RESEARCH ARTICLE

PPAR-Gamma Pro12Ala Polymorphism and Gastric Cancer Risk in a Turkish Population

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Abstract

Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand dependent transcription factor involved in various processes, including carcinogenesis. We aimed to investigate any possible association of the PPARγ Pro12Ala (rs1801282) polymorphism with risk of developing gastric cancer (GC). Patients and Methods: A hospital based case control study was designed covering 50 patients with GC and 120 healthy controls. The frequencies of PPARγ Pro12Ala (rs1801282) were determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Results: The Ala12 allele of the PPARγ Pro12Ala G gene was associated with a 1.95 fold increased risk of GC development (p: 0.022; 95% CI: 1.58-2.40). Subgroup analyses showed that the same allele was also associated with metastasis (p: 0.000; OR:4.09; 95%CI:2.273-7.368) and differentiation (p: 0.004; OR:1.95; 95%CI:1.335-2.875) in patients with GC. Conclusion: This study suggests that the PPARγ Pro12Ala G (Ala12) allele might be associated with development, differentiation and metastatic process of GC in the Turkish population. Further studies conducted in larger study groups and in different ethnic populations will be needed to clarify the exact role of the PPARγ Pro12Ala polymorphism in GC.

Keywords: PPARγ Pro12Ala polymorphism - gastric cancer - Turkish population

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Introduction

Gastric cancer (GC) is the fourth most common human malignant disease and second leading cause of cancer related death in both sexes worldwide (Ferlay et al., 2010). Recently, there has been intense interest in the search for common genetic variants as biomarkers for genetic susceptibility to GC development, namely single nucleotide polymorphisms (SNPs) (Milne et al., 2009; Yin et al., 2009). Beside associations between some of SNPs in genes and GC risk have been reported in previous studies, definition of susceptibility genes for GC risk remains to be determined.

Peroxisome proliferator-activated receptor γ (PPAR γ) is a member of the nuclear hormone receptor superfamily that plays an important role in cellular differentiation and carcinogenesis as well as regulation of metabolism, glucose and lipid homeostasis, and intracellular insulin-signaling events (Michalik et al., 2004). PPAR γ exists as two isoforms, γ1 and γ2, generated by alternative promoters and differential splicing of at least three different transcripts from the PPAR γ gene on chromosome 3p25. Several polymorphisms in PPAR γ2 have been identified so far and one of the common structural polymorphism in the PPAR γ2 gene was identified as CCA-to-GCA (Pro12Ala) missense mutation in codon 12 of exon B (Yen et al., 1997). This substitution possibly results in a conformational change in protein structure and reduced function of the PPAR γ2 gene. The role of Pro12Ala polymorphism of PPAR γ gene has been recently studied in cancers. Individuals with the Ala12 allele are found to have an increased risk of GC in Chinese (Liao et al., 2006), Indian (Prasad et al., 2008) and Japanese (Tahara et al., 2008) populations. As PPARγ Pro12Ala polymorphism is found to be associated with GC risk we examined the potential association between Pro12Ala polymorphism in the PPARγ gene and GC in Turkish population

Materials and Methods

Study Groups

Two hundred fifteen individuals included in this

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study. Sixty eight patients with GC at their initial staging admitted to Department of General Surgery, Istanbul Medical Faculty, Istanbul University were included in this study. One hundred twenty nine healthy controls were selected from individuals who came for routine health check during the same period of time. The patient and control groups were matched for age and sex. All participants signed an informed consent before enrollment and Institutional Ethical committee approval was obtained for the study.

Polymorphism Analysis

Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood according to salting-out technique (Miller et al., 1998). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism the procedures of PCR-RFLP are given in Table 1 (Liao et al., 2006). The appropriate primers were used to amplify the corresponding gene of the subjects by PCR and the reaction products were digested by using the appropriate enzyme at 60°C. The digested products were analyzed on 2.5% agarose gel stained with ethidium bromide and examined under transillumination. Each gel was read by two observers unaware of the subject's status. If there is any conflict, samples were repeated. The expected results after restriction for each gene were also given in Table 1.

