Tropical Gastroenterology 2010;31(1):30–33

Original Article

# Cytokine gene polymorphisms in Irritable bowel syndrome in Indian population - A pilot case control study

S Santhosh*, AK Dutta*, P Samuel#, AJ Joseph*, J Ashok Kumar* and George Kurian*

Department of Gastrointestinal* Sciences and Biostatistics#, Christian Medical College, Vellore 632 004, Tamil Nadu

**Correspondence:**
*Dr. S Santhosh*
*Email: sunsanthosh@gmail.com, santhosh@cmcvellore.ac.in*

*ABSTRACT*

Aim: This study aimed at evaluating the single nucleotide polymorphisms (SNPs) in five cytokine genes regulating inflammation at altogether 8 different loci and compared their frequencies in patients with Irritable bowel syndrome (IBS) versus healthy age and sex matched controls.
Methods: Peripheral blood was collected for DNA cytokine analysis from 23 patients with IBS and 20 healthy controls. The cytokine SNPs studied include TNF-á (-308G/A), TGF-â1 (codon10T/C, codon25G/C), IL-10 promoter (-1082A/G; -819T/C; -592A/C), IL-6 promoter (-174G/C), and IFN-ã (+874T/A).
Results: There was a significant difference between a SNP in IL-10 (-592A/C) among cases and controls. There was also a trend to significance as regards to IL-6 promoter (-174G/C). Frequencies of other SNPs were not significantly different between the two groups.
Conclusion: This pilot study shows that there are polymorphism differences in cytokine genes between patients with IBS and healthy controls from India.

*KEYWORDS*: Irritable bowel syndrome, cytokine gene, polymorphisms

## Introduction

Irritable bowel syndrome is a common yet enigmatic entity. Despite its frequent occurrence, the exact pathophysiology remains to be defined. Proposed mechanisms include visceral hypersensitivity, abnormal motility, bowel inflammation and stress[1-3]. The clustering of IBS in families as well as twin studies has provided evidence for role of hereditary factors[4,5]. The hereditary pattern is complex rather than simple Mendelian pattern[6]. Genetic factors may play a role in modulation of gut inflammation or altered central processing of gut signals.

There is evidence supporting the role of inflammation in IBS[7]. These include the occurrence of IBS following gastroenteritis and higher frequency of IBS (than expected) like symptoms in inflammatory bowel disease patients in remission[7,8]. Animal models suggest that inflammatory response alters function of gut smooth muscle, enteric nerve and interstitial cells of Cajal[9-11]. In some patients with IBS an increase in the number of inflammatory cells in gut has been demonstrated[12-14]. Apart from infections, genetic factors may also predispose to inflammation. This has led to the hypothesis that enteric infection, genetic predisposition and stress factors may lead to a low grade inflammatory state which causes gut sensory motor dysfunction resulting in IBS symptoms[15].

Individuals differ in their ability to produce cytokines[16,17]. The maximal capacity of cytokine production has an important genetic component[18,19]. Polymorphism in the coding and/or regulatory region of cytokine genes which affects their overall expression or a different expression from normal are possible potential mechanisms[18-21]. Interleukin-10 (IL10), interleukin-6 (IL-6), transforming growth factor â1 (TGF-â1), interferon-ã (IFN-ã), and tumor necrosis factor-á (TNF-á) are important cytokines regulating inflammation[22-26]. Based on above evidence, it may be hypothesized that polymorphisms in these genes resulting in higher level of pro-inflammatory (IL-6, TNF-á, IFN-ã) and lower level of anti-inflammatory cytokines (TGF â1, IL-10) may be a risk factor for IBS or may make the response different from others.

We aimed to study the single nucleotide polymorphisms (SNP) in the above five cytokine genes regulating inflammation at altogether 8 different loci and compared their frequencies in patients with IBS versus healthy age and sex

© Tropical Gastroenterology 2010

matched controls. The cytokine SNPs studied include TNF-á (-308G/A), TGF-â1 (codon10T/C, codon25G/C), IL-10 promoter (-1082A/G; -819T/C; -592A/C), IL-6 promoter (-174G/C), and IFN-ã (+874T/A).

