Gly395Arg Polymorphism of PPARα Gene Was Not Detected in Japanese Population of 729 Individuals

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(Received September 2, 2005)

Summary Peroxisome proliferator-activated receptor α (PPARα) is a member of the nuclear receptor superfamily and participates in the regulation of key proteins involved in lipid metabolism, fatty acid oxidation, homeostasis, and inflammation. Several polymorphisms of the human PPARα gene, such as Leu162Val polymorphism and Val227Ala polymorphism, have been described in different races. Recently, another PPARα polymorphism Gly395Arg polymorphism has been reported in Caucasian and African subjects. Using the Invader assay, we searched for this polymorphism in 729 Japanese adults randomly selected in a rural population. Although the synthesized oligonucleotides of each polymorphism could be distinguished clearly, all 729 individuals had the Gly (G) allele and none had the Arg (C) allele. These data suggest that there is racial variability in the frequencies of PPARα gene polymorphisms.

Key Words polymorphism, PPARα, Invader assay, Japanese

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily, including receptors for retinoic acid (vitamin A), vitamin D and other lipophilic molecules (1). Three subtypes of the receptor (PPAR α, δ, γ) are expressed in mammals including humans. PPARα is most commonly expressed in organs and tissues in which fatty acid oxidation is active, such as the liver, kidney and muscle as well as adipose tissue. Previous studies indicate that the genes regulated by PPARα participate in the regulation of key proteins involved in lipid metabolism, fatty acid oxidation, homeostasis, and inflammation. Fibrates are a widely used class of hypolipidemic agents that act through PPARα activation. In addition, fatty acids can activate PPARα, suggesting its activity is closely related to nutritional status. Indeed, fasting conditions increased the expression and activity of PPARα (1). In the liver, fatty acids are oxidized to acetyl coA and subsequently to ketone bodies. These processes are strongly stimulated by PPARα. Moreover, dietary fish oils containing eicosapentaenoic acid and docosahexaenoic acid can regulate gene transcription through the activation of PPARα. Fish oils increase the transcription of regulatory genes of fatty acid oxidation, such as lipoprotein lipase and acyl coA oxidase via PPARα, and fish oils can decrease the blood triglyceride concentration in hypertriglyceremic patients (1). Thus, PPARα appears to be a nutritional sensor and an important regulator of fat metabolism in response to nutritional changes.

The gene (PPARα) encoding PPARα is located on the long arm of chromosome 22. Several polymorphisms of the human PPARα gene, such as Leu162Val polymorphism and Val227Ala polymorphism, have been described (2–7). The Leu162Val site is located in the DNA-binding domain, which includes the zinc finger. The Val162 isoform increases PPAR-responsive element-dependent transcriptional activity compared with the Leu162 isoform in in vitro transient transfection assay (2, 3). The Val227Ala site is located in the region between the DNA binding and ligand binding domain of the PPARα gene, which is also thought to contain the dimerization domain. Leu162Val polymorphism was identified in Caucasians. Vohl et al. found an association between the Leu162Val polymorphism and hyper-apobeta-lipoproteinaemia in non-diabetic subjects (4). Flavell et al. reported associations between the Leu162Val polymorphism and total cholesterol, HDL-cholesterol, and apolipoprotein levels in type II diabetic subjects (2). However, this polymorphism was not detected in a study conducted on 401 Japanese subjects (7). In contrast, the Val227Ala polymorphism has not been detected in Caucasians (2–4), suggesting that there is variability in the frequencies of PPARα gene polymorphisms between ethnic groups.

Recently, another PPARα polymorphism, Gly395Arg polymorphism (GGA, Gly and CGA, Arg), was reported (8). The Gly395Arg site is located in the ligand-binding domain of the PPARα gene. The substitution of Gly for Arg at amino acid 395 may cause a functional change in ligand binding and PPARα activation. However, the association of this polymorphism with other factors has...
not been studied yet. The aim of our study was to search for the genetic polymorphism of Gly395Arg in the PPARα gene in the Japanese population, and if found, to examine its role in the Japanese. We conducted a cross-sectional study on 729 Japanese adults randomly selected in a rural population to investigate the association of the PPARα gene with obesity-related factors.

**Methods**

Lifestyle assessments, blood examinations, and DNA analyses were performed as described previously (9–11). The present study was approved by the ethics review committee of the National Institute of Health and Nutrition, Japan. All subjects gave written informed consent. The allele frequency was determined by direct counting.

**Study subjects.** In 1999 to 2000, the subjects were randomly selected from all residents aged 40–69 y in Shiso, a rural county located in the northwestern part of Hyogo Prefecture, Japan. All participants were examined at local community halls. Lifestyle assessments, blood examinations, and DNA analyses were performed as described below.

**Laboratory methods.** Venous blood samples were drawn into EDTA-tubes and serum tubes. Serum was then obtained by centrifugation at 3,000 rpm for 10 min at 4°C and subsequently used for biochemical assays. The blood cells were frozen and kept at −20°C until DNA extraction. DNA was extracted from white blood cells by a standard method (Gentra System, Inc.).

The Invader assay. The Invader assay was performed as described elsewhere (12). The primary probes (G polymorphism and C polymorphism) and the Invader oligonucleotides (Invader probe) are as follows:

- Primary probe for G (Gly): ecggcgagctacgttta GAAGGCCAG (FAM, Green dye), Primary probe for C (Arg): acggcgagctacgtttagaaggccag (RED, Red dye). Invader probe: AGCACATGCTACATCGGCTCT CGCATTTTTCTAATGTTTCT. The small letters indicate the flap sequences of primary probes. The Invader assay was performed at the Research Department, R&D Center, BML (Saitama, Japan).

