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MICA*A4 protects against ulcerative colitis, whereas MICA*A5.1 is associated with abscess formation and age of onset

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Summary

Ulcerative colitis (UC) is one of the two major forms of inflammatory bowel disease, the aetiology of which remains unknown. Several studies have demonstrated the genetic basis of disease, identifying more than 130 susceptibility loci. The major histocompatibility complex class I chain-related gene A (MICA) is a useful candidate to be involved in UC pathogenesis, because it could be important in recognizing the integrity of the epithelial cell and its response to stress. The aim of this study was to analyse the relationship between polymorphisms in the transmembrane domain of MICA and susceptibility to develop UC. A total of 340 patients with UC and 636 healthy controls were genotyped for MICA transmembrane polymorphism using a polymerase chain reaction (PCR) combined with fluorescent technology. Different MICA alleles were determined depending on the PCR product size. The allele MICA*A4 was less frequent in patients than in controls ($P = 0{\cdot}003$; OR = 0·643), and this protective role is higher when it forms haplotype with B*27 ($P = 0{\cdot}002$; OR = 0·294). The haplotype HLA-B*52/MICA*A6 was also associated with UC [$P = 0{\cdot}001$; odds ratio (OR) = 2·914]. No other alleles, genotypes or haplotypes were related with UC risk. Moreover, MICA*A5.1 is associated independently with abscesses ($P = 0{\cdot}002$; OR = 3·096) and its frequency is lower in patients diagnosed between ages 17 and 40 years ($P = 0{\cdot}007$; OR = 0·633), meaning an extreme age on onset. No association with location, extra-intestinal manifestations or need for surgery was found.

Keywords: autoimmunity, cell surface molecules, inflammation, MHC, molecular biology

Introduction

Ulcerative colitis (UC) is one of the two major forms of inflammatory bowel disease (IBD). UC shares some clinical features with Crohn's disease (CD) and it is sometimes difficult to distinguish one from the other. Both are complex diseases, and several factors are involved in pathogenesis: environmental factors (diet, microflora, tobacco, antibiotic treatment, etc.) and genetic background [1]. A total of 163 IBD loci have been identified to date: 110 are associated with both diseases, 30 are CD-specific and 23 UC-specific [2]. The genes located in the susceptibility loci are implicated in several pathways, many of them shared with host responses to infection [2]; they are involved in the impairment of the mucosal barrier function, autophagy, deregulation or dysfunction of innate and adaptive immunity as well as in the regulation and cross-talk between commensal microbiota and the immune system [3].

Major histocompatibility complex (MHC) class I chain-related gene A (MICA) is a non-classical MHC class I gene [4–6] located between human leucocyte antigen (HLA)-B and tumour necrosis factor (TNF)-α [7]. To date, more than 100 different MICA alleles have been described [8]. This polymorphism can affect the protein structure, location on the cell [9] or the affinity of the interaction with its receptor [10]. MICA gene is composed of six exons encoding a cytoplasmic tail, a transmembrane region and three extracellular domains (α1, α2 and α3) [7,11]. There is a short tandem repeat (STR) polymorphism described by Mizuki *et al.* [12] in exon 5 (that encodes the transmembrane domain), consisting of four to 10 trinucleotide GCT repetitions [13–15]: the alleles differ in the number of

