ELSEVIER

Association of TNF-α Polymorphisms in Crohn Disease

Katrin Zipperlen, Lynette Peddle, Bill Melay, Donna Hefferton, and Proton Rahman

ABSTRACT: Clinical and molecular studies implicate tumor necrosis factor alpha (TNF-α) as a key mediator in the initiation and propagation of Crohn disease (CD). Genetic associations have been documented between promoter polymorphisms of TNF-α and CD; however, these associations have not been universally replicated. In this study, we set out to examine the association of five promoter TNF-α polymorphisms in CD subjects from a founder population. In total, 128 CD subjects and 103 ethnically matched healthy controls were genotyped with time-of-flight mass spectrometry for the following five single nucleotide polymorphisms (SNPs) in the 5′ flanking region of TNF-α gene: -1031 (T→C), -863 (C→A), -857 (C→T), -308 (G→A), and -238 (G→A). Primer sequences, termination mixes, and multiplexing were determined with Sequenom SpectroDESIGNER software v1.3.4. The minor allele frequency for the TNF-α SNPs in subjects with CD and healthy controls, respectively, were -238 (5.5% vs. 5.3%); -308 (17.6% vs. 18.9%); -857 (5.1% vs. 7.8%); -863 (19.1% vs. 17.5%), and -1031 (24.6% vs. 22.8%). Thus, none of the TNF-α variants was associated with CD. Furthermore, no genotype/phenotype correlations were observed for the mutant allele of the TNF-α variants and selected clinical outcomes. In conclusion, there was no significant association for any of the TNF-α promoter polymorphism tested and CD in the Newfoundland population. *Human Immunology 66, 56-59 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: Crohn disease; TNF-α; association studies

ABBREVIATIONS

CD Crohn disease

TNF-α tumor necrosis factor alpha

INTRODUCTION

Crohn disease (CD) is a complex multifactorial disease that is likely due to an interplay of genetic and environmental factors. It has been estimated that approximately one-third of the genetic contribution to CD arises from the major histocompatibility region [1]. A key cytokine that resides within this region is tumor necrosis factor alpha (TNF-α) because it plays an essential role in the initiation and propagation of CD. This is evidenced by an increased amount of TNF-α in intestinal tissues and peripheral phagocytes and a marked clinical response of TNF antagonists in patients with active CD [2, 3]. Genetic associations have also been observed between promoter polymorphisms of TNF-α and CD [3–5]. However, these associations have been variable because they are not universally replicated [6, 7]. These conflicting results may be attributed to genetic variation of the different populations or systemic differences in the ancestry of cases and controls.

Because Newfoundland represents a relatively homogenous population [8], there may potentially be a greater signal-to-noise ratio to assess the impact of high priority candidate genes such as TNF-α in the Newfoundland population. In this study, we set out to examine the association of five promoter TNF-α polymorphisms in subjects with CD from Newfoundland.

MATERIALS AND METHODS

Patients

This study was approved by the local ethics committee at Memorial University of Newfoundland, and informed

From Newfound Genomics (K.Z., L.P., B.M., D.H.) and Division of Rheumatology (P.R.), Memorial University of Newfoundland, Newfoundland, Canada.

Address reprint requests to: Dr. Proton Rahman, St. Clare's Mercy Hospital, 1 South, 154 LeMarchant Road, St. John's, Newfoundland, Canada A1C 5B8; Tel: (709) 777-5732; Fax: (709) 777-5212; E-mail: prahman@mun.ca.

The first two authors contributed equally to this article.

Received June 21, 2004; revised October 8, 2004; accepted October 14, 2004.

