association of haplotype combinations with obesity indices was observed (Table 1). In women, however, significant associations with age-adjusted values for BMI, waist and hip circumference, and body fat, with or without adjustment for lean body mass, were observed (Table 2). Abdominal visceral and subcutaneous fat showed borderline significant differences. Age-adjusted plasma levels of glucose, triglycerides, and HDL cholesterol also differed, but these differences were not maintained after adjustment for BMI. Adjustment for postmenopausal hormone use had no substantial effect on results. Possible confounding by menopause was excluded, because BMI and other obesity indices differed significantly among haplotype combinations in women >52 years of age and not using hormone replacement therapy (all $P < 0.03$).

In women, the haplotype combination of the double-variant and double–wild-type alleles was associated with significantly lower BMI, waist and hip circumferences, fat mass, triglyceride levels, and fasting blood glucose in comparison to the haplotype combination of two wild-type alleles, whereas HDL cholesterol was increased (Table 2, all $P < 0.05$). However, subjects homozygous for the double-variant or double–wild-type alleles displayed similar obesity indices, as exemplified for BMI in Table 3. To determine associations with obesity in this population, we compared genotype and haplotype frequencies between obese (BMI >30 kg/m^2) and nonobese (BMI <30 kg/m^2) women. Frequencies of *G482S* genotypes and haplotype combinations restricted to double–wild-type and variant alleles significantly differed between obese and nonobese women. Estimated risks for heterozygous subjects were lower than for wild-type subjects (online appendix, Table 4).

The apparent association of heterozygosity at two polymorphic sites with reduced obesity indices in women are likely to reflect linkage disequilibria with putative causative site(s). Our allele-specific expression studies showing tissue-specific differences in +2,962 A/G transcript ratios within and among *G482S* wild-type or heterozygous subjects imply functional polymorphisms in regulatory gene regions that are under tissue-specific control and may therefore confer tissue-specific advantages. Interestingly, several reports have shown that heterozygous PPAR-γ–deficient mice are protected from high-fat diet–induced or aging-induced adipocyte hypertrophy, obesity, and insulin resistance (9–11).

While this manuscript was in review, Ek et al. (12) reported the association of the *G482S* polymorphism with type 2 diabetes. These authors observed no association with BMI in their male/female type 2 diabetes and control populations. Because sex-specific data were not reported in this study, these results are difficult to compare with our findings. The association of the PPARGC1 gene locus with obesity indices in women suggests, but does not prove, that this locus may contribute to the genetic susceptibility for the multifactorial disorder of obesity. Our results are consistent with concepts emerging from animal studies and recent reports from the Quebec family study (7,13–15). We have no clear explanation why the associations were only observed in women. There is, however, precedence for sex-specific associations of genetic markers with obesity indices (16–18). In addition, PPARGC1 has been shown to interact with the estrogen receptor and enhances its transcriptional activity (3,19). Moreover, animal experiments have demonstrated sex-specific effects of other nuclear hormone receptors whose transcriptional activity is regulated by PPARGC1. In PPAR-α null mice, inhibition of cellular fatty acid flux caused hepatic and cardiac lipid accumulation, hypoglycemia, and death in all male mice but in only 25% of female mice, and treatment with β-estradiol rescued the metabolic phenotype in male PPAR-α null mice (20). Clearly, identification of the functional sites at the PPARGC1 gene locus will be required to gain insight into the associations at the molecular level. If the associations reported are confirmed in other populations, PPARGC1 may be a target for obesity treatment.

RESEARCH DESIGN AND METHODS

Subjects. We studied 40- to 65-year-old men and 45- to 70-year-old women participating in SAPHIR, a population-based prospective study that investigates genetic factors contributing to atherosclerotic vascular disease. Recruitment procedures for the study have been detailed (8). The population

TABLE 1
Obesity indices and metabolic parameters in middle-aged men by PPARGC1 haplotype combination

