Immunogenicity of a rheumatoid arthritis protective sequence when acquired through microchimerism

Sami B. Kanaana,1, Oyku Sensoya, Zhen Yanab, Vijayakrishna K. Gadiab, Michael L. Richardsonc, and J. Lee Nelsonad

*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; 1Department of Medicine, Division of Oncology, University of Washington, Seattle, WA 98195; 1Department of Radiology, University of Washington, Seattle, WA 98195; and 1Department of Medicine, Division of Rheumatology, University of Washington, Seattle, WA 98195

Edited by Lawrence Steinman, Stanford University School of Medicine, Stanford, CA, and approved August 19, 2019 (received for review March 19, 2019)

HLA class II genes provide the strongest genetic contribution to rheumatoid arthritis (RA). HLA-DRB1 alleles encoding the sequence DERAA are RA-protective. Paradoxically, RA risk is increased in women with DERAA* children born prior to onset. We developed a sensitive qPCR assay specific for DERAA, and found 53% of DERAA−/− women with RA had microchimerism (Mc; pregnancy-derived allogeneic cells) carrying DERAA (DERAA-Mc) vs. 6% of healthy women. DERAA-Mc quantities correlated with an RA-risk genetic background including DERAA-binding HLA-DQ alleles, early RA onset, and aspects of RA severity. CD4+ T cells showed stronger response against DERAA* vs. DERAA− allogeneic cell lines in vitro, in line with an immunogenic role of allogeneic DERAA. Results indicate a model where DERAA-Mc activates DERAA-directed T cells that are naturally present in DERAA−/− individuals and can have cross-reactivity against joint antigens. Moreover, we provide an explanation for the enigmatic observation that the same HLA sequence differentially affects RA risk through Mendelian inheritance vs. microchimeric cell acquisition.

microchimerism | rheumatoid arthritis | HLA | noninherited genetic risk | DERAA

The heritability of complex autoimmune diseases remains only partially explained (1); however, a remarkable feature in most autoimmune diseases is an increased prevalence in females (2, 3) and a strong risk association with specific human leukocyte antigen (HLA) class II alleles (4). Rheumatoid arthritis (RA), a chronic autoimmune disease affecting synovial joints, follows this rule, with allelic polymorphisms in the HLA class II region accounting for the highest genetic risk and a female:male ratio of 3:1 (3). The HLA-DRB1 strongly RA-associated alleles code for the “shared epitope” (SE): a 5-amino acid motif, (Q or R)-K or R)-R-A-A13 in the third hypervariable region (HV3) of the DRβ1 molecule, most notably DRB1*01:01, *04:01, *04:04, *04:05, *04:08, *10:01, and *14:02 (5). At the same positions, the D-ERA-A24 sequence is RA-protective, encoded by HLA-DRB1 alleles including *01:03, *04:02, *11:02, *11:03, *13:01, *13:02, and *13:04 (6–11). Of incidental note, HLA-DRB1*15 (the allele group that contains the most frequent DERAA-encoding HLA alleles) is protective not only against RA but also against systemic lupus erythematosus, psoriasis, and systemic sclerosis (12, 13). Interestingly, the protective benefits of DERAA are reversed when women are exposed to fetal cells expressing DERAA during pregnancy; a 2017 study unexpectedly found that RA risk was increased when the HLA genotype of children born prior to RA onset encoded DERAA but the mother’s did not (14).

HLA molecules frequently present self-peptides themselves derived from HLA molecules (15, 16). The HLA-derived DERAA sequence can be presented on a variety of HLA molecules [e.g., peptide DERAAADTY presented on HLA-B (16)], but is notable for its affinity to DQ heterodimers encoded by HLA-DQA1 and -DQB1 alleles that are in very strong linkage disequilibrium with RA risk-associated SE-encoding -DRB1 alleles (mainly DQ molecules referred to as DQ7, DQ8, and DQ5) (17–25). A model to explain RA protection when DERAA is inherited was initially proposed in 1996 based on presentation of DERAA by particular DQ molecules (17). More recently, elegant studies by van Heemst et al. (25) identified naturally occurring DERAA-directed T cells with the potential to react against both endogenous DERAA (e.g., from synovium-expressed autoantigen vinculin) and microbial-derived DERAA (present in 66% of bacteria and 4% of viruses). Accordingly, protection against autoimmunity of RA would be maintained by thymic negative selection of DERAA-specific autoreactive T cells in DERAA* individuals. Such T cells are not deleted in DERAA−/− individuals and a break in tolerance could occur in the periphery by molecular mimicry after endogenous/exogenous DERAA encounter, facilitated by presence of the SE and DQ molecules that bind DERAA, in linkage disequilibrium with the SE (25).

