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Polymorphisms in the IL-1 receptor antagonist gene VNTR are possible risk factors for juvenile idiopathic inflammatory myopathies

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SUMMARY

Although HLA-DRB1 and -DQA1 alleles have been associated with adult and juvenile idiopathic inflammatory myopathies (JIIM), they only partially account for the genetic risk for these autoimmune disorders. Because IL-1α and IL-1β, and the anti-inflammatory competitive inhibitor, IL-1 receptor antagonist (IL-1Ra), have been implicated in the pathogenesis of myositis, we assessed the role of variable number tandem repeat (VNTR) polymorphisms of the IL-1Ra gene (IL-1RN) in the aetiology of JIIM: IL-1RN VNTR polymorphisms were performed on 250 JIIM patients and 471 race-matched controls and were correlated with clinical characteristics. The IL-1RN A1 allele, associated with increased proinflammatory activity, was found to be a risk factor for Caucasians with JIIM (96·0% carriage rate *versus* 90·2% in race-matched controls, $P_{corr} = 0{\cdot}037$, odds ratio (OR) = 2·5, confidence interval (CI) = 1·1–5·8), but not for African-Americans, in whom the A3 allele was a possible risk factor (7·0% *versus* 1·1% in race-matched controls, $P_{corr} = 0{\cdot}07$, OR = 6·5, CI = 1·1–40·3). IL-1RN genotypes did not correlate with circulating levels of IL-1Ra, which were higher in patients than in controls. The polymorphic IL-1RN locus could be the first non-MHC genetic risk factor identified for JIIM, and different alleles may confer susceptibility for different ethnic groups.

Keywords IL-1 receptors (genetics) polymorphism genetic risk factor myositis juvenile dermatomyositis

INTRODUCTION

Recent studies have suggested important roles for IL-1α and IL-1β, and the anti-inflammatory competitive inhibitor, IL-1 receptor antagonist (IL-1Ra), in the pathogenesis of infectious and autoimmune diseases [1,2]. In the gene encoding IL-1Ra, known as IL-1RN, five allelic polymorphisms (A1–A5) of a 86 base pair variable number tandem repeat (VNTR) in intron 2 have been described, representing two to six copies of the repeat sequence [3]. Allele A2, which contains two copies of the VNTR, has been reported to be a risk and severity factor for a number of autoimmune diseases, including systemic lupus erythematosus (SLE), ulcerative colitis, psoriasis, alopecia areata, and Graves' disease [4–8]. Some studies however, have failed to identify the A2 allele as a risk or severity factor for autoimmune diseases, while Sciacca *et al*. have found the A1/A1 genotype of IL-1Ra to be a possible risk and severity factor for multiple sclerosis [9–12].

The juvenile idiopathic inflammatory myopathies (JIIM) are a heterogeneous group of rare systemic autoimmune disorders characterized by chronic muscle inflammation. The long-term prognosis of JIIM is not well defined, but based on small retrospective series, approximately one-third of patients recover from illness within 2 years, one-third have a relapsing course, and one-third suffer from chronic uncontrollable inflammation. Mortality is high in patients who develop cutaneous or gastrointestinal ulcerations, 20–30% [13]. In addition, approximately one-third of JIIM patients develop dystrophic calcification [14]. African-American and other minority ethnic groups are believed

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to carry a poorer prognosis compared with Caucasians, which might be a consequence of delay in diagnosis and initiation of therapy [14].

Human leucocyte antigen (HLA) DRB1*0301, the linked allele, DQA1*0501, and HLA-DMA *0103 have been defined as genetic risk factors for JIIM in Caucasians [15,16]. These and other data however, suggest additional genetic risk factors, including non-MHC loci, should exist and may differ among different racial groups [17,18].

Markedly elevated serum levels of IL-1Ra and IL-1α have been detected in adult dermatomyositis (DM) and polymyositis (PM) with active disease, and have been reported to be elevated in three juvenile dermatomyositis (JDM) patients [19,20]. Immunohistochemical studies of muscle biopsies from adult DM and PM patients have demonstrated abundant IL-1α and IL-1β located in muscle capillary endothelium, mononuclear inflammatory cell infiltrates, and ischaemic fibres [21].

