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MICA, a gene contributing strong susceptibility to ankylosing spondylitis

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Abstract

Objective—The human major histocompatibility complex class I chain-related gene A (*MICA*) controls the immune process by balancing activities of natural killer cells, δ T cells and αβ CD8 T cells, and immunosuppressive CD4 T cells. *MICA* is located near *HLA-B* on chromosome 6. Recent genomewide association studies indicate that genes most strongly linked to ankylosing

spondylitis (AS) susceptibility come from the region containing *HLA-B* and *MICA*. While HLA-B27 is a well-known risk genetic marker for AS, the potential effect of linkage disequilibrium (LD) shields any associations of genes around *HLA-B* with AS. The aim of this study was to investigate a novel independent genetic association of *MICA* to AS.

Methods—We examined 1543 AS patients and 1539 controls from two ethnic populations by sequencing *MICA* and genotyping *HLA-B* alleles. Initially, 1070 AS patients and 1003 controls of European ancestry were used as a discovery cohort, followed by a confirmation cohort of 473 Han Chinese AS patients and 536 controls. We performed a stratified analysis based on HLA-B27 carrier status. We also conducted logistic regression with a formal interaction term.

Results—Sequencing of *MICA* identified that *MICA*007:01 is a significant risk allele for AS in both Caucasian and Han Chinese populations, and that *MICA*019 is a major risk allele in Chinese AS patients. Conditional analysis of *MICA* alleles on *HLA-B27* that unshielded LD effect confirmed associations of the *MICA* alleles with AS.

Conclusions—Parallel with *HLA-B27*, *MICA* confers strong susceptibility to AS in US white and Han Chinese populations.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterised by inflammation of the axial skeleton, including the spine and sacroiliac joints, as well as extraspinal involvement such as uveitis, enthesitis and peripheral arthritis. The prevalence of AS in Western European, Chinese and North American populations ranges between 0.2% and 0.7%.[1–4] Genetic factors play major roles in the pathogenesis of AS, particularly HLA-B27, which has persisted as one of the best examples of a hereditary marker with disease susceptibility. About 90% of AS patients of European ancestry have at least one *HLA-B27* allele in contrast to only 7.5% of normal individuals, which confers the strongest HLA association among all human chronic diseases reported thus far.[5,6] On the other hand, over 90% *HLA-B27* positive individuals do not develop AS, which suggests that this is not the sole risk factor.

Indeed, recent genomewide association studies (GWAS) of AS have identified several novel AS-associated polymorphisms on the genes including *ERAP1, IL23R, IL1R2, ANTXR2, IL12B, PTGER4, TNFR1* and *TRADD*, although some of them still need confirmation.[7–9] However, most of the strongest genetic variants reported from the GWAS of AS come from the HLA class I region of the major histocompatibility complex, especially those polymorphisms around the *HLA-B* locus.[7,8] Comparing the levels of relative risk, the non-major histocompatibility complex (MHC) genetic associations contribute only a small fraction of susceptibility to AS compared to that from the MHC.[7–9] It is entirely plausible that the HLA class I region contains additional genes influencing susceptibility to AS. *MICA* is located next to *HLA-B* at the centromeric end of the HLA class I region. MICA is normally expressed on the cell membrane, and functions in immune activation in response to stimuli through binding with the natural killer (NK) cell receptor NKG2D that is expressed on the surface of NK cells, γδ T cells and αβ CD8 T cells.[10] Our recent GWAS identified the single nucleotide polymorphisms (SNPs) in MICA tagged to HLA-B27 that is linked to

AS susceptibility.[9] This raises the further possibility that specific sequence changes of *MICA* may contribute to the pathogenesis of AS. However, previous confirmation studies of *MICA* associations with AS were hindered by linkage disequilibrium (LD) with *HLA-B*.

To address the confounding effect of *HLA-B27* due to LD, we examined two ethnic cohorts of AS patients and non-diseased populations, including North American Caucasian and Han Chinese by sequencing *MICA* and typing the *HLA-B* locus. Sequencing *MICA* provides a comprehensive view of *MICA* alleles in association with AS, and cross-examining two ethnic groups with distinct ancestry origins and looking in *HLA-B27* positive and negative individuals would capture the genetic diversity with distinct allele frequencies and LD patterns, which may facilitate the identification of true disease associations from LD effect.

