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Non Inherited Maternal HLA Antigens in Susceptibility to Familial Rheumatoid Arthritis

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Abstract

Objectives—Some rheumatoid arthritis (RA) patients lack RA-associated HLA alleles. Prior studies investigated non-inherited maternal HLA alleles (NIMA) in RA risk with conflicting results.

Methods—We examined NIMA in a large cohort of families from the North American Rheumatoid Arthritis Consortium.

Results—Among 620 patients with one or both parents HLA-genotyped, RA patients informative for analysis included 176 without HLA-DRB1*04 and 86 without the HLA shared epitope (SE). The frequency of NIMA encoding HLA-DR4 or the SE was compared to the non-inherited paternal allele (NIPA). DR4-encoding NIMA vs. NIPA revealed no significant difference (27% vs. 20%). However, parity is known to modulate RA risk and analyses stratified by sex and age of onset showed significant variation among women. Interestingly, among women with onset <45 years DR4-encoding NIMA was increased compared to NIPA; among women ≥45 years at onset the reverse was observed (31% vs. 16% compared to 10% vs. 60%, p=0.008). DR4 encoding NIMA vs. NIPA did not differ in men. The SE did not differ in men or women.

Conclusions—Risk of RA was associated with HLA-DR4 encoding NIMA in younger-onset women but not in older-onset women or men. These observations could help explain conflicting prior results of NIMA in RA.

Keywords

rheumatoid arthritis; HLA alleles; HLA-DRB1; gender

Introduction

Specific HLA class II molecules increase risk of rheumatoid arthritis (RA). An increased frequency of HLA-DRB1*04 has been reported among RA patients compared to controls in populations worldwide.[1] Further studies found an increase of DRB1*0401, *0404/8, *0405, *0101, *1402 and *1001 alleles in RA patients. These alleles have similarity of a DRβ1 sequence called the shared epitope (SE).[2] HLA-DRB1*0401 and *0404 are the most common RA-associated alleles in Caucasians.

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While most RA patients have one or more copies of DRB1*04 and SE-containing alleles, some are DRB1*04 and/or SE-negative. Other investigators have asked whether exposure to non-inherited maternal HLA antigens ("NIMA") might explain RA in these individuals. Lending support to the "NIMA hypothesis" in RA, an immunomodulatory effect of NIMA has been reported among adult recipients of allografts.[3] Exposure to NIMA in utero could explain this effect. However, maternal cells have recently been found to persist in her progeny into adult life providing additional rationale for investigating NIMA in RA.[4,5]

Six studies have investigated the NIMA hypothesis, all among European RA patients, both sporadic and familial, with controversial findings. Overall results have tended toward a positive association, although no individual study yielded a statistically significant outcome.[6–11] However, two different pooled analyses found significant DR4-positive and/or SE-positive NIMA associations among patients lacking the relevant alleles.[7,11] We investigated NIMA in the North American Rheumatoid Arthritis Consortium (NARAC) familial RA study and considered analyses according to gender and age because parity is thought to modulate RA risk among women.[12,13]

Methods

Patients and families

RA patients were recruited to NARAC across the USA as follows: 1) two or more siblings satisfy American College of Rheumatology RA criteria, 2) hand radiograph erosions for at least one sibling, and 3) age of onset 18–60 years for at least one sibling.[14] Reproductive history was not collected from NARAC probands.

HLA genotyping

DNA-based HLA-typing defined the basic allele groups DRB1*01, *15, *16, *03, *04, *11, *12, *13, *14, *07, *08, *09 and *10 for patients and parents. Next, specific alleles were determined for DRB1*04 and some, though not all, patients with DRB1*01. The following were considered SE-positive in analysis: DRB1*0101, *0401, *0404, *0405, *0408, *1402, *1001 or *10. (DRB1*10 has two alleles but DRB1*1002 has the same SE sequence so high resolution DRB1*10 typing is not required.) When SE status was uncertain data were excluded from analysis whether in an RA patient or in NIMA or NIPA. HLA-DRB1*04 and/or SE-positive non-inheritance could be determined for both parents for 162 and for one parent for 458 RA patients.