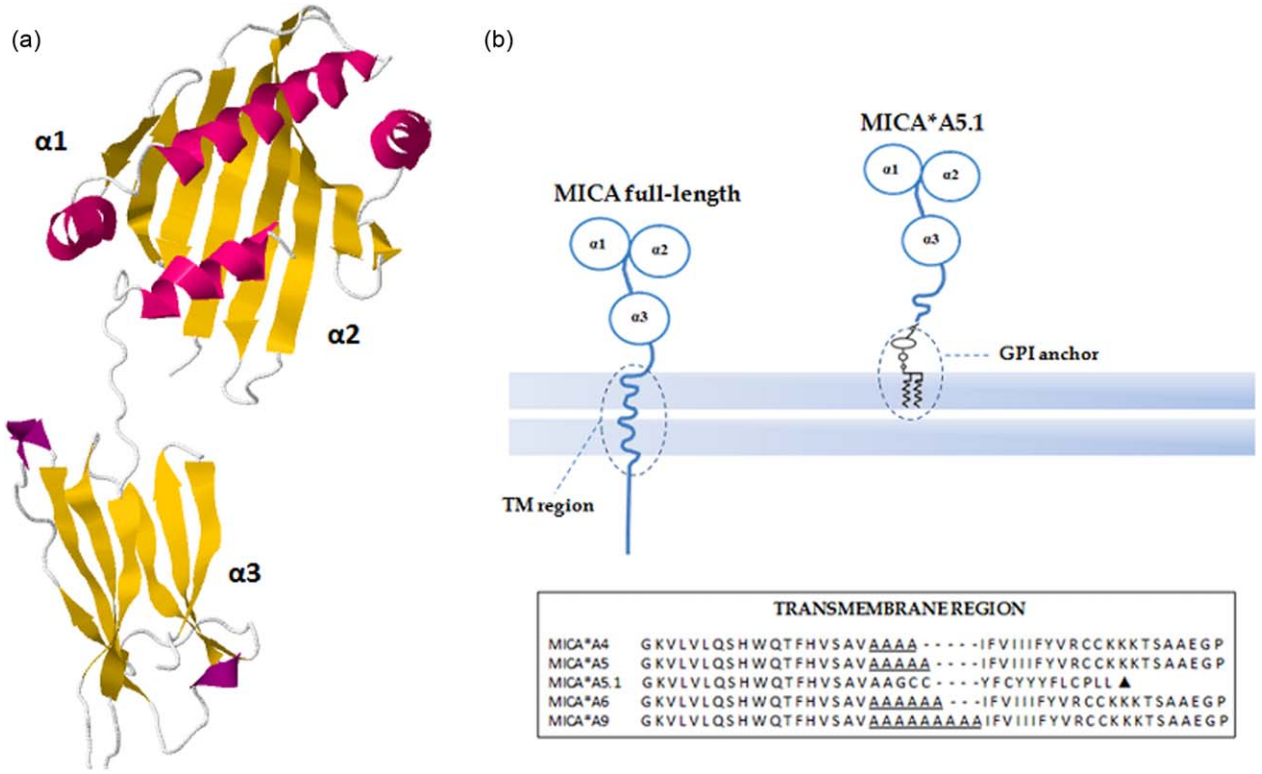

Fig. 1. Structure of the major histocompatibility complex class I chain-related gene A (MICA). (a) View of a ribbon representation of the crystal structure of MICA. Only the extracellular region is shown, which consists of two structural domains: α1α2 platform and α3. The α1α2 platform consists of four α-helices arranged on eight anti-parallel β-sheets. The α3 domain, a C-type immunoglobulin-like domain, is not in contact with the platform. Image from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (www.rcsb.org) (ID 1B3J) [18]. (b) Diagram of the transmembrane region and the cytoplasmic tail of MICA. Full-length MICA molecules (coded by MICA*A4, MICA*5, MICA*A6 and MICA*A9) are transmembrane (TM) proteins, whereas MICA*A5.1 attaches to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor and has no cytoplasmic tail. The sequence of amino acids of the TM region is shown in the lower box. The alleles differ in the number of trinucleotide GCT repetitions that code alanine (underlined); the frameshift in MICA*A5.1, due to a guanine insertion after the second GCT triplet, leads to a premature stop codon (black triangle).

polyalanine repeats inserted at position 296 [16]. Moreover, the allele MICA*A5.1 consists of five GCT repetitions plus a guanine insertion in the second triplet [12], leading to the appearance of a premature stop codon and the expression of a truncated protein. MICA*A5.1 differs from the other MICA proteins in several aspects, as the cell location or the attachment to membrane [9,16,17]. A crystal structure of extracellular domains of MICA from the Protein Data Bank [18] and a diagram of the transmembrane region can be seen in Fig. 1.

Although MICA expression has been demonstrated, at transcript level at least, in most tissues, it is important mainly in gastrointestinal epithelium [19]. MICA expression is very low in normal conditions and an overexpression as response to stress has been demonstrated [20]. MICA is a ligand for the NKG2D receptor [21], located in natural killer (NK), γδ T cells and αβ T $CD8^+$ cells. MICA–NKG2D engagement acts as activator/costimulatory signal in NK cells and T lymphocytes, respectively [22]. MICA can also bind directly to the T cell receptor (TCR) on Vδ1 γδ T cells [23,24].