MATERIALS AND METHODS

AS patients and controls

Two ethnic cohorts, including 1070 North American AS patients and 1003 local-matched controls of European ancestry, and 473 AS patients and 536 controls from China of Han Chinese ancestry were examined in these studies. The average ages of AS patients and controls for US Caucasian were 41 and 25 respectively, and for Han Chinese were 43 and 46, respectively. There were 70% men versus 30% women in US patients and 52% men versus 48% women in US controls, and 68% men versus 32% women in Chinese patients and 51% men versus 49% women in Chinese controls. The North American patients were examined as a discovery cohort, with associations confirmed in the Chinese cohort. White patients who were participants in the Prospective Study of Outcomes in AS,[11] as well as from the North American Spondylitis Consortium,[12] were examined in this study. Controls of Caucasian European ancestry were enrolled in the studies in the University of Texas Houston and Stanford School of Medicine. It is worth noting that majority of AS patients came from Texas and California. Any related patient and controls were excluded from the studies. Han Chinese patients and controls were enrolled from the clinics and hospitals in southern cities of China using a translated enrolment form of US standard that was used for North American patients. All patients met the modified New York criteria for this disease.[13] All participants underwent a clinical evaluation by one of the study rheumatologists, and had pelvic and spinal radiographs to confirm their diagnosis. Unrelated controls were examined without any history of rheumatic disease. Chinese controls were obtained from a study project of Chinese population genetics in Fudan University, Shanghai, China. The ethical approvals of the studies were obtained from Institutional Review Boards in both The University of Texas Medical School at Houston and Fudan University.

Sequencing and genotyping

Genomic DNA was extracted from peripheral blood cells from subjects. The *HLA-B* genotyping was performed with Dynal-sequence-specific oligonucleotide (SSO) methods and confirmed by direct PCR sequencing of exon 2. *MICA* was examined by PCR sequencing exon 2, 3, 4 and 5 region using bidirectional Sanger sequencing methods. The primers used for PCR and sequencing were based on previous reports.[14,15] Sequence data were analysed using SeqMan-II (DNA Star, Madison, Wisconsin, USA). Since all allelic

genotypes corresponding to polymorphisms of the *MICA* are known from the *MICA* database at IMGT/HLA (http://www.ebi.au.uk/imgt/hla), the polymorphisms of the *MICA* in each sample were first extracted from the sequencing data, and then were compared with specific polymorphisms in reference sequences of *MICA* alleles. Allelic genotypes of the *MICA* in each sample were obtained according to reference sequence of specific *MICA* alleles.

For quality control in DNA typing, all sequencing processes were performed using robotic automation system to minimise sample mislabelling and misplacing. We also had blind duplicates in each sample plate. In addition, *MICA* polymorphisms are in tight LD on given haplotypes or alleles that were used as an internal check for the genotyping while we performed alignment on different segments of the *MICA* gene.

Data analysis

Tests of association between genetic variants and AS were performed for each allele (marginally) assuming genetic dominance. Exact p values were obtained (Fisher's test) from 2×2 tables of allele counts and disease status. To explore the possibility of interactions, we considered combinations of alleles (diplotypes), which were also tabulated with 2×2 tables; we also conducted logistic regression with a formal interaction term.

Since *MICA**007:01 and *019 showed a strong marginal association with AS, we sought to condition out the effect from B-27, a known risk factor. We performed a stratified analysis based on HLA-B27 carrier status, analysing separately those without any B27 alleles. Within each stratum, we created 2×2 tables based on *MICA**007:01 or *019 and AS frequencies.

Since we utilised a separate (Chinese) cohort for replication, we did not consider multiple testing in the usual way, particularly given the dependent nature of the marker data. Instead, although we performed a considerable number of tests in both the European and Chinese samples, and as such, p values should be considered in this light, our recommended procedure is to identify putatively interesting results and then examine these in the replication set. In addition, among our study participants, only AS patients of US Caucasian origin were examined in the previous GWAS, [7–9] and this study was not found to have population stratification. For our US controls and Chinese cohorts, we did not have GWAS data for use in conducting an investigation of population stratification.

