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Frequency and Significance of Pro12Ala and Pro115Gln Polymorphism in Gene for Peroxisome Proliferation-Activated Receptor-γ_2 Regarding Metabolic Parameters in a Caucasian Cohort

Andreas Schäffler*,[1] Nicole Barth,[1] Gerd Schmitz,[2] Bettina Zietz,[2] Klaus-Dieter Palitzsch,[2] and Jürgen Schölmerich[2]

[1]*Department of Internal Medicine I,* [2]*Institute of Clinical Chemistry, University of Regensburg, Germany*

Peroxisome proliferation-activated receptor-γ_2 (PPARγ_2) is exclusively expressed in adipose tissue and belongs to the transcriptional regulators of adipocyte differentiation. Recently, two missense single-point mutations have been described in the PPARγ_2 gene: Pro12Ala and Pro115Gln. It was our aim to determine the frequency of these polymorphisms in a Caucasian cohort and to investigate their possible role in the pathogenesis of obesity, type 2 diabetes, and related metabolic disorders. The genotypes of 359 subjects (149 males, 210 females) with varying degrees of obesity and with or without type 2 diabetes were determined. Subsequent to genomic polymerase chain reaction amplification, the *Hpa*II restriction fragment length polymorphism (RFLP) analysis and the *Hind*II RFLP analysis were used for genotyping the Pro12Ala and Pro115Gln polymorphism, respectively. For the Pro115Gln polymorphism, all 359 subjects showed wild-type sequence, emphasizing the very rare occurrence of the mutated allele. For the Pro12Ala polymorphism, 276 subjects (76.9%) were homozygous for the wild-type allele, 80 (22.3%) were heterozygous, and only 3 (0.8%) were homozygous for the mutated allele. Genotype frequency was calculated to be 0.88 for the wild-type allele and 0.012 for the mutated allele. No significant differences were found in age; gender; body mass index; total cholesterol; low-density, high-density, and very low density lipoproteins; triglycerides; Lp(a); uric acid; and diabetes manifestation by comparing the different genotypes. Therefore, a major role of these polymorphisms in the pathogenesis of obesity and diabetes can be excluded.

Key Words: Peroxisome proliferation-activated receptor-γ_2; adipocyte; polymorphism; mutation; obesity; diabetes.

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Author to whom all correspondence and reprint requests should be addressed: Andreas Schäffler, Department of Internal Medicine I, University of Regensburg, D-93053 Regensburg, Germany. E-mail: andreas.schaeffler@klinik.uni-regensburg.de

Introduction

Human obesity occurs frequently and is estimated to be a complex metabolic disorder *(1)* with strong genetic components *(2)*. Common obesity is thought to represent a multigenic disease with numerous candidate genes *(3–5)* that might play a pathophysiologic role. Whereas monogenetic forms of obesity are well known in rodents, only a few cases have been reported in humans. Mutations within the genes for proopiomelanocortin *(6)*, MC-4-receptor *(7–9)*, leptin *(10,11)*, leptin receptor *(12)*, prohormone convertase-1 *(13)*, and peroxisome proliferation-activated receptor-γ_2 (PPARγ_2) *(14)* have been claimed to cause monogenetic obesity in humans.

PPARγ_2 belongs to the family of nuclear transcription factors and directs adipocyte differentiation in addition to the transcription factors C/EBP-α, -β, and -δ and ADD-1/SREBP-1. PPARγ_2 can be regarded as the master regulator of adipocyte differentiation *(15–17)*. PPARγ_2 is specifically expressed in adipose tissue and plays a key role in insulin action, glucose/lipid metabolism *(18,19)*, and adipocyte-specific gene regulation.

Activation of PPARγ_2 upregulates the expression of lipoprotein lipase *(19)*, aP2 (fatty acid transporter) *(20)*, phosphoenolpyruvate carboxykinase *(21)*, acylCoA synthase *(22)*, stearyl-CoA desaturase *(23)*, fatty acid synthase *(24)*, UCP-1 *(25)*, and C/EBP-α *(23,26,27)* and downregulates leptin *(28–31)* and tumor necrosis factor-α *(32)* gene expression. Thus, PPARγ_2 is clearly a candidate gene for regulation of adipose tissue metabolism in humans.

The thiazolidinediones represent a new class of antidiabetic, insulin-sensitizing agents that have been shown to improve insulin sensitivity and to reduce plasma glucose and blood pressure in subjects with type 2 diabetes *(33)*. They are highly specific ligands for PPARγ_2, leading to an activation of the nuclear receptor on binding *(34)*. PPARγ and retinoid X receptor (RXR) form a heterodimer that can be activated by both PPARγligands and RXR-specific ligands *(35)*. Because of their permissive effect on adipogenesis and their antihyperglycemic activity in vivo *(36)*, it is interesting to speculate that mutations within the PPARγ_2 gene might impair insulin sensitivity or influence body fat mass.

