Effects of polymorphism in G2677T/A triallelic region of MDR1 gene in Turkish patients with inflammatory bowel disease

MDR1 geni G2677T/A triallelik bölgesi polimorfizminin inflamatuvar barsak hastalığı olan Türk hastalarındaki etkisi

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Background/aims: *Crohn's disease and ulcerative colitis are both chronic inflammatory disorders of the gastrointestinal tract, the main causes of which remain unknown. Crohn's disease and ulcerative colitis are characterized by cell-mediated immune response against the luminal bacteria. It is suggested that expression levels and function of P-glycoprotein, encoded by the MDR1 gene, are important for protection of the gut against xenobiotics and bacterial toxins. Therefore, the mutations of the MDR1 gene are thought to be related with the pathogenesis of inflammatory bowel disease. The aim of this study was to investigate the G2677T/A polymorphism in the MDR1 gene in Turkish patients with inflammatory bowel disease and a healthy control group.* ***Methods:*** *In our study, the genotypes of endoscopically or histopathologically diagnosed Crohn's disease (n: 35; 14 F, 21 M) and ulcerative colitis (n: 82; 36 F, 46 M) patients and of 70 healthy individuals (39 F, 31 M) were compared. In the patient and control groups, polymerase chain reaction-restriction fragment length polymorphism analysis was performed for two polymorphisms (G2677T and G2677A) of the MDR1 gene.* ***Results:*** *In this study, the frequency of alleles at position 2677 of the MDR1 gene, which has a triallelic polymorphism, was not found to be significantly different between the patient and the healthy control groups. Moreover, the 2677A allele was not detected in either the patient group or the healthy control group.* ***Conclusions:*** *In this study, the G2677T/A polymorphism observed in the MDR1 gene was not found to be a risk factor for Crohn's disease or ulcerative colitis.*

Key words: Crohn's disease, inflammatory bowel disease, MDR1, P-glycoprotein, single nucleotide polymorphism, ulcerative colitis

Amaç: *Chron ve ülseratif kolit temel nedeni tam olarak bilinmeyen gastrointestinal sistemin kronik hastalıklarıdır. Ülseratif kolit ve Crohn hastalığı intestinal lümende yaşayan bakterilere karşı hücre aracılı immün cevap ile karakterize edilir. Barsağın ksenobiyotiklere ve bakteri toksinlerine karşı korunmasında, MDR1 geni tarafından kodlanan P-glikoproteininin fonksiyonunun ve ekspresyon seviyesinin önemli olduğu ileri sürülmektedir. Bu nedenle MDR1 genindeki mutasyonların inflamatuvar barsak hastalığı patogenezi ile ilişkili olduğu düşünülmektedir. Bu çalışmanın amacı MDR1 geninde görülen G2677T/A polimorfizminin Türk inflamatuvar barsak hastaları ve sağlıklı bireylerde incelenmesidir.* ***Yöntem:*** *Çalışmamızda endoskopik veya histopatolojik olarak tanı almış olan 35 Crohn (14 kadın + 21 erkek), 82 (36 kadın + 46 erkek) ülseratif kolit hastasının ve 70 (39 kadın + 31 erkek) sağlıklı bireyin genotipleri karşılaştırılmıştır. Bu çalışmada aday bölgedeki polimorfizmleri (G2677T ve G2677A) saptamak için polimeraz zincir reaksiyonu-restriksiyon parçacığı uzunluk polimorfizmi yöntemi kullanılmıştır.* ***Bulgular:*** *Bu çalışmada, MDR1 geninin triallelik bir polimorfizm taşıyan 2677 pozisyonundaki allel frekansı hasta ve sağlıklı kontrol grupları arasında anlamlı bir farklılık göstermemiştir. Ayrıca 2677A alleline her iki grupta da rastlanılmamıştır.* ***Sonuç:*** *Bu çalışmada, MDR1 geninde incelenen G2677T/A polimorfizmi Crohn veya ülseratif kolit için risk faktörü olarak bulunmamıştır.*

Anahtar kelimeler: Crohn Hastalığı, inflamatuvar barsak hastalığı, MDR1, P-glikoproteini, tek nükleotit polimorfizmi, ülseratif kolit

