A Common Polymorphism in the Interleukin-8 Gene Promoter Is Associated with an Increased Risk for Recurrent Clostridium difficile Infection

Kevin W. Garey,¹,²,³ Zhi-Dong Jiang,² Shashank Ghantoji,²,³ Vincent H. Tam,¹,³ Vaneet Arora,¹ and Herbert L. DuPont¹,²,³,⁴

¹University of Houston College of Pharmacy, ²University of Texas School of Public Health, ³St. Luke’s Episcopal Hospital, and ⁴Baylor College of Medicine, Houston, Texas

Background. Neutrophil recruitment coordinated by intestinal interleukin (IL)–8 secretion is a key component in the pathogenesis of Clostridium difficile infection (CDI). We hypothesized that a common single-nucleotide polymorphism (SNP) in the −251 region of the IL-8 gene promoter may be predictive of recurrent CDI.

Methods. This was a prospective cohort study of hospitalized adult patients with CDI who were admitted to a large, university-affiliated medical center from 2007 through 2008. Patients were monitored for 3 months after diagnosis of CDI and assessed for recurrent CDI (defined as a return of diarrhea that required treatment after initial symptom resolution). DNA was isolated from blood samples, and genetic sequencing was performed using polymerase chain reaction and pyrosequencing. The association between IL-8 genotype and recurrent CDI was assessed using univariate and multivariate statistics.

Results. Ninety-six patients with a mean (± standard deviation) age of 61 ± 16 years (54% of whom were female and 63% of whom were white) were identified. The overall incidence of recurrent CDI was 24%. IL-8 allele frequency was similar to previously reported findings (for A/A, 27%; for A/T, 53%; and for T/T, 20%). The incidence of recurrent CDI was 38% in patients with the A/A allele and 19% in all other patients (relative risk, 2.1; 95% confidence interval, 1.04–4.13) (P = .043).

Conclusions. This study indicates that a common SNP in the IL-8 gene promoter is an independent predictor of recurrent CDI. Our results could offer risk stratification for patients at high risk for recurrent CDI.

Clostridium difficile infection (CDI) is the leading cause of infectious diarrhea in hospitalized patients, with increased associated mortality rates noted in the United States and elsewhere [1, 2]. The pathogenesis of CDI involves colonization of the organism in the colon, with production of 1 or, usually, 2 toxins: toxins A and B. Toxin A, in particular, is a potent chemotactant for phagocytes, and both toxins cause the release of pro-inflammatory cytokines into the large intestine. This inflammatory response increases colonic vascular permeability, neutrophil recruitment, and opening of colonic epithelial cell junctions. A total of 20%–40% of patients with primary C. difficile infection experience recurrent disease. Lack of a host IgG antibody response is a key immunologic risk factor for primary and recurrent infections [3, 4]. Key clinical risk factors include advanced age, continued use of antibiotics, and use of gastric acid suppressive agents [5].

Interleukin (IL)–8 is an inflammatory cytokine and potent attractant of neutrophils and is a key component in the pathogenesis of C. difficile infection. Nine sequence variants in the IL-8 gene regions have been reported, although only one in the −251 promoter region has been associated with increased fecal levels of IL-8 [6]. Subjects with the A/A allele are known to produce high concentrations of fecal IL-8 in response to intestinal infections. Our group previously demonstrated that hospitalized patients with the IL-8 −251 A/A polymorphism were at higher risk for primary C. difficile infection [7], with lower rates of host immunoglobulin response to toxin A [8]. Kyne et al [4]
demonstrated that the development of a serum antibody to toxin A in patients with CDI was associated with protection against recurrent CDI. We hypothesized that the IL-8 −251 A/A polymorphism will be a predictor for recurrent C. difficile infection. A secondary aim was to assess whether this same polymorphism may be predictive of refractory CDI or the mortality rate after 3 months.