Pregnancy creates a long-term legacy of microchimerism (Mc), generating a source of acquired alloantigens (26). Mc of fetal origin is unique to women, and Mc with DERAA* HLA (DERAA-Mc) is compelling as an additional source driving molecular mimicry and explaining the paradoxical increase of RA when a child had DERAA in his/her HLA genotype. Mc occurs naturally as a result of fetal–maternal cell exchange (27), with long-term persistence of allogeneic cells including immunologically relevant cell types (28–30). These microchimeric cells express nonshared familial antigens with the potential for significant immunological

Significance

HLA genes confer the strongest genetic autoimmune disease risk. Specific HLA-DRB1 alleles predispose to rheumatoid arthritis (RA), while others encoding the sequence DERAA are RA-protective. Unexpectedly, having given birth to children with DERAA* HLA-DRB1 alleles prior to onset was found to increase RA risk in women. We show that microchimerism (Mc; allogeneic cell long-term legacy of pregnancy) carrying DERAA* HLA increases the odds of RA 17-fold and that allogeneic cells carrying DERAA stimulated DERAA−/− T cells in vitro. Microbial-derived DERAA peptides (among others) are known to stimulate DERAA-directed T cells naturally present in DERAA−/− individuals. Our data indicate that Mc with DERAA alloantigens can potentially activate similar adaptive immunity pathways and mechanistically link naturally occurring Mc to an autoimmune disorder.

Author contributions: S.B.K. and J.L.N. designed research; S.B.K. and O.S. performed research; S.B.K., Z.Y., V.K.G., M.L.R., and J.L.N. analyzed data; and S.B.K. wrote the paper.

Conflict of interest statement: S.B.K. and J.L.N. are cofounders of Chimerocyte, Inc., which develops highly sensitive chimerism analysis technologies. Chimerocyte, Inc. had no role in funding this research project.

This article is a PNAS Direct Submission.

Published under the PNAS license.

1To whom correspondence may be addressed. Email: skanaan@fredhutch.org.

2Present address: Diagnostic Pathology Medical Group, Inc., Sacramento, CA 95816.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1904779116/-/DCSupplemental.

First published September 9, 2019.

19600–19608 | PNAS | September 24, 2019 | vol. 116 | no. 39

www.pnas.org/cgi/doi/10.1073/pnas.1904779116
consequences for their host (31). Associations of Mc have been described with a number of autoimmune diseases (27) and, in risk of RA, Mc strongly depends on the allelic specificity of the acquired alloantigens (26, 32, 33). Nevertheless, a mechanistic basis for Mc involvement in RA or in the pathogenesis of other autoimmune diseases has been lacking.

The current studies address this knowledge gap and investigate the role of DERAA-Mc in RA biology. We first developed a highly sensitive and specific fluorescence-based qPCR assay for DERAA-encoding HLA-DRB1 alleles and then employed the assay to determine the prevalence and quantities of DERAA-Mc in women with RA and healthy women. We explored the immunogenicity of DERAA, evaluating T cell reactivity in studied individuals in vitro studies. Further, we examined DERAA-Mc in the context of the women’s HLA class II genotype and clinical characteristics in recent-onset RA and assessed measures of RA severity after RA had become chronic many years after onset.