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD), the exact causes of which remain unknown, are inflammatory disorders of the gastrointestinal tract. Although the cause of inflammatory bowel

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Manuscript received: 08.10.2007 **Accepted:** 08.05.2008

disease (IBD) remains unclear, the phenotype of the disease is widely accepted to be determined by the interactions between genetic and environmental factors (1). The principal difference between UC and CD is associated with the extension of the disease. Common symptoms of UC include rectal bleeding and diarrhea, and the main focus of the inflammation is in the rectum, which can be prolonged proximally affecting the entire colon (2). CD is a non-infectious chronic inflammatory disease in which the inflammation can extend to transmural layers and can affect any area of the gastrointestinal tract, from esophagus to the anus (3). The studies performed in monozygotic and dizygotic twins indicated that genetic factors contribute to CD and UC pathogenesis (4, 5). Moreover, the relative risk for development of IBD is 4-10 fold higher in first-degree relatives when compared with unrelated people. Although many hypotheses have been suggested for the pathogenesis of IBD, it is known to be characterized by dysregulated mucosal immune response of the gut (5). The intestinal microflora and products of luminal bacteria are thought to be important factors in the pathogenesis of intestinal inflammation and disruption of tissue. Disruption of the protective epithelial cell barrier leads to development of IBD (6), which was supported by a study performed on the MDR1 (Multidrug Resistance 1) gene encoding P-glycoprotein in P-glycoprotein knockout mice (7). This study, performed with knockout mice, implied that functional loss of the MDR1 gene, which is responsible for the xenobiotic efflux mechanism, may promote the development of colitis. The protein called P-glycoprotein that is encoded by the MDR1 gene plays a major role in disposition and in protecting the organism against toxins. P-glycoprotein transports the toxins from the intracellular area and membrane to extracellular area with its ATP-dependent efflux transporter pump function. It is thought that the physiologic role of intestinal P-glycoprotein might be to prevent entry of bacterial toxins into the intestine wall mucosa, avoiding development of IBD. A previous study showed that the apical surface of superficial columnar epithelial cells of the colon and distal small intestine have a high level of P-glycoprotein (8). Therefore, the studies have recently focused on the MDR1 gene that encodes P-glycoprotein.

The MDR1 gene, also known as ABCB1, consists of one promoter region and 29 exons (9). The MDR1 gene is located on chromosome 7q21.1, which has been identified as IBD loci in genome-wide scan studies (10-12). The study performed by Satsangi and his colleagues (10) suggested an evidence for a linkage between chromosome 7q and the D7S669 marker in a European cohort. This linkage region contains the MDR1 gene. Although there are two distinct multidrug resistance genes in humans, MDR1 and MDR3, three different genes in rodents, mdr1a, mdr1b, and mdr2, have been identified (13, 14). Human MDR1 and rodent mdr1a and mdr1b genes are related to drug transport and drug resistance (15). The product of the MDR1 gene, P-glycoprotein, is a 170 kDa integral membrane protein, which is a member of the ATP-binding cassette super family of membrane transporters (16, 17). P-glycoprotein, an ATP-dependent efflux pump, transports inflammatory material and xenobiotic toxins from the intracellular to extracellular region. Phosphorylated and glycolyzed P-glycoprotein consists of 1280 amino acids and two homologous halves, each of which contains 610 amino acids connected to each other with a flexible region of 60 amino acids (18).

Two of the important factors that affect the activity of the MDR1 gene are the expression level of the MDR1 gene and the activity of P-glycoprotein encoded by this gene. Some studies have indicated that individual allelic changes alter the expression of the MDR1 gene and activity of P-glycoprotein (19-22). Furthermore, some previous studies have shown that the expression level of the MDR1 gene is associated with SNPs in the MDR1 gene (23, 24). In these studies, it has been shown that alteration of P-glycoprotein expression and function may contribute to the pathogenesis of gastrointestinal inflammatory diseases. A study carried out to search the relation between MDR1 gene expression level and occurrence of gastrointestinal inflammatory disorder suggested that low MDR1 levels may cause more severe intestinal inflammation (25). In addition, these studies have shown that G2677T/A polymorphism at exon 21 may also be associated with transport, function or expression of P-glycoprotein (23-25). These results indicate that polymorphisms and mutations of the MDR1 gene may change both the level of expression and function of P-glycoprotein, which may cause IBD (19), and also some other diseases including colorectal cancer (20), leukemia (21) and Parkinson disease (22). Thus, MDR1 has become an important candidate gene for recent case-control

