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Short communication

Homozygosity of the *Pro12Ala* variant of the peroxisome proliferation-activated receptor-γ2 (*PPAR-γ2*): divergent modulating effects on body mass index in obese and lean Caucasian men

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Abstract

Aims/hypothesis. The objectives of the present investigation were to examine: 1) whether a *Pro115Gln* variant in the peroxisome proliferator-activated receptor-γ2 (*PPAR-γ2*) is associated with juvenile-onset obesity among Danish Caucasianmen and 2) whether the relation of a *Pro12Ala* polymorphism in *PPAR-γ2* with BMI and long-term weight regulation differ between lean and obese subjects within the same cohort.
Methods. The *Pro115Gln* and *Pro112Ala* variants were examined using PCR and RFLP in a group of 752 subjects with a Body Mass Index (BMI) of 31.0 kg/m^2 or more and in 869 non-obese control subjects.
Results. We did not find *Pro115Gln* in any of the 1621 male subjects we examined. Among the males with juvenile-onset obesity, the allelic frequency of the *Pro12Ala* polymorphism was 14 % (95 % confidence interval: 12–16 %) compared with 16 % (14–17 %) among the non-obese control subjects (NS). Heterozygosity of the *codon 12* variant was not associated with differences in BMI or changes in body weight regulation during follow up in lean or obese subjects. In the group of obese subjects, 21 homozygous *Ala12Ala* carriers had, however, a higher BMI (38.9 ± 5.4 kg/m^2 (means ± SD) vs 35.5 ± 5.5 kg/m^2, $p = 0.008$) and a higher weight gain (0.27 ± 0.24 kg · m^{-2} · year^{-1} vs 0.10 ± 0.24 kg · m^{-2} · year^{-1}, $p = 0.004$), compared with wild-type carriers. Moreover, within the control group of 869 men the 14 homozygous carriers of the variant had a lower BMI (24.4 ± 2.7 kg/m^2 vs 26.2 ± 3.7 kg/m^2, $p = 0.005$) and a slower increase in BMI (0.11 ± 0.11 kg · m^{-2} · year^{-1} vs 0.17 ± 0.11 kg · m^{-2} · year^{-1}, $p = 0.002$) compared with wild-type carriers.
Conclusion/interpretation. The *codon 12* variant of *PPAR-γ2* is not intrinsically associated with juvenile obesity. The variant may in its homozygous form interact, however, with various combinations of genetic and environmental factors in lean and obese subjects to cause divergent modulating effects on BMI and long-term body weight control. [Diabetologia (1999) 42: 892–895]

Keywords PPAR-γ2, mutations, obesity, body mass index changes, epidemiology.

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Abbreviations: PPAR-γ, Peroxisome proliferation-activated receptor-γ; PPRE, peroxisome proliferator response element.

Peroxisome proliferation-activated receptor-γ (PPAR-γ) is a transcription factor abundantly expressed in adipose tissue and to a lesser extent in liver, kidney and small intestines [1]. It has been shown to be involved in adipogenesis and in the regulation of adipocyte gene expression [2]. In humans, the *PPAR-γ* gene consists of nine exons. Alternate use of promotors and differential splicing of the human

form of *PPAR-γ* result in two different isoforms: PPAR-γ1 and PPAR-γ2. The predicted human PPAR-γ2 protein contains an additional 28 amino acids at the N-terminus compared with human PPAR-γ1 [1].

Recently, natural ligands (fatty acids and prostanoids) and agents that enhance sensitivity to insulin in vivo (thiazolidinediones) were shown to activate PPAR-γ [3]. In addition, PPAR-γ has a ligand-independent activation domain within the N-terminal domain, common in the two isoforms, where activity is potentiated by insulin [4]. Activation of PPAR-γ changes the rates of transcription of genes, especially genes with a peroxisome proliferator response element (PPRE) within their promotors (e.g. uncoupling protein, phosphoenolpyruvate carboxykinase) and it has been shown that the effect of PPAR-γ2 in this respect is more potent than the effect of PPAR-γ1. This finding is possibly explained by functional differences in their N-terminal domain [4].