MATERIALS AND METHODS

Patient Population and Definitions

This was a prospective cohort study of hospitalized patients ≥18 years of age between 2007 and 2008 at a large (≥500-bed) university-affiliated hospital in Houston, Texas. All patients with primary CDI were defined as patients who received an antimicrobial agent during their current hospitalization with diarrhea (defined as ≥3 more loose stools in a 24-h period) and who had a positive C. difficile cytotoxin B tissue culture assay result identified by the clinical microbiology laboratory [9]. Patients were identified by daily review of the C. difficile stool toxin tests from the clinical microbiology laboratory. Treatment of CDI occurred at the discretion of the primary medical team. Patients were excluded from the study if they had CDI within the previous 6 months or received antibiotic therapy other than metronidazole or oral vancomycin for CDI. Patients were monitored daily during hospitalization and were telephoned weekly after hospital discharge for recurrent CDI, for a total of 3 months. Recurrent CDI was defined as a new episode of diarrhea lasting for at least 2 days, with a positive toxin assay test result confirmed after resolution of the initial episode for at least 48 h and after discontinuation of C. difficile antibiotics. Refractory CDI was defined as continued assessment of diarrhea after 6 days of CDI antibiotic therapy. This study was approved by the institutional review boards of St. Luke’s Episcopal Hospital, the University of Texas, and the University of Houston. All patients provided signed informed consent.

Data collection

Data collected on all patients included demographic characteristics (age, sex, and race), medical history, comorbid conditions (as determined using the Charlson comorbidity scale), seriousness of underlying disease (as determined using Horn’s index), use of gastric acid suppressive agents, or continued use (after diagnosis of CDI) of the antibiotic (ie, the causative antibiotic) that the patient was receiving prior to CDI development [10, 11].

Laboratory Studies

DNA isolation. Human genomic DNA was extracted from patient blood samples by use of PureGene DNA isolation kits, as recommended by the manufacturer (Gentra Systems)

Polymerase chain reaction and pyrosequencing protocol. Detection of the single-nucleotide polymorphism (SNP) at the −251 region of the IL-8 promoter genes was performed as described elsewhere [7, 8, 12]. The primers used in the reaction included IL-8 SNP-251R 5′-cttataaatagctgactgtactatg-3′ and pyrosequencing primer 5′-ctagaatatttaaatcacta-3′. Primers were synthesized by Integrated DNA Technologies. Polymerase chain reaction was performed using HotStar Taq DNA polymerase (Qiagen). DNA samples of known genotype were included as controls in each experiment. With use of the conditions recommended by the manufacturer, SNP assays were performed on a Pyrosequencer with PSQ 96 SNP Reagent Kit (Pyrosequencing AB) at the genetics core laboratory of the University of Texas General Clinical Research Center Houston Medical School.

Statistical Analysis

Association of the IL-8 genotype with recurrent CDI was analyzed by univariate analysis and multivariate logistic regression. Analyses were performed using SAS software (version 9.1;
SAS Institute). Univariate statistics included Student’s t test for normally distributed continuous data and the Mann-Whitney U test for nonnormally distributed continuous data. χ² or Fisher’s exact tests were used for categorical data. To assess the censoring effect of death on the rate of recurrent CDI, a competing risks analysis was conducted by plotting observed cumulative incidence for recurrence and death before recurrence [13]. A stacked probability plot and cumulative incidence functions stratified by genotype plot were constructed. All tests were 2 tailed, and P<.05 was considered to denote statistical significance. The P values, odds ratios, and 95% confidence intervals are included in the data tables.