RESULTS

The discovery cohort of North American with AS showed significant associations with polymorphisms in the coding region of *MICA* across exon 2–5 (see online supplementary table S1). Among these, polymorphisms at the codon 6, 14, 24, 36, 64, 91, 122, 129, 173, 175, 181, 206, 210, 213, 215 and 306 represent amino acid (AA) substitutions. Some of these AA changes are distinct in side chain polarity and charges. The p values of these changes ranged from 9×10^{-7} to 10^{-270}. It is particularly interesting that the codon 295 has a tri-nucleotide microsatellite polymorphism $(GCT)_n$ that are designated as A_n, and a five

repetition of GCT may coexist with a guanosine insertion that is designated as A5.1. A significant increase of A4, but a decrease of A5.1 was observed in patients (table 1).

According to IMGT/HLA database (http://www.ebi.ac.uk/imgt/hla/align.html), there are 78 identified *MICA* alleles. Fifteen *MICA* alleles were found in US AS patients (table 2). Among these alleles, MICA*007:01 was found to be only one significantly increased in the AS patients, and emerged as the major AS-associated allele in the North American cohort (86.8% in AS vs 9.9% in controls). *MICA*008, particularly *MICA*008:01 or *008:04, appeared to be the most likely encountered allele in the North American white controls (65%). Comparison of allelic pairs of *MICA* indicated that any pairs containing *MICA*007:01 are increased in the AS patients, and that the genotype pair of *MICA*007:01/ *009:02 and *MICA*018:02/*008 are observed only in the North American AS cohort (see online supplementary table S2). Age and gender adjusted results for the codon 295 and MICA alleles were shown in online supplementary tables S3 and S4, respectively.

The Han Chinese confirmation cohort also showed significant associations with AS at multiple *MICA* polymorphism sites that were consistent with North American cohort (see online supplementary table S5). Importantly, associations with A4 (increased) and A5.1 (decreased) at codon 295 are consistent between two cohorts, while A5 was increased only in Chinese AS patients (table 3).

Eleven *MICA* alleles were found in Chinese cohort (table 4). *MICA*007:01 was consistently and significantly more common in the AS patients (25.4%) compared with that in controls (2.8%), but the major *MICA* allele encountered in the Chinese AS patients was *MICA*019, which occurred in 64.3% of the patients versus 11% in the controls. There were 12 Chinese AS patients with both *MICA*007:01 and *019 alleles, 19 with both *MICA*007:01 and *009:01 or *049, and 11 with both *MICA*007:01 and *027. These combinations were not found in the controls. The presence of either *MICA*007:01 or *019 is 87.1% in the Chinese AS patients versus 13.8% in Chinese controls. In contrast to US controls, the major *MICA* alleles in Chinese controls were *MICA*010:01 (40.3%), *008 (39.6%) and *002:01 (30.6%).

HLA-B27 is a well-defined risk factor for AS, and is in strong LD with *MICA*. However, this LD is not complete; that is, with our sample sizes and multiple populations, we can address and confirm independent risk effects from these two loci. Therefore, several conditional analyses were performed whose results are displayed in table 5. Particularly, in North American AS patients lacking HLA-B27, 19 of 100 AS patients (19%) versus 10 of 399 controls (2.5%) were carriers of *MICA*007:01, (p=4.3×10^{-8}, OR 9.13); in HLA-B27 negative Chinese AS patients, seven of 42 (16.7%) patients versus two of 424 (0.47%) controls were positive for *MICA*007:01 (p value of 9.4×10^{-7}, OR 42.2), and 21 of 42 (50%) AS patients versus 42 of 424 (9.9%) controls were positive for *MICA*019 (p=1.5×10^{-9}, OR 9.1). The presence of either *MICA*007:01 or *MICA*019 in HLA-B27 negative Chinese patients was 64.3% vs 10.38% in controls (p=1.3×10^{-14}, OR 15.55). A conditional analysis for *HLA-B27* association with AS was performed in non-carriers of *MICA*007:01 and *019. In *MICA*007:01 negative North American AS patients, 39 of 120 were B27 positive compares to one of 390 controls. In the Chinese cohort, *HLA-B27* was observed in 82 of 117 *MICA*007:01-negative AS patients versus 26 of 448 controls, and in

