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PPAR-γ2 Pro12Ala polymorphism in the population of obese and non-obese men of the city of Wroclaw

Polimorfizm PPAR-γ2 Pro12Ala w populacji otyłych i nieotyłych mężczyzn z populacji Wrocławia

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Abstract

Introduction: The aim of this study was to examine the association of Pro12Ala PPARγ2 polymorphism with anthropometric and biochemical parameters defining the risk for the development of metabolic syndrome in a healthy population of men

Material and methods: The study group consisted of 176 healthy men, aged 25–65 years (average 54.16 years). Polymorphisms of the PPAR-g gene (Pro12Ala, Ala12Ala, Pro12Pro) were explored using the PCR-RFLP method. Plasma glucose, insulin, total cholesterol, LDL, HDL and TG were measured using commercially available kits.

Results: The genotypic distribution of the Pro12Ala polymorphism was as follows: Pro/Ala 69.8% (n = 123), Ala/Ala 28.4% (n = 50) and Pro/Pro 1.8% (n = 3). The Pro12Ala and Ala12Ala subjects did not differ in any of the measured variables. The non-obese (BMI < 30 kg/m^2, n = 117) and obese subpopulations (BMI > 30 kg/m^2, n = 56) did not significantly differ in the distribution of the genotypes. In the non-obese subpopulation, the homozygous Ala12 carriers (n = 38, 32.4%) had higher systolic blood pressure, plasma triglycerides, insulin levels and HOMA-IR.

Conclusions: We conclude that despite the high frequency of the Ala allele at the *PPAR-γ2* gene in our population of Polish men, the Ala12 allele does not appear to improve insulin sensitivity or have an influence on the occurrence of obesity. It remains to be explained by larger studies if this polymorphism carries any risk of the development of metabolic abnormalities in non-obese men.

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Key words: *PPAR-γ2 polymorphism, Pro12Ala, metabolic syndrome*

Streszczenie

Wstęp: Celem pracy była ocena związku miedzy polimorfizmem Pro12Ala PPAR-γ2 a biochemicznymi i antropometrycznymi czynnikami ryzyka zespołu metabolicznego w populacji zdrowych mężczyzn z Wrocławia.

Materiał i metody: Grupa badana składała się ze 176 zdrowych mężczyzn, w wieku 25–65 lat (średnio 54,16 roku). Polimorfizm genu PPAR-γ (Pro12Ala, Ala12Ala, Pro12Pro) oceniano za pomocą metody PCR-RFLP. Stężenie glukozy, insuliny, cholesterolu całkowitego, cholesterolu frakcji LDL, frakcji HDL, triglicerydów (TG) w osoczu było oceniane przy użyciu standardowych zestawów.

Wyniki: Częstość poszczególnych polimorfizmów była następująca: Pro/Ala — 69,8% (n = 123), Ala/Ala — 28,4% (n = 50), Pro/Pro — 1,8% (n = 3). Pacjenci z polimorfizmem Pro12Ala i Ala12Ala nie różnili się pod względem wartości wszystkich ocenianych parametrów. Częstości genotypów w subpopulacji mężczyzn nieotyłych (BMI < 30 kg/m^2, n = 117) i otyłych (BMI > 30 kg/m^2, n = 56) nie różniły się istotnie statystycznie. W subpopulacji mężczyzn nieotyłych homozygoty Ala12 (n = 38, 32,4%) charakteryzowały się wyższym ciśnieniem skurczowym krwi, wyższym stężeniem triglicerydów w osoczu, wyższym stężeniem insuliny oraz wyższą wartością HOMA-IR.

Wnioski: Wyniki pracy pozwalają na wyciągnięcie wniosku, że pomimo wysokiej częstości allelu Ala genu *PPAR-γ2* w ocenianej populacji mężczyzn z Wrocławia, allel Ala12 nie wpływa na poprawę insulinowrażliwości oraz na występowanie otyłości. Konieczne jest potwierdzenie obserwacji, że polimorfizm ten sprzyja wystąpieniu zaburzeń metabolicznych u nieotyłych mężczyzn.

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Słowa kluczowe: *polimorfizm PPAR-γ2, Pro12Ala, zespół metaboliczny*

Introduction

Polymorphisms in peroxisome proliferator-activated receptor (PPAR)-γ2, a member of the nuclear hormone receptor family involved in adipocyte differentiation and gene expression, has been linked to the pathogenesis of type 2 diabetes mellitus, insulin resistance and atherosclerosis. The most common polymorphism, Pro12Ala genotype (proline to alanine substitution in exon B) has been well studied in association with type

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2 diabetes mellitus, obesity and other conditions. The Pro12Ala polymorphism in PPAR-γ2 was first identified in 1997 in some Caucasian populations, the ethnic group with its highest frequency. The carrier prevalence of the polymorphism can be close to 25%. The Ala12 allele, which has been found to decrease its transcriptional activity, has been clinically associated with improved insulin sensitivity and reduced risk for type 2 diabetes but increased long-term weight gain (1–3).