Because of the emerging role of IL-1 and IL-1Ra in the pathogenesis of adult IIM, we examined VNTR polymorphisms of IL-1RN as risk factors for susceptibility to and outcomes within JIIM in a large cohort of patients and race-matched controls. Our confirmation in this study that circulating levels of IL-1Ra are elevated in JIIM patients compared with controls also prompted us to examine whether there is a genetic contribution to differences in IL-1Ra levels.

PATIENTS AND METHODS

Subjects

Two hundred and fifty-nine JIIM patients meeting criteria for probable or definite myositis [22], with illness onset prior to 18 years of age, were enrolled in a protocol which required completion of a detailed clinical questionnaire and donation of a blood sample. Of these 259 JIIM patients, 250 had DNA samples available for IL-1RN VNTR polymorphism investigation and nine had plasma samples without a DNA source for measurement of IL-1Ra levels. The 250 JIIM patients in whom IL-1RN VNTR polymorphisms were examined included 201 Caucasians, 43 African Americans, and six patients from other racial groups; 177 were female and 73 were male. Two-hundred and one patients had JDM, 25 had juvenile polymyositis (JPM) and 24 had myositis overlapping with another connective tissue disease (CTM).

Disease course was classified into three categories for patients who had an established diagnosis for at least 2 years: a monocyclic illness course was defined on the basis of full recovery, with or without drug therapy within 2 years of diagnosis without clinical relapse; a polycyclic course had one or more relapses occurring between periods of inactive disease; and a chronic continuous course was defined as persistent disease activity for >2 years [13]. The presence of calcinosis as well as cutaneous or gastrointestinal ulceration were clinically determined; at least 2 years of follow-up data were required to define the presence of dystrophic calcification.

Two-hundred and ninety-five Caucasian and 176 African-American control DNA samples were obtained from unrelated healthy blood bank donors at the National Institutes of Health (Bethesda, MD) and Thomas Jefferson University (Philadelphia, PA) [23]. Additional control DNA samples were obtained from 38 Caucasian and five African-American unrelated healthy children who provided informed consent. Control subjects included in ELISA measurements had neither recent illnesses nor history of immunizations for at least 2 months prior to venepuncture.

Analysis of IL-1RN VNTR polymorphisms

Genomic DNA from patients was isolated from peripheral blood mononuclear cells (PBMC) by the QiAmp blood kit (Qiagen, Chatsworth, CA). Polymorphism analysis of IL-1RN VNTR in intron 2 was by the method of Tarlow [3]. Standard primer pairs were used ($5' =$ ctc agc aac act cct at; $3' =$ tcc tgg tct gca ggt aa). Ethidium bromide-stained polymerase chain reaction (PCR) products were visualized on a 2% Trevigel (Trevigen, Gaithersburg, MD) or a 1% agarose gel.

Measurement of circulating IL-1Ra

Plasma IL-1Ra levels were measured in duplicate for 71 childhood control subjects (median age 9·0 years) and 25 JIIM patients (median age 7·2 years) using a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

Statistical analysis

Data were analysed using PC SAS software (SAS Institute, Cary, NC). Allele frequencies (number of occurrences of the investigated allele in the population divided by the total number of alleles) and carriage rate (proportion of individuals who have at least one copy of the investigated allele) were calculated [4]. Differences in proportions between JIIM patient groups and controls were compared using the χ^2 statistic or Fisher's exact test. Wilcoxon and Kruskall–Wallis rank sum tests were used for examining differences in IL-1Ra concentrations. Because multiple allelic associations were investigated, Westfall's resampling method (PROC MULTTEST) was used to correct P values for the number of IL-1RN alleles present in the carriage rate and allele frequency analyses [24]. This resampling method makes critical use of the estimated correlation of joint outcomes, while the Bonferroni method of correcting for multiple comparisons is insensitive to the possible dependence of multiple outcomes. The Bonferroni method was used to correct P values for multiple comparisons (i.e. the number of IL-1RN alleles present in patients or controls) for the genotype analyses, because the PROC MULTTEST can currently only be used for 2×2 tables. In this study, a corrected P value (P_{corr}) of $<0{\cdot}05$ was considered a definite association, and an uncorrected P value of $<0{\cdot}05$ in association with a $P_{corr} > 0{\cdot}05$ and an odds ratio (OR) in which the 95% confidence interval (CI) was $>1{\cdot}0$ was considered a possible association worthy of further investigation [25].

