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Clustering of Disease Features Within 512 Multicase Rheumatoid Arthritis Families

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Objective. **To determine whether specific rheumatoid arthritis (RA) disease features demonstrate the presence of significant familial clustering.**

Methods. **We studied 1,097 individuals with RA from 512 multicase families enrolled in the North American Rheumatoid Arthritis Consortium. All patients were interviewed and examined to collect standardized information about demographic and clinical characteristics. Affected individuals also underwent radiography of the hands and wrists and were genotyped for the HLA–DRB1 shared epitope. Familial clustering of disease features was assessed using contingency table analysis and Pearson correlation coefficients. Multivariate logistic and linear regression analyses were used to account for other characteristics that might influence familial clustering, such as disease duration, sex, and age at diagnosis.**

Results. **Several disease characteristics exhibited significant familial clustering, including seropositivity (multivariate odds ratio [OR] 4.3, $P < 0.0001$), nodules (OR 2.3, $P < 0.0001$), and age at RA diagnosis (multivariate regression coefficient [β] 0.44, $P < 0.0001$). Other characteristics demonstrated statistically significant but modest degrees of familial clustering (Joint Alignment and Motion score, Health Assessment Questionnaire score, and year of RA diagnosis) or modest but nonsignificant familial clustering (other extraarticular manifestations, other autoimmune diseases).**

Conclusion. **The clustering of certain disease characteristics implicates specific genetic or nongenetic causes. These results highlight the importance of considering disease phenotype in future genetic and epidemiologic studies of RA.**

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease that affects the peripheral synovial joints. Although the etiology of the disease remains unknown, it is clear that both genetic and environmental factors play important roles (1). The early evidence for a genetic component to RA was derived from twin and family studies (2,3). Recently, the study of multicase families, particularly families containing multiple affected siblings, has been a popular approach for mapping RA genes (1).

Although the importance of genetic factors in RA susceptibility is undisputed, whether primarily genetic, environmental, or stochastic processes influence specific disease features remains unclear. Multicase families provide an opportunity to address this issue through examination of familial clustering of specific disease features. For the current study we examined the familial clustering of RA disease features utilizing a unique national resource of multicase families. Because some researchers have suggested that the clustering of RA cases within families is an artifact of large sibships (4–6), we also examined the distribution of siblings affected by RA and those without RA within this large sample of multicase families.

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PATIENTS AND METHODS

Patients. We studied 1,097 individuals with RA from 512 multicase families recruited as a collaborative effort of the North American Rheumatoid Arthritis Consortium (NARAC). This consortium was established to create a resource for RA gene mapping studies (7,8). Families were recruited nationwide through a variety of sources; most subjects were referred directly from rheumatologists.

In order to be eligible for entry into the NARAC collection, families had to meet the following criteria: 1) 2 or more siblings satisfying the 1987 American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria for RA (9); 2) at least 1 sibling having documented erosions on hand radiographs; and 3) at least 1 sibling having disease onset between the ages of 18 and 60 years. Since psoriasis, inflammatory bowel disease, and systemic lupus erythematosus can be associated with articular symptoms resembling RA, the presence of these diseases in affected individuals was a criterion for exclusion of the sibpair. Informed consent was obtained from every subject, including all participating family members, and approval of the local institutional review board was secured at every recruitment site prior to the start of enrollment. For the current study, we examined the first 512 families enrolled in the NARAC collection. A genome-wide screen to identify genetic markers linked to disease susceptibility in these families has been performed, and the results are reported elsewhere (8,10).

Telephone interviews. All patients with RA were interviewed by telephone to collect demographic and clinical information. This included date and location of birth, ethnicity, age at onset of RA symptoms, and age at RA diagnosis. Patients were also interviewed about their RA medication history and joint replacement surgery. Each proband provided information about parents and siblings, including the presence of RA among any of these relatives.

