

Pro12Ala Polymorphism in the Peroxisome Proliferator-Activated Receptor-gamma (PPAR γ) Gene in Inflammatory Bowel Disease

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Abstract

Background & Aims. Peroxisome proliferator-activated receptor-gamma (PPAR- γ) has recently been implicated as an endogenous regulator of cellular proliferation and inflammation. Impaired expression of PPAR- γ in colonic epithelial cells in ulcerative colitis (UC) and increased expression in hypertrophic mesenteric adipose tissue in Crohn's disease (CD) have been reported. Furthermore, PPAR- γ ligands have been shown to inhibit tissue injury associated with immune activation in UC. Any mutation in PPAR- γ gene may be responsible for the increase in inflammatory mediators and hence the perpetuation of inflammation in inflammatory bowel disease (IBD) patients. One common polymorphism in PPAR- γ gene is proline to alanine substitution (Pro12Ala) which results from a CCA to GCA missense substitution in codon 12 of exon 2 of the PPAR- γ gene. In this study, we aimed to explore Pro12Ala polymorphism in PPAR- γ gene in IBD in Turkish patients. **Methods.** 69 patients with CD, 45 with UC and 100 controls of similar age and sex were studied. Genomic DNA was isolated from peripheral blood leucocytes and mutagenically separated-polymerase chain reaction (PCR) analyses were performed to determine the Pro12Ala polymorphism of the PPAR- γ gene. **Results.** We observed no significant differences in the frequency of the Pro12Ala polymorphism in the PPAR- γ gene among subjects with CD, UC and controls (15.9%, 15.5% and 13%, respectively, $p > 0.05$). **Conclusion.** These results suggest that Pro12Ala polymorphism in the PPAR- γ gene relates neither to the risk of the development of inflammatory bowel disease nor to the clinical subtypes of CD in the Turkish population.

Key-words

PPAR γ – Pro12Ala polymorphism – Crohn's disease – ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD) is a group of chronic diseases of the gastrointestinal tract such as Crohn's disease (CD) and ulcerative colitis (UC) [1]. While the exact cause of IBD is still unknown, the most relevant explanation regarding its etiopathogenesis is that of an exaggerated immune response to normal intestinal flora in genetically susceptible subjects with the contribution of multiple internal and external factors [2]. Recent studies have revealed that the innate mucosal immune system has paramount importance in the pathogenesis of IBD, especially in the development of the CD [3].

Peroxisome proliferator-activated receptors (PPARs: PPAR- α , PPAR- β/δ , and PPAR- γ) are members of the nuclear hormone receptor family and have a significant role in the differentiation of adipocytes, regulation of lipid metabolism, and glucose homeostasis [4]. It has been shown that PPAR γ gene Pro12Ala polymorphism influences insulin sensitivity and risk of type II diabetes in various ethnic populations [5, 6]. Recent studies indicate that PPAR- γ has also a role in the pathogenesis of IBD and colon cancer [7-9].

Both animal and human studies have described PPAR- γ as an endogenous regulator of intestinal inflammation. In animal NASH and IBD models (Dextran sodium sulphate colitis, Trinitrobenzene sulphonic acid colitis), PPAR- γ ligands were shown to markedly decrease inflammation [8-12]. The combined result of these studies indicate an anti-inflammatory effect with PPAR- γ activation through inhibition of signal transduction pathways (Nuclear Factor kappa B "NF κ B", signal transducers and activators of transcription "STATs", activating protein 1 "AP1") regulating the expression of proinflammatory genes [13].

There are numerous human data that support the anti-inflammatory action of PPAR- γ in the liver and colon [14, 15]. In a double-blind, placebo-controlled, randomized clinical study, Lewis et al demonstrated clinical and

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endoscopic remission in patients with active UC treated with the PPAR- γ agonist rosiglitazone [15]. However, the decrease in PPAR- γ expression in the colonic epithelium cell, the lack of a genetic basis associated with PPAR- γ , and the anti-inflammatory efficacy of PPAR- γ ligands suggest that PPAR- γ ligands may be active independently from the receptor. Indeed, studies have shown that 15d-PGJ2, a PPAR- γ ligand, directly inhibits the expression of the NF κ B gene [16].

