Clinical and Experimental Medicine (2019) 19:367–375
https://doi.org/10.1007/s10238-019-00562-x

ORIGINAL ARTICLE

Functional polymorphisms of the receptor for the advanced glycation end product promoter gene in inflammatory bowel disease: a case–control study

Rachele Ciccocioppo[1] · Sara Bozzini[2] · Elena Betti[3] · Venerina Imbesi[4] · Catherine Klersy[5] · Lucia Sukovska Lakyova[6] · Lukas Sukovsky[6] · Jozef Benacka[7] · Peter Kruzliak[8,9] · Gino Roberto Corazza[3] · Antonio Di Sabatino[3] · Colomba Falcone[2]

Received: 27 April 2019 / Accepted: 24 May 2019 / Published online: 7 June 2019

Abstract

The receptor for the advanced glycation end products (RAGE) is a multiligand transmembrane receptor involved in chronic inflammation whose specific polymorphisms of the promoter gene were found to increase its transcriptional activity. We investigated the association of both allelic and genotypic -374T/A and -429T/C polymorphisms with inflammatory bowel disease. The STREGA guidelines were applied for planning and reporting. We enrolled 133 patients with Crohn's disease (CD), 149 with ulcerative colitis (UC), and 128 blood donors. Genomic DNA was extracted from peripheral blood leukocytes collected from each patient and control. RAGE polymorphisms were analyzed by PCR-restriction fragment length polymorphism assay. The Hardy–Weinberg equilibrium was first assessed, and then, the Kruskal–Wallis test and the Fisher exact test were used for etiologic group comparisons. Distribution of patients' characteristics across genotypes was evaluated by the Fisher exact test, while that across alleles was analyzed with a probit model. A 2-sided value of $p < 0.05$ was considered significant. Following the evidence of the Hardy–Weinberg equilibrium, we found a higher prevalence of the allele A of the -374T/A haplotype in UC ($p = 0.043$), and of the allele C of the -429T/C haplotype in CD ($p < 0.001$) with respect to the other groups. Moreover, the homozygous AA genotype of the -374T/A polymorphism resulted associated with late onset of CD, while its TT genotype with early onset ($p = 0.049$). The allele C of the 429T/C haplotype was associated with early onset of UC ($p = 0.03$), while a higher frequency of the heterozygous TC haplotype was found in those with pancolitis ($p = 0.026$). The differing distribution of these polymorphisms in healthy donors and CD/UC patients suggests a role in the development and outcome of these pathological conditions.

Keywords Gene polymorphisms · Inflammatory bowel disease · RAGE

✉ Rachele Ciccocioppo
rachele.ciccocioppo@univr.it

✉ Lucia Sukovska Lakyova
lucia.lakyova@upjs.sk

✉ Peter Kruzliak
kruzliakpeter@gmail.com

[1] Gastroenterology Unit, Department of Medicine, AOUI Policlinico G.B. Rossi and University of Verona, Piazzale L.A. Scuro, 10, 37134 Verona, Italy

[2] Interdipartimental Center for the Molecular Medicine Research and Cardiology Unit, Istituto di Cura Città di Pavia and University of Pavia, Pavia, Italy

[3] Clinica Medica I, Department of Internal Medicine and Therapeutics, I.R.C.C.S. Policlinico San Matteo Foundation and University of Pavia, Pavia, Italy

[4] Gastroenterology and Digestive Endoscopy Unit, Legnano Hospital, ASST Ovest Milanese, Legnano, Italy

[5] Service of Clinical Epidemiology and Biostatistics, Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia, Italy

[6] 1st Department of Surgery, Faculty of Medicine, Pavol Jozef Safarik University and Louis Pasteur University Hospital, Trieda SNP1, 040 11 Kosice, Slovakia

[7] Faculty of Health and Social Work, Trnava University, Trnava, Slovakia

[8] Department of Internal Medicine, Brothers of Mercy Hospital, Polni 3, 63900 Brno, Czech Republic

[9] 2nd Department of Surgery, Faculty of Medicine, Masaryk University and St. Anne's University Hospital, Brno, Czech Republic

Abbreviations

CD	Crohn's disease
CAI	Clinical activity index
CDAI	Crohn's disease activity index
IBD	Inflammatory bowel diseases
RAGE	Receptor for the advanced glycation end products
UC	Ulcerative colitis

Introduction

A complex interplay between a peculiar genetic background and an imbalanced immune response toward components of the gut microbiota accounts for the development of inflammatory bowel diseases (IBD), namely Crohn's disease (CD) and ulcerative colitis (UC) [1]. In recent years, great strides have been made to unravel the players responsible for triggering and sustaining tissue damage, whose targeted block is considered the best therapeutic strategy [2]. In parallel, the genome-wide association studies have led to the identification of more than 200 *loci* [3, 4], of which the most important is the NOD2 region encoding an intracellular pattern recognition receptor with a potent ability to induce autophagy [5], a pathway playing a crucial role in the host–microbiota interaction [6]. Nonetheless, the full cellular and molecular network involved in the inflammatory cascade is still largely unknown.