Certain MICA–STR alleles have been associated with immune-mediated diseases [12,25–30], including IBD: some revealed an association of MICA*A6 [31,32] or MICA*A5.1 with UC [33,34], while these results could not be replicated in other cohorts [35–37]. Moreover, MICA–STR alleles have been associated with UC phenotype, as location of disease [32,38], age at onset [32,39] or extra-intestinal manifestations (EIMs) [7,33,39]. Kamoun *et al.* [39] suggested the association of MICA with CD, but in many studies there was no evidence of this association [7,35–37].

The MICA gene is a useful candidate for involvement in genetic susceptibility to UC, as the cells that interact directly with MICA are involved in the maintenance of intestinal mucosa integrity. Although genome-wide association studies (GWAS) have found no association of MICA with IBD [2,40], the transmembrane (TM) microsatellite is

not a single nucleotide polymorphism (SNP). Therefore, it is interesting to study how MICA could be involved in IBD pathogenesis and, moreover, its relation with the phenotype. The aim of this study is to determine if the polymorphism in the transmembrane domain of MICA gene predisposes to develop UC or determines any clinical feature in our population.

Material and methods

Subjects

A total of 340 UC patients from the Digestive Section, Hospital Virgen de las Nieves, Granada were included into the study. The UC diagnosis was performed using standard clinical, radiological, endoscopic and histological criteria. Moreover, a total of 636 blood samples from regional blood bank donors in Granada were included as healthy controls. Controls and patients were checked in order to eliminate subjects without Spanish ancestry, gypsies and family members. Written informed consent was received from patients and controls. This project was approved by the hospital's ethics committee.

MICA and HLA-B genotyping

DNA was isolated from anti-coagulant-treated peripheral blood using standard methods. The STR polymorphism in exon 5 of MICA was analysed by polymerase chain reaction (PCR), using the method described above by Ota *et al.* [41]. PCR products were electrophoresed in an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and their size was analysed using the GenMapper version 4·0 software (Applied Biosystems). HLA-B was typed by sequence-specific oligonucleotide (SSO) with the Lifecodes® HLA-SSO typing kit (Immucor, Norcross, GA, USA), according to the manufacturer's protocol. SSO products were read by Luminex and results interpreted with Match it! DNA version 1·1 (Lifecodes, Immucor).

Statistical analysis

Statistical analysis was performed to compare allelic, genotypical and haplotypical distributions among patients and controls using the χ^2 test or two-tailed Fisher's exact test when necessary, with 2×2 contingency tables. In order to control the increased error from multiplicity, *P*-values were adjusted for multiple testing using a false discovery rate (FDR)-based method, described by Benjamini and Liu (BL) [42], instead of Bonferroni's correction. The FDR can be defined as the expected proportion of type I errors among the rejected hypotheses. The BL correction does not generate a new *P*-value for each finding, but rather indicates only whether the finding is significant at the prespecified level of statistical significance after the correction; using this method, the FDR is restricted to 0·05. The risk estimation was determined by calculating odds ratio (OR) with a confidence interval (CI) of 95%. The Kruskal–Wallis test was performed for finding association with age at onset. Logistic regression was used to prove the influence of MICA polymorphism as well as other risk factors and to correct the *P*-values. Data were analysed using SPSS version 15 (SPSS, Inc., Chicago, IL, USA) and PopGene version 1·32 (University of Alberta, Edmonton, Alberta, Canada) for testing Hardy–Weinberg Equilibrium (HWE).

Haplotype HLA-B/MICA frequencies were estimated by the maximum likelihood method using an expectation–maximization algorithm for multi-locus genotypical data when the gametic phase is not known under the assumption of HWE [43]; to measure the linkage disequilibrium (LD), the coefficients D, D′ and correlation coefficient (r^2) were calculated after gametic phase estimation using a pseudo-Bayesian approach (ELB algorithm) [44]. A haplotypically based hypothesis test was performed in order to investigate the difference among patients and controls or clinical subgroups. As described above, significance was determined at the $\alpha = 0{\cdot}05$ level. The estimation of haplotype frequencies, LD and haplotypically based hypothesis test were calculated using Arlequin version 3·1, the software package for population genetics [45].

Results

Cohort

Demographic and clinical features of the UC patients are shown in Table 1. The median age at diagnosis was 34 years and there were significantly more men than women ($P = 3.92 \times 10^{-8}$, OR = 1.689, 95% CI = 1·400–2·038). More than 40% of UC patients had a pancolonic location and in most cases evolution was intermittent. Only 12·4%

Table 1. Demographic and clinical features of the patients with ulcerative colitis (UC).

