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Original Article

Genotyping of peroxisome proliferator-activated receptor gamma (PPAR-γ) polymorphism (Pro12Ala) in Iranian population

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Abstract

BACKGROUND: The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear hormone receptor. It is predominantly expressed in adipose tissue and as a receptor for thiazolidinediones, it has drawn attentions towards itself as a key molecule to trigger pathways involving in some diseases such as cancers, type 2 diabetes, inflammations and osteoporosis. A proline changed to alanine in codon 12 of PPAR-γ gene (Pro12Ala) has been known to be responsible for decreased risk of type 2 diabetes. The aim of the present study is to investigate the frequency of Pro12Ala polymorphism in PPAR-γ in healthy Iranian population to compare with other populations. Understanding this polymorphism may help us in better diagnosis, prevention, and therapeutic approaches toward a better management of diseases such as type 2 diabetes and osteoporosis.

METHODS: 128 healthy volunteers were enrolled in this study. To determine single nucleotide polymorphisms (SNPs), we did real time polymerase chain reaction (RT-PCR), using TaqMan allelic discrimination assays.

RESULTS: Genotype frequencies for PPAR-γ gene Pro12Ala (rs1801282) polymorphism were 0.86 for CC, 0.14 for CG, 0.00 for GG while allelic frequencies were 0.93 and 0.0.07 for C and G, respectively.

CONCLUSIONS: There are statistical differences between the distribution of the PPAR-γ-2 Pro12Ala polymorphism in other populations and Iranian population.

KEYWORDS: PPAR Gamma, Polymorphism, Genetic, Iran.

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Peroxisome proliferator-activated receptor (PPAR) which is a transcription factor from superfamily of nuclear receptors regulates the expression of genes.[1,2] It is activated by fatty acids, prostanoids, and thiazolidinediones (insulin-sensitizing antidiabetic agents)[3,4] and plays an important role in regulation of cellular differentiation (adipocyte differentiation), development, metabolism (carbohydrate, lipid, protein), and tumorigenesis.[5] PPAR-gamma (PPAR-γ) is mostly expressed in adipose tissue.[5]

PPAR-γ has strong effects on various diseases including osteoporosis,[6,7] atherosclerosis, inflammation, carcinogenesis, type-2 diabetes mellitus, insulin resistance and obesity.

Hereditary disorders mutation in PPAR, rare gain-of-function mutation (Pro115Gln) and two loss-of-function mutations (Val290Met and Pro467Leu) have been recognized. The first genetic variant that is highly prevalent was Pro12Ala polymorphism which was identified by Yen et al.[8] It is CCA-to-GCA missense mutation which is associated with type-2 diabetes mellitus, insulin resistance and obesity. There are also some reports from Pro12Ala polymorphism effects on the growth of cancer cells.[9,10] This mutation in codon 12 of exon B of the PPAR gene encodes the NH2-terminal residue which defines the adipocyte-specific PPAR-γ-2 isoform and decreases the risk of insulin resistance with allele frequency 0.03-0.12 in some populations.[11]

The initial publication reported a 75% risk

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reduction for diabetes conferred by the Ala allele,[12] and it is said that persons with the Ala12 allele have a reduced risk of colorectal cancer,[13] prostate cancer,[14] endometrial cancer,[15] sporadic colorectal adenoma,[16] gastric cancer,[17] and adenocarcinoma and peptic ulcer disease (PUD).[10]

The rare allele frequencies (Ala) has been reported to range from 2% to 18% in healthy populations,[18] 5.9% to 21.6% (median: 12.7%) in Caucasians, and 1.7% to 9.3% (median: 4.5%) in Eastern Asian descent (Chinese or Japanese).[19] In some studies, the rare allele frequencies are reported 12% in Caucasians, 10% in Native Americans, 8% in Samoans, 4% in Japanese, 3% in African-Americans, 2% in Nauruan people, and 1% in Chinese.[20,21]

Full understanding of distribution and genotype frequency of this polymorphism might have clinical utility. In this study, we analyzed 128 healthy individuals to determine PPAR-□ (Pro12Ala) variants and identify the frequency of alleles in Iranian population.

Methods

128 healthy volunteers who were selected from Blood Transfusion Organization were enrolled in this study. In addition, Ethics Committee's approval and participants' consent were obtained. They were selected according to World Health Organization protocol for blood donation including all laboratory and clinical tests with normal parameters.