Furthermore, the expression of *PPAR-γ2* mRNA in adipose tissue is increased in human obesity and a strong positive correlation exists between the ratio of PPAR-γ2/γ1 and the body mass index (BMI) [5]. This suggests that PPAR-γ2 may have a pivotal role in the adipocyte differentiation and in the control of BMI.

In a German study a *Pro115Gln* mutation in *PPAR-γ2* was suggested to be a rare cause of morbid obesity [6]. Four morbidly obese subjects out of 121 patients had this missense-mutation compared with none of 237 subjects with normal body weight. Furthermore, overexpression of the mutant gene in murine fibroblasts led to increased protein expression and an accelerated differentiation into adipocytes and an accumulation of triglyceride, compared with wild-type *PPAR-γ2* [6]. This mutation has so far not been detected in other populations. Also a *Pro12Ala* polymorphism of *PPAR-γ2* has been reported [7]. The *Ala allele* was shown to be associated with lower BMI ($p = 0.027$) and improved insulin sensitivity among 333 lean to moderately obese Finnish subjects [8]. In contrast, the *Pro12Ala* variant was related to higher BMI among 169 morbidly obese middle-aged American Caucasians, suggesting different mechanisms of action of the variant in these two population samples [9].

The objectives of the present investigation were to examine: 1) whether the *Pro115Gln* variant is associated with juvenile-onset obesity among Danish Caucasians and 2) whether the relation of the *Pro12Ala* polymorphism with BMI and long-term weight regulation differ between lean and obese subjects within the same cohort.

Subjects and methods

Subjects. The cohort of obese men comprised 752 subjects who had a body mass index (BMI) of 31.0 kg/m^2 or more at their draft board examination during 1943–1977. As a control cohort 869 draftees were selected at random (every two hundredth) from the same group. Weight and height were measured at the time of the draft examination and in 1992–1994 after an average follow-up period of 24 years. At follow-up their mean BMI was 25 kg/m^2. All reported themselves to be Caucasians of Danish ancestry [10]. Genomic DNA was obtained from blood samples drawn at the last examination.

Prior to participation informed consent was obtained from all subjects. The study was approved by the ethics committee of Copenhagen and was in accordance with the principles of the Helsinki Declaration II.

Preparation of genomic DNA from whole blood. Genomic DNA was isolated from human leucocyte nuclei isolated from whole blood by proteinase K digestion followed by phenol-chloroform extraction on an Applied Biosystems 341 Nuclei Acid Purification System (Foster City, Calif., USA) or proteins were precipitated by addition of a saturated NaCl solution. Subsequently, genomic DNA was precipitated in ethanol.

Screening for the Pro12Ala polymorphism and the Pro115Gln mutation in PPAR-γ2. We carried out PCR amplification of the segment with the *Pro12Ala* mutation in a volume of 25 μl, containing 100 ng of genomic DNA, 0.2 μmol/l of each primer, 10 × PCR Buffer II (Perkin-Elmer/Cetus, Norwalk, Conn., USA), 0.0625 U of Taq DNA polymerase (Perkin-Elmer/Cetus) and 3.0 mmol/l of $MgCl_2$. The PCR conditions were: denaturation at 94 °C for 3 min followed by 40 cycles of denaturation for 30 s, annealing at 53 °C for 30 s, extension at 72 °C and a final extension at 72 °C for 9 min. Primers were designed from the genomic *PPAR-γ2* sequence (Genebank accession numbers: AB005520) as followed: forward primer 5′-CAAGCCCAGTCCTTTCTGTG-3′ and the reverse primer 5′-AGTGAAGGAATCGCTTTC**C**G-3′. Restriction fragment length polymorphism (RFLP) was detected after digestion overnight with 2 U of *Hpa*II (New England Biolabs, Beverly, Mass., USA) which cuts the mutant allele at a site introduced by the reverse primer (mismatched base indicated by bold letter).