Two missense mutations within the coding region of the PPARγ gene, Pro12Ala *(37,38)* and Pro115Gln *(14)*, have been reported to be associated with obesity. Therefore, it was the aim of the present study to investigate the frequency and possible association of these polymorphisms with body mass index (BMI), type 2 diabetes, and related metabolic disorders in a Caucasian cohort.

Results

Study Population

A total of 359 subjects, 149 males (41.5%) and 210 females (58.5%), participated in the study. The mean age was 40.0 ± 0.8 yr. Of these, 14 (3.9%) with type 2a diabetes and 19 (5.3%) with type 2b diabetes were registered. According to BMI, subjects were divided into five groups: group 1 = 29 subjects (8.1%), <20 kg/m^2; group 2 = 136 subjects (37.9%), 20–25 kg/m^2; group 3 = 93 subjects (25.9%), 25–30 kg/m^2; group 4 = 58 subjects (16.2%), 30–35 kg/m^2; group 5 = 43 subjects (11.9%), >35 kg/m^2. Polymerase chain reaction (PCR) amplifications and subsequent restriction digestion with the appropriate enzymes were successfully performed in all subjects.

Determination of Pro115Gln Polymorphism

All subjects showed wild-type sequence, homozygous for the Pro/Pro genotype at codon 115.

Determination of Pro12Ala Polymorphism

Two hundred seventy-six (76.9%) subjects were found to be homozygous for the wild-type allele Pro/Pro at codon 12, 80 (22.3%) were heterozygous (Pro/Ala), and only 3 (0.8%) were homozygous for the mutated allele (Ala/Ala). According to the Hardy-Weinberg equilibrium, the allelic frequency was calculated to be 0.88 for the wild-type allele and 0.12 for the mutated allele. No differences in age and gender distribution were found. Therefore, a Mendelian segregation pattern can be concluded.

Body Mass Index

The mean BMI was similar in all three genotypes with no significant differences (Table 1). In addition, genotype frequency was not significantly different within the five BMI groups in both genders.

Metabolic Parameters

Mean age was associated with BMI ($p < 0.001$; $r = 0.4$), low-density lipoprotein (LDL) ($p < 0.001$; $r = 0.26$), triglycerides ($p = 0.001$; $r = 0.3$), very low density lipoprotein (VLDL) ($p < 0.001$; $r = 0.3$), and uric acid ($p < 0.001$; $r = 0.24$). BMI was associated with LDL ($p < 0.001$; $r = 0.2$), triglycerides ($p < 0.001$; $r = 0.4$), VLDL ($p < 0.001$; $r = 0.33$), and uric acid ($p < 0.001$; $r = 0.4$). Lp(a) was associated with total cholesterol ($p = 0.001$; $r = 0.18$) and LDL ($p < 0.001$; $r = 0.21$).

No significant differences were found concerning the mean values of total cholesterol, LDL, high-density lipoprotein (HDL), VLDL, Lp(a), triglycerides, and uric acid (Table 1).

Diabetes

The occurrence of type 2 diabetes did not differ significantly between genotypes, and genotype frequency was not significantly different between patients with and without diabetes.

Discussion

Until now, five mutations have been described within the human PPARγ$_2$ gene (Table 2). In three subjects with insulin resistance, type 2 diabetes, and hypertension, two heterozygous, missense germline mutations have been detected: Pro467Leu and Val290Met *(39)*. Pro467Leu is located in helix 12, and Val290Met in helix 3 of of the ligand-binding domain (LBD) of PPARγ. The γ$_1$ and γ$_2$ receptor isoforms share common DNA-binding and ligand-binding domains linked to divergent N-terminal regions. Whereas the Val290Met mutation occurs in the center of the LBD, the Pro467Leu mutation is located at the origin of a C-terminal amphiphatic α-helix. This region is important for ligand-dependent transcription activation and coactivator recruitment *(39,40)*. Both mutations represent loss of function mutations. The Pro467Leu mutation severely impairs ligand binding and exhibits a strongly reduced activation profile upon stimulation with the thiazolidinedione BRL49653 *(39)*. The Val290Met mutation also exhibits an impaired activation profile on stimulation with BRL49653 *(39)*. The frequency of these mutations has not been investigated in further cohorts of patients.

The silent CAC1431CAT polymorphism has been found to be associated with obesity *(41)* and higher leptin levels *(41)*. However, the mechanisms responsible for this association need further investigation.