Statistical Analysis

Statistical analyses were performed using the SPSS software package (revision 16.0 SPSS Inc., Chicago, IL, USA.). Data are expressed as means+SD. Differences in clinicopathological characteristics between patients and controls were tested by chi-square test for categorical data and Student's t-test for numerical data. Odds ratio (OR) and 95% confidence interval (CI) for the association between genotype and GC was computed. A two-sided p-value of less than 0.05 was considered statistically significant.

Results

Characteristics of study group

Sixty eight patients with GC and 129 controls were studied. Characteristics of patients with GC and controls were given in Table 2. There was no significant difference in the baseline characteristics between patients and controls.

PPARγ Pro12Ala polymorphism

Genotypic distributions of patients with controls were in Hardy-Weinberg equilibrium for PPAR γ pro12ala (C12G) genotypes (p: 0.28) but it was not consistent with

Table 2. Characteristics of Patients With Gc. P Value Less Than 0.05 was Considered as Significant

Parameters	Patients (n=86)	Controls (n=129)	*p* value
mean age (year±SD)	58.6±8.6	57.1±8.14	0.384
Sex	female	27	50	0.169
	male	59	79	
Smoking status	yes	39	66	0.243
	no	47	63	
Alcohol consumption	yes	22	12	0.340
	no	107	74	
Tumor Size	T1	20		
	T2	22		
	T3	25		
	T4	19		
Nodal Status	N0	6		
	N1	15		
	N2	40		
	N3	25		
Metastasis	yes	25		
	no	61		
Differentiation	well/moderately	45		
	poor	41		

Table 3. Frequencies of PPAR γ Genotype Distributions in Patients with GC and Controls

Genotypes/Alleles PPARg Pro 12 Ala	Patients (n=86)	Controls (n=129)	p value
CC	68	116	0.047*
CG	14	12	
GG	4	1	
G+	18	13	0.022*
C+	82	128	0.085

*p value less than 0.05 was considered as significant.

Table 4. Comparison of Characteristic of GC Patients (n=86) with PPARγ Pro12Ala G Alleles

Parameters		G allele +	G allele -	P value
Age		18	68	0.685
Sex	Female	4	23	0.26
	Male	14	45	
Smoking consumption				
	Yes	8	31	0.573
	No	10	37	
Alcohol consumption				
	Yes	2	10	0.519
	No	16	58	
Tumor size	T1	4	16	0.928
	T2	4	18	
	T3	5	20	
	T4	5	14	
Nodal Status	N0	1	5	0.604
	N1	5	10	
	N2	8	32	
	N3	4	21	
Metastases	Yes	13	12	0.000*
	No	5	56	OR:4.09 (95%CI:2.273-7.368)
Differentiation	Well/moderately	4	41	0.004*
	Poor	14	27	OR:1.95 (95%CI:1.335-2.875)

*p value less than 0.05 was Considered as Significant

Table 1. PCR and RFLP Procedures and Products of PPARγ Pro12Ala Gene

Gene	Primers (forward and reverse)	PCR product	Restriction enzyme	Restriction products
PPAR γ Pro12Ala (Liao et al., 2006)	5'-GCC AAT TCA AGC CCA GTC-3' 5'-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC-3'	270bp	BstUI	CC: 270 bp GG: 43 bp GC: 270 bp, 227 bp,43 bp

genotypes of GC patients (p: 0.01). Genotype frequencies of PPAR γ Pro12Ala among patients and controls are shown in Table 3. The frequencies of PPAR γ gene was significantly different in patients compared with controls (p: 0.047, χ^2:6.12). Frequencies of PPAR γ Pro12Ala CG genotype in patients were higher than the controls (16.3% and 9.3% respectively). Individuals carrying G allele had a 1.57-fold increased risk for GC (p: 0.026, χ^2:4.925, 95%CI: 1.103-2.238).