Statistical analysis

To determine whether RA risk is affected by NIMA, we compared the frequency with which NIMA was DRB1*04 and/or encoded the SE to NIPA. Logistic regression models of non-inheritance for each parent of a proband were fit with parent gender as the predictor of interest; probands were included in the analysis whether contributing information on one or both parents. The resulting odds ratio estimates, for HLA-DRB1*04 for example, can be interpreted as the odds of a patient having DRB1*04-positive NIMA relative to the odds of having DRB1*04-positive NIPA. Robust standard errors were calculated via generalized estimating equations to account for correlation between siblings. Tests for trend in the NIMA vs. NIPA odds ratios across proband subsets were performed by fitting an interaction term. Rheumatoid factor positivity, and anti-cyclic citrullinated peptide or protein antibody positivity (≥20) were considered as potential confounders.

Results

The analysis dataset included 620 RA patients from 306 families. Patient characteristics are described in Table 1.

Among the 176 RA patients with no copy of HLA-DRB1*04, NIMA was DRB1*04 for 27% compared to 20% for NIPA. The difference was not statistically significant (Table 2). NIMA contained the SE for 32% compared to 31% for NIPA, among the 86 RA patients who had no copy of the SE (6 probands were excluded as SE status of parents was uncertain).

Because parity has been reported to modulate RA-risk, analysis was next stratified by sex and age of onset (Table 2). Interestingly, among women diagnosed within reproductive years (< 45) NIMA was DRB1*04 more often than NIPA (31% vs. 16%, OR 2.39, p=0.09) whereas among women who were 45 years or older NIMA was less likely to be HLA-DRB1*04 than NIPA (OR 0.08, p=0.03).

The two odds ratio estimates were statistically significantly different (p=0.008). Among male patients the prevalence of HLA-DRB1*04-positive NIMA did not vary from NIPA nor vary by age. The pattern across strata was similar for SE positive NIMA vs. NIPA, but no result was statistically significant in this analysis (Table 2).

There were no differences in the prevalence of HLA-specificities of NIMA or NIPA among RA probands overall, by sex or by age of onset. Among RA patients positive for HLA-DRB1*04 and/or the SE, the proportion of patients with DR4-positive NIMA was lower with each additional proband copy of DRB1*04 (27% in DRB1*04 negatives, 19% with 1 copy, and 17% with 2 copies, not significant). A similar pattern was observed for SE-positive NIMA (32% in SE negatives, 28% with 1 copy, and 24% with 2 copies).

Discussion

In Caucasian populations RA is predominantly associated with DRB1*04, consisting primarily of SE-encoding alleles DRB1*0401 and *0404.[1,2] Nevertheless a substantial minority of RA patients lack DRB1*04 and/or the SE. Prior studies have investigated the hypothesis that NIMA contributes to RA-risk among patients lacking RA-associated HLA alleles. A previously invoked explanation was exposure to NIMA during fetal life. Better tolerance to HLA mismatched allografts when the donor carried the recipient's NIMA lent support to this supposition.[3] Offering another explanation and further support, recent studies have described persistence of maternal cells acquired by the fetus into adult life, referred to as maternal microchimerism.[4,5]

The first study of NIMA in RA examined patients who lacked HLA DRB1*04 and found that NIMA encoded DRB1*04 more often than NIPA, although results were not significant.[6] A second study was similar and the combined analysis of these two Netherlands studies was statistically significant.[7] However, three other studies in European populations found no significant increase of NIMA encoding HLA-DR4 or the SE vs. NIPA.[8–10] Results were similar to those from the Netherlands in a sixth study from the UK with increased DRB1*04 and SE NIMA vs. NIPA.[11] RA patients were sporadic cases in some studies and familial in others, however differing results could not be explained on this basis. In the current study we investigated NIMA for DRB1*04 and the SE in a North American RA population and, because fetal-maternal cell trafficking results in both maternal and fetal microchimerism, but the latter is unique to women, we analyzed data stratified by sex and age of RA onset.