Results

Development of DERAA-Specific qPCR Assay. We developed a real-time qPCR assay with specific forward and reverse primers and fluorogenic probe targeting the DERAA-encoding HV3 sequences of the HLA-DRB1 locus. To assure specificity of the assay, testing was conducted against an extensive panel of DNA from well-characterized human B cell lymphoblastoid cell lines ranked at the International Histocompatibility Working Group project. Because the cells are clonally expanded, they are assumed Mc-free and safe for assay validation. The DERAA qPCR assay amplified DNA from HLA-DRB1 alleles *01:03, *04:02, *11:02, *11:03, *13:01, *13:02, and *13:04 but not other alleles (Fig. 1A). DERAA standard curves were generated as previously described (28) and run on background DNA (DERAA−/−) at varying concentrations to determine optimal sensitivity. The DERAA qPCR assay could detect a single DNA target in a background of up to 60,000 human cell genome equivalent (gEq) per amplification well (Fig. 1B). Because the assays are typically run in 6 replicates per study sample, a practical limit of detection for the assay was determined at ≤1,360,000. In the current study, a median (and interquartile range) of 161,581 [120,349 to 191,456] gEq of sampled DNA from 138 participants was run in DERAA qPCR assays.

DERAA-Mc Is Increased in RA Compared with Healthy Controls and Correlates with Risk-Associated Genetic Background. Subjects derived from a total population of 320 women consisting of 167 who met the 1988 American College of Rheumatology criteria for RA and 153 healthy women with no history of autoimmune disease (SI Appendix, Table S1), from which 135 selected who lacked a DERAA-encoding HLA allele in their genotype (65 healthy controls and 70 patients with an RA onset of ≥2 y). These 135 participants were randomly assigned to an initial cohort (40 controls and 32 RA) and a validation cohort (25 controls and 38 RA). DNA extracted from peripheral blood mononuclear cells (PBMCs) was tested with the DERAA qPCR assay. DERAA-Mc was present in nearly half of the patients and only in 5 to 8% of controls in the initial and replication groups (SI Appendix, Fig. S1). It was thus possible to merge, respectively, RA and control data from both initial and replication groups for all subsequent analyses (Fig. 2). The odds ratio (OR) of RA in the presence of DERAA-Mc was 17.1 and the 95% confidence interval was [5.7 to 46.9]. Quantitatively, concentrations of DERAA-Mc were higher in RA patients than in controls. Ranked values of DERAA-Mc were significantly higher among RA women (P < 0.0001, Mann–Whitney U) (Fig. 2A).

RA-risk alleles, specifically SE-encoding HLA alleles, were present in 80% of RA women and 46% of controls. The immunogenetics of RA indicates risk-associated alleles are not all equivalent, as some combinations of different SE-encoding alleles in a genotype synergistically compound RA risk (34). Such “compound heterozygosity” was extensively studied by Balandraud et al. (35), resulting in a table of OR values translating the risk of RA according to HLA-DRB1 genotypes. This genetic risk OR score ranges from 0.2 for DRB1*05/03, the most protective combination, to 28.2 for *04:01/*10, the most susceptible combination (35). We found that ranked DERAA-Mc concentrations in RA women positively correlated with the risk score (Fig. 2B). This HLA risk score analysis was obtained for anti-citrullinated protein antibody (ACPA)-positive RA because of its well-recognized diagnostic and predictive value, alongside the rheumatoid factor (RF) test (36). Considering autoantibody-positive (ACPA and/or RF) RA patients only, our DERAA-Mc data trended toward an increase with the risk score (SI Appendix, Fig. S2).

Together, our data showed a striking increase of DERAA-Mc prevalence and quantities in women with RA compared with controls. This is consistent with the unexpected increase of RA risk reported in women for whom children born prior to onset had DERAA-encoding “protective” HLA (14). Moreover, increased DERAA-Mc correlated with an individual’s risk-associated HLA genotype. This points to the functional significance of DERAA-Mc in RA pathogenesis, consistent with and extending upon a proposed model of molecular mimicry (25) where DERAA-carrying allogeneic cells may become immunogenic in DERAA−/− subjects and contribute to autoimmunity.
time. The cell-surface markers, some known to be early-expressed and short-lived (38), tended to fluctuate with time and were dropped from further analysis (SI Appendix, Fig. S5). T cell activation was measured as the fold increase of CFSElow: the ratio of CFSElow percentage when mixed with a stimulator and CFSElow percentage at baseline (Fig. 3B and SI Appendix, Fig. S6).