Peripheral blood was used for isolation of buffy coat (rich source of DNA) with use of Ficoll gradient (Fresenius Kabi Norge AS, Norway). DNA was extracted according to Commercial DNA extraction Kit DNP protocol (DNP Extraction Kit, Sinagen Company, Tehran, Iran). To determine single nucleotide polymorphisms (SNPs), we did real time polymerase chain reaction (RT-PCR), using TaqMan (MetaBion, Germany) allelic discrimination assays based on the procedure presented by Doney et al with some modifications.[22]

The primer and probes were as:

Pro12 Forward: TCCATGCTGTTATGGGTGAAACT

Pro12 Reverse: CTTTACCTTGTGATATGTTTGCAGACA

Pro12 Probe (Fam labelled): TCTCCTATTGACCCAGAAAGCGATTCCTT

Ala12 Probe (Fam labelled): TCTCCTATTGACGCAGAAAGCGATTCCTT

PCR premix consisted of commercial premix (Takara Bio Inc, Japan) with primer and TaqMan probe. Cycling was performed in a real time thermal cycler (BioRad, USA) as:

Stage 1 (initial denaturation): 95ºC for 10 sec

Stage 2 (PCR**):** 95ºC for 5 sec and 60ºC for 20 sec. This stage was repeated 45 times.

One product from each genotype was sequenced with ABI sequencer Genetic Analyzer (Applied Biosystems, Foster City, CA) to confirm the results.

Sample size was calculated based on Allel C in European population (p = 92.4%) and α = 0.05 and the result was n ≥ 108. Genotype and Allel frequencies were calculated and the Hardy-Weinberg equilibrium was tested using Arlequin 313 software. The frequencies of all genotypes and alleles in our population were compared with other reports. All calculations were done by SPSS statistical package (version 15.0, SPSS) using chi-square and Fisher's exact test. P value ≤ 0.05 was considered significant.

Results

Allele and genotypes frequencies for PPAR-□ (rs1801282) were deviated from Hardy-Weinberg equilibrium (HWE). HWE was used for checking the normal distribution of these genotypes in our population. Its p value was ≤ 0.05 that is not significant. It might be because

Table 1. The genotypic characters of studied individuals

Gene	Allelic frequencies n (%)		Genotypic Frequencies n (%)			HWE
PPAR	C (Pro) 238 (92.97)	G (Ala) 18 (7.03)	CC (Pro/Pro) 110 (85.94)	CG (Pro/Ala) 18 (14.06)	GG (Ala/Ala) 0	≤ 0.05

HWE: Hardy–Weinberg equilibrium,

Table 2. A comparison of allelic and genotypic frequencies (Pro12Ala) between Iranian population and others

		Allele frequencies		Genotype frequencies						
Population	**n**	**C Pro n (%)**	**G Ala n (%)**	**CC Pro/Pro n (%)**	**CG Pro/Ala n (%)**	**GG Ala/Ala n (%)**	**P (A)**	**P (G)**	**HWE**	**Ref**
Iranians (this study)	128	238 (92.97)	18 (7.03)	110 (85.94)	18 (14.06)	0 (0.0)	-	-	≤ 0.05	-
Asian	90	176 (97.8)	4 (2.2)	86 (95.6)	4 (4.4)	0 (0.0)	0.018	0.015	1.000	28
Asian	90	170 (94.4)	10 (5.6)	80 (88.9)	10 (11.1)	0 (0.0)	0.341	0.335	-	28
Asian	88	166 (94.3)	10 (5.7)	78 (88.6)	10 (11.4)	0 (0.0)	0.363	0.335	0.752	28
Asian	48	90 (93.8)	6 (6.2)	42 (87.5)	6 (12.5)	0 (0.0)	0.504	0.305	0.752	28
Uygurs	111	196 (89.0)	25 (11.0)	86 (77.0)	25 (23.0)	0 (0.0)	0.071	0.345	Yes	32
Kazaks	80	146 (91.0)	14 (9.0)	66 (83.0)	14 (17.0)	0 (0.0)	0.323	0.295	Yes	32
Hans	102	193 (95.0)	11 (5.0)	91 (90.0)	11 (10.0)	0 (0.0)	0.302	0.057	Yes	32
Hans	180	346 (96.1)	14 (3.9)	166 (92.2)	14 (7.8)	0 (0.0)	0.062	0.234	NM	30
Korea	427	809 (94.7)	45 (5.3)	384 (89.9)	41 (9.6)	2 (0.5)	0.179	0.323	NM	29
Indian	241	424 (88.0)	58 (12.0)	186 (77.2)	52 (21.6)	3 (1.2)	0.021	0.254	Yes	31
African-American	48	92 (95.8)	4 (4.2)	44 (91.7)	4 (8.3)	0 (0.0)	0.234	0.335	1.000	28
African-American	46	88 (95.7)	4 (4.3)	42 (91.3)	4 (8.7)	0 (0.0)	0.263	0.335	1.000	28
African-American	12	24 (100.0)	0 (0.0)	12 (100.0)	0 (0.0)	0 (0.0)	0.335	0.335	NM	28
African-American	1005	1964 (98.0)	46 (2.3)	959 (95.4)	46 (4.6)	0 (0.0)	0.00	0.00	Yes	37
European	44	82 (93.2)	6 (6.8)	38 (86.4)	6 (13.6)	0 (0.0)	0.345	0.345	0.752	28
European	48	84 (93.8)	6 (6.2)	42 (87.5)	6 (12.5)	0 (0.0)	0.876	0.335	0.752	28
European	120	222 (92.5)	18 (7.5)	104 (86.7)	14 (11.7)	2 (1.7)	0.295	0.335	0.251	28
European	118	218 (92.4)	18 (7.6)	102 (86.4)	14 (11.9)	2 (1.7)	0.335	0.335	NM	28
European (Caucasian)	22	40 (90.9)	4 (9.1)	18 (81.8)	4 (18.2)	0 (0.0)	0.335	0.335	0.752	28
French (Caucasian)	318	555 (87.0)	81 (13.0)	246 (77.4)	63 (19.8)	9 (2.8)	0.008	0.046	Yes	34
Ukraine	39	61 (72.6)	17 (27.4)	24 (63.0)	13 (33.0)	2 (4.4)	0.00	0.00	NM	33
Greece	140	266 (32.0)	14 (68.0)	126 (90.0)	14 (10.0)	0 (0.0)	0.335	0.335	Yes	35
Prague Czech	97	152 (79.0)	42 (21.0)	61 (63.0)	30 (31.0)	6 (6.0)	0.00	0.00	NM	36
Sub-Saharan African	120	240 (100.0)	0 (0.0)	120 (100.0)	0 (0.0)	0 (0.0)	0.00	0.00	NM	28