Growing evidence indicates the receptor for the advanced glycation end products (RAGE) as a key molecule involved in chronic inflammation and cancer [7]. It is a transmembrane receptor normally expressed at low levels on a wide range of differentiated cells, having the ability to bind a broad spectrum of ligands by virtue of the structure of its extracellular domain which recognizes a three-dimensional configuration rather than a specific amino acid sequence [8]. Upon activation, RAGE induces the synthesis of several pro-inflammatory molecules which, in turn, increase its expression on cellular surface, with the end result of magnifying rather than dampening inflammation [9]. The cleavage of its extracellular domain by matrix metalloproteinases causes the release of its soluble form, which plays a protective role by preventing the engagement of RAGE by its ligands [10]. Among them, the high-mobility group B-1 proteins and S100/calgranulins have already been found up-regulated in IBD [11, 12], and the inhibition of RAGE–ligand interaction was proved successful in an experimental model of CD [13].

The human RAGE gene is located on chromosome 6p21.3, in the so-called class III of the major histocompatibility complex [14], and several polymorphisms have been identified, although most of them are located in non-coding regions and, then, not affecting its transcriptional activity [15]. Within those with functional aftermath properties, the single-nucleotide polymorphisms at the -374 (A allele, wild-type T allele) and -429 (C allele, wild-type T allele) of the promoter region have been shown to increase protein synthesis threefold and twofold, respectively [16]. It is conceivable, therefore, that subjects carrying these genetic variations are at a higher risk of developing chronic inflammation, as already found in patients suffering from multiple sclerosis [17] and lupus [18]. So far, little information is available on the possible association of such polymorphisms with IBD. Specifically, the only evidence available in a Caucasian population (from Germany and USA) is related to the -374T/A polymorphism, which was found to be negatively associated with CD and its stricturing phenotype [19]. A further study was carried out in an Asian population (from China) where three RAGE single-nucleotide polymorphisms (-429T/C, -374T/A, G82S) were assessed in a large cohort of UC patients with respect to control subjects [20]. The results showed that the 82(GS + SS) genotype of the G82S haplotype represents a risk factor for UC, while no association was found with the -374T/A and -429T/C polymorphisms [20]. We sought to investigate, therefore, the distribution of both -374T/A and -429T/C genetic and allelic frequencies within a cohort of Italian CD and UC patients in comparison with healthy population, and searched for a possible correlation with relevant clinical features, in order to eventually use these polymorphisms as new biomarkers to predict disease course and severity.

Methods

Study population

All cases admitted at the IBD Unit of the Department of Internal Medicine of I.R.C.C.S. Policlinico San Matteo Foundation (Pavia, Italy) were consecutively screened until at least the prefixed sample size for each cohort was achieved. In all patients enrolled, the diagnosis of either CD or UC had been established according to widely accepted criteria [21, 22] and confirmed at pathology. Specifically, according to the Montreal classification [23], in CD we considered four categories of disease location (L1: terminal ileum; L2: colon; L3: ileo-colon; L4: upper intestine), and three phenotypes (B1: non-stricturing–non-penetrating; B2: stricturing; B3: penetrating), while in UC, three categories of disease extension were used (E1: proctitis; E2: left-sided; E3: pancolitis). Moreover, in both CD and UC patients, the presence of extraintestinal manifestations, i.e., arthropathy, metabolic bone disease, cutaneous and ocular manifestations, hepatobiliary disease, venous thromboembolism, and others according to the European Consensus statements [24], as well as the clinical indexes of disease activity, i.e., the CD activity index (CDAI) [25] and the clinical activity index (CAI) [26] for UC, was also routinely evaluated. In

parallel, blood donors admitted at the Immunotransfusional Unit of the I.R.C.C.S. Policlinico San Matteo Foundation (Pavia, Italy) on each Monday morning (e.g., when a qualified person of the IBD Unit was available to go there for recruitment) were consecutively screened in order to find race-matched control cases until at least the prefixed sample size was achieved. Exclusion criteria included the presence of neurological or cardiovascular diseases, diabetes, acute systemic illnesses, and previous or current history of cancer. Each patient and control gave written informed consent to participate in the study, and approval by the ethics committee was obtained.

Polymorphism genotyping

Peripheral whole blood samples harvested in Vacutainer tubes containing ethylenediaminetetraacetic acid as anticoagulant were collected from each IBD patient and control subject and used to isolate mononuclear cells by means of Ficoll density gradient centrifugation (Ficoll-Paque PLUS, Amersham Biosciences, Bucks, UK). Genomic DNA was then obtained from each cellular suspension by using a DNA extraction kit (GFX, Amersham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instructions. The A-T and the C-T transversion polymorphisms, at position -374 and -429, respectively, in the promoter region of the RAGE gene, were performed at the Interdepartmental Center for Research in Molecular Medicine of the University of Pavia (Italy) by applying the polymerase chain reaction-restriction fragment length polymorphism technique. Briefly, a 671-bp fragment of the RAGE promoter gene that contains both polymorphisms was amplified using the following primers: forward 5′-CCTGGGTTTAGTTGAGAATTTTTT -3′ and reverse 5′-GAAAGGCACTTCCTCGGGTTCT-3′. The thermocycling conditions were as follows: a denaturing step of 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, a cycle of 58 °C for 30 s, followed by 72 °C for 1 min, and a final incubation at 72 °C for 10 min. After amplification, the products were digested using *Tsp509 I* (New England Biolabs, Beverly, MA, USA) for 16 h at 65 °C and then run on an ethidium bromide-stained 3% agarose gel and read using an UV transilluminator. The *Tsp509 I* digestion of the 671-bp fragments were cut into 5 fragments of 284, 217, 110, 44, and 16 bp for the T allele, or 4 fragments of 327, 284, 44, and 16 bp for the A allele. The -429C/T polymorphism was analyzed by applying the same primers and technique and thermocycling program as the -374 polymorphism. The amplified segment of 671 bp was digested with *Alu I* (New England Biolabs) overnight at 37 °C and run on an ethidium bromide-stained 3% agarose gel and read using an UV transilluminator. The fragment of 671 bp was cut into two fragments (590 bp and 81 bp) only in the presence of the C allele. To reduce the possibility of genotyping errors, all results were independently scored by two blinded investigators. In case of ambiguous result, the sample was analyzed a second time.