48 of 69 *MICA**019-negative AS patients versus 24 of 406 controls, as well as in 10 of 25 patients versus 13 of 393 controls of who lacked both *MICA**007:01 and *MICA**019. Of note, we have typed only a portion of US controls for HLA-B alleles. The controls were randomly selected for the HLA-B typing. We noticed that there were only 100 AS patients who were HLA-B27 negative. We obtained 399 B27-negative controls in comparing with 100 B27-negative AS patients.

To assess statistical independence of effects from these two loci, we performed a logistic regression of AS on HLA-B27 and the *MICA* alleles (007:01, 019, or either). Logistic regression analysis was performed to fit a standard full model with three variables: indicator of *MICA* allele, indicator of B27 allele, and an interaction term. We found putatively significant interactions (p<0.05) for the following three analyses: B27*07:01 in Caucasians (β=−1.4, p=0.01); B27*07:01 in Chinese (β=−1.7, p=0.0001); and B27*019 in Chinese (β= −0.6, p=0.03). We note the coefficients for the interaction terms were consistently negative, that is, the total risk of both alleles is lower than what would be predicted based on adding their marginal effects.

In addition, the co-incidence of the MICA alleles and HLA-B27 subtypes in US Caucasian and Han Chinese cohorts were reported in online supplementary table S6.

DISCUSSION

This is the first report to suggest an association of MICA alleles with AS in both HLA-B27-positive and B27-negative AS patients, with confirmation in a different ethnic group. Genetic associations of *MICA* in several immune-mediated diseases have been reported previously. In particular, *MICA* polymorphisms have been associated with psoriatic arthritis, rheumatoid arthritis, Sjögren's syndrome, Behçet's disease and inflammatory bowel disease.[16–21] However, previous studies examined a limited number of polymorphisms and small cohorts, often with lack of confirmation in other ethnic populations. The studies of single nucleotide polymorphisms in a small cohort of Algerian AS patients indicated that *MICA*-129 was associated with early onset disease,[22] but two other reports suggested that the *MICA* association with AS was secondary to LD with *HLA-B27*.[23,24] Tsuchiya et al examined MICA with PCR-single-strand conformation polymorphism (PCR/SSCP) method in 36 HLA-B27 positive Japanese including 18 patients with AS, one patient with Reiter's syndrome and 17 healthy controls. *MICA**007 was found in six AS patients (35.3%),[23] which appeared similar in frequency in the Chinese AS patients examined here. However, the authors assumed *MICA**007 was in LD with B27. Martinez-Borra *et al*[24] examined *MICA* in HLA-B27 positive mixed populations including 161 AS patients and 161 controls. The mixed ethnic populations contained Spaniards, Slavic Siberians, Jews, West Africans and Asian Indians. They also reported that the HLA-B27 subtypes were associated with *MICA**007. Both studies did not examine HLA-B27 negative individuals, and only studied a relatively small number of patients.

Herein, we examined polymorphisms of *MICA* in two large AS cohorts from different ethnic populations and identified that multiple changes in the coding region of *MICA* are strongly associated with AS. The association levels are similar to those seen with the *HLA-B27*, and

largely surpass all other non-MHC genetic factors reported previously. Many of these polymorphisms encode AA substitutions with changes in side chain polarity and charges. Some of these changes may be associated with specific biological functions of MICA molecules. For instance, a previous report demonstrated that the presence of methionine versus valine at codon 129 confers stronger affinity of MICA to NKG2D.[25]

Comparisons between European whites and Han Chinese cohorts showed that AS patients from two different ethnic populations share many disease-associated polymorphisms, especially, at codons *MICA*-6, -14 and -306. On the other hand, the two ethnic AS cohorts also displayed distinct features of certain *MICA* polymorphisms. *MICA* alleles in Chinese AS patients are more likely to encode AA with more polar and positively charged side chains. In European Caucasians, the changes of specific *MICA* polymorphisms appeared more robust, especially at codon *MICA*-191 (see online supplementary table S1). An increased prevalence of the methionine encoding sequence at codon *MICA*-129 was observed in North American AS patients, which is in accordance with previous reports of this polymorphism in AS,[24] as well as in anterior uveitis[26] and psoriatic arthritis,[27] two diseases that frequently occur in AS patients.