	Haplotype combination							
	All*	0,0/0,0	0,1/0,0	0,1/0,1	0,0/1,1 (0,1/1,0)	0,1/1,1	1,1/1,1	*P*†
n	591	152	81	15	208	59	70	
Age (years)	49.9 (49.4–50.3)	49.8 (48.9–50.6)	49.6 (48.4–50.7)	51.3 (48.7–53.9)	49.9 (49.0–50.6)	49.1 (47.8–50.4)	50.6 (49.3–51.9)	0.561
BMI (kg/m^2)	27.2 (26.9–27.5)	27.2 (26.6–27.8)	27.5 (26.6–28.4)	26.1 (24.0–28.1)	27.2 (26.8–27.7)	27.1 (26.2–28.1)	27.0 (26.0–27.9)	0.703
Waist circumference (cm)	98.0 (97.2–98.9)	98.4 96.8–100.2)	98.7 (96.4–101.0)	96.4 (90.7–102.0)	98.1 (96.7–99.5)	98.2 (95.5–101.0)	97.0 (94.2–99.7)	0.767
Hip circumference (cm)	104.3 (103.6–105.1)	104.0 (102.5–105.4)	105.3 (103.1–107.5)	102.3 (97.5–107.1)	104.2 (103.0–105.3)	104.9 (102.7–107.1)	104.8 (102.6–106.9)	0.739
Waist-to-hip ratio	0.941 (0.935–0.946)	0.947 (0.937–0.958)	0.938 (0.932–0.952)	0.942 (0.913–0.971)	0.943 (0.935–0.952)	0.939 (0.913–0.965)	0.925 (0.909–0.941)	0.262
Body fat (kg)	19.8 (19.2–20.5)	19.9 (18.5–21.1)	21.5 (19.8–23.2)	17.9 (14.6–21.1)	19.7 (18.7–20.7)	19.4 (17.3–21.4)	19.5 (17.7–21.4)	0.400 0.304‡
Abdominal visceral fat (cm^2)§	93.3 (89.2–97.4)	91.2 (82.8–99.6)	101.7 (91.2–112.2)	77.5 (57.9–97.2)	95.2 (88.0–104.2)	88.3 (77.5–99.2)	92.9 (79.5–106.2)	0.250
Abdominal subcutaneous fat (cm^2)§	189.1 (181.0–197.2)	198.9 (183.4–214.4)	191.0 (165.8–217.5)	157.8 (130.0–185.7)	183.8 (170.3–197.3)	193.3 (166.7–220.0)	189.0 (163.6–214.3)	0.415
Glucose (mmol/l)‖	5.16 (5.11–5.20)	5.12 (5.04–5.20)	5.25 (5.13–5.37)	5.38 (4.98–5.73)	5.18 (5.09–5.24)	5.02 (4.87–5.17)	5.14 (5.05–5.31)	0.138
Insulin (pmol/l)‖	47.4 (44.4–49.8)	48.0 (41.4–54.6)	48.0 (41.4–54.6)	52.2 (28.8–75.0)	47.4 (42.6–52.2)	46.8 (37.8–55.2)	43.2 (37.2–48.6)	0.956
Cholesterol (mmol/l)	5.95 (5.87–6.05)	5.92 (5.74–6.10)	5.89 (5.66–6.16)	5.97 (5.35–6.57)	6.02 (5.87–6.15)	6.02 (5.74–6.28)	5.92 (5.66–6.15)	0.919
Triglycerides (mmol/l)	1.65 (1.55–1.75)	1.68 (1.50–1.86)	1.68 (1.39–1.99)	1.62 (1.21–2.03)	1.60 (1.46–1.75)	1.73 (1.33–2.12)	1.61 (1.31–1.89)	0.882
HDL cholesterol (mmol/l)	1.40 (1.37–1.42)	1.37 (1.32–1.42)	1.40 (1.33–1.45)	1.37 (1.22–1.53)	1.42 (1.37–1.45)	1.40 (1.32–1.47)	1.42 (1.34–1.50)	0.693
Type 2 diabetes (yes/no)	24/567	4/148	3/78	2/13	7/201	4/55	3/67	0.340¶

Data are *n* of observations or untransformed means (95% CI). Haplotypes are assigned numbers in the order of polymorphisms along the PPARGC1 gene locus. 0, more-common allele (482glycine, +2962A); 1, less-common allele (482serine, +2962G). *Includes six study subjects with rare haplotype combinations; †comparison among haplotype combinations, ANOVA adjusted for age; ‡adjusted for lean body mass; §performed in 512 men, numbers for each haplotype combination shown are 129, 68, 14, 185, 50 and 60; ‖subjects with type 2 diabetes are excluded; ¶χ^2 test.