Until now, polymorphisms of the genes encoding mucosal innate immune response have been described in both UC and CD, such as NOD2 CARD gene, TLR4 gene, MDR gene, proinflammatory cytokine IL-1RA, TNF-alpha genes and some of them have been related to the clinical course of the disease [17]. This suggests that polymorphism of PPAR- γ gene which takes place in the control of NF κ B expression might be involved in the clinical pattern of IBD. One common polymorphism in PPAR- γ gene is proline to alanine substitution (Pro12Ala) which results from a CCA to GCA missense substitution in codon 12 of exon 2 of the PPAR- γ gene which has a potential inhibitory effect on inflammatory signal pathways in patients with IBD, especially in CD. In this study, we aimed to explore Pro12Ala polymorphism in PPAR- γ gene in IBDs in Turkish patients.

Patients and methods

Patient groups

Sixty-nine patients with CD and 45 patients with UC, diagnosed by clinical, endoscopic, histological and radiological criteria in the Gastroenterology Clinic of Marmara University School of Medicine and the Marmara University Gastroenterology Institute were included in the study. Patients with indeterminate colitis were not included. Regarding the possible influence of the Pro12Ala polymorphism in PPAR- γ 2 gene on the risk of development of the type 2 diabetes mellitus, we excluded diabetics from the study and control groups.

The control group consisted of 100 healthy volunteers matched for age and gender. Phenotypic characteristics of CD were stricturing, fistulizing and inflammatory.

All of the 214 subjects were informed and gave written consent to participate in the study and to allow their blood samples to be genetically analyzed, according to the Helsinki Declaration. The study protocol was approved by the Ethical Committee of the Marmara University School of Medicine

Methods

Genomic DNA isolation

After the perusal and signing of an informed consent by patients and the control group, 2 ml venous whole blood samples were obtained in tubes with EDTA for genomic DNA isolation. Samples were stored at +4°C until DNA extraction. The time interval at +4°C from collection of blood samples to DNA extraction was 1 to 6 days. Genomic DNA was extracted from peripheral blood leukocytes using a phenol/chloroform method. All 214 individuals were screened for the presence of the G/C exon 2 of PPAR- γ gene.

Mutagenically separated polymerase chain reaction (MS-PCR)

The Pro12Ala polymorphism was detected by the mutagenically separated polymerase chain reaction (MS-PCR) method. Two different length allele specific reverse primers (P1 and P2) and a common forward primer were used to perform the MS-PCR. The PCR was carried out in a 25 μ l reaction volume containing 50ng DNA, 50mM Tris HCl, 10mM KCl, pH:8.3, 0,3 mM each dNTP, 3mM MgCl₂, 10 pmol P1, 5 pmol P2, 10 pmol P3 and 1,5U Fast Start Taq polymerase (Roche Diagnostics GmbH, Mannheim, Germany). The PCR condition included initial denaturation step at 94°C for 3 minutes, 35 cycles of amplification with denaturation at 94°C for 45s, primer annealing at 62°C for 45 s, extension of primer at 72°C for 45s followed by final extension step at 72°C for 3 min. The sequence of primers is indicated in Table I. CC genotypes (Pro12Pro) expressed in exon 2 are evaluated as normal, while CG (Pro12Ala) is heterozygous and GG (Ala12Ala) is homozygous mutant. The PCR products were then subjected to electrophoresis in a 3% ethidium bromide-stained nusieve gel and visualized under UV light. The Pro12 and 12Ala allele give bands of 230 and 250, respectively [18].