Confirmation of RA diagnoses. Confirmation of RA diagnoses was obtained from patients' rheumatologists, who provided information about which components of the 1987 ACR criteria were met and about the presence of extraarticular manifestations, including rheumatic lung disease, rheumatoid vasculitis, rheumatic eye disease, and Felty's syndrome. They also provided information about the presence of other autoimmune diseases, including autoimmune thyroid disease, Sjögren's syndrome, polymyositis/dermatomyositis, polyarteritis nodosa, idiopathic thrombocytopenic purpura, myasthenia gravis, multiple sclerosis, scleroderma, and undifferentiated connective tissue disease.

Physical examination. Patients were examined for joint tenderness and swelling using the Joint Alignment and Motion (JAM) instrument (range 0–112) (11), and for the presence of subcutaneous nodules. At the time of these examinations, patients also completed a Health Assessment Questionnaire (HAQ) (12) and visual analog scales for pain and fatigue, and answered questions about current and past arthritis activity, joint pain, and morning stiffness.

Radiography. Radiographs of the hands and wrists of all affected individuals were obtained at the time of study entry, unless films taken within 2 years prior to entry were available for review. All radiographs were read by a single radiologist who was blinded to patients' clinical and genetic information, to document the presence or absence of erosions.

Rheumatoid factor testing. For all affected individuals, rheumatoid factor (RF) testing was performed at the University of Washington Department of Laboratory Medicine (Seattle, WA), using a latex-enhanced nephelometric assay (Behring Diagnostics, San Jose, CA) with human and rabbit IgG–coated latex beads as antigen. This assay was calibrated to the World Health Organization international standard for RF (13). RF values <12 IU were considered negative.

Statistical analysis. We examined the following disease features for evidence of familial clustering: RF, nodules, other extraarticular manifestations (vasculitis, rheumatic lung disease, Felty's syndrome, scleritis, and scleromalacia), autoimmune thyroid disease, other autoimmune diseases (Sjögren's syndrome, polymyositis/dermatomyositis, polyarteritis nodosa, idiopathic thrombocytopenic purpura, myasthenia gravis, multiple sclerosis, scleroderma, and undifferentiated connective tissue disease), JAM score, HAQ score, age at disease onset, and calendar year of disease onset. Nodules, other extraarticular manifestations, autoimmune thyroid disease, and other autoimmune diseases were considered to be present if reported by the patient's rheumatologist.

Clustering of categorical disease features according to the probands' characteristics was assessed using contingency tables and chi-square tests. This involved comparing siblings of probands who had a specific manifestation with siblings of probands who lacked that manifestation. Pearson correlation coefficients were generated for analysis of clustering of continuous outcomes. These analyses involved comparisons of continuous or ordinal outcomes between probands and their affected siblings. Multivariate logistic or linear regression was used to examine familial clustering of disease features, controlling for probands' disease duration, sex, and age at RA onset. These analyses involved examination of a series of models in which all covariates were initially included, followed by their serial removal from the model. All statistical analyses were performed using SAS version 8.2.

RESULTS

Demographic and clinical features. A total of 1,097 individuals with RA from 512 multicase families

Table 1. Clinical and demographic features of 1,097 affected siblings with rheumatoid arthritis (RA) included in the study

Race, % white	91.5
Sex, % female	77
Age at RA diagnosis, mean ± SD years	41.0 ± 13.1
Disease duration, mean ± SD years	14.3 ± 11.1
Erosions, %	95.3
Rheumatoid factor positive, %	81.1
Health Assessment Questionnaire score, mean ± SD	1.0 ± 0.8
Joint Alignment and Motion scale score, mean ± SD	32.8 ± 30.5
Nodules, %	38.8
Other extraarticular manifestations, %*	4.9
Autoimmune thyroid disease, %	6.3
HLA–DRB1 shared epitope positive, %	83.5

* Vasculitis, rheumatic lung disease, Felty's syndrome, scleritis, or scleromalacia.