RESULTS

IL-1Ra circulating levels

Circulating levels of IL-1Ra were significantly elevated in JIIM patients compared with control subjects (median 818 pg/ml in JIIM *versus* 88 pg/ml in controls; $P \leq 0{\cdot}001$). IL-1Ra concentrations were also higher in each clinical group compared with controls (JDM, median 592 pg/ml, $P \leq 0{\cdot}0001$; JPM, median 818 pg/ml, $P \leq 0{\cdot}001$; and CTM, median 225 pg/ml, $P = 0{\cdot}026$). IL-1Ra concentrations did not significantly differ between Caucasian and African-American control subjects (median 100 *versus* 79 pg/ml, respectively, $P = 0{\cdot}47$). IL-1Ra levels were not associated with the presence of calcinosis or ulcerations.

Table 1. Carriage rates of IL-1 receptor antagonist–VNTR polymorphism alleles in Caucasian and African-American juvenile idiopathic inflammatory myopathy (JIIM) patients and in race-matched normal controls*

IL-1RN VNTR allele	Caucasian controls *n* (%)	Caucasian JIIM *n* (%)	*P*	P_{corr}	Odds ratio (95% CI)	African-American controls *n* (%)	African-American JIIM *n* (%)	*P*	P_{corr}	Odds ratio (95% CI)
A1	266 (90·2%)†	193 (96·0%)	0·015	0·037	2·5 (1·1–5·8)	170 (96·6%)†	42 (97·7%)	NS		
A2	137 (46·4%)‡	88 (43·8%)	NS			29 (16·5%)‡	9 (20·9%)	NS		
A3	8 (2·7%)	5 (2·5%)	NS			2 (1·1%)	3 (7·0%)	0·038	0·07	6·5 (1·1–40·3)
A4	2 (0·7%)	1 (0·5%)	NS			5 (2·8%)	0 (0%)	NS		

*Carriage rate is the proportion of individuals who have at least one copy of the investigated allele. A5 was not present in any controls or patients of either race. Total numbers of subjects studied: Caucasian controls = 295; Caucasian JIIM = 201; African-American controls = 176; African-American JIIM = 43.

†A1, Caucasian *versus* African-American controls: $P = 0{\cdot}01$, $P_{corr} = 0{\cdot}018$.

‡A2, Caucasian *versus* African-American controls: $P = 0{\cdot}001$, $P_{corr} = 0{\cdot}001$.

IL-1RN VNTR polymorphism analysis

Carriage rate (Table 1) and genotype frequency (Table 2) analyses of IL-1RN alleles demonstrated a decrease in A1 and increase of A2 in Caucasian compared with African-American controls. The frequency of the A1 allele was 74·1% in Caucasian and 88·1% in African-American controls ($P_{corr} < 0{\cdot}004$) and the frequency of the A2 allele was 27·4% in Caucasian compared with 9·9% in African-American controls ($P_{corr} < 0{\cdot}004$). Examination of the distribution of IL-1RN genotypes confirmed a decrease in the frequency of A1A1, as well as an increase in the frequency of A1A2 and possibly of A2A2 in Caucasian compared with African-American control subjects (Table 2).

Based on carriage rate analysis (Table 1), the A1 allele was a risk factor for JIIM in Caucasians ($P_{corr} = 0{\cdot}037$, OR = 2·5). In comparing the distribution of IL-1RN genotypes between JIIM patients and race-matched controls (Table 1), no specific genotype was identified as a risk factor for JIIM in Caucasians. The A3 allele was a possible risk factor for JIIM in African-American patients ($P = 0{\cdot}038$, $P_{corr} = 0{\cdot}07$, OR = 6·5). This appeared to be related to the genotype A1A3, which was associated with the risk in African-Americans (Table 2). Notably, A2 was not increased in frequency in Caucasian or African-American JIIM patients compared with race-matched controls. Despite the differences in carriage rates of the IL-1RN polymorphisms in Caucasian or African-American JIIM patients compared with controls, no differences in allele frequencies were detected among these groups.