Statistical evaluation

Continuous variables, expressed as mean \pm SD, were compared using parametric or nonparametric t tests as appropriate. The comparison of the distribution of the PPAR γ genotypes (Pro12Pro, Pro12Ala, Ala12Ala) between the study groups and the influence of PPAR γ genotypes in demographic and phenotypic characteristics of CD were done by χ^2 statistics or 2-tailed Fisher's exact test. Odds ratios (ORs) as estimates of relative risk of the disease were calculated based on 95% confidence intervals (CI). The

Table I. MS-PCR primers

MS primers	Nucleotide Sequence	
P1 (reverse)	5'GTGTATCAGTGAAGGAATCGCTTTCT <u>T</u> G-3'	Pro allele specific primer
P2 (reverse)	5'TTGTGATATGTTTGCAGACAAGGTATCAGTGAAGGAATCGCTTTGTGC-3	Ala allele specific primer
P3 (reverse)	5' TTTCTGTGTTTATCCCATCTCTCCC-3	Common Primer

Note: The reverse primers contained mutagenic mismatch nucleotides (underlined) which made them separate the polymorphic alleles specifically in the same PCR reaction.

InStat statistical program was used. A “p” value less than 0.05 was considered statistically significant.

Polymorphism was tested in controls to ensure that it fitted the Hardy-Weinberg equilibrium.

Results

The characteristics of the patients with UC, CD and of the healthy controls are shown in Table II. There was no statistical difference between groups in terms of gender and age distribution ($p > 0.05$).

The results of genotyping for the PPAR- γ Pro12Ala in CD and UC patients and matched healthy controls are presented in Table III.

Of the 69 patients in the CD group, 11 (15.9%, M/F: 4/11) had Pro12Ala heterozygous polymorphism in the PPAR- γ gene. Of the 11 patients with CD that had polymorphism, 4 (36.36%) had stricturing type, 3 penetrating (27.27%) type, and 4 (36.36%) inflammatory type disease. There was no difference between CD subgroups in terms of PPAR- γ Pro12Ala polymorphism ($p=0.98$; OR:1.08, 95% CI 0.52-2.23).

Of the 42 patients in the UC group 7 (15.5%) had Pro12Ala polymorphism. Of the 100 healthy subjects in the control group, 13 (13%) had PPAR- γ Pro12Ala polymorphism.

The Pro12Ala genotype frequency of PPAR- γ 2 was not significantly different between either CD patients and matched controls [$p=0.9$; OR: 0.98 (95%CI 0.67-1.44)] or UC patients and matched controls [$p=0.7$; OR: 0.85 (95% CI 0.45-1.62)] or CD patients and UC patients [$p=0.5$; OR: 0.83 (95% CI 0.48-1.42)].

PPAR- γ genotypes were found to be in the Hardy-Weinberg equilibrium in both the study and control

groups.

Figures 1-3 demonstrate polymorphic PCR products of the CD, UC patients and controls, respectively.

Discussion

Many studies support that IBD develops in genetically predisposed individuals with the contribution of multiple internal and external factors as a result of excessive immune response to the intestinal flora [3]. IBD subgroups have different immunoregulation despite similar tissue damage. Excessive immune response due to undetermined reasons in individuals with genetic predisposition causes chronic inflammation, tissue damage, and fibrosis. Currently, the basic inclination concerning this disease group is the investigation of environmental and genetic factors to determine clinical subgroups.

Through work in several animal models of colitis, it is now recognized that PPAR- γ ligands also inhibit tissue injury associated with immune activation [8, 11, 12, 19-21]. Furthermore, recent studies showed that treatment with PPAR- γ ligands of mild to moderate active UC patients was efficacious [22, 23].

The study by Dubuquoy et al was the first to demonstrate that, unlike patients with CD, patients with UC had impaired expression of PPAR- γ in the colonic epithelium cell [19]. In order to investigate the genetic basis of this finding, they made a DNA sequence analysis of the PPAR- γ gene, and determined only the presence of Pro12Ala polymorphism in the 12th codon of exon 2 previously described. They reported a similar prevalence of this variant in the healthy population (12% vs. 11%). However, a major weakness of that study was that PPAR- γ Pro12Ala polymorphism, investigated in patients with UC, was not explored in patients with CD.