## Methodology

Twenty three patients with IBS and 20 healthy hospital based controls with similar age and sex distribution were enrolled in the study during March-April 2008 in Christian Medical College and Hospital, Vellore, India. IBS was diagnosed using ROME II criteria. After obtaining informed consent, peripheral blood was drawn for genetic study. Patient information was recorded in a standardized data form using structured questionnaire. The study was approved by institutional ethics committee

DNA was extracted from peripheral leukocytes using Qiagen DNA extraction kit as per the manufacturer's instructions. In this study, the known 8 polymorphisms in 5 genes including 8 wild type and 8 variant allele having human â globin gene as internal control primer were genotyped.

Polymerase Chain Reaction- Sequence Specific Primers (PCR-SSP) was employed to amplify the target sequence using One lambda cytokine genotyping kit as per manufacturer's instruction.

PCR programming was performed as follows; In step 1, denaturation was performed for 2.10 minutes at 96°C. In step 2, brief annealing at 63°C was performed for 1 minute. In step 3, denaturation as performed for 10 seconds. In step 4, brief annealing at 63°C was performed for 1 minute. Step 3 and 4 was performed for 8 times. This step is to ensure that there is homogeneous distribution of primers annealing to the target DNA fragment. In step 6, denaturation was performed for 10 seconds at 96°C. In step 7, annealing was performed at 59°C for 50 seconds. In step 8, extension was performed at 72°C for 30 seconds. Steps 6, 7 and 8 was performed for 19 cycles and the programe ends. The entire programe was performed in Gene Amp PCR System 9600 (Perkin-Elmer Corporation, Foster City, CA).

## Statistical Analysis

Fisher's exact test or chi square test was used to estimate significant differences between categorical variables (genotype, allele and phenotype frequencies) as appropriate. Mann Whitney's U test was used for comparing continuous variables. A two tailed p value of < 0.05 was considered significant. Analysis was done using STATA 8.0.

## Results

Twenty three patients with IBS and twenty healthy controls were studied. The age and sex distribution between cases and controls were similar (Table 1). Twelve patients had constipation predominant IBS (IBS-C), while four had diarrhoea predominant IBS (IBS-D). Seven patients had alternating diarrhoea and constipation (IBS-A).

Table 2 shows the allelic frequencies among patients and controls. There was a significant difference between a SNP in IL-10 (-592A/C) among cases and controls.

**Table 1: Baseline characteristic of patients and controls**

| | IBS patients (n=23) | Controls (n=20) | P |
|---|---|---|---|
| Age (Mean±SD) | 46.3+10 | 46.7+11 | 0.9 |
| Sex (Males) | 69.6% | 65% | 1 |
| IBS-D | 4 (17.4%) | NA | NA |
| IBS-C | 12 (52.2%) | NA | NA |
| IBS-A | 7 (30.4%) | NA | NA |

*IBS-D - Diarrhea predominant IBS*
*IBS-C - Constipation predominant IBS*
*IBS-A - IBS with alternating diarrhea and constipation*
*NA- not applicable*

**Table 2: Allelic frequencies of cytokine genes among patients and controls**

| Gene | Allele | Patients (n=23) | Controls (n=20) | P |
|---|---|---|---|---|
| IL-10 | -1082*G | 4 (8.7%) | 3 (7.5%) | 0.99 |
| | -1082*A | 42 (91.3%) | 37 (92.5%) | |
| IL-10 | -819* C | 20 (43.5%) | 17 (42.5%) | 0.99 |
| | -819 *T | 26 (56.5%) | 23 (57.5%) | |
| IL-10 | -592* A | 27 (58.7%) | 11 (27.5%) | 0.004 |
| | -592 *C | 19 (41.3%) | 29 (72.5%) | |
| TGF-â1 | Codon10*T | 24 (52.2%) | 23 (57.5%) | 0.62 |
| | Codon25*C | 22 (47.8%) | 17 (42.5%) | |
| TGF-â1 | Codon25*G | 42 (91.3%) | 36 (90.0%) | 0.99 |
| | Codon25*C | 4 (8.7%) | 4(10.0%) | |
| TNF-á | -308*G | 43 (93.5%) | 35 (87.5%) | 0.46 |
| | -308*A | 3 (6.5%) | 5 (12.5%) | |
| IL-6 | -174*G | 36 (78.3%) | 36 (90.0%) | 0.16 |
| | -174*C | 10 (21.7%) | 4 (10.0%) | |
| IFN-ã | +874 *T | 15 (32.6%) | 7 (17.5%) | 0.11 |
| | +874 *A | 31 (67.4%) | 33 (82.5%) | |