**Results and discussion**

PPARα is important for human lipid metabolism. We examined the relationship between Gly395Arg polymorphism and various factors related to lipid metabolism and nutritional conditions. The sample we used was obtained from healthy participants who completed a standardized questionnaire concerning dietary intake, physical activity, smoking and alcohol drinking habits. A total of 729 Japanese (341 men and 388 women) 40 to 69 y of age, were selected from all the residents by satisfied random sampling based on gender and decade of age. Serum total cholesterol, HDL cholesterol, triglyceride, hemoglobin A1c, and glucose levels were also determined. The mean body mass index (kg/m²) for all the 728 individuals, the 340 men and the 388 women was 23.1±3.0, 23.4±3.0 and 22.8±3.0 kg/m², respectively, and a percentage with a body mass index of or more 25 was 25.1, 31.3 and 19.6%, respectively. The clinical lipid parameters possibly related to PPARα functions for 592 individuals (missing values for blood parameters were excluded), 281 men and 311 women, were 99.6 and 1.7, 112.6 and 1.7, 89.1 and 1.6 mg/dL (geometric mean and geometric SD), respectively, for fasting blood triglycerides, 207.7±35.4, 200.9±34.4 and 213.8±35.3 mg/dL (mean±SD), respectively, for fasting total cholesterol, 59.6±15.0, 55.9±14.5 and 62.9±14.6 mg/dL, respectively, for fasting HDL cholesterol, 5.03±0.71, 5.08±0.90 and 4.98±0.48%, respectively, for hemoglobin A1c: the percentage with a triglyceride level of 150 mg/dL or higher was 22.3, 30.1 and 15.1%, respectively, the percentage with a cholesterol level of 220 mg/dL or higher was 35.3, 29.5 and 40.5%, respectively, the percentage with an HDL-cholesterol of less than 40 mg/dL was 5.1, 8.5 and 1.9%, respectively, and the percentage with a hemoglobin A1c of 6.1% or higher was 5.4, 8.1 and 3.5%, respectively.

The frequency of the PPARG Gly395Arg polymorphism in the Japanese population is unknown. First, we set up the ‘Invader method’ to analyze Gly395Arg polymorphism. The Invader assay was recently developed to determine the genotyping of a single nucleotide polymorphism (SNP) and has been used for high-throughput genotyping.Briefly, the Invader assay, a non-PCR based assay, consists of the hybridization of two oligonucleotides (a discriminatory primary probe and an Invader oligonucleotide) to the target DNA forming an overlapping structure, that is, the substrate for a structure-specific 5’ nuclease termed Cleavase using two different discriminatory primary probes, each with a spectrally distinct fluorophore, which allows for the detection of alleles of a given SNP in a single reaction. This method is highly sensitive and specific for detection of alleles of a given SNP in a single reaction (12). To set up the assay system of Gly395Arg polymorphism of PPARG gene, we performed the Invader assay using synthesized 64-mer oligonucleotides (Oligonucleotide [G]: GTCCTGCGCTTATAACGTA[G]GACACATTGAAA AAATGCAGGAGGGTTATTGTACATGTGCTCAT and Oli-
No Gly395Arg Polymorphism of PPARα Gene in Japanese

Table 1. Ratio of population with indicated genotype. Numbers in parentheses are actual number of samples.

|              | Leu162 (homozygote) | Val162 (hetero- and homozygote) | Race                  | References |
|--------------|----------------------|----------------------------------|-----------------------|------------|
| Gly395       | 65% (15)             | 35% (8)                          | Caucasians+Africans   | 8          |
|              | 100% (729)           | 0% (0)                           | Japanese              | this study |
| Leu162       | 93.4% (369)          | 6.6% (26)                        | Caucasians            | 4          |
|              | 86.1% (971)          | 13.9% (157)                      | Caucasians            | 5          |
|              | 100% (401)           | 0% (0)                           | Japanese              | 7          |
| Val227       | 96% (356)            | 4.0% (16)                        | Japanese              | 6          |
|              | 90.3% (362)          | 9.7% (39)                        | Japanese              | 7          |
|              | 100% (395)           | 0% (0)                           | Caucasians            | 4          |
| Arg395       | 65% (15)             | 35% (8)                          | Caucasians+Africans   | 8          |
|              | 100% (729)           | 0% (0)                           | Japanese              | this study |

Platko showed that the genotype frequency of the Gly395Arg polymorphism was G/G=0.609, C/C=0.348 and C/C=0.043 in Caucasian and African subjects (8). Although the number of alleles examined was not large (n=46), their results seem reliable, because 1) PCR results were confirmed in multiple reactions, 2) homozygotes were detected in individual genotype data, and 3) the Hardy-Weinberg probability appears to be sufficient: ($\chi^2=0.01$, df=1, $p=1.0$). The source of their samples was mainly Caucasian and African (8). Thus, the Gly395Arg polymorphism is not common in this Japanese population. As found in Leu162Val and Val227Ala, there is variability in the frequency of PPARα gene polymorphisms between ethnic groups. The Gly395Arg polymorphism does not appear to play a role in the Japanese. Further epidemiological and genetic studies using non-Japanese subjects are needed to elucidate the role of the Gly395Arg polymorphism of PPARα gene in different ethnic populations.

Acknowledgement

This study was supported, in part, by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

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