		Ulcerative colitis *n* (%)
Median age at diagnosis (Q1–Q3)		34 (24–47)
Male gender		187 (55·0)
Family history of IBD		47 (13·8)
Perianal disease		12 (3·5)
Location	Proctitis	62 (18·2)
	Distal Colitis	134 (39·4)
	Pancolitis	144 (42·4)
Evolution	Intermittent	268 (78·8)
	Continuous	71 (20·9)
Colon resection surgery		19 (5·6)
Immunomodulators		120 (35·3)
Anti-TNF treatment		38 (11·2)

Q1 = first quartile; Q3 = third quartile; IBD = inflammatory bowel disease; TNF =tumour necrosis factor.

Table 2. Allelic and genotype frequencies of major histocompatibility complex class I chain-related gene A (MICA) in the patients with ulcerative colitis (UC) and healthy controls (HC).

Allele	HC ($2n$ = 1272) n (%)	UC ($2n$ = 680) n (%)	Genotype	HC (n = 636) n (%)	UC (n = 340) n (%)
*MICA*A4*	193 (15·4)	73 (10·7)*	*A4/A4*	12 (1·9)	2 (0·6)
			A4/A5	18 (2·9)	5 (1·5)
			A4/A5·1	58 (9·4)	21 (6·2)
			A4/A6	67 (10·9)	29 (8·5)
			A4/A9	26 (4·2	14 (4·1)
*MICA*A5*	138 (11·0)	65 (9·6)	*A5/A5*	16 (2·6)	4 (1·2)
			A5/A5·1	22 (3·6)	18 (5·3)
			A5/A6	43 (7·0)	25 (7·4)
			A5/A9	23 (3·7)	9 (2·6)
MICA A5.1*	310 (24·7)	192 (28·2)	*A5·1/A5·1*	41 (6·4)	30 (8·8)
			A5·1/A6	107 (17·3)	64 (18·8)
			A5·1/A9	41 (6·6)	29 (8·5)
MICA A6*	423 (33·8)	244 (35·9)	*A6/A6*	77 (12·5)	45 (13·2)
			A6/A9	52 (8·4)	36 (10·6)
MICA A9*	170 (13·6)	106 (15·6)	*A9/A9*	14 (2·3)	9 (2·6)

*P-value remains significant after the correction for multiple testing using the false discovery rate (FDR)-based Benjamini and Lui (BL) method. BL critical value = 0·010, P = 0·003 ($P <$ BL value); odds ratio (OR) = 0·643, 95% confidence interval = 0·482–0·858). HC = healthy controls; UC = ulcerative colitis.

of UC patients suffered some digestive complications (as fistulae, abscesses, perforation, perianal disease, toxic megacolon or microcolon). Thirty-three per cent of UC patients developed EIMs, where joint manifestations were most frequent (19·4%). Only 19 UC patients (5·6%) suffered colon resection and the need for immunomodulators and biological treatment was 35·3 and 11·2%, respectively. There were only seven documented cases of colorectal cancer.

MICA allele frequencies in UC patients and healthy controls

MICA–STR alleles were identified according to their size. Five MICA–STR alleles were found: MICA*A4 [179 base pairs (bp)], MICA*A5 (182 bp), MICA*A5.1 (183 bp), MICA*A6 (185 bp) and MICA*A9 (194 bp). The control population is in HWE for this polymorphism and in both populations (UC patients and healthy controls) there was less than 5% of missing data. Allelic and genotypical frequencies were obtained by direct count assuming homozygosity when only one allele could be identified, as shown in Table 2. MICA*A6 was the most frequent allele in the two populations, followed by MICA*A5.1. MICA*A4 frequency was significantly lower in UC patients than in controls and the OR suggested a protective role. No statistical differences were found at genotypical level, although the frequency of homozygous genotype MICA*A4*/A4 is lower in patients than in controls (0·6 *versus* 1·9%). MICA*A5.1 and MICA*A5.1/A5.1 were more frequent in patients; however, no statistical differences were found.