**Table 3: Genotypic frequencies of cytokine genes among patients and controls**

| Gene | Genotype | Patients (n=23) | Controls (n=20) | P |
|---|---|---|---|---|
| IL-10 (-1082) | G/G | 0 | 0 | |
| | G/A | 4 (17.4%) | 3 (15.0) | 0.99 |
| | A/A | 19 (82.6%) | 17 (85.0) | |
| IL10 (-819) | C/C | 3 (13.0%) | 5 (25.0%) | |
| | C/T | 14 (60.9%) | 9 (45.0%) | 0.51 |
| | T /T | 6 (26.1%) | 6 (30.0%) | |
| IL-10 (-592) | A/A | 7 (30.4%) | 2 (10.0%) | |
| | A/C | 12 (52.2%) | 11 (55.0%) | 0.18 |
| | C/C | 4 (17.4%) | 7 (35.0%) | |
| TGF-â (Codon10) | T/T | 6 (26.1%) | 4 (20.0%) | |
| | T/C | 12 (52.2) | 13 (65.0) | 0.76 |
| | C/C | 5 (21.7) | 3 (15.0) | |
| TGF-â1 (Codon25) | G/G | 19 (82.6%) | 16 (80.0%) | |
| | G/C | 4 (17.4%) | 4 (20.0%) | 0.99 |
| | C/C | 0 | 0 | |
| TNF-á (-308) | G/G | 21 (91.3%) | 15 (75.0%) | |
| | G/A | 1 (4.4%) | 5 (25.0%) | 0.08 |
| | A/A | 1 (4.4%) | 0 | |
| IL-6 (-174) | G/G | 16 (69.6%) | 17 (85.0%) | |
| | G/C | 4 (17.4%) | 2 (10.0%) | 0.59 |
| | C/C | 3 (13.0%) | 1 (5.0%) | |
| IFN-ã (+874) | T/T | 3 (13.0%) | 1 (5.0%) | |
| | T/A | 9 (39.1%) | 5 (25.0%) | 0.34 |
| | A/A | 11 (47.8%) | 14 (70.0%) | |

**Table 4: Phenotypic frequencies of cytokine genes among patients and controls**

| Gene | Phenotype | Patients (n=23) | Controls (n=20) | p |
|---|---|---|---|---|
| IL-10 | High | 0 | 0 | |
| | Intermediate | 4 (17.4%) | 3 (15%) | 1 |
| | Low | 19 (82.6%) | 17 (85%) | |
| TGF-â1 | High | 14 (60.9%) | 13 (65%) | |
| | Intermediate | 8 (34.8%) | 6 (30%) | 0.95 |
| | Low | 1 (4.3%) | 1 (5%) | |
| TNF-á | High | 2 (8.7%) | 5 (25%) | 0.22 |
| | Low | 21(91.3%) | 15(75%) | |
| IL-6 | High | 20 (87%) | 19(95%) | 0.61 |
| | Low | 3 (13%) | 1(5%) | |
| IFN-ã | High | 3 (13%) | 1 (5%) | |
| | Intermediate | 9 (39.1%) | 5 (25%) | 0.32 |
| | Low | 11 (47.8%) | 14 (70%) | |

Frequencies of other SNPs were not significantly different between the two groups.

Table 3 compares the frequency of cytokine genotypes in the two groups and none reached statistically significant difference. Table 4 depicts the phenotypic frequencies of the two groups and they were similar.