Table II. Characteristics of the study groups

Groups	Crohn's disease	Ulcerative colitis	Control
Total number (n)	69	45	100
Sex ratio (Male/Female)	32/37	21/24	51/49
Age, years (mean \pm SD)	39 \pm 3	36.5 \pm 2.5	41.5 \pm 1.5
Duration of the disease, years (Mean \pm SD)	4.6 \pm 3.2	5.5 \pm 1.9	—
Co-morbid disease	None	None	None

Table III. PPAR- γ 2 genotype frequencies in the study groups

Groups	Crohn's disease patients n=69	Ulcerative colitis patients n=45	Healthy controls n=100
Pro 12 Pro (Wild tip)	58 (84.1%)	38 (84.5%)	87 (87%)
Pro 12 Ala (Heterozygote)	11 (15.9%)	7 (15.5%)	13 (13%)
Ala 12 Ala (Homozygote)	0	0	0

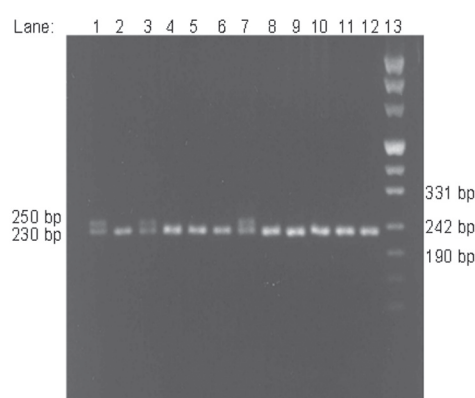


Fig 1. Electrophoresis patterns for the Pro12Ala allele of the PPAR-gene analyzed by MS-PCR in the Crohn's Disease group. Lane 13: pUC Mix Marker, 8, DNA Ladder, Lanes 1, 3 and 7: electrophoresis patterns in case of three individuals heterozygous for the Pro12Ala polymorphism *Pro/Ala). The other lanes, electrophoresis patterns in case of nine individual displaying the wild type (Pro/Pro) genotype.

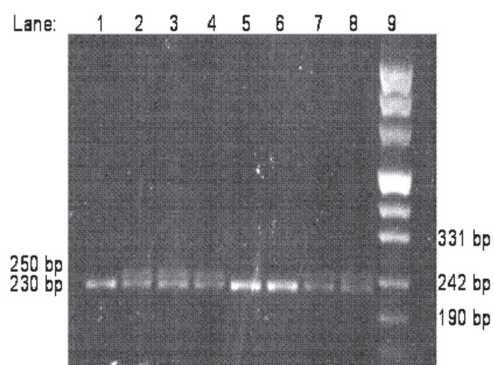


Fig 2. Electrophoresis patterns for the Pro12Ala allele of the PPAR- γ gene analyzed by MS-PCR in the ulcerative colitis. Lane 9: pUC Mix Marker 8, DNA Ladder, Lanes 2, 3, 4, 7 and 8: electrophoresis patterns in case of five individuals heterozygous for the Pro12Ala polymorphism (Pro/Ala). The other lanes; electrophoresis patterns in case of three individuals displaying the wild type (Pro/Pro) genotype.

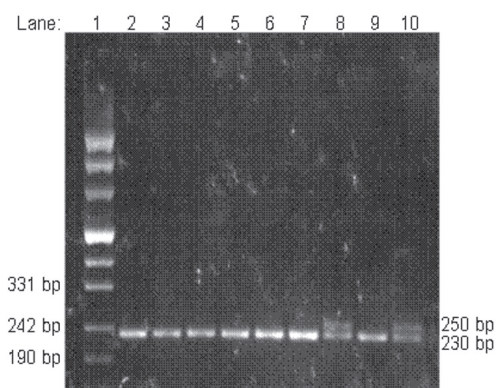


Fig 3. Electrophoresis patterns for the Pro12Ala allele of the PPAR- γ gene analyzed by MS-PCR in control group. Lane 1: pUC Mix Marker 8, DNA Ladder, Lanes 8 and 10: electrophoresis patterns in case of two individuals heterozygous for the Pro12Ala polymorphism (Pro/Ala). The other lanes; electrophoresis patterns in case of seven individuals displaying the wild type (Pro/Pro) genotype.