In paired comparisons, activation of CD4+ T cells was significantly more pronounced when the stimulator cell line’s non-shared allele was DERAA+ vs. when it was DERAA−, and this was observed whether the shared allele was DQ8 or DQ6.2 (P < 0.0001 and = 0.03, respectively, Wilcoxon signed-rank test) (Fig. 3C and SI Appendix, Fig. S6). We report both RA and control
results combined; the observation, however, is similar when analyzed separately (SI Appendix, Fig. S6C). On the other hand, activation of CD8+ T cells in response to DERAAs did not differ significantly vs. DERAAs (Fig. 3C); this was expected because responder-stimulator HLA matching was based on class II and not class I.

Of the 26 subjects from the T cell activation studies, 11 healthy controls and 13 RA patients could be evaluated for correlation with their corresponding DERAAs concentrations. No correlation was detected between DERAAs-Mc levels and the T cell activation ratio of a DERAAs stimulation over a DERAAs stimulation (SI Appendix, Fig. S7).

Together, these data showed that, when controlling for the HLA class II genotypes of responders and stimulators, allogeneic cells that carried DERAAs-encoding HLA induced a stronger in vitro helper T cell activation in both patients and controls, compared with cells that did not. The data support an immunogenic role of DERAAs when carried by allogeneic cells, as also strongly implicated by results of familial HLA genotyping reported by others (14). However, we cannot exclude alternative explanations for the in vitro observations, including, for example, intrinsic differences of the cell lines or other experimental variables known or unknown.

**DERAAs-Mc Data Are Not Correlated with Clinical Features or Pregnancy History.** In our study, DERAAs-Mc appeared to be independent of age at RA onset. DERAAs-Mc did not correlate with patients’ ACPA and RF statuses, nor with patients’ swollen joint count (SI Appendix, Fig. S8). Some patients were taking disease-modifying antirheumatic drugs (DMARDs) at the time blood was obtained, including hydroxychloroquine, gold, methotrexate, sulfasalazine, and azathioprine; however, use of these drugs appeared unrelated to DERAAs-Mc levels (SI Appendix, Fig. S8). The most common sources of naturally acquired Mc are fetal-origin Mc (FMc) acquired from pregnancies resulting in birth, miscarriage, or elective termination (27, 39) and maternal Mc (MMc) acquired during fetal life. Most of our study subjects were parous women (SI Appendix, Table S1); however, DERAAs-Mc results did not appear to correlate with parity or gravidity (SI Appendix, Fig. S8).

**The DERAAs-Mc Observations Differ from Those of Other Mc Specificities.** Because DERAAs-Mc levels differed sharply between RA patients and controls, we asked if this phenomenon was similar with other types of Mc. One such type is Mc carrying the SE. Indeed, the hypothesis that RA patients who are SE− can acquire RA risk through SE-Mc was addressed in a study that found a higher occurrence of Mc-carrying HLA-DRB1*04 and *01 allele groups (among which a majority although not all alleles encode SE sequences) in RA women who were not HLA-DRB1*04 and *01 (32). This was subsequently confirmed and extended with direct demonstration of the SE sequences OKRRAA and ORRRAA as Mc (33). These studies, considered together with the recent report of increased RA risk in SE-negative women for whom a previously born child had an SE allele (14), bring support to the “minigene transfer” hypothesis whereby patients without risk-associated alleles may nevertheless acquire them through Mc (26, 27).

In our SE-Mc studies, we included subjects who participated in the earlier study of Yan et al. (33) as well as additional subjects, for a total of 64 women with RA and 41 healthy women, all of whom lacked an SE-encoding HLA allele in their genotype. DNA from PBMCs of these study subjects was tested for both OKRRAA and ORRRAA markers and, with the additional participants, results remained similar to previous findings (SI Appendix, Fig. S9) (33). We evaluated overall SE-Mc, defined as having either OKRRAA-Mc or ORRRAA-Mc or both. Therefore, results of both assays were added to generate quantitative values of SE-Mc (Methods). SE-Mc detection was significantly increased in patients vs. controls, and ranked values of SE-Mc were significantly higher among RA women (Fig. 4/4). When positive, Mc exclusively originated from either a OKRRAA or a ORRRAA marker, except in 3 RA patients and 1 control positive for both markers simultaneously. Bearing in mind the hypothesis of Mc-derived DERAAs peptide involvement in RA pathogenesis of SE+ individuals, we asked if the minigene transfer hypothesis could involve SE+ microchimeric immune cells reacting against peptides from a DERAAs host. However, we found no suggestion of a difference in SE-Mc results if a subject had DERAAs (SI Appendix, Fig. S10).