The codon *MICA*-295 is of particular interest. The *MICA**A5.1 results in a premature stop codon, and leads to formation of soluble form of MICA.[19,28] In contrast to the MICA protein that is expressed on the membrane, which stimulates activation of NK and T cells, soluble MICA is likely to induce immunosuppressive NKG2D CD4 T cells.[29] Therefore, balance between membrane MICA and soluble MICA may control the immune process in response to stimuli. Interestingly enough, the presence of *MICA**A5.1 is substantially low, but the *MICA**A4 microsatellite carrying the shortest repetition of GCT is significantly increased in AS patients from both cohorts. Although, it is still unknown whether such shorter repetition of GCT affects stability and/or functional changes of MICA, both *MICA**A9 and *MICA**A6 microsatellites that carry relatively longer repetitions of GCT are significantly less frequent in the AS patients from these two ethnic groups.

Further analysis of *MICA* alleles based on combinations of all polymorphisms indicated that the *MICA**007:01 is a susceptibility allele for AS in European whites. Although two ethnic populations have distinct frequencies of individual alleles, the *MICA**007:01 also confers susceptibility to Chinese AS patients along with predominant *MICA**019. Predominance of the *MICA**019 in Chinese AS patients, but the lack of this in those of European ancestry suggests that evolution of *MICA* alleles differ in these two populations.

By conditioning on *HLA-B27*, it appears that both *MICA**007:01 and *019 contribute to susceptibility to AS, independent of the LD effect of *HLA-B27*. Interestingly, the presence of either *MICA**007:01 or *019 in Chinese AS patients is 87.1% versus 13.8% in Controls, that is similar to the occurrence rate of the *MICA**007:01 in white AS patients from the US (86.8% AS vs 9.9% control), which suggests that both ethnic populations are consistent in the level of the *MICA* contribution to susceptibility to AS. Of note, both the *MICA**007:01 and *MICA**019 carry shorter repetitions of GCT, known as the microsatellite markers *MICA**A4 and *MICA**A5, respectively, at codon *MICA*-295. In contrast, the most common *MICA* allele in normal control is *MICA**008 (table 2) that contains *MICA**5.1.

In addition to possession of specific AS-associated *MICA* alleles, uncommon *MICA* allelic combinations were observed in US whites or Han Chinese, including the *MICA**007:01/ *009:02, *007:01/*019, *007:01/*009:01 or *049 and *007:01/*027. A common feature of these genotypes is the lack of the *MICA**A5.1 microsatellite at codon *MICA*-295.

The MHC class I region genes comprise the most polymorphic region in the human genome. Considering the complexity of extensive LD across this region, further confirmation of a direct and independent contribution of *MICA* alleles to AS susceptibility may require further analysis of other genes in this region and other cohorts of AS.

It is worth noting that the increased possessions of *MICA**007 and *MICA**019 have been previously reported in whites with ulcerative colitis (UC)[21] and Spanish patients with Behçet's disease,[30] respectively. Both diseases share clinical features with AS, which suggests that *MICA**007 and *019 may be share common pathogenic features in these diseases. It is particularly interesting that MICA molecules are preferentially expressed in the epithelial cells of intestine, and chronic intestinal inflammation is commonly encountered in AS patients.[31,32] Therefore, a gut microbe-driven inflammatory process involving the *MICA**007 and *019 may be associated with the development of chronic inflammatory arthritis and spinal inflammation in AS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Comparison of the presence of specific *MICA* variants at codon 295 in US AS patients and controls