Statistical analysis

The study protocol and reporting were developed according to the STREGA guidelines for case–control studies [27]. Continuous variables were described by using the median and 25th–75th percentiles, while categorical data as counts and percentage. The presence of Hardy–Weinberg equilibrium within each cohort was first tested. The Kruskal–Wallis test and the Fisher exact test were applied for comparisons between etiologic groups (controls, CD, UC). We also compared patients' characteristics distribution across genotypes with the Fisher exact test and across alleles with a probit model; in this case, we computed Huber–White robust standard errors to account for intra-patient correlation of measures. We used Stata 15.1 (StataCorp, College Station, TX, USA) for computation. A 2-tailed p value < 0.05 was considered statistically significant. For post hoc comparisons between etiologies, we set significance at 0.017, according to the Bonferroni correction.

Results

Study population

A final cohort of 133 CD and 149 UC adult patients, all of Caucasian origin, were consecutively recruited between January 1, 2015, and December 31, 2018 (see the flow diagram in Fig. 1). A race-matched cohort of 161 adult blood donors were recruited as control population. Their demographic and clinical features were assessed at the time of enrollment and are shown in Table 1. A statistically significant difference in age at enrollment among the three groups and between CD versus UC patients, being UC cases older than all the others, was clearly evident. By contrast, no significant difference was found for age at diagnosis between CD and UC patients, thus highlighting that UC patients had a longer disease duration. However, when considering the proportion of cases experiencing disease onset over the age of 40 years, a greater although not statistically different percentage of UC patients (44%) had had disease onset over the age of 40 in comparison with CD (32%). Moreover, a prevalence of male in the UC group was found with respect to both CD ($p = 0.012$) and control ($p < 0.001$) groups. As far as CD location [23] is regarded, as expected [28], a prevalence of the ileo-colonic site (54%) was found, followed by the colonic (31%) and terminal ileum (13%) localization, with the upper gastrointestinal involvement affecting only a few patients (2%). When evaluating the CD behavior [23], almost half cases showed the inflammatory