Among DRB1*04-negative RA patients, we found a non-significant increase of DRB1*04-positive NIMA compared to NIPA. However, interestingly, when RA patients were stratified

according to sex and age of onset we found contrasting results for women aged less than 45 years at onset compared to 45 or older at onset. Among DRB1*04-negative probands, women with RA onset during their reproductive years showed an increased whereas women older at onset showed a decreased prevalence of DRB1*04-positive NIMA compared to NIPA and the difference between the two groups was statistically significant. Further, there was no apparent difference in DRB1*04-positive NIMA vs. NIPA among male RA patients, and no variation according to age, although our sample contained few male subjects. No significant differences were observed for RA patients lacking the SE. The DRB1*04 family of molecules has a distinguishing amino acid sequence that is proximal to the SE on the DRβ1 chain, but why a difference was observed for DRB1*04-positive NIMA but not the SE is not known.

While some HLA-DR molecules are associated with RA risk others are thought to be RA-protective. Interestingly, a recent study found that the RA-protective DRβ1 amino acid sequence “DERAA” was significantly underrepresented as NIMA among RA patients in two independent populations [15]. This novel observation indicates NIMA may contribute risk or protection from RA depending upon its specificity. We were not able to conduct a similar analysis because all DRB1 alleles were not routinely high resolution typed in the NARAC cohort. However, this observation by analogy suggests a potential explanation for differences observed in the current study between men and women, and in women by whether RA occurred in younger (reproductive) vs. older years. That is, pregnancies prior to RA onset *and* the HLA-specificity of acquired fetal microchimerism could potentially impact RA risk in women. If parous women acquire fetal microchimerism with RA-protective HLA alleles more often than RA-risk associated HLA alleles this could also potentially explain the putative reduction in RA-risk in parous vs. nulliparous women.[12,13].

In conclusion, RA risk was associated with HLA-DRB1*04 NIMA in women but not in men, and differed significantly among women by whether RA onset occurred during or after reproductive years. Interpretation of the current results must be cautious due to small sample sizes. Our observations indicate further investigation of NIMA is needed among women with RA for whom pregnancy history is known as well the HLA-genotypes of children born prior to RA onset.

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Table 1

Patient characteristics

Number	620
Sex: N (%)	
Female	488 (79)
Male	132 (21)
Race/Ethnicity: N (%)	
Caucasian	576 (93)
Hispanic	35 (6)
Other or unknown	9 (1)
Age at onset: median (range)	35 (2–75)
Age at onset unknown: N	5
Erosive disease: N (%)	
Yes	575 (93)
No	37 (6)
Unknown	8 (1)
RF positive: N (%)	
Yes	504 (81)
No	116 (19)
ACPA positive (≥20): N (%)	
Yes	471 (76)
No	124 (20)
Unknown	25 (4)
HLA-DRB1*04: N (%)	
No copy	176 (28)
One copy	300 (48)
Two copies	144 (23)
Shared epitope: N (%)	
No copy	92 (15)
One copy	261 (42)
Two copies	207 (33)
Unknown	60 (10)

Abbreviations: RF = rheumatoid factor, ACPA = anti-cyclic citrullinated peptide or protein antibody.

Table 2

Prevalence of NIMA and NIPA in probands lacking the relevant alleles

Probands	DR4+ NIMA	DR4+ NIPA	OR (95% CI)
All DR4 negative[1]	27% (44/163)	20% (14/70)	1.48 (0.65–3.36)
Women, < 45 years at onset	31% (32/104)	16% (8/51)	2.39 (0.87–6.56)
Women,≥ 45 years at onset	10% (3/29)	60% (3/5)	0.08 (0.01–0.81)
Men, all ages	30% (8/27)	27% (3/11)	1.12 (0.22–5.72)
	SE+ NIMA	SE+ NIPA	
All SE negative[2]	32% (26/82)	31% (11/36)	1.06 (0.36–3.06)
Women, < 45 years at onset	30% (17/57)	24% (6/25)	1.35 (0.35–5.18)
Women,≥ 45 years at onset	31% (4/13)	50% (1/2)	0.44 (0.02–9.32)
Men, all ages	40% (4/10)	57% (4/7)	0.50 (0.06–4.42)

[1] DR4 non-inheritance was known for both parents in 57 probands, for the mother only in 106 probands and for the father only in 13 probands.

[2] SE non-inheritance was known for both parents in 32 probands, for the mother only in 50 probands and for the father only in 4 probands.