The aim of our study was to investigate, in a Polish population of men, the relationship between the *Pro12Ala*, *PPARγ-2* gene polymorphism and obesity and its metabolic parameters.

Material and methods

One hundred and seventy-six male subjects aged 25–65 years were recruited by advertising. Only non-diabetic subjects were included, according to current diagnostic criteria of the American Diabetes Association. Before participation in the study, informed consent was obtained from all subjects. The study protocol was approved by the local ethic committee of Wroclaw Medical University.

After overnight bed-rest fasting, blood samples were taken from each subject in the early morning and immediately frozen until further analysis. Body weight was measured to the nearest 0.1 kg on a calibrated balance. The BMI was estimated by dividing the body weight (in kilograms) by the square of the height (in metres). Subjects in this study were classified as normal weight, overweight or obese, according to BMI cut-off point 30 kg/m^2. Waist and hip circumferences were measured to the nearest 0.5 mm with a plastic tape measure and the waist/hip ratio (WHR) was calculated. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) formula, defined as fasting insulin × fasting glucose/22.5.

Detection of the Pro12Ala polymorphism in the PPAR-γ *gene*

Genomic DNA was obtained from human leukocyte nuclei isolated from whole blood. PCR amplification of the DNA segment containing the variant was carried out in a volume of 30 μl, containing 10 ng DNA, 10 mmol/l of each dNTP, 1.5 mmol/l $MgCl_2$, 100 pmol of each primer (PPAR12-F: 5′-CAA GCC CAG TCT TTC TG TG-3′; PPAR12-R: 5′-AGT GAA GGA ATC GCT TTC CG-3′), 0.8 IU of Taq DNA polymerase and PCR buffer. PCR conditions were: denaturation at 94°C for 3 min, 40 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec), extension (72°C, 45 sec), and a final extension at 72°C for 9 min. Restriction fragment length polymorphism was detected after digestion with Hpa II, which cuts the wild-type allele at a site introduced by the reverse primer.

Glucose, cholesterol, triglyceride (TG), low and high-density lipoproteins (LDL, HDL) and insulin were measured by routine laboratory methods.

Statistical analyses

Characteristics of the group variables were expressed as the mean and SD. Allele frequencies were estimated by gene counting. Comparisons between genotypic groups were tested with ANOVA. The calculations were performed by Statistica software (version 4.5 win, StatSoft Inc., USA). A *P*-value less than 0.05 was considered statistically significant.

Results and discussion

The association between the substitution of alanine for proline at codon 12 of *PPAR-γ2* gene and the risk for obesity, insulin resistance and type 2 diabetes has been widely studied giving inconsistent results [1–3]. The majority of studies found that the Ala 12 allele carried a lower risk of insulin resistance [2, 4, 5]. However, not all studies have found this association. So far, no studies have reported an association between Pro12Ala polymorphism and greater insulin resistance [1, 6]. In our study, 69.8% subjects (n = 123) had the variant in its heterozygous form (Pro12Ala), whereas 28.4% of the population (n = 50) were homozygous for the Ala allele (Ala12Ala), and only 1.8% (n = 3) had Pro12Pro genotype. Tables I–III illustrate the clinical and biochemical characteristics of the study group in obese and non-obese men.

There were no significant differences between wild-type and Ala12 carriers regarding adiposity, body fat distribution, plasma lipids or insulin sensitivity in our population of men (Table I). The non-obese (BMI < 30 kg/m^2, n = 117, Table II) and obese subpopulations (BMI > 30 kg/ms, n = 56, Table III) were not significantly different regarding the distribution of genotypes. In the non-obese subpopulation, the homozygous Ala12 carrier had higher systolic blood pressure ($P < 0.05$) and higher plasma triglycerides and insulin levels ($P < 0.02$, $P < 0.03$, respectively) associated with higher measurement of insulin resistance HOMA-IR ($P < 0.01$) compared with heterozygous Pro12Ala. The correlations were significant after adjustment for age. If confirmed by larger studies, the association of the Ala12 allele with some metabolic abnormalities is a new and interesting observation. In the obese subpopulation there were no differences found in the two genotype subgroups regarding all the parameters investigated.