We carried out PCR amplification of the segment with the Pro115Gln mutation as described above (except for 1.5 mmol/l $MgCl_2$, 55 °C annealing, 35 cycles) using forward primer: 5′-TGCAATCAAAGTGGAGCCTGCATGTC-3′ and reverse primer: 5′-CAGAAGCTTTATCTCCACAGAC-3′. The RFLPs were detected after digestion overnight with 2 U of *Hinc*II (New England Biolabs) which cuts the mutant allele. Primer sequence together with a mutant plasmid for positive control were kindly provided by Dr. M. Ristow and Dr. C.R. Kahn, Joslin Diabetes Center, Boston, USA. All fragments were resolved on a 3 % agarose gel and made visible by staining with ethidium bromide.

Statistics. When appropriate Chi-squared analysis and Fisher's exact test were applied to test for significant differences in allele frequencies. Differences in continuous variables between groups of subjects were tested with Student's t test when the distribution of the variable or of the logarithmically transformed variable approached a normal distribution and the variances of the variables were equal in the groups compared. Otherwise the Mann-Whitney rank sum test was used. Data are means and standard deviation (SD). A p-value less than

Table 1. Clinical characteristics of 752 obese and 869 lean Caucasian men classified in accordance with their genotype of the *Pro12Ala* polymorphism of the *PRAR-γ2* gene

	Pro12Pro	Pro12Ala	p	Ala12Ala	p
Obese					
n	540	191		21	
Age at follow-up (year)	43.1 (6.2)	43.1 (6.2)	0.77	42.1 (3.6)	0.43
BMI at draft board (kg/m^2)	33.3 (2.7)	33.4 (2.6)	0.56	32.8 (1.8)	0.99
BMI at follow-up (kg/m^2)	35.5 (5.5)	36.0 (5.9)	0.50	38.9 (5.4)	0.008
Δ BMI/year (kg · m^{-2} · year^{-1})	0.10 (0.24)	0.12 (0.27)	0.16	0.27 (0.24)	0.004
Non-obese					
n	641	214		14	
Age at follow up (year)	47.2 (8.5)	49.1 (8.2)	0.18	48.9	0.93
BMI at draft board (kg/m^2)	21.7 (2.4)	21.5 (2.1)	0.56	21.7 (2.1)	0.44
BMI at follow-up (kg/m^2)	26.2 (3.7)	26.0 (3.2)	0.25	24.4 (2.7)	0.005
Δ BMI/year (kg · m^{-2} · year^{-1})	0.17 (0.1)	0.16 (0.1)	0.45	0.11 (0.1)	0.002

Values are mean (SD). The *p* value compares subjects heterozygous *(Pro12Ala)* or homozygous *(Ala12Ala)* for the polymorphism with subjects carrying the wild-type *(Pro12Pro)*

0.05 (two-tailed) was considered significant. Statistical Package of Social Science (SPSS) for Windows, version 7.5, was used for statistical analysis.

Results

The Pro115Gln mutation was not found in any of the 1621 subjects examined.