## Discussion

The purpose of this study was to look at a wide variety of genetic polymorphisms relating to inflammatory molecules in patients with Irritable bowel syndrome (IBS). This was a pilot study to determine if it was worth pursuing this area in a larger series in Indian population. The breadth of inflammatory molecules covered in this study have not been used in earlier studies on polymorphisms in IBD. We found a significant difference in polymorphism with regards to IL 10 (-592A/C) and a trend to significance as regards to IL-6 promoter (-174G/C).

Most studies on genetics of IBS have focused on polymorphisms of serotonin transporter gene and no association was found in a meta-analysis[27]. Cytokines studied previously include IL-10, TNF-á and TGF- â1[28-30]. As IL-10 downregulates production of Th1 cytokines, lower level in patients may be less effective in downgrading inflammatory response[22]. Genotypes GCC/GCC corresponds to high level of IL-10 production while GCC/ACC and GCC/ATA with intermediate levels[31]. Genotype ACC/ACC, ACC/ATA and ATA/ATA correlate with low levels of IL-10 production[31]. Chan, et al, in a study on 140 IBS patients showed significantly reduced frequency of IL-10 promoter (-1082G) allele (associated with higher production of IL-10) as compared with healthy controls[30]. Another study on 230 IBS patients and 450 healthy controls from UK showed significantly reduced frequencies of the high producer genotype for interleukin 10 promoter than controls[28]. However, a study from The Netherlands on IL-10 in 111 IBS patients and 162 healthy controls failed to show any association[29]. In our study we demonstrated an increased frequency of IL-10 promoter (-592A) allele in IBS patients. This variation across the world might be inferred to be due to differences in the various ethnic group.

TGF- â1 has an inhibitory effect on T-cell/dendritic cell maturation and macrophage activation[24]. Five combinations of SNPs at codon 10 and 25 were seen in this study (T/T & G/G – high production, T/C & G/G – high production, T/C & G/C – intermediate production, C/C & G/C – intermediate production, T/T & G/C – low production)[32] The allelic, genotype and phenotype frequency was not significantly different between patients and controls. The UK study also showed no difference in TGF-â1 genotype between cases and controls[28]. In contrast, Chan et al have shown the frequency of TGF- â1 high producer allele to be less in IBS patients[30].

TNF-á is a potent pro-inflammatory cytokine[26]. The A/A and G/A genotypes are associated with the production of high levels of TNF-á while the G/G genotype is associated with low levels[19]. The Netherlands study showed a significantly increased number of patients with high producer phenotype of IL-10 than healthy controls[29]. However, we did not find any difference between the two groups.

IL-6 is a pro-inflammatory Th2 cytokine produced by T cells and macrophages[23]. For IL-6 promoter, G/G and G/C genotypes are associated with the production of high levels of IL-6 while the C/C genotype is associated with low levels[20]. IFN-ã is a Th1 cytokine which has multiple effects including macrophage activation, antigen presentation and leukocyte adhesion[25]. Genotype T/T is associated with high levels, T/A with intermediate levels and A/A with low levels of IFN-ã production[21]. The allelic, genotypic and phenotypic frequencies of IL-6 and IFN-ã were not significantly different between IBS patients and controls.

In conclusion, this pilot study found a significant difference in polymorphism with regards to IL 10 (-592A/C) and a trend to significance as regards to IL-6 promoter (-174G/C) in patients with IBS , however these polymorphisms need to be validated in studies of much larger sample size.