P (A): P value of x^2 test between allele frequencies; P (G): P value of x^2 test between genotypic frequencies; HWE: Hardy Weinberg equilibrium; NM: Not mentioned

of small sample size, and also same ethnic compositions that could be disappear if random sampling would be done. Also, the accuracy of our method was confirmed by sequencing; indicating decrease of possibilities in personal and instrumental errors.

The allelic frequencies were 0.93 and 0.07 for Pro and Ala, respectively. Distributions of genotypes were 0.86 for Pro/Pro, 0.14 for Ala/Pro and 0.00 for Ala/Ala which is shown in table 1. A comparison of allelic and genotypic frequencies in Iranian population and those in others is shown in table 2.

Discussion

The first understanding of the importance of PPAR-γ in human occurred in 1995, when it was identified as the receptor for the Thiazolidinedione (TZD) insulin-sensitizing drugs.[23] Three potent and highly PPAR-γ-selective TZDs as the first new class of insulin-sensitizing agents have been used in large-scale clinical practice to date. Understanding the relation between response to drugs such as Pioglitazone and these SNPs is necessary and may be useful in individualized treatments in clinics. Pro-to-Ala exchange would reduce transcriptional activity of PPAR,[24] and several studies reported possible involvement of Pro12Ala polymorphism in the pathogenesis of insulin resistance, atherosclerosis, adipocyte differentiation, lipid metabolism, inflammation, and malignancy. It may be associated with decreased insulin resistance and decreased risk of type 2 diabetes.

Genotypic frequencies were 85.94% for CC, 14.06% for CG, and 0% for GG and allelic frequencies were 0.93 and 0.07 for C and G, respectively. Comparing allelic and genotypic frequencies of our data with previous reports on several ethnic groups, such as reports from Asians,[25] Indians,[10] African Americans,[26] Sub-Saharan Africans,[25] Frenchs,[27] Ukarians,[28] and Prague Czechs,[29] showed significant differences.

Five reports from European-Caucasians,[25] Greeks,[30] three reports from Asians, three reports from African Americans,[25] some reports from Hans,[31,32] Uygurs,[32] Koreans,[33] and Kazaks [32] showed no significant differences between our data and their findings.

This gene might be an important thrifty gene. The frequency of the minor alanine allele in Caucasians (0.10) was comparable to the ones previously reported in other Caucasian populations.[34,35] We found significant differences in minor allele frequencies between African Americans (0.04) and American Indians (0.19) compared to Caucasians. In Asians, the frequency of the minor alanine allele differs from 0.022 to 0.062 when compared to our population (0.073); it is deviate from the findings in Asia.

It was also showed that the frequency of the minor alanine allele in Uygurs (11.0) was significantly higher than that in Hans (3.9-5.0).[32] Also There are some controversies between different studies in Europe in minor allele frequencies.[25,27-30]

Conclusions

Statistical differences in the distribution of the PPAR-γ-2 Pro12Ala polymorphism between various populations and Iran showed the importance of studying this SNP as well. Iran is an ethno-linguistically diverse country located on southwest Asia, with 67 million populations. It seems that Iranians are genetically different from some other populations in Asia and other continents. Our study confirms this difference regarding the PPAR-γ-2 polymorphism.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

FN was participated in writing the proposal and the manuscript. NA and PRM were contributed in proposal and article writing. All authors have read and approved the content of the manuscript.

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