RESULTS

Patient characteristics. Ninety-six patients with a mean (± standard deviation [SD]) age of 61 ± 16 years (54% of whom were female and 63% of whom were white) were recruited during the study. Patients were classified, by use of Horn’s index, as having moderate (frequency, 22%), severe (60%), or extremely severe (15%) underlying disease. The average (± SD) Charlson score was 2.8 ± 2.2. Patients were commonly given a proton pump inhibitor (frequency, 49%), and most continued receiving the causative antibiotic after diagnosis of CDI (frequency, 76%). The most common comorbid conditions present in at least 10% of the population included diabetes (39%), congestive heart failure (28%), chronic obstructive pulmonary disease (17%), chronic renal failure requiring hemodialysis (17%), oncologic tumors (16%), previous myocardial infarction (15%), solid-organ transplantation (14%), obesity (13%), and peripheral vascular disease (10%). Ninety-two percent of patients were given metronidazole as initial CDI therapy. Twenty-three (24%) of 96 patients experienced recurrent CDI during the 3-month study. Confounders with a nonsignificant trend (\(P > .05\)) toward increasing the risk of recurrent CDI included nonwhite race, use of proton pump inhibitors, and a lack of certain chronic conditions (diabetes mellitus and previous myocardial infarction). Risk of recurrent CDI decreased from 28% to 29% (as determined by a Horn’s index of 2) to 3%–7% in patients with a Horn’s index of 4 (Table 1).

Association between IL-8 promoter gene polymorphism and recurrent CDI. Of the 96 patients, 26 (27%) were identified as having the A/A genotype, 51 (53%) were identified as having the A/T genotype, and 19 (20%) were identified as having the T/T genotype. Recurrence rates were lowest in association with the T/T genotype (16%), followed by the A/T genotype (20%) and the A/A genotype (39%) (\(P = .063\), for trend). Patients with the A/A genotype were more likely to experience recurrent CDI (\(P = .043\)) (Figure 1). Ten (39%) of 26 patients with the A/A genotype experienced recurrent CDI, compared with 13 (19%) of 70 patients with the A/T or T/T genotypes (odds ratio [OR], 2.7; 95% confidence interval [CI], 1.01–7.4).

IL-8 promoter gene polymorphism and refractory CDI, recurrent CDI, and the mortality rate after 3 months. Thirty (31%) of 96 patients experienced refractory CDI. There was no difference in IL-8 genotype and refractory CDI (\(P = .85\)). The incidence of refractory CDI was 32% in patients with the T/T genotype, 33% in patients with the A/T genotype, and 27% in patients with the A/A genotype.

The overall mortality rate after 3 months was 22%. Mortality rates were lowest in patients with the A/T genotype (16%), compared with patients with the T/T genotype (26%) or the A/A genotype (31%), although this was not statistically significant (\(P = .12\)). Cumulative incidence curves of CDI recurrence and of death before recurrence are presented in Figure 2. For patients with the AA polymorphism and the AT or TT polymorphism, the cumulative incidence curves for CDI recurrence and death before recurrence are shown in Figures 3 and 4.

Demographic predictors of the IL-8 A/A genotype. An exploratory analysis was undertaken to assess whether patients of the

Figure 1. Rates of recurrence of Clostridium difficile infection stratified by the interleukin (IL)-8 genotype. Patients with the A/A genotype were much more likely to experience recurrent C. difficile infection, compared with patients with the A/T or T/T genotype (odds ratio, 2.7; 95% confidence interval, 1.01–7.4) (\(P = .043\)). SNP, single-nucleotide polymorphism.

Figure 2. Cumulative incidence curves for recurrence of Clostridium difficile infection and death before recurrence. The cumulative incidence functions are stacked. The distance between the 2 curves denotes the probability of the 2 events.
DISCUSSION

Approximately 1.42 million SNPs in the human genome have been identified; many have important clinical implications [14]. A number of polymorphisms in inflammatory cytokine genes, such as tumor necrosis factor-α, IL-1β, IL-1ra, IL-4, IL-6, and IL-8, have shown an effect on the level of immune response that occurs after infectious stimuli [15]. Previous studies have shown that the production of IL-8 is also genetically determined and that neutrophils from individuals who are homozygous for the A/A genotype at the −251 position demonstrated a trend toward higher levels of IL-8 in response to lipopolysaccharide than did those without the allele. Our research group demonstrated that the chances of having enteroaggregative Escherichia coli (EAEC)–associated diarrhea, a gastrointestinal disease associated with neutrophil infiltration, were significantly increased among 69 US students who remained in Mexico for 5 weeks with the A/A genotype at the −251 position, compared with those with the T/T genotype at the −251 position [6].