Another type of Mc is HLA specificities other than DERAAs or SE. To identify non-DERAAs non-SE Mc (DERAAs/SE− Mc), we conducted HLA genotyping for family members of RA and healthy women and were able to identify 25 women with RA and 47 healthy women to target a nonshared marker that was not a DERAAs- or an SE-encoding allele. Because familial HLA was known, the origin of DERAAs/SE− Mc could be identified as maternal and/or fetal-origin (SI Appendix, Fig. S11A). DNA from

![Fig. 2.](image-url)
PBMCs of these study subjects was tested in assays specific to HLA-DRB1*15/16, *03, *07, *08, DRB4*01, DQA1*01, *03, DQB1*02, *03, *04, *06, and B*44 or non-HLA polymorphisms in the GSTT1, TG, and ATIII genes in case HLA of the subject and the Mc source was indistinguishable (28, 40). DERAA−/SE−Mc was significantly increased in patients vs. controls, and ranked values were significantly higher among RA women (Fig. 4A). The trends were similar when considering DERAA+/SE− that was FMc or that was MMc separately (SI Appendix, Fig. S1B). In a few cases there was overlap, namely a woman’s mother and the woman’s child shared the same marker (SI Appendix, Fig. S1A).

Neither SE-Mc nor DERAA+/SE− Mc concentrations correlated with the genetic risk OR score in either RA patients or controls (Fig. 4B and C). However, there was a tendency toward
lower levels of SE-Mc with increasing RA-risk score ($P = 0.063$) (Fig. 4 B, Right) in agreement with the minigene transfer hypothesis (26, 27).

It is worth noting that, among healthy subjects across the 3 groups (of different Mc specificities), both prevalence and quantities of DERAA-Mc were significantly decreased compared with SE-Mc and DERAA"SE" Mc [including some non-DERAA markers associated with RA protection (35), e.g., $DRB1^{*}03$ and $DRB1^{*}07$ (Fig. 4 A)]. Moreover, our results of SE-Mc and DERAA"SE" Mc in healthy controls generally agreed with Mc findings from previous studies in the healthy adult population, when testing PBMC samples using HLA and other polymorphism-specific assays (29, 40, 41).

Together, our data indicate that Mc of any kind, consistently prevalent in about half of RA patients, is naturally increased in RA compared with the healthy population. Such consistent patterns
are not observed in healthy subjects where DERAA-Mc is very uncommon, contrasting SE-Mc and DERAA-+/SE- Mc that are prevalent in about a quarter of controls.

**DERAA-Mc Is Greater in Recent-Onset than in Established RA.** Considering the time-dependent effects of parity on disease onset (42, 43), we asked whether DERAA-Mc differed according to time since RA onset. Of the 70 patients with an RA onset between 0.1 and 2 y (median 0.8 y) tested in the DERAA-Mc arm of our study, we obtained a sample to test the same individual for 15 of them after onset. To these, we added 8 additional patients with established RA of onset >2 y for a total of 23 RA patients for which time since onset ranged from 2.2 to 11.3 y (median 7.3 y). DERAA-Mc was positive at early RA, and dropped to undetectable at follow-up in 5 patients, and in 2 cases remained detectable but without increase (Fig. 5A); DERAA-Mc was undetectable in early RA and remained undetectable at follow-up for 7 patients. DERAA-Mc was detected in 22% of the RA patients tested at both time points, and a Wilcoxon signed-rank test on 15 paired values determined this decrease was statistically significant (P = 0.016) (Fig. 5A).

DERAA-Mc–ranked concentrations tended to correlate inversely with the genetic risk OR score of patients when tested after RA was established (P = 0.05) (Fig. 5B), and DERAA-Mc was not detected when patients were SE-/+ (SI Appendix, Fig. S12A). The SE, which is associated with high genetic RA-risk scores (35), in some studies has been considered a marker of severe disease when on an appropriate background, including cartilage erosion and bone destruction, regardless of therapy (34, 44, 45). DERAA-Mc concentrations were significantly correlated with the joint-space narrowing score, disability score, and pain score of RA patients who were seen at onset and again after RA was established (SI Appendix, Fig. S12B).