MICA and phenotype in UC

After classification of the patients into three groups according to age at onset (aged 0–16, 17–40 and > 40 years), differences in MICA frequencies were observed. MICA*A5.1 frequency was lower in patients diagnosed between ages 17 and 40 years (P = 0·007; OR = 0·633, 95% CI = 0·452–0·886) or the frequency was higher before age 16 years and after age 40 years (Table 3). The P-value was not significant when the Kruskal–Wallis test was performed (P = 0·485), and the mean and median ages at onset were very similar whether or not MICA*A5.1 was present. This allele was also associated with abscesses in UC patients (Table 3): more than 85% of patients who suffered abscesses carried MICA*A5.1 (P = 0.002; OR = 3·096, 95% CI = 1·445–6·638). Fistulae were also more frequent in the patients carrying MICA*A5.1, but no significant differences were found. There were few with abscesses, so these results should be interpreted with caution. Both associations, age at onset and abscesses, were not found at genotypical level. Only seven of our patients developed colorectal cancer; five of them carried MICA*A4 and two the MICA*A4/A9 genotype. However, there were no differences after statistical analysis, due perhaps to the small sample size.

We did not find any association of MICA–STR with the location, evolution of disease, EIMs, need for surgery or treatment.

Linkage disequilibrium analysis between MICA and HLA-B and multivariable analysis

It is known that, given the physical proximity, MICA and HLA-B are in LD. Therefore, to confirm if the differences found are due to MICA or HLA-B, both genes must be

Table 3. Some clinical features and its association with major histocompatibility complex class I chain-related gene A (MICA) polymorphism.

	MICA*A5.1 *n* (%)	Other alleles *n* (%)	*P*	BL critical value	*P* < BL value?	OR (CI 95%)
<16 or >40 years	98 (33·6)	194 (66·4)	0·007	0·010	Yes	0·633 (0·452–0·886)
17–40 years	94 (24·2)	294 (75·8)				
Abscesses	15 (53·6)	13 (46·4)	0·002	0·010	Yes	3·096 (1·445–6·638)
No abscesses	177 (27·1)	475 (72·9)				
Fistulae	12 (42·9)	16 (57·1)	0·079	0·010	No	
Not fistulae	180 (27·6)	472 (72·4)				

The false discovery rate (FDR)-based Benjamini and Lui (BL) method was used to the correction for multiple testing. *P*-value was considered significant only when it was smaller than its corresponding BL critical value. OR = odds ratio; CI = confidence interval.

studied together. HLA-B was not associated with UC risk or phenotype in our population (Table 4). MICA/HLA-B haplotypes were constructed and analysed: we found 23 possible haplotypes considering only haplotypes with a frequency of 1% or higher in healthy controls. Moreover, some factors, such as age, gender or disease location, could be associated with the variables studied and a multivariable analysis was necessary.

MICA*A4 frequency was significantly lower in UC patients so, presumably, MICA*A4 played a protective role. MICA*A4 was in LD with HLA-B*18 and HLA-B*27 and, as shown in Table 5, the value of r^2 was much higher in HLA-B*18/MICA*A4. B*18 and B*27 frequencies were lower in patients (Table 4), but these differences were not significant after adjustment by the FDR-based BL method. The frequencies of both haplotypes were lower in patients, although there were differences only in the case of HLA-B*27/MICA*A4 after correction ($P = 0{\cdot}002$; OR = 0·294): these data suggest a real protective role of MICA*A4. Nevertheless, the OR was lower in HLA-B*27/MICA*A4 than in MICA*A4, so the protective role of MICA*A4 was higher when co-segregated with HLA-B*27.

We also analysed haplotypes and performed logistic regression to prove the association of MICA*A5.1 with abscesses and age at onset. After a multivariable analysis with gender, HLA-B and MICA as independent variables, MICA*A5.1 was still associated with diagnosis before age 16 years or after age 40 years ($P = 0{\cdot}044$, OR = 1·580, 95% CI = 1·129–2·211). A second multivariable analysis was performed with gender, age at onset, location of disease, HLA-B and MICA as independent variables: MICA*A5.1 ($P = 0{\cdot}004$, OR = 3·140, 95% CI = 1·454–6·780) and pancolonic location ($P = 0{\cdot}003$, OR = 3·626, 95% CI = 1·565–8·397) were associated with the presence of abscesses. MICA*A5.1 was in LD with HLA-B*07, but this allele was not associated with either age at onset or abscesses. HLA-B*07/MICA*A5.1 and the other MICA*A5.1 haplotypes were not associated with any clinical feature.

Table 4. Comparison of frequencies of human leucocyte antigen (HLA)-B alleles among the patients with ulcerative colitis (UC) and healthy controls (HC).

