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# Association between bactericidal/permeability increasing protein (BPI) gene polymorphism (Lys216Glu) and inflammatory bowel disease

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**KEYWORDS**
Inflammatory bowel disease;
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**Abstract**

*Background:* Increasing evidence suggests that innate immune system may have a key role in the pathogenesis of the inflammatory bowel disease (IBD). Bactericidal/permeability increasing protein (BPI) has an important role in the recognition and neutralization of gram-negative bacteria by host innate immune system. The polymorphism on BPI gene called Lys216Glu is on the suspected list of IBD pathogenesis.
*Methods:* We studied the Lys216Glu polymorphism on BPI gene, in a Turkish IBD patient population. A total of 238 IBD patients; 116 Crohn's disease (CD) and 122 ulcerative colitis (UC), besides 197 healthy controls were included in this study.
*Results:* The Glu/Glu genotype and allele frequencies were found to be statistically higher compared to healthy control group not only in CD patients [P: 0.03, OR: 1.87 (CI 95% 1.02–3.42) and P: 0.00001 (OR: 2.07 CI 95% 1.47–2.91) respectively] but also in UC patients [P: 0.0002, OR: 2.71 (CI 95% 1.53–4.80) and P: 0.00002 (OR: 2.71 CI 95% 1.53–4.80) respectively].
*Conclusions:* BPI polymorphism (Lys216Glu) is associated both to CD and UC. Our findings differ from the two Western European studies; one without any association and the other indicating an association only with CD. Our study is the first one reporting a novel association between BPI gene mutation (Lys216Glu) and UC.
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## 1. Introduction

Inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are chronic relapsing inflammatory diseases predominantly affecting the gastrointestinal tract and yet the underlying pathophysiology is unidentified. In the pathogenesis of both diseases, genetic tendencies and triggering environmental factors are suspected. We have some information about the importance of the integrity of the intestinal wall and the recognition of the pathogenic intestinal bacteria by the host.[1] It has been shown that if the environment can be kept germ-free in the spontaneous colitis models, no colitis will happen.[2] Defective balance and tolerance between the host immune system and intestinal germs of intestinal microbes, especially gram-negative bacteria (GNB), seem to be important in the pathogenesis of IBD. Innate immune system is in the key position for the recognition of microbial pathogens especially the GNB.[3] CARD 15 (Caspase recruitment domain 15), as part of the innate immune system, is functioning in recognition of bacterial components and results in the activation of nuclear factor kB. For the first time, tree mutations of CARD 15 gene (R702W, G908R and 1007fsinsC) were found to be increasing susceptibility for CD previously.[4–7] Toll Like Receptors (TLR) are expressed on the host cell membrane and involved in the recognition of microbial components. They are the membrane sense receptors of the host for the recognition of GNB via Lipopolysaccharide (LPS) portion and also the link pathway to the adaptive immune system.[8] Recently, two polymorphisms related to TLR4 genes (Asp299Gly and Thr 399Ile) were found to be associated to IBD.[9] Besides TLR4, LPS is also bonded by LPS-binding protein (LBP) and becomes a complex which is recognized by monocytes via a molecule called as CD 14.[3]

Bactericidal/permeability increasing protein (BPI) is a potent antimicrobial protein which is both structurally and also functionally closely related to LBP.[10] BPI has high affinity for the conserved Lipid A/inner core region of the endotoxin. BPI also promotes the delivery of GNB outer membrane antigens to dendritic cells. By that pathway adaptive immune system can be activated via T lymphocytes. BPI and other antimicrobial peptides like defensins are areas of increasing interest in IBD pathogenesis. Decreased antimicrobial activity in the intestinal mucosal extracts was found to be associated not only with low antibacterial expression in the colonic CD[11] and but also diminished levels of Paneth cell $\alpha$-defensins in ileal CD patients.[12] On the other hand BPI helps to constrain local tissue infection besides prevent systemic infection and inflammation. BPI expression on mRNA and protein level has been demonstrated for human gastrointestinal epithelial cells. BPI is produced on the apical and apicobasal surfaces of the human GIS epithelial cells.[13] BPI protein concentrations found to be increased in tissue specimens of ulcerative colitis patients.[13–15] Auto-antibodies against BPI (p-ANCA) were described and found to be increased with increasing severity of clinical status in IBD.[16] Recombinant BPI (rBPI) found be useful against lethal challenges with endotoxin and also GNB at least in animal models.[17] Previously a polymorphism of BPI gene (Glu216Lys) was described.[18] Recently that polymorphism is found to be associated with Crohn's disease.[19] We want to study that BPI gene polymorphism in the Turkish population of IBD patients.