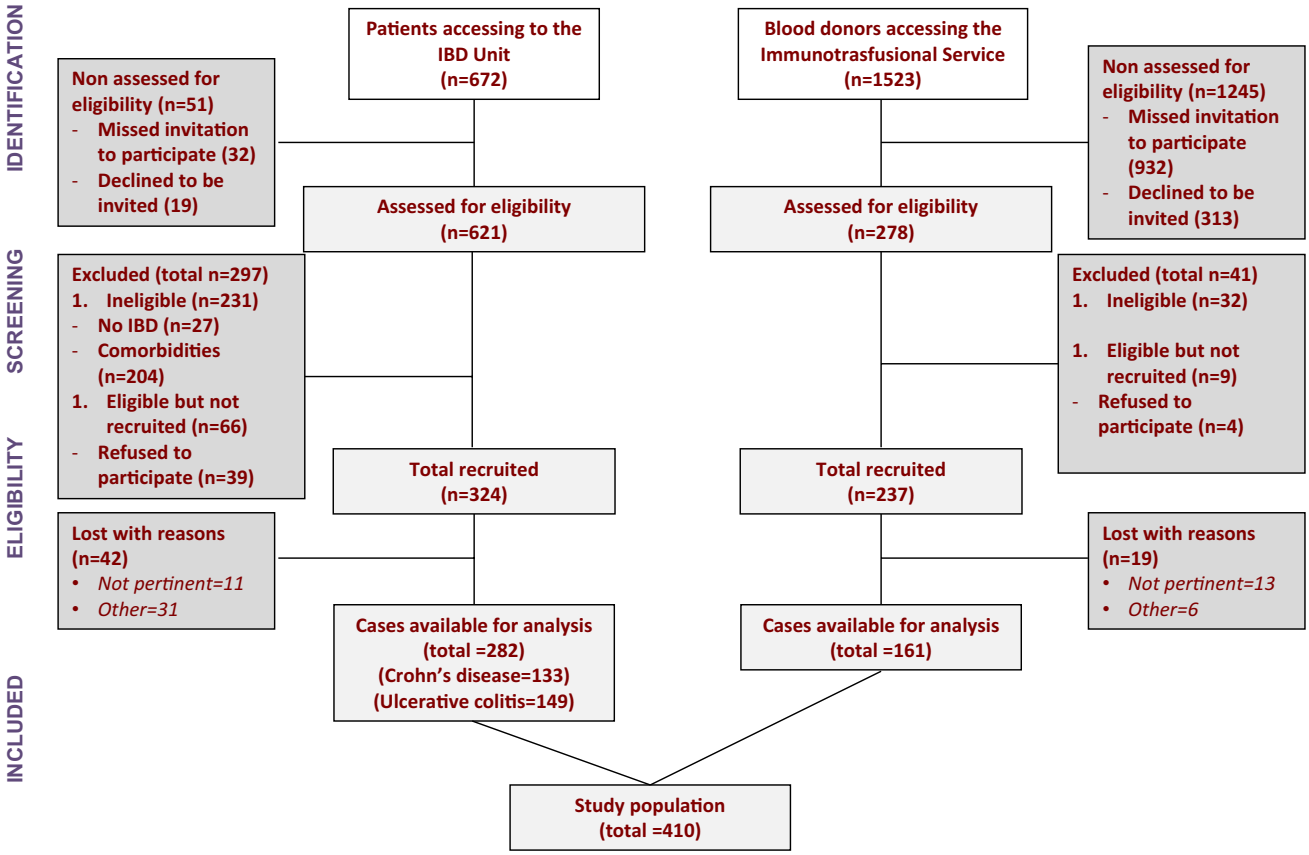

Fig. 1 STREGA flowchart showing study population recruitment

phenotype (B1) and more than one-third displayed the stricturing one (B2), with only a small proportion displaying the penetrating pattern (B3). Moreover, in both IBD groups, the clinical activity scores were coherent with an active phase of the disease (median CDAI value: 213, 110–322, range 76–438—median CAI: 8.1, 4.8–12.1, range 1–16). In the UC cohort, half cases suffered from pancolitis (48%) and almost all the other had left colitis (46%), with a small proportion having proctitis (6%); this is why we chose to pool together these latter two subgroups (Table 1). The presence of only a few patients with proctitis is consistent with the expected distribution of UC extension [29] in patients with a long-lasting duration of the disease approaching 10 years (121 months), since the surface of involved bowel naturally progresses proximally [22]. Finally, a similar frequency of extraintestinal manifestations between the two diseased groups was also evident (Table 1).

Polymorphisms frequency in the study population

The genotypic distribution of both -374T/A and -429T/C polymorphisms of the RAGE promoter gene in IBD patients and controls was in Hardy–Weinberg equilibrium (Table 2).

As shown in Table 3, we found a significant difference of the genotype distribution of the -374T/A haplotype within IBD and control groups ($p=0.043$), with a higher prevalence of the homozygous AA genotype in IBD patients and of the homozygous TT genotype in controls, while the prevalence of the heterozygous AT genotype was comparable between groups. Correspondingly, the T allele frequency was higher in controls, while the A allele was associated with IBD ($p=0.012$). When splitting the IBD group according to the diagnosis, we did not elicit a significant association with the genotype ($p=0.130$), while a weak association was observed with the allele frequency, being the allele A prevalent in UC and the allele T prevalent in CD and control groups ($p=0.043$). After Bonferroni correction, a statistical significant difference was found between UC versus both CD ($p=0.040$) and control ($p=0.024$) groups.

As regards the -429T/C polymorphism (Table 4), we did not observe any association of single genotypes with IBD or control group ($p=0.104$), while a higher frequency of the allele T in IBD was found ($p=0.032$). When considering each etiologic group separately, we observed a highly significant association ($p<0.001$) with both genotype and

Table 1 Demographic and clinical features of study cohort

	Controls ($n=161$)	Crohn's disease ($n=133$)	Ulcerative colitis ($n=149$)	p value (all)	p value (CD vs UC)
Age in years	37 (24–50)	40 (28–51)	48 (36–60)	**0.003**	**<0.001**
Age at diagnosis in years	–	29 (22–45)	35 (25–43)	–	0.082
Disease duration in months	–	72 (25–168)	121 (37–216)	–	**0.028**
Body mass index as weight in kilos/height^2	22.4 (19.6–25.1)	21.7 (18.9–23.4)	22.0 (19.3–24.0)	0.427	0.387
Male	62 (38%)	64 (48%)	94 (63%)	**<0.001**	**0.012**
Age at onset > 40 years		34 (32%)	40 (44%)	–	0.779
Crohn's disease location	–		–	–	–
L1		16 (13%)			
L2		37 (31%)			
L3		66 (54%)			
L4		2 (2%)			
Crohn's disease behavior	–		–	–	–
B1		65 (49%)			
B2		52 (39%)			
B3		16 (12%)			
Ulcerative colitis extension	–	–		–	–
Proctitis and left colitis			70 (52%)		
Pancolitis			64 (48%)		
Extraintestinal manifestation	–	41 (31%)	37 (25%)	–	0.287

Bold values indicate statistically significant results

Data are expressed as median (25th–75th percentiles) for continuous variables and as counts (percentages) for categorical data. Crohn's disease location and behavior are referred according to Montreal classification, as follows: L1: terminal ileum; L2: colon; L3: ileo-colon; L4: upper gastrointestinal location; B1: non-stricturing–non-penetrating; B2: stricturing; B3: penetrating (see Reference no. [23])

CD Crohn's disease, *UC* ulcerative colitis

Table 2 Hardy–Weinberg equilibrium assessment

Polymorphisms	Controls	Crohn's disease	Ulcerative colitis
RAGE-374T/A	0.743	0.107	0.412
RAGE-429T/C	0.742	0.270	0.482

Data are expressed as p values

allele frequency. In both cases, post hoc comparisons elicited significant differences in distribution between CD and UC ($p=0.001$ for both genotype and allele distribution) and between CD and controls ($p=0.001$ for both genotype and allele distribution), but not between controls and UC. Specifically, CD patients had a lower rate of the TT genotype and a higher frequency of the C allele than both controls and UC patients.