In our study we investigated whether there were any relationships between PPAR- γ polymorphism and risk of developing IBD in the Turkish population. Because this is the first study concerning CD patients, we also searched whether PPAR γ Pro12Ala polymorphism has any influence on the clinical course of CD. We found a prevalence of 15.5% in Pro12Ala polymorphism of the PPAR- γ gene in the UC patient group. This result was similar to the frequency in the healthy and CD populations. In our population bearing PPAR γ , Pro12Ala did not influence the clinical course of CD.

The size of our study groups were comparable with the relevant studies reporting [24, 25]. Furthermore, the study

by Erdogan et al in the Turkish population showed that the frequency of homozygote G allele of PPAR- γ was 0%, similar to our results.

Studies that exposed the importance of the hypertrophic mesenteric adipose tissue in CD have particularly highlighted the PPAR- γ receptors, which contribute to the control of adipocyte differentiation [26]. The increased PPAR- γ expression in the mesenteric adipose tissue despite increased levels of TNF- α in CD suggests the polymorphism in the PPAR- γ gene. There may be a polymorphism in the PPAR- γ gene in CD, one major property of which is genetic predisposition. Thus we investigated Pro12Ala polymorphism in the PPAR- γ gene in 69 patients with CD, and found a prevalence of 15.9%. This was not statistically different from the prevalence in patients with UC or the healthy Turkish populations.

In previous studies, the frequency of Pro12Ala polymorphism in the PPAR- γ gene was reported as 12% in Caucasians, 10% in Native Americans, 4% in the Japanese, 3% in African Americans, and 1% in the Chinese [27, 28]. In our study, the prevalence of Pro12Ala polymorphism of the PPAR- γ gene was 13% in a random Turkish population of 100 healthy, non-obese subjects without a history of diabetes. This is similar to the frequency reported in Caucasian populations.

In **conclusion**, although the limited number of patients studied might have contributed to the negative results, our study suggests that Pro12Ala polymorphism in the PPAR- γ gene is not associated with the risk of developing IBD in the Turkish population.

Conflicts of interest

None to declare.

References

- Rubin PH, Marion J, Present DH. Differential diagnosis of chronic ulcerative colitis and Crohn's disease of the colon. In: Kirsner JB, editor. Inflammatory bowel disease, fifth edition. Philadelphia: W.B. Saunders; p: 315-325, 1999.
- Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; 115: 182-205.
- Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol* 2003; 3: 521-533.
- Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. *Nature* 2000; 405: 421-424.
- Hara K, Okada T, Tobe K, et al. The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 2000; 271: 212-216.
- Frederiksen L, Brødback K, Fenger M, et al. Comment: studies of the Pro12Ala polymorphism of the PPAR-gamma gene in the Danish MONICA cohort: homozygosity of the Ala allele confers a decreased risk of the insulin resistance syndrome. *J Clin Endocrinol Metab* 2002; 87: 3989-3992.
- Gupta RA, Dubois RN. Controversy: PPAR gamma as a target for treatment of colorectal cancer. *Am J Physiol Gastrointest Liver Physiol* 2002; 283: G266-269.
- Su CG, Wen X, Bailey ST, et al. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response.