## 2. Materials and methods

### 2.1. Study population

We collected clinical data and also blood samples for genetic analysis from the IBD patients following the IBD clinics of our hospital. The clinical, radiological, endoscopic, and histological criteria were used for the conventional diagnosis of inflammatory bowel diseases. Inflammatory bowel diseases were classified as Crohn's disease or ulcerative colitis, whereas undetermined colitis was excluded from the study. A total of 238 IBD patients (116 CD and 122 UC), and 197 healthy controls were included into the study. The clinical data documented for the study was as follows; the type of the disease (Crohn's disease (CD) or ulcerative colitis (UC)), age, sex, date of the diagnosis, disease localization, phenotype, corticosteroid usage, smoking, and family history. For control group we included healthy unrelated blood donors. The baseline characteristics of the study population are given in Table 1.

### 2.2. DNA extraction

We collected peripheral venous blood samples into sterile tubes containing $K_3$EDTA solution. Genomic DNA was extracted by using phenol–chloroform method in our laboratory.[20]

### 2.3. Genotyping

Single nucleotide polymorphism was studied: the Lys216Glu (645A/G) polymorphism in the BPI gene. We used polymerase chain reaction (PCR) restriction fragment length polymorphism analysis (RFLP) for the typing of the polymorphism.[25] 100 ng of genomic DNA, 1× PCR buffer (Fermentas, 830 Harrington Court, Burlington, Ontario, Canada), 0.2 mM of each dNTP (Fermentas), 0.5 U of FastStart Taq DNA polymerase (Fermentas) and 5 pmol of the primer i.e.;

**Table 1** Characteristics of the study population.

| | | Ulcerative colitis (UC) | Crohn' s disease (CD) |
|---|---|---|---|
| N | | 122 | 116 |
| Age: mean ± SD | | 43.4 ± 15.5 | 39.2 ± 13.9 |
| (Range) | | (18–80) | (19–86) |
| (% Female) | | 47.5 | 45.7 |
| (%) Localization UC | Distal colitis | 45.1 | – |
| | Pan-colitis | 39.3 | – |
| | Proctitis | 15.6 | – |
| (%) Phenotype CD | Inflammatory | – | 68.9 |
| | Fibrostenotic | – | 19.8 |
| | Fistulizing | – | 11.3 |
| (%) Localization CD | Ileal | – | 36.2 |
| | Ileocolonic | – | 37.1 |
| | Colonic | – | 26.7 |
| (%) Smoking | | 23 | 26.7 |

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F:5′-CACTATGGGAAGACCTTACTGATTAC-3′ and R:5′-CAGAGTCTGGAAATAAGGTTGAAGC-3′ were included to the total 20 μl volume of the PCR solution. The final $MgCl_2$ concentration was 2.5 mM. PCR conditions were as follows; 15 min of initial denaturizing at 95 °C, 35 cycles of 94 °C for 30 s, annealing for 30 s at 60 °C and 72 °C for 30 s, additionally at 72 °C for 10 min. *H*ind III restriction enzyme (Fermentas) was used for the PCR products for overnight digestion at 37 °C. 2.5% agarose gels stained with ethidium bromide were used for the analyzation of the fragments after electrophoresis. The lengths of restriction fragments were for Glu allele: 104 bp and Lys Allele: 78 + 26 bp.

### 2.4. Statistics

SPSS 11.5 version was used for data entry and statistical analysis. Chi-square ($\chi^2$) test with Yates correction was used to compare the genotype and allele frequencies between disease and control groups. The Fisher's exact test was used when appropriate. For multiple comparisons, Bonferroni correction was applied. *P* values less than 0.05 were considered significant. The Hardy–Weinberg equilibrium was used for the comparison of the predicted frequencies and observed genotype frequencies.

## 3. Ethical considerations

The study was approved by the Ethic Committee of the Marmara University Faculty of Medicine, Istanbul, Turkey. Consent was taken from each patient that was included to the study after appropriate information was given.

## 4. Results

After statistical analysis, the Glu/Glu genotype and allele frequencies of the BPI Lys216Glu polymorphism are found to be significantly increased compared to healthy control group not only in CD patient group [P: 0.03, OR: 1.87 (CI 95% 1.02–3.42) and P: 0.00001 (OR: 2.07 CI 95% 1.47–2.91) respectively] but also in UC patient group [P: 0.0002, OR: 2.71 (CI 95% 1.53–4.80) and P: 0.00002 (OR: 2.71 CI 95% 1.53–4.80) respectively] (Table 2). No association can be determined with univariate or multivariate analyses for age, sex, date of the diagnosis, disease localization, phenotype, corticosteroid usage, smoking, and family history.