Association with disease clinical features

As shown in Table 3, in the CD group the homozygous AA genotype and the allele A of the -374T/A polymorphism appeared significantly associated with an age older than 40 years at onset ($p=0.049$ and 0.012, respectively), whereas the homozygous TT genotype and the allele T were associated with age younger than 40 years at onset. By contrast, no association was found between these polymorphisms and corresponding alleles with age of onset in our cohort of UC patients. As far as the -429 T/C haplotype and its alleles are regarded (Table 4), while similar frequencies in the two subgroups of age at onset were found in CD, in UC the allele C appeared associated with an age at onset below 40 years ($p=0.03$). Moreover, no association of either -374T/A or -429T/C polymorphisms and their relative alleles was found with either CD location or behavior (Tables 3, 4), while in UC, a higher frequency of the heterozygous TC haplotype of the -429T/C polymorphism was found in those with pancolitis ($p=0.026$; Table 4). Considering the chronic and remitting/relapsing character of IBD, we did not search for any association of genetic polymorphisms of the RAGE promoter gene with disease clinical activity indexes, since these latter may change at any time, whereas the genetic susceptibility is stable throughout the life of the patients. Finally, no association with the presence or absence of extraintestinal manifestations was found for both haplotypes and their alleles in both pathological conditions (Tables 3, 4).

Table 3 Genotype distribution and allelic frequency of RAGE-374T/A haplotype according to clinical features in Crohn's disease, ulcerative colitis, and control groups

	AA ($n=92$)	AT ($n=199$)	TT ($n=152$)	p value	Allele A ($n=383$)	Allele T ($n=503$)	p value
Control/IBD				**0.043**			**0.012**
Control	53 (18)	130 (44)	111 (38)		236 (40)	352 (60)	
IBD	39 (26)	69 (46)	41 (28)		147 (49)	151 (51)	
Etiology				0.130			**0.043***
Control	27 (17)	75 (46)	59 (37)		129 (40)	193 (60)	
Crohn's disease	26 (20)	55 (41)	52 (39)		107 (40)	159 (60)	
Ulcerative colitis	39 (26)	69 (46)	41 (28)		147 (49)	151 (51)	
Crohn's disease age at onset				**0.049**			**0.012**
≤40 years	8 (11)	30 (41)	35 (48)		46 (32)	100 (68)	
>40 years	9 (26)	16 (47)	9 (27)		34 (50)	34 (50)	
Crohn's disease location				0.977			0.842
L1	4 (25)	6 (38)	6 (37)		14 (44)	18 (56)	
L2	7 (19)	17 (46)	13 (35)		31 (42)	43 (58)	
L3	11 (17)	30 (45)	25 (38)		52 (39)	80 (61)	
L4	0 (0)	1 (50)	1 (50)		1 (25)	3 (75)	
Crohn's disease behavior				0.463			0.955
B1	11 (17)	29 (45)	25 (38)		51 (39)	79 (61)	
B2	13 (25)	17 (33)	22 (42)		43 (41)	61 (59)	
B3	2 (13)	9 (56)	5 (31)		13 (41)	19 (59)	
Ulcerative colitis age at onset				0.384			0.231
≤40 years	21 (26)	36 (46)	22 (28)		78 (49)	80 (51)	
>40 years	6 (15)	21 (52)	13 (33)		33 (41)	47 (59)	
Ulcerative colitis extension				0.663			0.434
Proctitis and left colitis	15 (22)	33 (47)	22 (31)		63 (45)	77 (55)	
Pancolitis	18 (28)	28 (44)	18 (28)		64 (50)	64 (50)	
Extraintestinal manifestation				0.692			0.826
No	46 (22)	93 (46)	65 (32)		185 (45)	223 (55)	
Yes	19 (24)	31 (40)	28 (36)		69 (44)	87 (56)	

Bold values indicate statistically significant results

Data are expressed as count (percentage). Crohn's disease location and behavior are referred according to Montreal classification, as follows: L1: terminal ileum; L2: colon; L3: ileo-colon; L4: upper gastrointestinal location; B1: non-stricturing–non-penetrating; B2: stricturing; B3: penetrating (see Reference no. [23])

Allele frequency: CD versus control $p=0.97$; CD versus UC $p=$ **0.040**; UC versus control $p=$ **0.024**

IBD inflammatory bowel disease

*Post hoc comparisons (significance at 0.017 after Bonferroni correction)