Allele	HC ($2n$ = 1272) *n* (%)	UC ($2n$ = 680) *n* (%)	*P* HC *versus* UC	BL critical value	*P* < BL value?
B*07	124 (9·7)	80 (11·8)	0·165	0·011	No
B*08	64 (5)	20 (2·9)	0·030	0·005	No
B*13	26 (2)	22 (3·2)	0·105	0·005	No
B*14	68 (5·3)	56 (8·2)	0·013	0·004	No
B*15	66 (5·2)	31 (4·6)	0·542	0·050	No
B*18	131 (10·3)	44 (6·5)	0·005	0·003	No
B*27	43 (3·4)	8 (1·2)	0·004	0·003	No
B*35	131 (10·3)	64 (9·4)	0·534	0·036	No
B*38	32 (2·5)	26 (3·8)	0·105	0·006	No
B*39	18 (1·4)	11 (1·6)	0·725	0·050	No
B*40	38 (3)	17 (2·5)	0·535	0·050	No
B*44	196 (15·4)	91 (13·4)	0·228	0·014	No
B*45	20 (1·6)	10 (1·5)	0·862	0·050	No
B*49	33 (2·6)	26 (3·8)	0·131	0·009	No
B*50	40 (3·1)	15 (2·2)	0·232	0·018	No
B*51	117 (9·2)	71 (10·4)	0·375	0·025	No
B*52	20 (1·6)	22 (3·2)	0·016	0·004	No
B*57	29 (2·3)	24 (3·5)	0·106	0·007	No

Only the alleles with frequency higher than 1% are shown. The false discovery rate (FDR)-based Benjamini and Lui (BL) method was used to the correction for multiple testing. *P*-value was considered significant only when it was smaller than its corresponding BL critical value.

Table 5. Linkage disequilibrium analysis between major histocompatibility complex class I chain-related gene A (MICA) and human leucocyte antigen (HLA)-B in patients with ulcerative colitis (UC) and controls (HC) and haplotypic comparison.

	Healthy controls		Ulcerative colitis				
	HF	$\|D'\|/r^2$	HF	$\|D'\|/r^2$	*P* HC *versus* UC	BL critical value	$P <$ BL value?
B*07/A51	0·081	0·823/0·220	0·113	0·948/0·304	0·045	0·0030	No
B*08/A51	0·042	0·825/0·106	0·029	1/0·0770	0·139	0·0061	No
B*13/A51	0·015	0·640/0·026	0·031	0·937/0·075	0·023	0·0027	No
B*14/A5	0·011	0·108/0·005	0·022	0·188/0·029	0·066	0·0033	No
B*14/A6	0·039	0·637/0·045	0·054	0·471/0·036	0·163	0·0083	No
B*15/A5	0·026	0·472/0·092	0·031	0·642/0·180	0·601	0·0500	No
B*15/A51	0·018	0·182/0·005	0·012	0·086/0·0001	0·210	0·012	No
B*18/A4	0·098	0·945/0·571	0·063	0·975/0·555	0·005	0·0025	No
B*27/A4*	0·033	0·973/0·185	0·010	0·860/0·074	0·002	0·0022	Yes
B*35/A5	0·031	0·242/0·052	0·029	0·237/0·054	0·649	0·0500	No
B*35/A6	0·018	0·777/0·015	0·009	0·739/0·032	0·867	0·0500	No
B*38/A9	0·022	0·888/0·128	0·035	0·909/0·180	0·105	0·0042	No
B*39/A9	0·012	0·864/0·066	0·016	1/0·090	0·467	0·0245	No
B*40/A5	0·016	0·482/0·056	0·013	0·478/0·054	0·610	0·0500	No
B*40/A51	0·013	0·278/0·007	0·010	0·180/0·002	0·512	0·048	No
B*41/A6	0·011	0·899/0·019	0·007	1/0·013	0·399	0·0188	No
B*44/A51	0·045	0·070/0·003	0·053	0·158/0·010	0·511	0·0333	No
B*44/A6	0·098	0·458/0·074	0·079	0·366/0·037	0·129	0·0053	No
B*49/A6	0·024	1/0·049	0·038	1/0·071	0·106	0·0047	No
B*51/A6	0·080	0·852/0·140	0·102	0·956/0·190	0·167	0·0099	No
B*52/A6†	0·011	0·520/0·008	0·032	1/0·060	0·001	0·0021	Yes
B*53/A9	0·012	1/0·078	0·004	1/0·024	0·093	0·0037	No
B*57/A9	0·020	0·955/0·124	0·031	0·852/0·146	0·146	0·0071	No
B*58/A9	0·013	1/0·083	0·007	1/0·041	0·259	0·0235	No