## 5. Discussion

BPI is a single cationic 55 kDa protein having two domains mainly produced in the polymorph nuclear leukocytes (PMNL). BPI is expressed not only on the gastrointestinal mucosal cells but also on the fibroblasts as a second barrier to GNB.[10] BPI protein concentrations were found to be increased in tissue specimens of UC and CD patients.[15] Increased concentrations of BPI reported to be well correlated not only with histological inflammatory activity of UC[14] but also with the endoscopic inflammation score.[15] Auto-antibodies against BPI (p-ANCA) was described to be present[21] and found to be increased with increasing severity of clinical status and higher inflammatory score in IBD.[22,23] These auto-antibodies reported to impair antibiotic activity of BPI.[24] BPI-Anca is found to be related to colonic CD and disease activity in UC.[16]

Recently, Klein et al. from Germany reported that the polymorphism is found to be associated with Crohn's disease.[19] The Glu/Glu genotype was found to be significantly decreased in CD patients when compared to the healthy controls. No difference was found with UC patients. Török et al.[25] reported in another study from the same country which could not find any association. The frequencies of the genotypes and alleles were found to be very similar in the healthy control group in the two studies which were reported from the same country (Table 3). In our study, we found a statistically significant increase in the Glu allele and Glu/Glu genotype frequencies of the BPI Lys216Glu polymorphism in patients with UC and also CD when compared to the healthy control group (*P* = 0.00001 and *P* = 0.00001 for allele and P: 0.03 and P: 0.0002 for genotype frequencies respectively). Our result seems to be different from the previously mentioned studies. That may be due to ethnic differences between two countries. We still do not know how that sequence variation may play a role in the pathogenesis of UC and CD. Klein et al. proposed that amino

**Table 2** Genotype and allele frequencies.

| | Genotype frequencies (%) | | | Allele frequencies (%) | |
|---|---|---|---|---|---|
| | Lys/Lys | Lys/Glu | Glu/Glu | Glu (allele) | Lys (allele) |
| CD patients (n = 116) | 7.8 | 66.4 | 25.9<br>*P: 0.03* [a]<br>*OR: 1.87*<br>*(1.02–3.42)* | 59.1<br>*P: 0.00001* [a]<br>*OR: 2.07*<br>*(1.47–2.91)* | 40.9 |
| UC patients (n = 122) | 16.4 | 50 | 33.6<br>*P: 0.0002* [a]<br>*OR: 2.71*<br>*(1.53–4.80)* | 58.6<br>*P: 0.00001* [a]<br>*OR: 2.03*<br>*(1.45–2.84)* | 41.4 |
| Healthy controls (n = 197) | 33.5 | 50.8 | 15.7 | 41.1 | 58.9 |

[a] P value found to be statistically significant compared to healthy control group.

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**Table 3** Genotype and allele frequencies of all BPI216Glu polymorphism studies in IBD patient population.

| | | Genotype frequencies (%) | | | Allele frequencies (%) | |
|---|---|---|---|---|---|---|
| | | Lys/Lys | Lys/Glu | Glu/Glu | Glu (allele) | Lys (allele) |
| Akın | CD patients (n = 116) | 7.8 | 66.4 | 25.9<br>P: 0.03 | 59.1<br>P: 0.00001 | 40.9 |
| Török[25] | CD<br>N:102 | 18 | 57 | 25 | 54 | 46 |
| Klein[19] | CD<br>N = 265 | 25 | 54 | 21<br>P < 0.027 | 48 | 52 |
| Akın | UC patients (n = 122) | 16.4 | 50 | 33.6<br>P: 0.0002 | 58.6<br>P: 0.00001 | 41.4 |
| Török | UC<br>N = 98 | 17 | 51 | 32 | 57 | 43 |
| Klein | UC<br>N = 207 | 22 | 51 | 27 | 53 | 47 |
| Akın | Healthy controls (n = 197) | 33.5 | 50.8 | 15.7 | 41.1 | 58.9 |
| Török | HC<br>N = 145 | 22 | 50 | 28 | 53 | 47 |
| Klein | HC<br>N = 608 | 23 | 49 | 28 | 53 | 47 |

acid exchange caused by that genetic variation may result a functional change. This idea of functional change, resulting in a possible increased expression of BPI molecule, may also bring an explanation to the question; why tissue levels of BPI increased in tissue specimens of IBD patients. Further clinical studies searching possible association of BPI polymorphism and BPI molecules' increased expression in the tissue samples of IBD patients are needed. On the other hand, BPI is known to have many functions; the amino-terminal domain of BPI has antibiotic activity against GNB, and endotoxin neutralization. The carboxyl-terminal domain performs opsonophagocytosis. These two domains address the disposal of LPS/BPI. For complement activation function, both terminals are needed.[10] So a possible functional change may result in crucial defects to all of these functions. Furthermore, that sequence variation of BPI may cause a conformational change in the epitope recognized by p-ANCA directed against BPI which may alter effective BPI neutralization by p-ANCA. BPI also has recently been reported to have some antiangiogenetic effects,[26] through which it helps to constrain local tissue infection besides prevent systemic infection and inflammation. Altered BPI production may also worsen the inflammation via defective antiangiogenetic effects. Further molecular and clinical studies are needed to clarify the results of BPI polymorphism Lys216Glu effect on BPI molecule and IBD patient population.

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