0198-8859/05/$–see front matter
doi:10.1016/j.humimm.2004.10.004

TABLE 1 Primer sequences for TNF-α SNPs tested

SNP ID	Termination mix[a]	Forward primer	Reverse primer	Mass extend primer	Miltiplex with[b]
1031 rs1799964	ACT	5′-ACGTTGGATGGG GAAGCAAAGGAG AAGCTG-3′	5′-ACGTTGGATGTA CATGTGGCCATA TCTCCC-3′	5′-GACCCTGACTTT TCCTTC-3′	857, 238
857 rs1799724	ACT	5′-ACGTTGGATGCT ATGGAAGTCGAG TATGGG-3′	5′-ACGTTGGATGTA TTCCATACCTGG AGGTCC-3′	5′-CCTCTACATGGC CCTGTCTTC-3′	1031, 238
238 rs361525	ACT	5′-ACGTTGGATGAC ACAAATCAGTCA GTGGCC-3′	5′-ACGTTGGATGAT CAAGGATACCCC TCACAC-3′	5′-AGAAGACCCCCC TCGGAATC-3′	1031, 857
308 rs1800629	ACG	5′-ACGTTGGATGGG TCCCCAAAAGAA ATGGAG-3′	5′-ACGTTGGATGGA TTTGTGTGTAGG ACCCTG-3′	5′-GAGGCTGAACCC CGTCC-3′	
863 rs1800630	GCT	5′-ACGTTGGATGCT ATGGAAGTCGAG TATGGG-3′	5′-ACGTTGGATGTA TTCCATACCTGG AGGTCC-3′	5′-CGAGTATGGGGA CCCCC-3′	

[a] Termination mix indicates a combination of dNTP/ddNTPs; the label refers to the dideoxynucleotides present in the mix (*e.g.,* ACT -dGTP/ddATP/ddCTP/ddTTP).

[b] Reactions that are compatible using these specific primer combinations.

consent was obtained from all patients. Stringent diagnostic criteria were used to diagnose CD. Consecutive subjects who were referred from gastroenterologists and satisfied the diagnostic criteria of CD on the basis of the clinical, endoscopic, and histological findings were included in the study. Subjects with CD were assessed by a standardized protocol that included a detailed history ascertained by a specialized research nurse, and physical examination performed by gastroenterologists. Healthy control subjects were selected from the same population and had no recognizable autoimmune disease at the time of assessment. Data collected included basic demographic information, pattern and onset of bowel manifestations,extraintestinal manifestations, surgeries, concomitant diseases, and use of medications.

Laboratory Method

Blood samples were collected from patient volunteers with CD and healthy controls in EDTA anticoagulant. DNA was extracted from peripheral blood lymphocytes with the Wizard Genomic DNA Purification Kit from Promega (Madison, WI). DNA samples were genotyped for five TNF variants by time-of-flight mass spectrometry with the Sequenom platform. All five single nucleotide polymorphisms (SNPs) were in the 5′ flanking region of TNF-α gene at the following positions: -1031 (T→C), -863 (C→A), -857 (C→T), -308 (G→A), and -238 (G→A). Primer sequences, termination mixes, and multiplexing capabilities were determined with Sequenom SpectroDESIGNER software v1.3.4 (Table 1).

For each sample, 2.5 ng of genomic DNA was amplified under standard conditions with forward and reverse primer pairs. After DNA amplification, all unincorporated nucleotides in the polymerase chain reaction product were deactivated with shrimp alkaline phosphatase. A primer extension reaction was then carried out with the mass extend primer and the appropriate termination mix. The primer extension products were then cleaned and spotted onto a SpectroChip. The chip was scanned with a mass spectrometry workstation (Bruker), and the resulting spectra were analyzed with the Sequenom SpectroTYPER-RT software.

RESULTS

One hundred twenty-eight patients with CD (65% women) and 103 controls (69% women) were assessed. All subjects were white and of Newfoundland ancestry.

TABLE 2 Genotypes for five TNF-α variants for Crohn disease and controls

SNP 238	GG	AG	AA
Cases	114	14	0
Controls	93	9	1
SNP 308	GG	AG	AA
Cases	85	41	2
Controls	67	33	3
SNP 857	CC	CT	TT
Cases	115	13	0
Controls	88	14	1
SNP 863	CC	AC	AA
Cases	85	37	6
Controls	70	30	3
SNP 1031	TT	CT	CC
Cases	73	47	8
Controls	63	33	7