and meta-analysis studies, showing genetic differences among distinct populations (2, 26-30). We, therefore, decided to perform a study to investigate the association between IBD, CD and UC and the G2677T/A polymorphism of the MDR1 gene in the Turkish population, in which no such studies have been conducted previously.

MATERIALS AND METHODS

Subjects

In this study, endoscopically or histopathologically diagnosed UC (n: 82; 46 M, 36 F; mean age: 40.5±1.7) and CD (n: 35; 21 M, 14 F; mean age: 38.9±1.9) patients from Ankara University Ibn-i Sina Hospital and T.C. Yuksek Ihtisas Hospital were investigated. Diagnosis and classification of IBD were made according to previously established international criteria (31). Seventy healthy volunteers (31 M, 39 F; mean age: 32.5±1.6) citing no previous bowel disease were included in the study to serve as the healthy control group. The study protocol was approved by the Ethics Committee of the Medical School of Ankara University. Informed consent was obtained from all patients for the use of their samples for the study.

DNA Isolation

Samples of genomic DNA were obtained from peripheral lymphocytes using high salt concentration method (32).

Genotyping of MDR1 Gene G2677A/T Polymorphism

The genomic DNA samples were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods for the G2677T and G2677A variants of the MDR1 gene, exon 21. PCR method was used for amplification of the specific gene region, and the oligonucleotides (17) used for PCR were commercially synthesized at MWG Biotech, Germany. The SNPs, the restriction enzymes and sizes of the RFLs can be seen in Table 1. For the PCR reactions, 100 ng of genomic DNA was added in a total volume of 50µl PCR mixture consisting of 25 pmol/µl of each specific primer pair, 10X PCR buffer with 1 mmol/L magnesium chloride, 2 mmol/L of deoxynucleotide triphosphate mixture (QBiogeen, Germany), and 1.25U of Taq DNA polymerase enzyme (MBI Fermentas, Lithuania). The PCR was run with an initial denaturation for 5 minutes at 95°C followed by 35 cycles of denaturation for 1 minute at 95°C, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute. The final extension was performed at 72°C for 7 minutes. PCR reactions were performed in an Eppendorf MasterCycler Personal thermocycler (Eppendorf, Hamburg, Germany). PCR products were confirmed on a 1% agarose gel stained with ethidium bromide. Subsequent restriction enzyme digestion analysis was performed according to the manufacturer's recommendations (MBI Fermentas, Lithuania). After restriction enzyme digestion, the DNA fragments were analyzed on 3% agarose gel. A PCR product of 224 base pairs (bp) was obtained by using specific primers to examine the G→T polymorphism in nucleotide 2677 of the MDR1 gene. This product was digested with BsrI restriction enzyme. Undigested and digested (198 and 26 bp sized bands) were named as T allele and G allele, respectively. Similarly, a PCR product of 220 bp in length was amplified using specific primers for G→A polymorphism in nucleotide 2677 of the MDR1 gene. After digestion with Ban I restriction enzyme, undigested and digested (206 and 14 bp sized bands) were named as G allele and A allele, respectively.

Statistical Analysis

The case-control analysis was tested using chi-square statistics and the Fisher's exact tests. Allele frequencies were calculated as the percentages of variant alleles.