A heterozygous missense mutation, Pro115Gln, has been reported to be associated with morbid obesity in three unrelated subjects *(14)*. In contrast to the two missense mutations already mentioned, this mutation represents a gain of function mutation leading to an enhanced adipocyte differentiation by disruption of mitogen-activated protein kinase–dependent phosphorylation of a serine residue *(42)*. However, in large cohorts of lean and obese subjects, this sequence variation has not been found *(43,44)*. Confirming the literature, we also were not able to detect this mutation in our cohort of patients, suggesting that this mutation does not play a major role in the pathogenesis of common obesity or type 2 diabetes.

A relatively common Pro12Ala polymorphism within the N-terminal region of PPARγ$_2$ has been associated with increased *(37,38)* BMI, higher weight gain *(43)*, decreased BMI *(45)*, and higher insulin sensitivity *(45,46)* or has been

Table 1
Metabolic Parameters (mean values ± SE)
and Genotype of Pro12Ala Polymorphism of the PPARγ_2 Gene in 359 Caucasian Subjects[a]

Parameter	Total	Pro/Pro	Pro/Ala	Ala/Ala	p
n (%)	359 (100%)	276 (76.9%)	80 (22.3%)	3 (0.8%)	n.s.
Men (*n* [%])	149 (41.5%)	111 (40.2%)	37 (46.3%)	1 (33.3%)	n.s.
Women (*n* [%])	210 (58.5%)	165 (59.8%)	43 (53.7%)	2 (66.7%)	n.s.
Age (yr)	40.0 ± 0.8	39.6 ± 0.9	41.6 ± 1.6	44.7 ± 7.5	n.s.
BMI (kg/m^2)	27.3 ± 0.4	27.2 ± 0.4	27.5 ± 0.7	26.7 ± 3.7	n.s.
Cholesterol (mg/dL)	221 ± 3	220 ± 3	227 ± 5	188 ± 8	n.s.
LDL (mg/dL)	132 ± 2	130 ± 3	136 ± 5	109 ± 2	n.s.
HDL (mg/dL)	60 ± 1	60 ± 2	60 ± 2	67 ± 4	n.s.
Tg (mg/dL)	161 ± 12	161 ± 15	165 ± 20	87 ± 15	n.s.
VLDL (mg/dL)	30.4 ± 1.8	30.7 ± 2.1	30.2 ± 3.3	12.0 ± 2.0	n.s.
Lp(a) (mg/dL)	25.7 ± 2.0	25.0 ± 2.2	28.7 ± 4.8	11.3 ± 5.4	n.s.
Uric acid (mg/dL)	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.2	6.3 ± 1.3	n.s.
Diabetes type 2a (*n* [%])	14 (3.9%)	12	2	—	n.s.
Diabetes type 2b (*n* [%])	19 (5.3%)	12	6	1	n.s.

[a]n.s., nonsignificant; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Tg, triglycerides; VLDL, very low density lipoprotein.

Table 2
Mutations Within Gene for PPARγ_2 and Their Effects on Metabolic Parameters

Mutation	Specification	Cohort	Frequency	Association	Reference
Pro12Ala	Missense	Obese (296)	0.13	No association	*44*
		Lean (130)	0.14	No association	*44*
		Obese (752)	0.14	Higher weight gain in Ala12Ala	*43*
		Lean (869)	0.16	No association	*43*
		Diabetic (26)	0.1	No association	*48*
		Nondiabetic (333)	0.09	Pro/Pro associated with type 2 diabetes Ala associated with lower BMI and improved insulin sensitivity	*45*
		Obese (170)	0.14	Associated with obesity in women	*38*
		Type 1 diabetes (522)	0.14	No association	*47*
		Type 2 diabetes (503)	0.14	No association	*47*
		Nondiabetics (310)	0.15	No association	*47*
		Nondiabetics (108)	0.17	Higher insulin sensitivity	*46*
		Subjects (517)	0.11	Associated with higher BMI	
		Obese (169)	0.11	Associated with higher BMI	*37*
		Present data	0.12	No association	*37*
Pro115Gln	Missense	Obese (296)	Not observed	—	*44*
		Lean (130)	Not observed	—	*44*
		Obese (752)	Not observed	—	*43*
		Lean (869)	Not observed	—	*43*
		Subjects (358)	0.05	Gln associated with obesity	*14*
		Present data	Not observed	—	
P467L	Missense	Type 2 diabetes (3)	—	L associated with insulin resistance, type 2 diabetes, hypertension	*39*
V290M	Missense	Type 2 diabetes (3)	—	M associated with insulin resistance, type 2 diabetes, hypertension	*39*
CAC1431CAT	Silent	Subject (1)	—	?	*48*
		Obese (170)	0.19	T associated with obesity in women	*38*
		Subjects (820)	0.133	T associated with higher leptin levels	*41*