Codon 295	US AS	%	US Cont	%	OR	p Value	95% CI
A5.1	485	0.453	706	0.704	0.35	8.94×10^{-31}	0.29 to 0.42
A4	963	0.900	236	0.235	29.25	5.33×10^{-206}	22.84 to 37.46
A5	133	0.124	178	0.177	0.66	7.05×10^{-4}	0.52 to 0.84
A6	267	0.250	319	0.318	0.71	$5.36 \mathrm{E} \times 10^{-4}$	0.59 to 0.86
A9	161	0.150	271	0.270	0.48	1.41×10^{-11}	0.38 to 0.59

Table 2

Comparison of the presence of *MICA* alleles between US AS patients and controls

Allele	US AS	%	US Cont	%	OR	p Value	95% CI
*002:01	133	0.124	199	0.198	0.58	5.59×10^{-6}	0.45 to 0.73
*004	86	0.080	125	0.125	0.61	8.68×10^{-4}	0.46 to 0.82
*007:01	929	0.868	99	0.099	60.66	2.60×10^{-269}	46.26 to 79.71
*008	482	0.451	705	0.703	0.35	3.67×10^{-31}	0.29 to 0.42
*010:01	61	0.057	104	0.104	0.50	3.47×10^{-5}	0.36 to 0.70
*017	26	0.024	80	0.080	0.29	1.01×10^{-8}	0.18 to 0.45
*018:01	49	0.046	80	0.080	0.48	1.38×10^{-4}	0.33 to 0.71

Table 3

Comparison of the presence of specific *MICA* variants at codon 295 in Chinese AS patients and controls

Codon 295	Chinese AS	%	Chinese Cont	%	OR	p Value	95% CI
A5.1	119	0.222	212	0.396	0.51	1.18×10^{-6}	0.39 to 0.67
A4	171	0.319	124	0.231	1.88	5.71×10^{-6}	1.43 to 2.48
A5	372	0.694	309	0.576	2.71	1.2×10^{-12}	2.05 to 3.57
A6	72	0.134	127	0.237	0.58	7.38×10^{-4}	0.42 to 0.80
A9	76	0.142	173	0.323	0.40	2.53×10^{-9}	0.30 to 0.55

Table 4

Comparison of the presence of *MICA* alleles between Chinese AS patients and controls

MICA allele	Chinese AS	%	Chinese Cont	%	OR	p Value	95% CI
*002:01	71	0.150	164	0.306	0.40	7.3×10^{-9}	0.30 to 0.55
*007:01	120	0.254	15	0.028	11.81	7.78×10^{-26}	6.79 to 20.54
*008	118	0.249	212	0.396	0.51	8.02×10^{-7}	0.39 to 0.67
*010:01	100	0.211	216	0.403	0.41	2.71×10^{-10}	0.31 to 0.54
*019	304	0.643	59	0.110	14.54	2.84×10^{-69}	10.46 to 20.22

Table 5

Conditional analysis on *HLA-B27* negative subjects for associations of MICA alleles with AS, and on *MICA*007:01* or *019 negative subjects for associations of *HLA-B27* with AS

	AS patients	Controls	OR	p Value	95% CI
US cohort—HLA-B27 (−)					
MICA*007:01 (+)	19/100	10/399	9.12	4.28×10^{-8}	4.09 to 20.35
US cohort—MICA*007:01 (−)					
HLA-B27 (+)	39/120	1/390	187.3	4.33×10^{-26}	25.37 to 1387.99
Chinese cohort—HLA-B27 (−)					
MICA*007:01 (+)	7/42	2/424	42.2	9.35×10^{-7}	8.45 to 210.87
MICA*019 (+)	21/42	42/424	9.1	1.46×10^{-9}	4.59 to 18.02
MICA*007:01 or *019 (+)	27/42	44/424	15.55	1.3×10^{-14}	7.69 to 31.43
Chinese cohort—*MICA*007:01* (−)					
HLA-B27 (+)	82/117	26/448	38.03	3.8×10^{-47}	21.72 to 66.56
Chinese cohort-*MICA*019* (−)					
HLA-B27 (+)	48/69	24/406	36.38	3.04×10^{-31}	18.84 to 70.25
Chinese cohort—*MICA*007:01 and *019* (−)					
HLA-B27 (+)	10/15	13/393	19.49	5.94×10^{-8}	7.38 to 51.54