RESULTS

In the present study, a total of 82 UC, 35 CD patients and a healthy control group of 70 volunteers

Table 1. Primer oligonucleotides used for amplification of PCR products, restriction endonucleases used for RFLP analysis and RFLP fragment sizes produced according to the presence of wild type or variant allele

Exon	SNP	Primer Sequence	Restriction Enzyme	*Fragment Length (bp)
Exon 21	G2677A	TGC AGG CTA TAG GTT CCA GG	BanI	220
		GTT TGA CTC ACC TTC CCA G		206, 14
Exon 21	G2677T	TGC AGG CTA TAG GTT CCA GG	BsrI	198, 26
		TTT AGT TTG ACT CAC CTT CCC G		224

PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; Bp: Base pairs.
* upper line, fragments of wild type allele; lower line, fragments of variant allele.

were genotyped for triallelic polymorphism of G2677T/A in the MDR1 gene. The genotype distributions of G2677T/A variants were found to be similar in both patient and control groups (Table 2). Increased frequency of the 2677T allele and decreased frequency of the G2677 allele were identified in patients with CD when compared with healthy controls; however, the difference was not statistically significant. In addition, the frequency of alleles of G2677T, showing a high degree of polymorphism, was very similar in both the UC and healthy control groups, and no statistical significance was found when the groups were compared to each other (Table 2). Interestingly, the 2677A allele was not detected in either patient group or in the control group.

We did not find any statistical difference between the patient and healthy control groups regarding either the allele or genotype distributions of the G2677T/A polymorphism of the MDR1 gene ($p>0.05$) (Table 2).

DISCUSSION

Evaluation of gene polymorphisms with potential functional importance is essential for identifying susceptibility to IBD. The MDR1 gene is an attractive candidate gene for the pathogenesis of IBD. The physiological importance of P-glycoprotein, the encoded product of the MDR1 gene in the gastrointestinal tract, has been proven with a study of the MDR1 knockout mice model, which demonstrated development of a spontaneous colitis in a specific pathogen-free environment (7). Additionally, in a study performed to investigate the association between the MDR1 gene and IBD, the effects of a proinflammatory cytokine tumor necrosis factor (TNF)-α on MDR1 gene expression and activity of P-glycoprotein were investigated (33). The study showed that TNF-α has an effect on decreasing the m-RNA level of the MDR1 gene and expression of P-glycoprotein, without any other stimulating effect on P-glycoprotein. These results indicated that there is an association between TNF-α and IBD that may be due to increased susceptibility to IBD with the effect of decreased MDR1 gene expression. These variations in the gene expression may result from functionally important polymorphisms in the MDR1 gene. Thus, many studies were performed in order to establish a correlation between these polymorphisms and IBD, which have so far produced conflicting results (2, 26, 28, 29, 34). In a study performed to investigate the contribution of MDR1 gene polymorphisms to the efficacy of azathioprine, it was observed that the frequency of 2677TT genotype is higher in nonresponders than in responders to azathioprine. In addition, the 2677T/3435T haplotype was also found to be more abundant in nonresponders while the 2677G/3435C haplotype was more abundant in responders (35). These results revealed that the G2677T polymorphism may be important for the pathogenesis and the cure of IBD. Therefore, the relationship between the MDR1 gene polymorphism, G2677T/A, and IBD was investigated in the present study.

In a multicenter North American cohort with 444 IBD trios, the triallelic G2677T/A SNP (Ala893Ser/Thr) was investigated (2). Ala893 (G2677) variant, which was known to decrease transporter function of P-glycoprotein, was found to be significantly associated with IBD, especially in non-Jewish subjects. Furthermore, a statistically significant increase in 2677T allele frequency was shown in UC cases when compared with controls in a large British case-control study (26). In the same study, the TT genotype was found to be significantly associated with severe UC. In another study, an association with T allele, 893 Ser variant of G2677T/A polymorphism, was found in UC patients (27). However, subsequent studies did not find a correlation between these polymorphisms and IBD, supporting results of the present study (28, 30). In an Italian case-control study performed by Ardizzone and his colleagues (34), an association between MDR1 G2677T/A and C3435T

Table 2. Genotype counts (%) and allele frequencies of G2677T/A polymorphism in the control, ulcerative colitis and Crohn's disease groups

G2677T/A	Number (n)	Genotype Count						Allele Frequency		
		GG	GT	TT	GA	TA	AA	G	T	A
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	%	%	%
UC	82	25 (30.49)	35 (42.68)	22 (26.83)	0 (0)	0 (0)	0 (0)	52	48	0
CD	35	8 (22.86)	16 (45.71)	11 (31.43)	0 (0)	0 (0)	0 (0)	46	54	0
Control	70	21 (30.00)	31 (44.29)	18 (25.71)	0 (0)	0 (0)	0 (0)	53	47	0