Table 2. Familial clustering of rheumatoid arthritis disease features (categorical variables) among 512 multiplex families

Disease characteristic	Sibs of positive proband, no. (%)	Sibs of negative proband, no. (%)	Odds ratio (95% CI)*	*P*
Rheumatoid factor positive	402 (85)	62 (61)	3.6 (2.3–5.8)	<0.0001
Presence of nodules	117 (50)	103 (22)	2.4 (1.7–3.4)	<0.0001
Other extraarticular manifestations†	2 (6)	23 (4)	1.5 (0.3–6.8)	0.4
Autoimmune thyroid disease	4 (11)	33 (6)	2.0 (0.7–5.8)	0.2
Other autoimmune diseases‡	6 (13)	50 (9)	1.5 (0.6–3.6)	0.4

* The odds ratio describes the risk of each disease characteristic among affected siblings of probands with that characteristic versus the risk among affected siblings of a proband lacking that characteristic. 95% CI = 95% confidence interval.
† Vasculitis, rheumatic lung disease, Felty's syndrome, scleritis, or scleromalacia.
‡ Sjögren's syndrome, polymyositis/dermatomyositis, polyarteritis nodosa, idiopathic thrombocytopenic purpura, myasthenia gravis, multiple sclerosis, scleroderma, or undifferentiated connective tissue disease.

were included in this study. An additional 52 potentially affected siblings were not included because they declined to participate and we were therefore unable to confirm their RA diagnosis. Of the 512 families, 55 had 3 affected siblings, 7 had 4 affected siblings, and 1 had 6 affected siblings. Overall, 12.3% of the families had ≥3 affected siblings.

Table 1 displays demographic and clinical characteristics of the 1,097 affected individuals. The majority of subjects were white, and 77% were female. The mean age at RA diagnosis was relatively young, and most patients had well-established disease at the time of study entry. As expected based on our eligibility criteria, the majority of affected individuals had evidence of erosions. Physical examination revealed substantial joint damage and the frequent presence of nodules among these families. Approximately 5% of affected individuals had other extraarticular manifestations as reported by their rheumatologists. Laboratory testing revealed that 81.1% of affected individuals were seropositive for RF, and 83.5% were HLA–DRB1 shared epitope positive. A comparison of characteristics between probands and other affected siblings revealed that probands were significantly more likely to be female (82% versus 72%; $P = 0.0001$); however, other characteristics listed in Table 1 did not differ significantly between probands and other affected siblings (data not shown).

Familial clustering of disease features. Several disease features demonstrated striking familial clustering, as shown in Table 2. Among affected siblings of seropositive probands, the risk of RF seropositivity was significantly higher than for seronegative probands. The presence of nodules also demonstrated significant familial clustering. Similarly, age at RA diagnosis, calendar year of diagnosis, and JAM and HAQ scores demonstrated statistically significant familial correlation (Table 3). (Further analyses depicting the distribution of values are available from the authors.) Modest but nonsignificant degrees of familial clustering were observed for other extraarticular manifestations, autoimmune thyroid disease, and other autoimmune diseases. We also performed analyses restricted to the proband and 1 additional affected sibling (chosen randomly) to ensure that larger family size did not qualitatively influence the results. Results obtained in analyses including all affected individuals versus those obtained with 2 affected individuals per family were very similar (data not shown).

To determine whether exclusion of the 52 potentially affected siblings who declined study participation might have influenced our results, we included these subjects in sensitivity analyses, first assuming that all 52

Table 3. Familial clustering of RA disease features (continuous variables) among 512 multiplex families*

Disease characteristic	Correlation coefficient (r)	*P*
Age at RA diagnosis	0.46	<0.0001
Year of RA diagnosis	0.30	<0.0001
JAM score	0.24	<0.0001
HAQ score	0.22	<0.0001

* RA = rheumatoid arthritis; JAM = Joint Alignment and Motion scale; HAQ = Health Assessment Questionnaire.