Among the 64 RA patients tested in the SE-Mc arm of our study, 13 had an RA onset of ≤2 y and 51 an RA onset >2 y. However, we found no suggestion of a difference in SE-Mc results if RA was recent vs. established (SI Appendix, Fig. S13). As for the RA patients for whom we obtained family members and tested in the DERAA-+/SE- Mc arm, such comparison was not possible because all 25 had an RA onset >2 y (SI Appendix, Table S1).

Together, these data suggest involvement of DERAA-Mc in RA pathogenesis early on, after which DERAA-Mc levels decrease. This decrease is associated with more severe disease outcome (i.e., joint-space narrowing, disability, pain), reflecting a stronger chronic inflammation that could be naturally more efficient against allogeneic DERAA-Mc.

**Discussion**

In the current study, we report a striking difference of Mc with HLA alleles encoding the DRβ1 HV3 sequence DERAA in RA patients vs. healthy controls. The presence of DERAA-Mc increased the odds of RA ~17 times. Moreover, DERAA-Mc was greater in recent-onset RA vs. established RA, and this later decrease correlated with a more severe outcome, especially hand and wrist radiograph joint-space narrowing and standardized measures of disability and pain, and correlated with a higher genetic RA-risk score as well. Together, these observations strongly implicate DERAA-Mc in RA pathogenesis. That naturally acquired Mc has functional consequences is further supported by the consistent observation that CD4+ T cells had a significantly greater response against DERAA+ than DERAA− allogeneic cells in allosereactive in vitro cocultures in RA patients and controls.

Prevalence of DERAA-Mc was ~50% in RA. For comparison, we extended studies to evaluate naturally acquired Mc encoding for HLA specificities other than DERAA. Mc encoding the RA risk-associated SE as well as Mc encoding neither DERAA nor the SE were also detectable in ~50% of patients. However, the difference between RA patients and controls was much less for Mc with specificities other than for DERAA-Mc and did not correlate with RA-risk genotypes. Our interpretation of these results is that DERAA-Mc carries greater immunogenicity and potency as a contributor to autoimmunity in RA, and hence is rare in healthy individuals.

Our results add strength to the report by Cruz et al. (14), who unexpectedly found increased RA risk among women who had children prior to disease onset for whom the paternally inherited HLA allele encoded DERAA. At the same time, this report presented a paradox: Why would HLA alleles that are protective in a person’s own genotype be associated with RA risk when acquired as Mc? Our studies offer an explanation for this paradox. In an individual who has DERAA in their genotype, acquired as Mc? Our studies offer an explanation for this paradox. In an individual who has DERAA in their genotype, autorreactive T cells can be deleted in the thymus. However, in a DERAA− individual, naturally occurring DERAA-directed T cells remain present as previously described (25). In these subjects, DERAA peptides from Mc [self-presented on microchimeric cells]...
or presented by host antigen-presenting cells (16)) would activate DERAA-directed T cells that are part of a repertoire recognizing both endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of HLA specificities, including by DERAA-binding HLA-DQ alleles (25). When this initial immune activation becomes chronic, the risk of emergence of RA through molecular mimicry becomes apparent (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25). This immune activation can be promoted by a variety of endogenous and exogenous antigens containing the DERAA sequence (25).
**Statistical Analysis.** Categorical variables were reported as counts and percentages, and comparisons were performed using Fisher’s exact test. ORs were used to depict the association between risk factors and rheumatoid arthritis. Continuous variables were compared using the Mann–Whitney U test (for 2-group comparisons), Kruskal–Wallis test (for >3-group comparisons), Wilcoxon matched-pairs signed-rank test (for paired comparisons), and Spearman rank test (for correlations). Log-log best fit lines were used to illustrate trends. Analyses were performed using GraphPad Prism 7.

**ACKNOWLEDGMENTS.** This work was supported by NIH Grants HL-117737 and AI-45659 and the Won Foundation. We thank Whitney E. Harington, Nathalie C. Lambert, and Isabelle Auger, for helpful discussions; Jean Roudier in Marseille, France for insights regarding the analyses with the genetic risk odds ratio scores; Christine Luu, Tessa Aydelotte, and Alex M. Forsyth for technical assistance; and Judy Allen and Francesca Urselli for programmatic assistance.