Only the haplotypes with frequency higher than 1% in healthy controls are shown. The false discovery rate (FDR)-based Benjamini and Lui (BL) method was used to the correction for multiple testing. *P*-value was considered significant only when it was smaller than its corresponding BL critical value. HF = haplotypic frequency; D′ = standardized D; r^2 = correlation index. *Odds ratio (OR) = 0·294, 95% confidence interval (CI) = 0·131–0·659; †OR = 2·914, 95% CI = 1·481–5·732.

We could see some differences when we compared haplotype frequencies among both populations (Table 5). As already mentioned in this section, HLA-B*27/MICA*A4 frequency was significantly lower in patients; moreover, HLA-B*52/MICA*A6 was higher in UC patients than in healthy controls ($P = 0{\cdot}001$; OR = 2·194, 95% CI = 1·481–5·732). No differences were found in other MICA*A6 haplotypes, such as as HLA-B*51/MICA*A6 or HLA-B*44/MICA*A6; thus, this association could be due to B*52 or the HLA-B*52/MICA*A6 haplotype. HLA-B*52 frequency was twice that in patients than in controls (3·2 *versus* 1·6%), but there was no difference after correction for multiple comparison using the FDR-based method (Table 4).

Discussion

UC is a complex disease in which the genetic background has been demonstrated to play an important role [2]. The results of association studies of MICA with UC are heterogeneous and sometimes contradictory, due most probably to ethnic or geographic differences, dietary habits, technical mistakes, small sample size, statistical analysis with increased type I error, variable disease definition or the use of different classification criteria. In this paper we describe, for the first time, a protective allele to develop UC and some novel associations of MICA with phenotype.

MICA*A4 seemed to play a protective role against UC because in our patients its frequency was significantly lower. Although an important decrease in MICA*A4 frequency was observed in other UC populations [33], the protective role of this allele has been shown, to our knowledge, for the first time in this study. After haplotype analysis we could confirm these results: no protection of B*18 or B*27 (alleles in LD with MICA*A4) was described, although an increased protective role was observed when B*27 was present. MICA–STR is in LD with an SNP in exon 3 of MICA, consisting of an amino acid change (MICA-129^{Val} or MICA-129^{Val}) [10]. This polymorphism is located in the α2 domain that interacts directly with NKG2D receptor and, depending on the amino acid at position 129, the NKG2D|MICA affinity could be high (MICA-129^{Met}) or weak (MICA-129^{Val}) [10,46]. MICA*A4 is in LD with MICA-129^{Met} (high binder) [47], and the T

helper type 1 (Th1)/Th2 balance could tend towards Th1, with a predominant cellular response, instead of humoral Th2 response [48]. As the Th2 response is predominant in pathological UC mucosa [49], the protective role of MICA*A4 makes sense. López-Hernández and colleagues [37] described the protective role of the MICA-129Met/Val heterozygous genotype, but there were only 29 UC patients in their study and these results must be interpreted with caution. MICA*A4 is also in strong LD, with the amino acid substitution of glycine (Gly) by tryptophan (Trp) at position 14 in the α1 domain, and this change may affect the affinity of MICA with the NKG2D receptor [50]. Kopp *et al.* [47] described a MICA*A4 association with colorectal cancer and with poor prognosis. In our colorectal patients there was an increase of MICA*A4 frequency, but there were only seven patients in the study and no statistical differences were found. As MICA*A4 is a NKG2D high-binder, it should not be associated a priori with tumour escape and progression. However, MICA-129^{Met} has been related to a reduced surface expression and an increased MICA shedding; the limited cell surface expression of this high-binder MICA variant causes a strong NKG2D down-regulation and a modified NK, Tγδ and T CD8^{+} lymphocyte activation [51].