Relationship of IL-1Ra polymorphisms to demographic and prognostic factors

Because the myositis syndromes consist of different clinicopathologic and serologic groups, which may differ in their genetic risk

Table 2. Genotype frequencies of IL-1 receptor antagonist VNTR polymorphisms in Caucasian and African-American juvenile idiopathic inflammatory myopathy (JIIM) patients and in race-matched normal controls*

Group	A1,A1	A1,A2	A1,A3	A1,A4	A2,A2	A2,A3	A2,A4	A3,A3
Caucasian controls	150	110	6	0	25	0	2	2
n (%)	(50·8%)†	(37·3%)‡	(2·0%)	(0%)	(8·5%)¶	(0%)	(0·7%)	(0·7%)
Caucasian JIIM	107	80	6	1	8	1	0	0
n (%)	(53·2%)	(39·8%)	(2·8%)	(0·5%)	(3·8%)	(0·5%)	(0%)	(0%)
African-American controls	140	23	2	5	6	0	0	0
n (%)	(79·6%)†	(13·1%)‡	(1·1%)§	(2·8%)	(3·4%)¶	(0%)	(0%)	(0%)
African-American JIIM	31	8	3	0	1	0	0	0
n (%)	(72·1%)	(18·6%)	(7·0%)§	(0%)	(2·3%)	(0%)	(0%)	(0%)

*A5 was not present in any controls or patients of either race. Total numbers of subjects studied: Caucasian controls = 295; Caucasian JIIM = 201; African-American controls = 176; African-American JIIM = 43.

†A1A1, Caucasian *versus* African-American controls: $P = 0{\cdot}001$, $P_{corr} = 0{\cdot}005$.

‡A1A2, Caucasian *versus* African-American controls: $P = 0{\cdot}001$, $P_{corr} = 0{\cdot}005$.

§A1A3, African-American JIIM *versus* African-American controls: $P = 0{\cdot}053$, $P_{corr} = 0{\cdot}26$; OR = 6·5 (1·06–40·3).

¶A2A2, Caucasian *versus* African-American controls: $P = 0{\cdot}032$, $P_{corr} = 0{\cdot}224$.

factors [26], we assessed IL-1RN polymorphisms in these subgroups. In Caucasian patients, A1 was a possible risk factor for JDM, present in 95·8% of JDM patients compared with 90·2% of control subjects ($P = 0{\cdot}03$, $P_{corr} = 0{\cdot}077$, OR = 2·5 (95% CI 1·1–5·8). Genotype and carriage rate analysis did not reveal IL-1RN as a risk or protective factor for JPM or CTM in Caucasians, but confidence in the lack of associations is not strong because of the small numbers of patients in these subsets.

The relationship of IL-1RN polymorphisms to clinical outcomes was also examined, although some analyses were limited by small patient numbers. A2, and other IL-1RN alleles, were not found to be severity factors for the presence of gastrointestinal or cutaneous ulcerations in Caucasian (A2 carriage rate 55·0% in 40 Caucasian JIIM patients with ulcerations *versus* 40·2% in 164 patients without ulcerations) or in African-American JIIM patients (A2 carriage rate 30·0% in 10 African-American patients with ulcerations *versus* 20·0% in 30 patients without ulcerations). A2 and other IL-1RN alleles were also not found to be severity factors for the presence of calcinosis in Caucasian (A2 carriage rate 33·3% in 54 Caucasian JIIM patients with calcinosis *versus* 66·7% in 141 patients without calcinosis) or in African-American JIIM patients (A2 carriage rate 16·7% in 12 African-American patients with calcinosis *versus* 23·1% in 26 patients without calcinosis). IL-1RN alleles were not associated with disease course in Caucasian patients.