CD: Crohn's disease; UC: Ulcerative colitis.

polymorphisms and IBD was examined. Results of the study indicated that there were no significant differences between genotype frequencies of MDR1 G2677T/A and C3435T polymorphisms when either CD and/or UC patients were compared with the control group. On the other hand, a significant association was found between MDR1 C3435T polymorphism and patients with ileo-colonic CD, whereas no significant association between G2677T/A polymorphism and any specific subphenotypes was found (34). A haploid analysis performed by Ho and his colleagues (28) to investigate an association between C3435T and G2677T/A variants of the MDR1 gene and IBD showed a significant association between UC and 3435 TT genotype. However, no association was indicated between IBD and G2677T/A polymorphism. In a recent meta-analysis study, no significant association between G2677T/A polymorphism and IBD was demonstrated (29).

These conflicting results may depend on the ethnic divergence of populations in the different studies. The frequency of A allele at position 2677 was found to be 3.3-36% for Asians, 1.9-10% for whites, and 0.5% for blacks, showing that there is an ethnic difference for frequency of alleles (36). However, 2677A allele, which is significantly more common in the Japanese population (37), was not detected in either IBD or the control group in our study. Similarly, the different characteristics of the Turkish population have been documented in other polymorphism studies showing that the Turkish population does not share the same set of predisposing genes such as cytokine (38) and non-cytokine genes, including CARD15 gene (39), the major susceptibility factor in patients with CD in Western countries.

The identification of susceptibility genes for IBD, which is a complex genetic disease, is not ordinary. Consequently, G2677T/A variant of MDR1 gene was not found to be a risk factor for IBD in the present study. One reason for the conflicting results is genetic heterogeneity, in which different genes are thought to cause genetic susceptibility to IBD in different individuals. Some other explanations for this situation could be the small sample size of the studies and the different ethnic populations. The other polymorphisms in the MDR1 gene and other susceptibility candidate genes for IBD should be investigated in order to understand the molecular mechanisms underlying the genetic susceptibility to IBD.

REFERENCES

1. Loftus EV, Sandborn WJ. Epidemiology of inflammatory bowel disease. Gastroenterol Clin N Am 2002; 31: 1-20.
2. Brant SR, Panhuysen CI, Nicolae D, et al. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. Am J Hum Genet 2003; 73: 1282-92.
3. Louis E, Collard A, Oger AF, et al. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. Gut 2001; 49: 777-82.
4. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins: a study of heritability and the influence of smoking. Gut 1988; 29: 990–6.
5. Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-29.
6. Podolsky DK. Inflammatory bowel disease (first of two parts). N Engl J Med 1991; 323: 928-37.
7. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J Immunol 1998; 161: 5733-44.
8. Thiebaut F, Tsuruo T, Hamada H, et al. Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 1987; 84: 7735-8.
9. Bodor M, Kelly EJ, Ho RJ. Characterization of the human MDR1 gene. AAPS J 2005; 7: E1-5.
10. Satsangi J, Parkes M, Louis E, et al. Two-stage genome wide searches in inflammatory bowel disease provide evidence for susceptibility loci on chromosomes 3, 7, 12. Nat Genet 1996; 14: 199-202.
11. Van Heel DA, Fisher SA, Kirby A, et al. Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. Human Mol Genet 2004; 13: 763-70.
12. Hugot JP, Thomas G. Genome-wide scanning in inflammatory bowel diseases. Dig Dis 1998; 16: 364-9.
13. Hsu SI, Lathstein L, Horwithz SB. Differential overexpression of three mdr gene family members in multidrug-resistant J774.2 mouse cells. Evidence that distinct P-glycoprotein precursors are encoded by unique mdr genes. J Biol Chem 1989; 264: 12053-62.
14. Ueda K, Clark DP, Chen CJ, et al. The human multidrug resistance (mdr1) gene. cDNA cloning and transcription initiation. J Biol Chem 1986; 47: 381-9.
15. Fardel O, Payen L, Courtois A, et al. Regulation of biliary drug efflux pump expression by hormones and xenobiotics. Toxicology 2001; 167: 37–46.
16. Higgins CF. ABC transporter: from microorganisms to man. Ann Rev Cell Biol 1992; 8: 67-113.
17. Cascorbi I, Gerloff T, Johne A, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther 2001; 69: 169-74.