TABLE 2
Obesity indices and metabolic parameters in middle-aged women by PPARGC1 haplotype combination

	Haplotype combination								
	All*	0,0/0,0	0,1/0,0	0,1/0,1	0,0/1,1 (0,1/1,0)	0,1/1,1	1,1/1,1	P†	P‡
n	467	120	81	8	145	61	47		
Age (years)	56.3 (55.9–56.6)	56.4 (55.6–57.2)	56.4 (55.4–57.4)	54.8 (50.8–58.7)	56.2 (55.6–56.9)	56.0 (55.0–57.0)	56.5 (55.3–57.8)	0.926	
BMI (kg/m^2)	26.5 (26.1–26.9)	27.0 (26.1–27.8)	27.1 (26.0–28.1)	27.5 (24.7–30.3)	25.2 (24.6–25.9)	27.0 (25.9–28.0)	26.7 (25.3–28.1)	0.005	0.006
Waist circumference (cm)	88.9 (26.1–26.9)	90.1 (26.1–27.8)	90.5 (26.0–28.1)	89.0 (24.7–30.3)	85.3 (24.6–25.9)	90.8 (25.9–28.0)	90.5 (25.3–28.1)	0.010	0.012
Hip circumference (cm)	104.5 (103.4–105.5)	105.3 (103.1–107.5)	106.4 (103.9–108.9)	108.0 (100.7–116.3)	101.7 (99.9–103.6)	104.7 (101.7–107.7)	106.5 (103.3–109.6)	0.030	0.075
Waist-to-hip ratio	0.850 (0.843–0.857)	0.858 (0.944–0.872)	0.849 (0.832–0.866)	0.819 (0.747–0.881)	0.839 (0.828–0.851)	0.868 (0.848–0.886)	0.847 (0.821–0.874)	0.129	0.379
Body fat (kg)	23.3 (22.5–24.2)	24.5 (22.8–26.3)	24.2 (22.4–26.3)	27.1 (21.3–32.9)	20.9 (19.5–22.3)	24.7 (22.2–27.1)	23.1 (20.2–26.0)	0.005 0.028§	0.007 0.009§
Abdominal visceral fat (cm^2)‖	78.7 (74.4–83.0)	80.9 (72.8–88.8)	82.5 (70.9–94.1)	86.0 (40.0–131.9)	70.6 (63.5–77.7)	89.7 (76.6–102.8)	74.9 (61.8–88.1)	0.093	0.319
Abdominal subcutaneous fat (cm^2)‖	260.1 (249.1–271.1)	264.6 (242.1–287.2)	274.2 (247.0–301.4)	309.8 (211.3–408.4)	237.8 (219.0–256.6)	281.9 (250.9–312.9)	251.8 (212.2–291.3)	0.081	0.340
Glucose (mmol/l)¶	5.02 (4.96–5.08)	5.05 (4.96–5.13)	5.12 (5.01–5.23)	5.07 (4.11–5.94)	4.91 (4.82–4.98)	4.94 (4.83–5.11)	5.05 (4.86–5.25)	0.033 0.144#	0.117 0.090#
Insulin (pmol/l)¶	43.5 (41.4–46.2)	45.0 (40.8–49.2)	46.2 (41.4–51.4)	46.2 (29.4–61.8)	39.6 (36.6–43.2)	47.4 (40.2–55.2)	42.6 (36.0–49.2)	0.383	0.643
Cholesterol (mmol/l)	6.10 (6.00–6.20)	6.00 (5.82–6.18)	6.08 (5.87–6.31)	6.39 (5.43–7.34)	6.18 (5.99–6.38)	6.13 (5.82–6.44)	6.05 (5.79–6.34)	0.682	0.720
Triglycerides (mmol/l)	1.21 (1.15–1.27)	1.23 (1.12–1.34)	1.24 (1.13–1.34)	1.64 (0.81–2.46)	1.09 (1.01–1.19)	1.22 (1.07–1.37)	1.28 (1.01–1.53)	0.028 0.261#	0.146 0.318#
HDL cholesterol (mmol/l)	1.73 (1.71–1.78)	1.71 (1.63–1.78)	1.71 (1.63–1.81)	1.58 (1.32–1.84)	1.86 (1.78–1.91)	1.68 (1.58–1.76)	1.68 (1.55–1.78)	0.006 0.137#	0.028 0.015#
Type 2 diabetes (yes/no)	13/454	4/116	2/79	1/7	1/144	2/59	3/44	0.378	0.184**

Data are n of observations or untransformed means (95% CI). Haplotypes are assigned numbers in the order of polymorphisms along the PPARGC1 gene locus. 0, more-common allele (482glycine, +2962A); 1, less-common allele (482serine, +2962G). *Includes five study subjects with rare haplotype combinations; †comparison among haplotype combinations, ANOVA adjusted for age; ‡Tukey's post hoc comparison between haplotype combinations 0,0/0,0 and 0,0/1,1; §adjusted for lean body mass; ‖performed in 410 female subjects, numbers for each haplotype combination shown are 101, 74, 7, 129, 53 and 41; ¶subjects with type 2 diabetes are excluded; #adjusted for BMI; **χ^2 test.