MICA*A5.1 and MICA*A5.1/A5.1 frequencies were higher in our patients than in healthy controls, but the differences were not statistically significant. However, this allele was associated for the first time in our population with the occurrence of abscesses and diagnosis before age 16 years or after age 40 years. After haplotype analysis and logistic regression, we checked the association of MICA*A5.1 and not of HLA-B*07 (allele in LD with MICA*A5.1). The results of MICA*A5.1 association with disease or clinical features are not consistent between populations. The association of MICA*A5.1 with UC has been described [33,46] in the Chinese population, whereas no association was found by other authors [35,36]. The association of MICA*A5.1 with EIMs [7,33,39] and its relation with the location of disease [38] was also described. In our study, no differences were found with regard to location of disease and, although no differences were found after correction for multiple comparisons, the frequency of MICA*A5.1 homozygous genotype is higher in patients with EIMs: half of UC patients with the MICA*A5.1/A5.1 genotype suffered some EIMs, while the frequency of EIMs in patients with other genotypes was 33%.

There are some differences between MICA*A5.1 and the other MICA molecules, regarding protein length, cellular trafficking and location, membrane attachment and release from cells, among others. First, MICA*A5.1 is a truncated protein because a single nucleotide insertion in exon 5 leads to the appearance of a premature stop codon. It does not have a cytoplasmic tail and can be found in human cells in two forms: a surface protein attached to the membrane by glycosylphosphatidylinositol (GPI) anchor or soluble forms in intracellular compartments [17]. Secondly, MICA*A5.1 is located aberrantly on the apical surface of intestinal epithelial cells, instead of the basolateral surface where the interaction with intraepithelial T lymphocytes and NK cells takes place. Thus, MICA*A5.1 carriers may have an aberrant immunological surveillance by NK and T cells [9,52]. Furthermore, N-glycosylation requirement for cell trafficking of MICA*A5.1 is also different: there is an alanine residue at position 24 (α1 domain) in MICA*A5.1, instead of threonine; Thr^{24} N-glycosylation directly regulates MICA surface expression and, therefore, regulation of the expression of MICA*A5.1 allele is not affected by changes in N-glycosylation [53]. MICA*A5.1 is not released from cells by proteolytic shedding due to the lack of the two cysteines required, but rather as a membrane anchored full-length molecule in exosomes [50,54]. Moreover, MICA*A5.1 is released as a multimeric protein, instead of soluble monomeric MICA and, therefore, is more potent in down-regulating NKG2D [17,55]. Conversely, MICA*A5.1 is in LD with MICA-129^{Val}, a NKG2D weak binder [10]. All these features together could affect the immune response in the gut mucosa and the cross-talk with the microbiota in patients carrying MICA*A5.1 and, therefore, MICA*A5.1 could favour bacterial spreading in a previously damaged mucosa and abscess formation. Furthermore, it has been shown that the proliferation and composition of the microbiota could be different depending on age [56] and, thus, MICA*A5.1 could be important in antigen triggering according to age.

In our study, MICA*A6 was the most common allele in controls and patients; these results can also be observed in other studies from southern Spain [30,57]. In our UC patients there were no differences in MICA*A6 frequency among patients and controls. MICA*A6 was in LD with HLA-B*52 and, although its frequency was doubled in patients than in controls, there was no statistical association. However, the HLA-B*52/MICA*A6 haplotype was associated with UC, so B*52 was associated with UC, but only when it was accompanied with MICA*A6 in our population. MICA*A6 was also in LD with others alleles HLA-B, but these haplotypes were not associated with disease. B*52 and B*52/MICA*A6 UC association has already been described [31,32].

In conclusion, MICA transmembrane polymorphism is implicated in UC phenotype and susceptibility. MICA*A4 is a protective allele against disease and HLA-B*52/MICA*A6 is a risk haplotype to develop UC. Conversely, MICA*A5.1 is associated with abscess formation and extreme age at onset. MICA typing at diagnosis could help clinicians in disease management, prevention of complications and the adaptation of therapy. Moreover, although the B*52/MICA*A6 haplotype is not common in UC patients, it is much less frequent in the control population or CD patients: MICA and HLA-B typing could serve to

guide the diagnosis towards UC or CD in ambiguous patients.

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Disclosure

None of the authors have any disclosures to declare.

Author contributions

M. A. L. N., J. M. and A. M. C. conceived and designed the research. M. J. C. and M. G. G. monitored and diagnosed the patients. A. M. carried out sample collection and laboratory work. A. M. C. and A. M. carried out data acquisition and created the database. A. M. C. performed statistical analysis, interpreted results and wrote the manuscript. All authors contributed to the preparation of the manuscript, and approved the final version to be published.

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