Original Articles

The Frequency of Alleles of the Pro12Ala Polymorphism in PPARγ2 Is Different between Healthy Controls and Patients with Type 2 Diabetes

(PPARγ2 / Pro12Ala polymorphism / type 2 diabetes / allele frequency / lipids)

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Abstract. The aim of this initial case-control study was to determine the association between common Pro12Ala polymorphism in the PPARγ2 gene and type 2 diabetes in the Czech Republic. Furthermore, the effect of this polymorphism on phenotypic characteristics and on levels of lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) was studied. One hundred thirty-three patients with type 2 diabetes and 97 control subjects were investigated. PCR and RFLP analysis were used for identification of individual genotypes. In the group of patients, three samples (2.26%) were identified as homozygous for the Ala/Ala genotype and 99 samples (74.44%) were homozygotes for the Pro/Pro genotype. Thirty-one samples (23.31%) were identified as Pro12Ala heterozygous. In the control group, six samples (6.19%) were homozygous for the Ala/Ala genotype and 61 samples (62.89%) were homozygotes for the Pro/Pro genotype. Thirty samples (30.93%) were identified as Pro12Ala heterozygous. The allele frequency for the Ala allele was lower in the type 2 diabetic group than in the control group (13.91% vs. 21.43%, P = 0.022). There was no difference (at P < 0.05) between the phenotypic characteristics (BMI, sex) studied in the group of patients according to the Pro12Ala genotype. There was no significant effect of the Pro12Ala polymorphism on lipid levels.

Received August 23, 2004. Accepted September 13, 2004.

Funded by the 3rd Faculty of Medicine, Charles University Prague; Grant Number: J13/98: 111200001.

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Abbreviations: PCR – polymerase chain reaction, PPAR – peroxisome proliferator-activated receptor, RFLP – restriction fragment length polymorphism.

Peroxisome proliferator-activated receptor (PPAR) γ is a transcription factor that has among others an important role in adipocyte differentiation and expression of the adipocyte-specific genes (Deeb et al., 1998; Zietz et al., 2002). PPARγ is activated by naturally occurring fatty acids and fatty acid derivates (Debril and Renaud, 2001). The biomolecular action of PPARγ is well documented. This protein heterodimerizes with another intracellular protein, the retinoid X receptor, and binds to specific DNA sequences noted as PPERs (Debril and Renaud, 2001). PPARγ activation is linked to an increased differentiation of preadipocytes to adipocytes. There are three already known forms of PPARγ: PPARγ1, PPARγ2 and PPARγ3. These are products of an alternative splicing (Šrámková et al., 2001). The Pro12Ala polymorphism resides inside exon 2, which is just in the form called PPARγ2 (Yen et al., 1997). The protective impact of the Ala genotype is probably based on less efficient stimulation of target genes and lower accumulation of adipose tissue and improved insulin sensitivity (Deeb et al., 1998; Hara et al., 2000).

Recently reported data are very inconsistent about the association of Pro12Ala polymorphisms in the PPARγ2 gene with type 2 diabetes. There are two large studies suggesting a decreased risk of type 2 diabetes for the Ala12 genotype in PPARγ2 (Deeb et al., 1998; Altshuler et al., 2000). Several subsequent publications failed to confirm the association (Mori et al., 1998; Mancini et al., 1999; Ringel et al., 1999; Clement et al., 2000), whereas others supported the data (Hara et al., 2000; Jacob et al., 2000; Mori et al., 2001).

Material and Methods

Subjects

DNA samples were obtained from 133 unrelated Czech patients with type 2 diabetes (characterization:

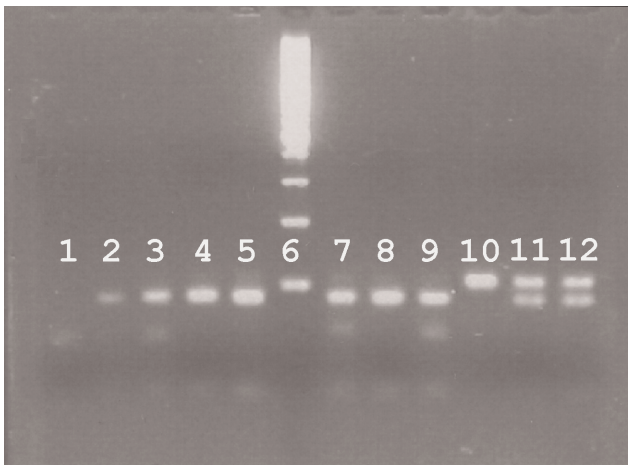

Fig. 1. Electrophoresis of PCR products after restriction on 2% agarose gel. Line 1: negative control. Lines 2,3,4,5,7,8,9: homozygotes for the Pro/Pro genotype. Line 6: DNA marker. Line 10: homozygotes for the Ala/Ala genotype. Lines 11,12: heterozygotes for the Pro/Ala genotype.

age > 35 years, C-peptide > 200 pmol/l, antiGAD < 50 ng/ml). The level of C-peptide was determined by an immunoradiometric method (Immunotech, Prague, Czech Republic). The presence of IgG antibodies against GAD was detected by ELISA (Roche Molecular Biochemicals, Mannheim, Germany). The levels of lipids were determined using automatic analyser KONELAB 60 (Labsystems CLD, Espoo, Finland) and commercially available kits (BioVendor, Brno, Czech Republic). Ninety-seven healthy subjects were used as a control. All of them were recruited from blood donors and no clinical details were available for this group. Informed consent was obtained from all subjects.

Genetic analysis

Genomic DNA was isolated from peripheral blood using a commercially available kit (QIAamp Blood Kit, Qiagen, Hilden, Germany). The DNA samples were stored at -20°C.