Discussion

Genes encoding for immunoregulatory molecules constitute important candidate susceptibility *loci* for IBD [3, 4], whose whole pathogenic mechanism is still under investigation [1]. Recently, a potential role of the RAGE pathway in chronic inflammation that sustains IBD has been suggested [30], and a significant increase in serum levels of its soluble form which correlates with disease activity in UC patients has been also found [31]. In physiological conditions, RAGE is constitutively expressed at low levels in all tissues including intestinal epithelium and immune cells [8], but upon engagement with its natural ligands, its expression becomes significantly enhanced with the end result of magnifying rather than dampening the inflammatory cascade [9]. Thus, it appears as a super-molecule involved in sustaining inflammatory cascade in any organ or tissue. Other than by the accumulation of its ligands, an up-regulation of RAGE on the cellular surface may be triggered by the presence of specific polymorphisms in the promoter region of its gene, which may prime tissues to an exaggerated immune response toward those antigens which are usually tolerated. In this regard, our study investigated the possible relationship between the two functional polymorphisms known to up-regulate RAGE expression [15], i.e., -374T/A and -429T/C, with IBD. We found that the frequency of both the A allele and AA homozygous genotype of the -374T/A variant was prevalent in IBD patients, and mostly in those with UC,

Table 4 Genotype distribution and allelic frequency of RAGE-429T/C haplotype according to clinical features in Crohn's disease, ulcerative colitis, and control groups

	CC ($n=18$)	TC ($n=121$)	TT ($n=304$)	p value	Allele C ($n=157$)	Allele T ($n=729$)	p value
Control/IBD				0.104			**0.032**
Control	14 (5)	88 (30)	192 (65)		116 (20)	472 (80)	
IBD	4 (3)	33 (22)	112 (75)		41 (14)	257 (86)	
Etiology				**< 0.001***			**< 0.001***
Control	2 (1)	41 (26)	118 (73)		45 (14)	277 (86)	
Crohn's disease	12 (9)	47 (35)	74 (56)		71 (27)	195 (73)	
Ulcerative colitis	4 (3)	33 (22)	112 (75)		41 (14)	257 (86)	
Crohn's disease age at onset							0.160
< 40 years	9 (12)	27 (37)	37 (51)		45 (31)	101 (69)	
≥ 40 years	3 (9)	8 (23)	23 (68)	0.279	14 (21)	54 (79)	
Crohn's disease location				0.444			0.219
L1	0 (0)	4 (25)	12 (75)		4 (13)	28 (87)	
L2	6 (16)	11 (30)	20 (54)		23 (31)	51 (69)	
L3	5 (8)	25 (38)	36 (54)		35 (27)	97 (73)	
L4	0 (0)	1 (50)	1 (50)		1 (25)	3 (75)	
Crohn's disease behavior				0.978			0.973
B1	6 (9)	22 (34)	37 (57)		34 (26)	96 (74)	
B2	5 (10)	18 (34)	29 (56)		28 (27)	76 (73)	
B3	1 (6)	7 (44)	8 (50)		9 (28)	23 (72)	
Ulcerative colitis age at onset				0.119			**0.030**
≤ 40 years	4 (5)	20 (25)	55 (70)		28 (18)	130 (82)	
> 40 years	0 (0)	6 (15)	34 (85)		6 (8)	74 (92)	
Ulcerative colitis extension				**0.026**			0.744
Proctitis and left colitis	4 (6)	11 (16)	55 (78)		19 (14)	121 (86)	
Pancolitis	0 (0)	19 (30)	45 (70)		19 (15)	109 (85)	
Extraintestinal manifestation				0.686			0.646
No	12 (6)	55 (27)	137 (67)		79 (19)	329 (81)	
Yes	4 (5)	25 (32)	49 (63)		33 (21)	123 (79)	

Bold values indicate statistically significant results

Data are expressed as count (percentage). Crohn's disease location and behavior are referred according to Montreal classification, as follows: L1: terminal ileum; L2: colon; L3: ileo-colon; L4: upper gastrointestinal location; B1: non-stricturing–non-penetrating; B2: stricturing; B3: penetrating (see reference no. 23)

Genotype distribution: CD versus control $p<$ **0.001**; CD versus UC $p=$ **0.001**; UC versus control $p=0.54$

Allele frequency: CD versus control $p<$ **0.001**; CD versus UC $p=$ **0.001**; UC versus control $p=0.94$

IBD inflammatory bowel disease

*Post hoc comparisons (significance at 0.017 after Bonferroni correction)

whereas the control and Crohn's groups preferentially carried the wild-type -374T allele. While the protective role of the AA genotype in the prevention of myocardial infarction has been already established in a population carrying a similar genetic background [32, 33], only scant information is available as regards its association with autoimmune diseases. For instance, in systemic lupus erythematosus, a chronic inflammatory condition [34] which shares several pathogenic mechanisms with CD [21], the T allele was significantly prevalent [18], whereas a higher frequency of the TT genotype was observed in patients suffering from multiple sclerosis [17]. In this regard, our evidence of a low prevalence of the allele T in UC represents a novel finding. As far as the -429T/C polymorphism is concerned, the frequency of both C allele and CC genotype was significantly higher in CD patients as compared to UC patients and controls who displayed similar values, and where the CC homozygous genotype was almost completely absent. This result was in keeping with the evidence of a higher frequency of the C allele in lupus and related nephritis [18], but at variance with multiple sclerosis where no difference with general population was found [17]. Remarkably, the