In our study we found no association of Pro12Ala polymorphism in exon B of PPAR-γ2 with the presence of obesity or clinical parameters defining metabolic syn-

Table I. *Clinical and biochemical characteristics of the study group regarding the Pro12Ala genotype*
Tabela I. *Kliniczna i biochemiczna charakterystyka badanej grupy w zależności od obecności genotypu Pro12Ala*

	Ala12Ala genotype (n = 50)	Pro12Ala genotype (n = 123)
Age [years]	52.53 ± 9.76	51.46 ± 11.24
Systolic blood pressure [mm Hg]	134.29 ± 16.65	130.87 ± 17.36
Diastolic blood pressure [mm Hg]	84.08 ± 16.79	84.96 ± 10.98
Body mass index	27.15 ± 3.53	28.44 ± 4.59
Waist-hip ratio	0.91 ± 0.08	0.91 ± 0.08
Total Cholesterol [mg/dl]	208.94 ± 47.7	214.52 ± 42.25
Triglycerides [mg/dl]	153.35 ± 76.91	142.36 ± 91.25
HDL-Cholesterol [mg/dl]	53.88 ± 12.13	54.32 ± 12.26
LDL-Cholesterol [mg/dl]	124.23108.5 ± 41.35	130.87 ± 43.31
HOMA-IR	2.48 ± 1.76	2.59 ± 2.7

Table II. *Clinical and biochemical characteristics of non-obese men (BMI < 30) regarding the Pro12Ala genotype*
Tabela II. *Kliniczna i biochemiczna charakterystyka nieotyłych mężczyzn (BMI < 30) w zależności od obecności genotypu Pro12Ala*

Genotype	Ala12Ala genotype (n = 38)	Pro12Ala genotype (n = 78)
Age (years)	51.59 ± 10.47	49.83 ± 11.97
Systolic blood pressure [mm Hg]	134.05 ± 17.63	127.44 ± 16.05*
Diastolic blood pressure [mm Hg]	83.78 ± 17.85	83.91 ± 11.04
Body mass index	25.57 ± 2.13	25.76 ± 2.36
Waist-hip ratio	0.9 ± 0.08	0.89 ± 0.07
Total Cholesterol [mg/dl]	210.14 ± 47.67	214.12 ± 42.26
Triglycerides [mg/dl]	147.76 ± 66.94	122.33 ± 66.47*
HDL-Cholesterol [mg/dl]	54.76 ± 13.14	57.52 ± 12.84
LDL-Cholesterol [mg/dl]	128.49 ± 37.12	133.3 ± 40.71
HOMA-IR	2.21 ± 1.08	1.82 ± 1.6*

* $P < 0.05$

Table III. *Clinical and biochemical characteristics of obese men (BMI > 30) regarding the Pro12Ala genotype*
Tabela III. *Kliniczna i biochemiczna charakterystyka otyłych mężczyzn (BMI > 30) w zależności od obecności genotypu Pro12Ala*

	Ala12Ala genotype (n = 12)	Pro12Ala genotype (n = 42)
Age (years)	55.42 ± 6.7	54.81 ± 8.93
Systolic blood pressure [mm Hg]	135 ± 13.82	137.02 ± 18.32
Diastolic blood pressure [mm Hg]	85 ± 13.65	87.02 ± 10.82
Body mass index	32.04 ± 2.25	33.42 ± 3.41
Waist-hip ratio	0.94 ± 0.05	0.96 ± 0.07
Total Cholesterol [mg/dl]	205.25 ± 47.88	214.76 ± 44.13
Triglycerides [mg/dl]	170.58 ± 103.53	177.93 ± 118.59
HDL-Cholesterol [mg/dl]	51.17 ± 8.11	48.15 ± 8.75
LDL-Cholesterol [mg/dl]	108.5 ± 53.64	126.35 ± 49.25
HOMA-IR	3.29 ± 2.96	3.94 ± 3.72

drome. This discrepancy with other studies may be explained by the differences in genetic background of the populations studied. Alternatively, environmental factors (*e.g.* food intake, nutrient composition of diet) or gene-environment/gene-nutrient interaction, as previously demonstrated for this variant, may be involved [7].

We conclude that despite the high frequency of the Ala allele at the PPAR-γ2 gene in our population of men, Ala12 allele does not appear to improve insulin sensitivity or have an influence on the occurrence of obesity. It remains to be explained by larger studies if this polymorphism carries any risk of the development of metabolic abnormalities in non-obese men.

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