In addition to these findings, frequency of G allele was higher in patients with metastatic disease (p: 0.000, χ^2:20.55, OR: 4.09; 95%CI: 2.273-7.368) and poor differentiation (p: 0.004, χ^2:8.27, OR: 1.95;95%CI: 1.335-2.875). Metastases and poorly differentiated tumors invasion were more common in G allele carriers of PPAR γ gene in GC patients.

Discussion

In this study we investigated the association between PPAR γ Pro12Ala polymorphism and GC risk in Turkish population.

In our study we demonstrated that PPAR γ Pro12Ala gene polymorphism was associated with GC. Individuals carrying G (Ala 12) allele had increased risk for GC. Moreover, G allele carriers were higher in GC patients with tumor harboring poorly differentiation and metastatic process.

PPARs represent a family of nuclear receptors that are related to thyroid and retinoid receptors. PPARγ is the most extensively studied of three PPAR subtypes. Ligand binding to PPAR activates the transcription of PPAR responsive genes including cellular development, differentiation and carcinogenesis. PPARγ gene expression was identified in both gastric cancerous and normal gastric epithelium (Leung et al., 2004). It has also been shown activation of PPARγ inhibits the growth and induces apoptosis of gastric cancer cells (Leung et al., 2004).

Presence of the Ala12 polymorphism which is associated with reduced PPAR γ functional activity was thought to increase the risk of GC (Stuvoll and Haring, 2002; Meirhaeghe and Amouyel, 2004). In our study, Ala12 allele was frequently present in GC when compared to control group. We found that the patients with Ala12 allele carriers had 1.57-fold (95%CI: 1.103-2.238) increased risk of progression to GC. Regarding the association between Ala 12 polymorphism of PPAR γ gene and GC risk, Liao et al. (2006) reported 2.5-fold increased risk in Chinese, Prasad et al. (2008) reported 2.14-fold increased risk in Indian, and Tahara et al. (2008). reported 2.4-fold increased risk in Japanese population of progression to GC. Bazargani et al. (2010) investigated the association between PPAR γ polymorphism and non-cardia GC risk in Iranian population. They have found that Ala 12 PPAR γ gene carriers had 3.28 fold increased risk for non-cardia GC development in the presence of *H.pylori* infection. It appears that the risk for development of GC related to Ala12 polymorphism of PPARγ gene in our Turkish population is similar with previous studies in other ethnic population. Furthermore, Xu et al. (2010) reported that Ala12 variant of PPARγ was associated with increased risk of GC (OR: 2.31, 95%CI: 0.95-1.23). When we analyzed the association of PPARγ gene polymorphism with patients' characteristics, the Ala12 carriers had higher in patients harboring poorly differentiation as well as with distant metastases. Our subgroup analyses support that PPARγ Ala12 polymorphism is associated with carcinogenesis and metastatic processes of GC in Turkish population. Functional role of this substitution is not clear but it possibly results in a conformational change in protein structure and reduced function of the PPAR γ2 gene. The functional role of Pro12Ala polymorphism of PPAR γ gene also needs to be studied with further studies.

Although the present study has a novel finding in Turkish population, it has some limitations such as small sample size and lack of tissue expression and association of PPAR γ polymorphism with presence of *H.pylori* infection in our patients. Indeed, GC risk was found to be increased in Ala12 allele carriers of PPARγ gene in the presence of *H.pylori* infection in previous studies (Liao et al., 2006; Prasad et al., 2008). Further analysis in GC patients with presence of *H.pylori* infection seems to be mandatory to clarify the association between *H.pylori* infection and PPARγ polymorphism in GC development. Beside this, the functional studies of this polymorphism will be clarified exact role of this polymorphism in GC.

In conclusion, this study suggests that PPARγ Pro12Ala polymorphism might be potential marker for genetic susceptibility to GC in Turkish population. We also report, PPARγ Pro12Ala polymorphism is associated with tumor characteristics suggesting the prognostic importance of this gene polymorphism in GC. Further studies on larger study group with expression of PPAR γ gene and determination of the presence of *H.pylori* infection and different ethnic groups are needed to confirm the association of this polymorphism with development and prognosis of GC.

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