The hypothesis underlying this study is that increased IL-8 production leads to more inflammation in the colonic epithelium, leading to increased risk of symptomatic disease in patients infected with C. difficile. In 2 separate studies that our group performed in hospitalized patients, the A/A genotype at the −251 position of the IL-8 gene was shown to be a significant risk factor for primary CDI [7, 8]. The present study builds on these findings by showing that this genetic trait is also a risk factor for recurrent CDI. Ten (39%) of 26 patients with the A/A genotype experienced recurrent CDI, compared with 13 (19%) of 70 patients with the A/T or T/T genotypes (OR, 2.7; 95% CI, 1.01–7.4) (P = .042). Patients with the A/A genotype also experienced recurrences later than patients with the A/T or T/T genotypes. The strengths of the study include a prospective study design, a large sample size of almost 100 patients, and an extended follow-up of 3 months. To the best of our knowledge, this is the first report of a host genetic trait that predicts recurrence of CDI. Although we did not measure fecal levels of IL-8 in the stools of our subjects, we have previously shown that the IL-8 A/A genotype is correlated with higher levels of IL-8 in feces in patients with CDI and traveler’s diarrhea [6, 7].

Treatment with monoclonal antibodies directed against toxins A and B in combination with CDI antibiotics has recently been shown to decrease rates of CDI recurrence by 72% [16]. We have previously shown that a defective humoral immune response and the IL-8 A/A genotype increased the risk for primary CDI [8]. Future research will focus on the association between IL-8 genotype and the host humoral immune to CD toxins in patients with primary and recurrent CDI. In subanalyses, we investigated whether the IL-8 genotype may affect refractory CDI or the mortality rate after 3 months. Although they were nonstatistically significant, trends for decreased mortality rates for patients with the A/T genotype were demonstrated. We also showed a trend for a higher proportion of nonwhites to have the A/A genotype, compared with whites. Previous research has shown different haplotype genealogy among Europeans and Africans [17]. However, whether a demographic variable such as race could predict which patients have a higher likelihood for the IL-8 A/A genotype with a subsequent increased risk for CDI or recurrent CDI will require further study.

This study has certain limitations. We did not perform strain typing and therefore do not know the proportion of the epidemic BI/NAP1 in our patient population. We did not measure the immunoglobulin G antibody response to CDI and will base future studies on investigating the immunoglobulin G antibody response to toxins A and B in association with the IL-8 genotype. We did not perform a multivariate analysis because of
the low number of recurrent cases \((n = 23)\). We had high rates of continued use of causative antibiotics and proton pump inhibitors in our elderly patient population. We hypothesize that these high incidence rates prevented us from seeing an association between these variables and recurrent CDI, as reported in the literature \([5]\). How these results are used with new therapies to prevent recurrent CDI, such as monoclonal antibody therapy or antibiotic therapy with rifaximin, fidaxomicin, tapered or pulsed vancomycin, or probiotics, will be areas for future research \([16, 18–23]\).

In summary, in a prospective cohort study of 96 patients with CDI, 10 (39%) of 26 patients with the A/A genotype in the IL-8 promoter gene experienced recurrent CDI, compared with 13 (19%) of 70 patients with the A/T or T/T genotypes \((\text{OR}, 2.7; 95\% \text{ CI}, 1.01–7.4) \quad (P = .043)\). This is the first report of a host genetic trait associated with increased risk of recurrent CDI. Our results could offer risk stratification for patients at high risk for recurrent CDI.

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