TABLE 3
BMI in middle-aged women by common PPARGC1 haplotype combination

	Allele 2		
	0,0	0,1	1,1
Allele 1			
0,0	27.0* (26.1–27.8) $n = 120$	27.1* (26.0–28.1) $n = 81$	25.2*†‡ (24.6–25.9) $n = 145$
0,1		27.5‡ (24.7–30.3) $n = 8$	27.0† (25.9–28.0) $n = 61$
1,1			26.7‡ (25.3–28.1) $n = 47$

Data are untransformed means (95% CI). Haplotype alleles 1 and 2 are assigned numbers in the order of polymorphisms along the PPARGC1 gene locus. 0, more-common polymorphism (482glycine, +2962A); 1, less-common polymorphism (482serine, +2962G). *$P <$ 0.05, †$P < 0.10$, ‡not significant; n, number of observations.

comprised only white Europeans, mainly of Bavarian and Austrian German descent. The prevalence of obesity in this sample is comparable with the reported prevalence in the same geographic region (21). BMI was calculated from measurements of weight and height. We classified subjects as diabetic if they were using hypoglycemic medications or had fasting plasma glucose concentrations ≥7.0 mmol/l.

Methods. Fat and lean body mass was determined by bioelectric impedance analysis using the phase-sensitive, fully digital multifrequence analyzer BIA-2000M (Data Input, Hofheim, Germany). Abdominal adipose tissue areas were assessed by computed tomography using a MX TWIN Picker CT scanner (Marconi Medical Systems, Cleveland, OH) in 512 men and 410 women. An abdominal scan was obtained between the fourth and fifth lumbar vertebrae (L4-L5), and the adipose tissue area was determined by calculating the pixel distribution with attenuation values between –190 and –30 Hounsfield units. The abdominal visceral fat area was determined by drawing a line within the muscle wall surrounding the abdominal cavity. The encircled area with attenuation values between –190 and –30 Hounsfield units was then calculated using the Voxel Q software package (Marconi Medical Systems). Abdominal subcutaneous fat was determined by subtracting visceral from abdominal total fat. After an overnight fast, plasma glucose, insulin, cholesterol, triglycerides, HDL and LDL cholesterol, and apolipoproteins A-I and B were measured (22). DNA was isolated from white blood cells using the QIAamp Blood Kit (Qiagen, Valencia, CA).

Multiplex restriction isotyping was used to type the *G482S* and +2962A/G polymorphisms. Primer pairs 5′-GAGCCGAGCTGAACAAGCAC-3′ (+1,496 − +1,515)/5′-GGAGACACATTGAACAATGAATAGGATTG-3′ (+1,705 − +1,733) and 5′-CAATAACAACAATGGTTTACATGA-3′ (+2,681 − +2,704)/5′-CGAACATTTTGAAGTTCTAGGTTTTACG-3′ (+2,963 − +2,990) were used to amplify PPARGC1 sequences harboring the *G482S* and +2962A/G polymorphisms. Numbers in parentheses refer to GenBank accession no. NM-013261. Optimum multiplex PCR conditions were achieved with 200 μmol/l dG/dA/dCTP, 400 μmol/l dUTP, 4.5 mmol/l $MgCl_2$, and 0.2 units AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primer concentrations were 200 nmol/l for the *G482S* and 400 nmol/l for the +2962A/G polymorphism. Uracil-*N*-Glycosylase (MBI Fermentas, Hanover, MD) was included in reactions to prevent contamination. PCR products were digested with *Msp*I and *Mlu*I (New England Biolabs, Beverly, MA) overnight at 37°C before separation of fragments in agarose gels.

Statistical analysis. Group differences of continuous variables were ascertained by ANOVA. Transformations were made if the equal variance and normality assumptions of ANOVA were rejected. Measurements were adjusted by multiple regression for the effects of age and BMI, as indicated. Allele frequencies were estimated by gene counting. Agreement with Hardy-Weinberg expectations was tested using a χ^2 goodness-of-fit test. The standardized pairwise linkage disequilibrium statistic (D′) and haplotype frequencies were estimated using methods described by Terwilliger and Ott (23). Tukey's multiple comparison procedure (24) was used to test for differences of obesity indices among individual genotypes and haplotype combinations.

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