[a]Number of individuals is given in parentheses.

shown to have no metabolic effect *(43,44,47,48)*. In the present group of patients, we found no association between the Pro12Ala genotype and obesity. Furthermore, the Pro12 Ala genotype was unrelated to age, gender, total cholesterol, LDL, HDL, VLDL, triglycerides, Lp(a), uric acid, and diabetes type 2 manifestation. In contrast to earlier studies *(37,38,43)*, our data provide evidence that the mutated allele is not associated with any metabolic effect and does not play a major role in the development of common obesity or type 2 diabetes. The allelic frequency of the mutated allele is within the range of that reported in the literature (Table 2). A functional relevance of the Pro12Ala mutation has not yet been demonstrated in the literature.

Materials and Methods

Study Population

Three hundred fifty-nine patients with varying degrees of obesity and with or without type 2 diabetes were referred from the endocrinologic outpatient clinic (Department of Internal Medicine I, University Hospital of Regensburg, Germany). Only healthy volunteers (staff of the clinic and patients suffering from nonendocrinologic diseases not affecting metabolic parameters) and patients who came for the assessment of diabetes, obesity, hypertension, or dyslipidemia were investigated. Patients with disorders affecting metabolic parameters such as hypercortisolism, thyroid diseases, and abnormalities in sex hormone regulation were excluded (for characteristics of the entire study population, see Table 1). Patients with type 2 diabetes were divided into two groups: type 2a (BMI $\leq$ 25 kg/m^2) and type 2b (BMI >2 5 kg/m^2).

All participants (for characteristics of the study cohort, see Table 1) were informed about the aim of the study and gave informed consent. The investigation was conducted according to the guidelines expressed in the Declaration of Helsinki, and the study was approved by the local ethics committee.

Preparation of Genomic DNA from Whole Blood

Genomic DNA was isolated from whole blood samples by standard procedures (QuiAmp Blood Kit^R; Quiagen, Hilden, Germany).

Determination of Pro12Ala Polymorphism in PPARγ_2 Gene

PCR was performed using standard procedures for genomic PCR analysis (annealing temperature = 60°C). A 237-bp PCR fragment encompassing the polymorphic *Hpa*II restriction site was amplified in a GeneAmp9600^R thermal cycler (Perkin Elmer) using the upstream primer 5'-CAAGCCCAGTCCTTTCTGTG-3'and the downstream primer 5'-AGTGAAGGAATCGCTTTCCG-3' (mismatched base underlined). The mutant allele is cut at a site introduced by the downstream primer. PCR products were purified and then digested with the restriction enzyme *Hpa*II (Boehringer-Mannheim, Germany), loaded onto a 3% agarose gel, and analyzed for the corresponding pattern of bands. Subjects homozygous for the wild type show two bands of 217 and 20 bp; heterozygous subjects show three bands of 237, 217, and 20 bp; and subjects homozygous for the mutation show one single band of 237 bp.

Determination of Pro115Gln Polymorphism in PPARγ_2 Gene

A 129-bp PCR fragment ecompassing the polymorphic *Hind*II restriction site was amplified in a GeneAmp9600 thermal cycler (Perkin Elmer) using the modified upstream primer 5'-TGCAATCAAAGTGGAGCCTGCATGTC-3' (mismatched base underlined) and the downstream primer 5'-CAGAAGCTTTATCTCCACAGAC-3'. The mutant allele is cut at a site introduced by the upstream primer. Subjects homozygous for the wild type show one single band of 129 bp, heterozygotes show three bands of 129, 104, and 25 bp; and subjects homozygous for the mutation show two bands of 104 and 25 bp. PCR products were purified and then digested with the restriction enzyme *Hind*II (Boehringer-Mannheim) loaded onto a 3% agarose gel, and analyzed for the corresponding pattern of bands. Since the mutated allele occurs extremely rarely, a plasmid construct carrying a *Hind*II restriction site was used as an internal control in order to evaluate proper enzyme digestion.

Statistical Analyses

Subjects were compared according to their genotypes for differences in anthropometric and biochemical data by two-tailed Mann-Whitney or Kruskal-Wallis tests for comparison of two or more independent samples and Pearson χ^2 tests for associations between classified variables. Data are expressed as means and standard deviations for simplicity. The significance level was set at 0.05 in all statistical tests. To examine associations as exactly as possible, tests were first performed with all three genotypes together in a one-way analysis of variance. When a significance level of <0.1 was achieved, we examined correlations of the possibly associated parameters to the genotypes in every possible combination. For comparison between metabolic parameters, the Spearman correlation analysis was used.

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