The part of exon 2 containing codon 12 was amplified by using forward primer (Deeb et al., 1998) 26-mer 5'-GACAAAATATCAGTGTGAATTACAGC-3' and reverse primer 25-mer 5'-GTATCAGTGAAGGAACCGCTTTCTG-3'. The used PCR mix contained: 1x polymerase chain reaction (PCR) buffer for Taq polymerase, 200 μM dNTP (each), 1.5 mM $MgCl_2$, 0.4 μM primers, 2 U of Taq polymerase and 30–100 ng of the DNA sample. The PCR conditions were: denaturation for 2 min at 94°C, followed by 30 cycles of denaturation at 94°C for 25 s, annealing at 54°C for 30 s and extension at 72°C for 30 s, final extension at 72°C for 5 min. The result of the PCR reaction was a 106-bp fragment. This PCR product was visualized by electrophoresis on a 2% agarose gel in 1x TBE buffer.

The *Bse*LI restriction endonuclease was used for digestion. We changed the sequence of DNA with the reverse primer and we prepared the digest site for this inexpensive restriction enzyme. The digestion was done at 55°C for 1 h.

Then the final results were obtained from the second electrophoresis on an agarose gel (Fig. 1).

Statistical methods

The statistical difference in allele frequencies between the group of patients and the control group was assessed by the binomial proportions test in the Statgraphics Plus software. To confirm the difference between groups of genotypes, the χ^2 test was used in the EpiInfo 2000 software. The influence of the genotype on the clinical parameters was estimated by the ANOVA test. The P value < 0.05 was considered as significant.

Results

Association of the Pro12Ala variant in the PPARγ2 with type 2 diabetes

Genotype distribution in the group of patients was: 2.3% homozygous for the Ala/Ala allele, 23.3% Ala/Pro heterozygous, and 74.4% were Pro/Pro homozygous. In the control group, 6.2% were homozygous for the Ala/Ala allele, 30.9% Ala/Pro heterozygous, and 62.9% were Pro/Pro homozygous. There was no significant difference in the proportions of the different genotypes at codon 12 between the group of patients and the control group (see Table 1 below). The Ala-allele frequency was 21.4% in the control group and 13.9% in the patient group. The allele frequency for the Ala allele was significantly lower in the type 2 diabetic group than in the control group (13.91% vs. 21.43%, P = 0.022). These data suggest that the polymorphism Pro12Ala plays some role in type 2 diabetes in the Czech population.

Table 1. Calculated P values for genotype frequencies

	No. of genotype			
	Pro/Pro	**Pro/Ala**	**Ala/Ala**	**Total**
Patients	99	31	3	133
Controls	61	30	6	97
P value	0.06	0.19	0.13	

The odds ratios are not mentioned in the table because none of P values is significant.

Correlation between the PPARγ2 genotype and clinic parameters of the type 2 diabetic subjects with and without the Ala12 variant

No relation between the polymorphism and BMI, sex or levels of total cholesterol, HDL- and LDL-cholesterol or triglycerides could be detected in the group of the patients.

Table 2. Clinical characteristics of patients with the type 2 diabetes

Parameter	Total	Pro/Pro	Pro/Ala +Ala/Ala	P value
N (%)	133 (100)	99 (74.4)	34 (25.6)	
Age [years]	65.3 ± 9.6	64.6 ± 10.0	66.3 ± 9.0	n.s.
BMI [kg/m]	30.9 ± 6.3	31.2 ± 4.9	31.5 ± 5.8	n.s.
Total cholesterol [mmol/l]	6.3 ± 1.1	6.3 ± 1.1	6.4 ± 0.9	n.s.
HDL [mmo/l]	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	n.s.
LDL [mmol/l]	3.9 ± 1.1	3.9 ± 1.1	4.0 ± 0.9	n.s.
Triglycerides [mmol/l]	2.7 ± 1.2	3.1 ± 1.7	2.4 ± 1.8	n.s.

n.s. – not significant

Discussion

This study supports the hypothesis that the Pro12Ala polymorphism of the PPARγ2 gene plays a significant role in type 2 diabetes of the Czech population. Our results showed that the frequency of the Ala12 variant of the PPARγ2 gene is higher in the control group than in the group of patients. This can be explained by the fact that the proline to alanine substitution in the codon 12 in PPARγ2 is associated with a decreased risk of the type 2 diabetes. These data are consistent with several previous studies carried out on German (Jacob et al., 2000), Finnish (Deeb et al., 1998), Japanese (Hara et al., 2000; Mori et al., 2001) or Caucasian (Altshuler et al., 2000) populations and inconsistent with others (Mori et al., 1998; Mancini et al., 1999; Ringel et al., 1999).

Some authors compared numerous clinical characteristics and the Pro12Ala polymorphism between type 2 diabetic subjects and control subjects or type 2 diabetic subjects with and without the Ala12 variant. They found many various associations of the Pro12Ala polymorphism with BMI (Deeb et al., 1998), insulin sensitivity (Deeb et al., 1998; Koch et al., 1999; Hara et al., 2000; Jacob et al., 2000), changed concentrations of total cholesterol (Mori et al. 2001; Zietz et al. 2002) and LDL-cholesterol (Zietz et al., 2002). But it is obvious from our analysis of clinical characteristics that there is no significant diference in the BMI or lipid levels. It thus seems that for studying the role of the Pro12Ala polymorphism of the PPARγ2 gene in the genetic background of dyslipidaemia, much larger studies are needed.

In summary, we can conclude from our results that the Pro12Ala polymorphism of the PPARγ2 gene is associated with reduced risk of type 2 diabetes. This protective effect is evident among Ala12 variant carriers. We have further demonstrated that the polymorphism is not associated with BMI and changed lipid levels.

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