Relationship between IL-1Ra levels and IL-1RN VNTR polymorphism

Controls with the A1 allele had similar circulating IL-1Ra levels (median 92 pg/ml for 53 subjects) compared with those without A1 (median 90·5 pg/ml for eight subjects, $P = 0{\cdot}59$). Controls who had the A2 allele also had comparable circulating IL-1Ra levels (median 71 pg/ml for nine subjects) to those without A2 (median 92 pg/ml for 51 controls, $P = 0{\cdot}36$). Analyses could not be performed with A3 or A4 due to the small number of subjects with these alleles.

DISCUSSION

While HLA class II alleles appear to be strong genetic risk factors for many forms of autoimmunity, additional non-HLA polymorphic loci have been identified as risk factors for some autoimmune diseases [17]. In examining a large American control population, we identified for the first time differences in the distribution of IL-1RN genotypes and carriage rates between Caucasian and African-American controls. By studying a sizeable JIIM population, we were also able to identify the A1 allele in Caucasian and the A3 allele in African-American patients as the first non-MHC risk factors for myositis. Although the strengths of the associations are modest, and the A1 allele is common in the normal population, the magnitudes of these associations are in the range expected for polygenic diseases in which multiple loci together define genetic risks for disease [27]. The variation in allelic distributions and risk factors between Caucasian and African-American controls and patients is not unexpected, as distinct racial groups often differ in their polymorphic genetic risk factors for particular autoimmune diseases [18,26]. The differences in susceptibility factors among ethnic groups may be due to population selection which occurs as distinct populations encounter different environmental agents, or to founder effects in small populations.

It is possible that IL-1RN VNTR variant alleles are not genetic risk factors themselves, but are linked to genes which confer risk, possibly including the polymorphic loci for IL-1α and IL-1β [28–31]. Consistent with a recent report in Italian patients with multiple sclerosis [9], we have found A1 to be a risk factor for JIIM in Caucasians. Other investigators have found an association of A2 with other autoimmune diseases based on allele frequencies, rather than carriage rate analyses [7,12,32,33]. Our study suggests that only a single copy of the A1 allele is needed to confer risk, and with either type of analysis we do not see an association with A2.

Our findings that in neither control subjects, who were free of confounding illnesses, nor in JIIM patients were there associations among circulating IL-1Ra levels and IL-1RN genotypes or alleles is consistent with a similar study of patients with SLE [32]. Other studies however, demonstrated that PBMC of the A1/A1 genotype, when stimulated with *Mycobacterium tuberculosis in vitro*, produced lower amounts of IL-1Ra compared with cells homozygous for A2 [9,34]. These findings suggest that the more proinflammatory A1 allele could result in less IL-1Ra production and a greater inflammatory response following certain environmental exposures. It is possible that an association between IL-1RN genotypes and circulating IL-1Ra levels was not evident in the present study because the normal subjects were not exposed to an environmental stimulus relevant to the pathogenesis of JIIM. Alternatively, it is also possible that an association between IL-1RN genotypes and circulating levels of the gene product were not evident because plasma levels do not reflect the IL-1Ra concentrations in involved tissues. Furthermore, VNTR polymorphisms might primarily influence the intracellular isoform, which is particularly abundant in keratinocytes and epithelial cells, rather than the secreted isoform of IL-1Ra, and this would not be apparent by measuring circulating IL-1Ra levels [2].

We found that the A3 allele was a possible risk factor for African-American patients overall, who notably have a poorer prognosis [14]. In contrast to previous studies in other autoimmune diseases, we did not find A2, or other IL-1RN alleles, to be severity factors for JIIM [4,6,8,9]. In our study, the number of patients with ulcerations, calcinosis or a chronic illness course was relatively small, particularly in African-American patients, and thus these analyses are preliminary and require confirmation in a larger study.

In conclusion, we have demonstrated that VNTR polymorphisms of IL-1RN are risk factors for JIIM and differ in Caucasian and African-American patients. In contrast to earlier studies, which reported A2 as a risk factor for a number of other autoimmune diseases, we identified A1 as a risk factor for the development of juvenile myositis in Caucasians. This is consistent with the recent finding that A1, under certain circumstances, can result in decreased production of IL-1Ra and may lead to unchecked inflammatory responses following certain environmental exposures [9,34].

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Appendix A

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