18. Nagata K, Nishitani M, Matsuo M, et al. Nonequivalent nucleotide trapping in the two nucleotide binding folds of the human multidrug resistance protein MRP1. J Biol Chem 2000; 275: 17626–30.
19. Hoffmeyer S, Burk O, Von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97: 3473-8.
20. Ho GT, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 17 0): an important determinant in gastrointestinal disease? Gut 2003; 52: 759-66. Review.
21. Kaya P, Gunduz U, Arpaci F, et al. Identification of polymorphisms on the MDR1 gene among Turkish population and their effects on multidrug resistance in acute leukemia patients. Am J Hematol 2005; 80: 26-34.
22. Tan EK, Chan DK, Ng PW, et al. Effect of MDR1 haplotype on risk of Parkinson disease. Arch Neurol 2005; 62: 460-4.
23. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001; 70: 189-99.
24. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 2001; 297: 1137-43.
25. Blokzijl H, Vander Borght S, Bok LI, et al. Decreased P-glycoprotein (P-gp/MDR1) expression in inflamed human intestinal epithelium is independent of PXR protein levels. Inflamm Bowel Dis 2007; 13: 710-20.
26. Onnie CM, Fisher SA, Pattni R, et al. Associations of allelic variants of the multidrug resistance gene (ABCB1 or MDR1) and inflammatory bowel disease and their effects on disease behavior: a case-control and meta-analysis study. Inflamm Bowel Dis 2006; 12: 263-71.
27. Potocnik U, Ferkolj I, Glavac D, Dean M. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. Genes Immun 2004; 5: 530-9.
28. Ho GT, Nimmo ER, Tenesa A, et al. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. Gastroenterology 2005; 128: 288-96.
29. Annese V, Valvano MR, Palmieri O, et al. Multidrug resistance 1 gene in inflammatory bowel disease: a meta-analysis. World J Gastroenterol 2006; 12: 3636-44.
30. Urcelay E, Mendoza JL, Martin MC, et al. MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. Inflamm Bowel Dis 2006; 12: 33-7.
31. Lennard-Jones JE. Classification of inflammatory bowel disease. Scand J Gastroenterol Suppl 1989; 24: 2–6.
32. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 1997; 25: 4692–3.
33. Belliard AM, Lacour B, Farinotti R, Leroy C. Effect of tumor necrosis factor-alpha and interferon-gamma on intestinal P-glycoprotein expression, activity, and localization in Caco-2 cells. J Pharm Sci 2004; 93: 1524-36.
34. Ardizzone S, Maconi G, Bianchi V, et al. Multidrug resistance 1 gene polymorphism and susceptibility to inflammatory bowel disease. Inflamm Bowel Dis 2007; 13: 516-23.
35. Mendoza JL, Urcelay E, Lana R, et al. MDR1 polymorphisms and response to azathioprine therapy in patients with Crohn's disease. Inflamm Bowel Dis 2007; 13: 585-90.
36. Yi SY, Hong KS, Lim HS, et al. A variant 2677A allele of the MDR1 gene affects fexofenadine disposition. Clin Pharmacol Ther 2004; 76: 418-27.
37. Moriya Y, Nakamura T, Horinouchi M, et al. Effects of polymorphisms of MDR1, MRP1, and MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. Biol Pharm Bull 2002; 25: 1356-9.
38. Celik Y, Dagli U, Kilic MY, et al. Cytokine gene polymorphisms in Turkish patients with inflammatory bowel disease. Scand J Gastroenterol 2006; 41: 559-65.
39. Ozen SC, Dagli U, Kilic MY, et al. NOD2/CARD15, NOD1/CARD4, and ICAM-1 gene polymorphisms in Turkish patients with inflammatory bowel disease. J Gastroenterol 2006; 41: 304-10.