- J Clin Invest 1999; 104: 383-389.
9. Desreumaux P, Dubuquoy L, Nutten S, et al. Attenuation of colon inflammation through activation of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPAR-gamma) heterodimer. A basis for new therapeutic strategies. *J Exp Med* 2001; 193: 827-838.
 10. Tahan V, Eren F, Avsar E, et al. Rosiglitazone attenuates liver inflammation in a rat model of nonalcoholic steatohepatitis. *Dig Dis Sci.* 2007; 52: 3465-3472.
 11. Dubuquoy L, Bourdon C, Peuchmaur M, et al. Peroxisome proliferator activated receptor-gamma (PPAR) gamma: a new target for the treatment of inflammatory bowel disease. *Gastroenterol Clin Biol* 2000; 24: 719-724.
 12. Tagaki T, Naito Y, Tomatsuri N, et al. Pioglitazone, a PPAR- gamma ligand, provides protection from dextran sulfate sodium- induced colitis in mice in association with inhibition of the NF-kappa B - cytokine cascade. *Redox Rep* 2002; 7: 283-289.
 13. Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator- activated receptor gamma. *J Mol Med* 2001; 79: 30-47.
 14. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003; 38: 1008-1017.
 15. Lewis JD, Lichtenstein GR, Deren JJ, et al: Rosiglitazone for Ulcerative Colitis Study Group. Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial. *Gastroenterology* 2008; 134: 688-695.
 16. Straus DS, Pascual G, Li M, et al. 15- Deoxy- delta 12,14 Prostaglandin J2 inhibits multiple steps in NF-kappa B signaling pathway. *Proc Natl Acad Sci U S A* 2000; 97: 4844-4849.
 17. Cho JH, Weaver CT. The genetics of inflammatory bowel disease. *Gastroenterology* 2007; 133: 1327-1339.
 18. Swarbrick MM, Chapman CM, McQuillan BM, Hung J, Thompson PL, Beilby JP. A Pro12Ala polymorphism in the human peroxisome proliferator-activated receptor-gamma 2 is associated with combined hyperlipidaemia in obesity. *Eur J Endocrinol* 2001; 144: 277-282.
 19. Dubuquoy L, Jansson EA, Deeb S, et al. Impaired expression of peroxisome proliferator- activated receptor gamma in ulcerative colitis. *Gastroenterology* 2003; 124: 1265-1276.
 20. Katayama K, Wada K, Nakajima A, et al. A novel PPAR gamma gene therapy to control inflammation associated with inflammatory bowel disease in a murine model. *Gastroenterology* 2003; 124: 1315-1324.
 21. Sugawara K, Olson TS, Moskaluk CA, et al. Linkage to peroxisome proliferator-activated receptor-gamma in SAMP1/YitFc mice and in human Crohn's disease. *Gastroenterology* 2005; 128: 351-360.
 22. Lewis JD, Lichtenstein GR, Stein RB, et al. An open-label trial of the PPAR-gamma ligand rosiglitazone for active ulcerative colitis. *Am J Gastroenterol* 2001; 96: 3323-3328.
 23. Lewis JD, Lichtenstein GR, Deren JJ, et al; Rosiglitazone for Ulcerative Colitis Study Group. Rosiglitazone for active ulcerative colitis: a randomized placebo-controlled trial. *Gastroenterology* 2008; 134: 688-695.
 24. Pollex RL, Mamakeesick M, Zinman B, Harris SB, Hegele RA, Hanley AJ. Peroxisome proliferator-activated receptor gamma polymorphism Pro12Ala is associated with nephropathy in type 2 diabetes. *J Diabetes Complications* 2007; 21: 166-171.
 25. Erdogan M, Karadeniz M, Eroglu Z, Tezcanli B, Selvi N, Yilmaz C. The relationship of the peroxisome proliferator-activated receptor-gamma 2 exon 2 and exon 6 gene polymorphism in Turkish type 2 diabetic patients with and without nephropathy. *Diabetes Res Clin Pract* 2007; 78: 355-359.
 26. Desreumaux P, Ernst O, Geboesk K, et al. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. *Gastroenterology* 1999; 117: 73-81.
 27. Vigoroux C, Fajas L, Khallouf E, et al. Human peroxisome proliferator-activated receptor-gamma2: genetic mapping, identification of a variant in the coding sequence, and exclusion as the gene responsible for lipotrophic diabetes. *Diabetes* 1998; 47: 490-492.
 28. Mori H, Ikegami H, Kawaguchi Y, et al. The Pro12 Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. *Diabetes* 2001; 50: 891-894.