in vitro evaluation of the RAGE gene promoter activity in cells transfected with the -429C allele was found to be higher as compared to cells transfected with the wild-type -429T allele [35]. However, since both the -374T/A and -439T/C polymorphisms have been shown to exert significant effects on the transcriptional activity of the RAGE gene [16], our results raise the question of how and how much these polymorphisms may affect the risk of developing IBD. It is conceivable that in the case of an abnormal accumulation of natural ligands in the gut mucosa, as already found in IBD [11, 12], the presence of a specific genetic susceptibility leading to an increased transcription of the receptor on both epithelial and immune cells might, in turn, cause an increased production of those pro-inflammatory molecules through the activation of the nuclear factor kappa B [36], such as tumor necrosis factor-α, interleukin-17, transforming growth factor-β, and nitric oxide, ultimately leading to tissue damage [1]. Accordingly, the use of laquinimod (TV-5600, Teva Pharmaceutical Industries Ltd., Israel), an oral quinolone-3-carboxide oral small molecule with the capability of inhibiting the interaction of S100A9 with both toll-like receptor-4 and RAGE, thus preventing the downstream release of inflammatory cytokines [2], has been successfully tested in immune-mediated conditions such as multiple sclerosis, lupus nephritis, and CD [37]. Thus, the possibility of blocking RAGE cognate interaction with its ligands seems an ideal therapeutic tool in those conditions characterized by chronic inflammation, independently from the target organ [38].

Finally, in order to assess whether these functional RAGE polymorphisms were clinically relevant, we investigated their relationship with those features usually evaluated in the management of IBD patients [39]. Specifically, we considered the activity, duration, behavior, localization, age at onset, and occurrence of extraintestinal manifestations. A prevalence of the AA homozygous genotype of the -374T/A polymorphism in patients with late onset of CD and of the TT haplotype with early onset of the disease is worthy of note, while no association with age at onset in the UC group was found. By contrast, the C allele of the -429T/C polymorphism was found robustly associated with those UC patients with young age at onset, while a higher frequency of the heterozygous TC haplotype was found in those with pancolitis; conversely, no association with age at onset of CD cases was found. It is arguable, therefore, that the presence of a double dose of the wild-type T allele could play a protective role against the development of the disease at an early age, a feature which is known to be associated with a more aggressive pattern. Moreover, it appeared to be more frequently associated with those UC patients with left-side colitis, thus reinforcing the idea that the wild type may also prevent the development of an extended disease. Also the lengthy duration of the illness (median of 121 months) in our cohort of UC patients substantiates this concept, since a progressive involvement of colonic mucosa characterizes the natural history of the disease [29]. Conversely, we did not find any association of the -429T/C polymorphisms with CD age at onset, location, and behavior. Our results slightly differ from those of Däbritz et al. who did not find any association between the -429T/C polymorphisms and CD, and showed a negative association of the -374T/A genotype with this illness in the German population, where a lower frequency of the A allele in comparison with the healthy population was also evident [19]. Finally, we did not find any relationship between these polymorphisms and the other clinical features considered, i.e., duration of the disease and extraintestinal manifestations.

Conclusions

Overall considered, our results, other than supporting a role of the two main functional polymorphisms of the promoter region of the RAGE gene in IBD, show a clear difference in both allelic and genotypic distribution between CD and UC, which seems to affect both the development and the severity of these illnesses. If these data will be confirmed, genotyping of the RAGE promoter gene may become a useful biomarker predictor of disease evolution.

Funding This study was funded by a grant from: Direzione Scientifica, Fondazione I.R.C.C.S. Policlinico San Matteo (Pavia, Italy) – Progetto di Ricerca Corrente entitled: "STUDIO DELLA ESPRESSIONE DEL RECETTORE PER I PRODOTTI FINALI DELLA GLICOSILAZIONE AVANZATA (RAGE) NELLE MALATTIE INFIAMMATORIE CRONICHE INTESTINALI" to R.C. The Direzione Scientifica did not take part in the study design, collection, analysis and interpretation of data, writing the manuscript, and decision to submit the paper for publication. The authors are grateful to Mrs. Sheila McVeigh for her thorough revision of the English text.