The allelic frequency of the *Pro12Ala* polymorphism in the *PPAR-γ2* gene was 14 % (95 % CI: 12–16 %) among 752 males with juvenile-onset obesity and 16 % (14–17 %) among 869 control subjects (NS). The observed genotypes were in the Hardy-Weinberg equilibrium. Neither in the lean nor in the obese group of subjects, did the heterozygous carriers of the *codon 12* polymorphism differ with respect to BMI at the draft board examination or to BMI at the end of the follow-up period compared with findings in wild-type carriers. Yet, within the cohort of obese men, 21 homozygous carriers (2.8 % of the population) of the *codon 12* variant were identified. These subjects had higher BMI at the time of re-examination (mean age 43 years) than wild-type carriers (38.9 ± 5.4 kg/m^2 vs 35.5 ± 5.5 kg/m^2, $p = 0.008$) (Table 1). Also ΔBMI/year was higher among *Ala12Ala* carriers than wild-type carriers (0.27 ± 0.24 kg · m^{-2} · year^{-1} vs 0.10 ± 0.24 kg · m^{-2} · year^{-1}, $p = 0.004$). Within the control group, 14 homozygous carriers (1.6 % of the group) of the variant had lower BMI (24.4 ± 2.7 kg/m^2 vs 26.2 ± 3.7 kg/m^2, $p = 0.005$) and lower ΔBMI/year (0.11 ± 0.11 kg · m^{-2} · year^{-1} vs 0.17 ± 0.11 kg · m^{-2} · year^{-1}, $p = 0.002$), compared with wild-type carriers (Table 1). There was no difference between the number of homozygous carriers in lean and obese subjects ($p = 0.072$).

Discussion

In contrast to a recent report [6], we were not able to detect the *Pro115Gln* mutation in *PPAR-γ2* in any of the 1621 subjects we examined, suggesting that this variant is highly unlikely to be involved in the pathogenesis of juvenile-onset obesity in Danish Caucasians. Thus, our finding supports the notion that the rare incidences of obese *Pro115Gln* carriers in the German study may reflect a founder effect. It should be emphasised that the probability of missing the detection of a *Pro115Gln* mutation is negligible, since a positive control sample was included in our screening assay.

Regarding the *Pro12Ala* polymorphism of the gene we found an allelic frequency similar to previous studies [7–9]. The variant was not intrinsically associated with obesity. Among 869 subjects with normal body weight at the draft board examination, the polymorphism in its homozygous form was, however, related to a lower BMI ($p = 0.005$) and a relatively lower increase in BMI at follow-up (average age: 48 years) ($p = 0.002$) which is in line with a previous study in Finnish non-obese subjects, where 5 subjects homozygous and 71 subjects heterozygous for the variant (mean age of 44 years) had lower BMI than 257 wild-type carriers (mean age of 45 years) ($p = 0.0027$). In the Finnish study the variant was also shown to be associated with an improved insulin sensitivity [8].

Interestingly, the present study also showed a higher BMI ($p = 0.008$) and a higher increase in BMI during follow-up ($p = 0.004$) in obese subjects homozygous for the *Pro12Ala* variant than wild-type carriers. This finding contrasts with our finding among the lean subjects but accords with the recent report from studies of American Caucasians with morbid obesity where a group of 28 subjects, homozygous and heterozygous for the variant, had higher BMI compared with 141 wild-type carriers (mean age of 41–43 years)

($p < 0.001$) [9]. There is no obvious explanation for the discrepancy between lean and obese subjects but the results could indicate a variable interaction of the *Ala allele* with other genetic and environmental factors with regulatory effects on BMI and long-term changes in body weight. Apparently, these unknown genetic and non-genetic factors differ greatly between obese and lean subjects. Thus, lean subjects carrying the *codon 12* polymorphism may, as suggested by the Finnish study [8], have an increased insulin sensitivity which is maintained due to specific combinations of lifestyle and genetic factors, whereas theoretically an increased insulin sensitivity of adipose tissue could contribute to fat accumulation and obesity in subjects predisposed for obesity. Given the intrinsic lack of association of the *Pro12Ala* polymorphism with obesity, it seems, however, equally likely that this genetic variant is not directly involved in the pathogenesis of obesity.

In conclusion, the *Pro12Ala* variant of *PPAR-γ2* does not itself influence the development of juvenile-onset obesity among Danish Caucasians. Yet, the homozygous *codon 12* variant may in the context of pre-existing obesity lead to a further increase in obesity whereas in groups not prone to weight gain the opposite effect is observed pointing to the importance of the genetic background for the impact of this amino acid polymorphism.

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