## References


1. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology*. 2002;**123**:2108–31.
2. Talley NJ, Spiller R. Irritable bowel syndrome: a little understood organic bowel disease? *Lancet*. 2002;**360**:555–64.
3. Mayer EA, Collins SM. Evolving pathophysiologic models of functional gastrointestinal disorders. *Gastroenterology*. 2002;**122**:2032–48.
4. Levy RL, Jones KR, Whitehead WE, Feld SI, Talley NJ, Corey LA. Irritable bowel syndrome in twins: heredity and social learning both contribute to etiology. *Gastroenterology*. 2001;**121**:799–804.
5. Saito YA, Zimmerman JM, Harmsen WS, De Andrade M, Locke GR 3rd, Petersen GM, et al. Irritable bowel syndrome aggregates strongly in families: a family-based case-control study. *Neurogastroenterol Motil*. 2008;**20**:790–7.
6. Saito YA, Talley NJ. Genetics of irritable bowel syndrome. *Am J Gastroenterol*. 2008;**103**:2100–4;
7. Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, et al. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut*. 2000;**47**:804–11.
8. Neal KR, Hebden J, Spiller R. Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome: postal survey of patients. *BMJ*. 1997;**314**:779–82.
9. Vallance BA, Galeazzi F, Collins SM, Snider DP. CD4 T cells and major histocompatibility complex class II expression influence worm expulsion and increased intestinal muscle contraction during Trichinella spiralis infection. *Infect Immun*. 1999;**67**:6090–7.

10. Galeazzi F, Haapala EM, van Rooijen N, Vallance BA, Collins SM. Inflammation-induced impairment of enteric nerve function in nematode-infected mice is macrophage dependent. *Am J Physiol Gastrointest Liver Physiol*. 2000;**278**:G259–65.
11. Der T, Bercik P, Donnelly G, Jackson T, Berezin I, Collins SM, et al. Interstitial cells of cajal and inflammation-induced motor dysfunction in the mouse small intestine. *Gastroenterology*. 2000;**119**:1590–9.
12. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology*. 2002;**122**:1778–83.
13. Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. *Dig Dis Sci*. 1993;**38**:1590–5.
14. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, et al. Increased mast cells in the irritable bowel syndrome. *Neurogastroenterol Motil*. 2000;**12**:449–57.
15. Collins SM, Piche T, Rampal P. The putative role of inflammation in the irritable bowel syndrome. *Gut*. 2001;**49**:743–5.
16. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation*. 1998;**66**:1014–20.
17. Bidwell JL, Wood NA, Morse HR, Olomolaiye OO, Laundy GJ. Human cytokine gene nucleotide sequence alignments, 1998. *Eur J Immunogenet*. 1998;**25**:83–265.
18. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*. 1997;**24**:1–8.
19. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci*. U S A 1997;**94**:3195–9.
20. Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest*. 1998;**102**:1369–76.
21. Pravica V, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol*. 2000;**61**:863–6.
22. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol*. 2004;**22**:929–79.
23. Diehl S, Rincon M. The two faces of IL-6 on Th1/Th2 differentiation. *Mol Immunol*. 2002;**39**:531–6.
24. Schuppan D, Krebs A, Bauer M, Hahn EG. Hepatitis C and liver fibrosis. *Cell Death Differ*. 2003;**10** Suppl 1:S59–67.
25. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol*. 2004;**75**:163–89.
26. Gaur U, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem Pharmacol*. 2003;**66**:1403–8.
27. Van Kerkhoven LA, Laheij RJ, Jansen JB. Meta-analysis: a functional polymorphism in the gene encoding for activity of the serotonin transporter protein is not associated with the irritable bowel syndrome. *Aliment Pharmacol Ther*. 2007;**26**:979–86.
28. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? *Gut*. 2003;**52**:91–3.
29. Van der Veek PP, van den Berg M, de Kroon YE, Verspaget HW, Masclee AA. Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am J Gastroenterol*. 2005;**100**:2510–6.
30. Chan J, Gonsalkorale W, Perrey M. IL-10 and TGF-beta genotype in irritable bowel syndrome: evidence to support an inflammatory component? *Gastroenterology* (Abstract). 2000;**118**:A184.
31. Zhang X, Hei P, Deng L, Lin J. Interleukin-10 gene promoter polymorphisms and their protein production in peritoneal fluid in patients with endometriosis. *Mol Hum Reprod*. 2007;**13**:135–40.
32. He B, Xu C, Yang B, Landtblom AM, Fredrikson S, Hillert J. Linkage and association analysis of genes encoding cytokines and myelin proteins in multiple sclerosis. *J Neuroimmunol*. 1998;**86**:13–9.