Compliance with ethical standards

Conflict of interest Dr. Ciccocioppo received a consulting (honorary) fee by Takeda Pharmaceuticals, and she is a member of the Advisory Board Takeda Italy. All the other authors of this manuscript have no conflict of interest to disclose.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. de Souza HSP, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol. 2016;13:13–27.
2. Coskun M, Vermeire S, Nielsen OH. Novel targeted therapies for inflammatory bowel disease. Trends Pharmacol Sci. 2017;38:127–42.
3. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491:119–24.
4. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47:979–86.
5. Fritz T, Niederreiter L, Adolph T, Blumberg RS, Kaser A. Crohn's disease: NOD2, autophagy and ER stress converge. Gut. 2011;60:1580–8.
6. Kabi A, Nickerson KP, Homer CR, McDonald C. Digesting the genetics of inflammatory bowel disease: insights from studies of autophagy risk genes. Inflamm Bowel Dis. 2012;18:782–92.
7. Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. Annu Rev Immunol. 2010;28:367–88.
8. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. Biochim Biophys Acta. 2000;1498:99–111.
9. Schmidt AM, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. J Clin Investig. 2001;108:949–55.
10. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE: the receptor for advanced glycation end products. J Mol Med. 2005;83:876–86.
11. Foell D, Kucharzik T, Kraft M, et al. Neutrophil derived human S100A12 (EN-RAGE) in strongly expressed during chronic active inflammatory bowel disease. Gut. 2003;52:847–53.
12. Vitali R, Stronati L, Negroni A, et al. Fecal HMGB1 is a novel marker of intestinal mucosal inflammation in pediatric inflammatory bowel disease. Am J Gastroenterol. 2011;106:2029–40.
13. Davé SH, Tilstra JS, Matsuoka K, et al. Ethyl pyruvate decreases HMGB1 release and ameliorates murine colitis. J Leukoc Biol. 2009;86:633–43.
14. Sugaya K, Fukagawa T, Matsumoto K, et al. Three genes in the human MHC class III region near the junction with the class II: gene for receptor of advanced glycosylation end products, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene int-3. Genomics. 1994;23:408–19.
15. Hudson BI, Stickland MH, Grant PJ. Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene: prevalence in type 2 diabetes and ethnic groups. Diabetes. 1998;47:1155–7.
16. Hudson B, Stickland MH, Futers S, Grant PJ. Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. Diabetes. 2001;50:1505–11.
17. Tiszlavicz Z, Gyulai Z, Bencsik K, et al. RAGE gene polymorphisms in patients with multiple sclerosis. J Mol Neurosci. 2009;39:360–5.
18. Martens HA, Nienhuis HL, Gross S, et al. Receptor for advanced glycation end products (RAGE) polymorphisms are associated with systemic lupus erythematosus and disease severity in lupus nephritis. Lupus. 2012;21:959–68.
19. Däbritz J, Friedrichs F, Weinhage T, et al. The functional -374T/A polymorphism of the receptor for advanced glycation and products (RAGE) may modulate Crohn's Disease. Am J Physiol Gastrointest Liver Physiol. 2011;300:G823–32.
20. Wang J, Zeng J, Wang H, Ye S, et al. Genetic polymorphisms of RAGE and risk of ulcerative colitis in a Chinese population. Immunol Lett. 2016;170:88–94.
21. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. Lancet. 2017;389:1741–55.
22. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389:1756–70.
23. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus and implications. Gut. 2006;55:749–53.
24. Harbord M, Annese V, Vavricka SR, et al. The first European evidence-based consensus on extra-intestinal manifestations in inflammatory bowel disease. J Crohns Colitis. 2016;10:239–54.
25. Best WR, Beckel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index: national cooperative Crohn's disease study. Gastroenterology. 1976;70:439–44.
26. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomized trial. Br Med J. 1989;298:82–6.
27. Little J, Higgins JPT, Ioannidis JPA, et al. Strengthening the Reporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. Plos Med. 2009;6:e22.
28. Munkholm P. Crohn's disease: occurrence, course and prognosis. An epidemiologic cohort-study. Dan Med Bull. 1997;44:287–302.
29. Langholz E, Munkholm P, Davidsen M, Nielsen OH, Binder V. Changes in extent of ulcerative colitis: a study on the course and prognostic factors. Scand J Gastroenterol. 1996;31:260–6.
30. Andrassy M, Igwe J, Autschbach F, et al. Posttranslationally modified proteins as mediators of sustained intestinal inflammation. Am J Pathol. 2006;169:1223–37.
31. Yilmaz Y, Yonal O, Eren F, Atug O, Hamzaoglu HO. Serum levels of soluble receptor for advanced glycation and products (sRAGE) are higher in ulcerative colitis and correlate with disease activity. J Chrons Colitis. 2011;5:402–6.
32. Falcone C, Geroldi D, Buzzi MP, et al. The -374T/A RAGE polymorphism protects against future cardiac events in non-diabetic patients with coronary artery disease. Arch Med Res. 2008;39:320–5.
33. Picheth G, Costantini CO, Pedrosa FO, da Rocha Leme, Martinez T, Maltempi de Souza E. The -374A allele of the receptor for advanced glycation end products (RAGE) gene promoter is a protective factor against cardiovascular lesions in type 2 diabetes mellitus patients. Clin Chem Lab Med. 2007;45:1268–72.
34. Alunno A, Bartoloni E, Bistoni O, et al. Balance between regulatory T and Th17 cells in systemic lupus erythematosus: the old and the new. Clin Dev Immunol. 2012;2012:823085.
35. Beucher J, Boëlle PY, Busson PF, Modifier Gene Study Investigators, et al. RAGE -429T/C is associated with an increased lung disease severity in cystic fibrosis. Plos One. 2012;7:e41913.
36. Moura FA, Goulart MOF, da Paz Martins AS, Campos SBG. Close interplay of nitro-oxidative stress, advanced glycation end products and inflammation in inflammatory bowel diseases. Curr Med Chem. 2018. https://doi.org/10.2174/0929867325666180904115633.
37. D'Haens G, Sandborn WJ, Colombel JF, et al. A phase II study of laquinimod in Crohn's disease. Gut. 2015;64:1227–35.
38. Body-Malapel M, Djouina M, Waxin C, et al. The RAGE signaling pathway is involved in intestinal inflammation and represents a promising therapeutic target for inflammatory bowel diseases. Mucosal Immunol. 2019;12:468–78.
39. Mowat C, Cole A, Windsor A, et al. Guidelines for the management of inflammatory bowel disease in adults. Gut. 2011;60:571–607.