Table 4. Results of multivariate analyses examining familial clustering of disease features in 512 multiplex RA families*

Disease characteristic	Odds ratio (95% CI) or regression coefficient†	*P*	Significant covariates‡
Categorical variables			
Rheumatoid factor positive	4.3 (2.6–7.2)	<0.0001	Disease duration, sex
Presence of nodules	2.3 (1.6–3.4)	<0.0001	Disease duration, sex
Other extraarticular manifestations§	1.7 (0.4–7.6)	0.5	None
Autoimmune thyroid disease	1.9 (0.6–5.8)	0.3	Sex
Other autoimmune diseases¶	1.5 (0.6–3.7)	0.4	None
Continuous variables			
Age at RA diagnosis	0.44	<0.0001	Disease duration, sex
Year of RA diagnosis	0.010	0.001	Disease duration, age at diagnosis
JAM score	0.12	0.001	Disease duration, age at diagnosis
HAQ score	0.16	0.0001	Disease duration, sex, age at diagnosis

* 95% CI = 95% confidence interval (see Table 3 for other definitions).
† Odds ratios (by logistic regression) are shown for categorical variables, and regression coefficients (by linear regression) for continuous variables.
‡ Covariates analyzed were disease duration, sex, and age at diagnosis. Statistically significant covariates for the analysis of each outcome (disease characteristic) are shown.
§ Vasculitis, rheumatic lung disease, Felty's syndrome, scleritis, or scleromalacia.
¶ Sjögren's syndrome, polymyositis/dermatomyositis, polyarteritis nodosa, idiopathic thrombocytopenic purpura, myasthenia gravis, multiple sclerosis, scleroderma, or undifferentiated connective tissue disease.

subjects were concordant with the proband for the disease manifestation and then using the assumption that all were discordant with the proband for the disease manifestation. The results of these analyses were not substantially different from our main results (data not shown). Although these analyses were necessarily confined to the univariate analysis of categorical variables, the findings suggest that exclusion of these 52 subjects did not substantially influence our results.

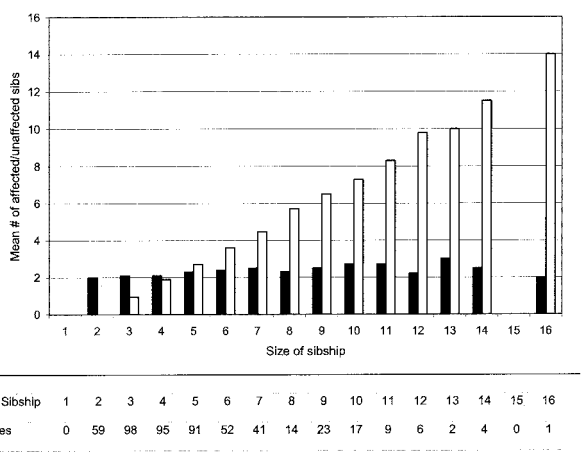

Figure 1. Mean number of affected siblings (solid bars) and unaffected siblings (open bars) per family, by size of sibship, among the 512 families studied.

Because several other characteristics might have influenced familial clustering, we repeated our analyses using multivariate logistic and linear regression. Covariates for these analyses included disease duration, sex, and age at diagnosis. As an example, these analyses entailed estimating the likelihood that an affected sibling was RF positive based on the RF status of the proband and other characteristics of the affected sibling (e.g., sex, disease duration). Thus, only the affected siblings (not the probands) were included in these analyses. These multivariate results are summarized in Table 4. Overall, adjustment for these covariates did not alter our results. The only exception was for year of RA diagnosis, for which the magnitude of familial clustering was markedly reduced after covariate adjustment. As with the univariate analyses, our results were essentially unchanged when we repeated the analyses including only 1 affected sibling per family, suggesting that the larger families did not influence the results.

Sibship size. The mean number of affected and unaffected siblings per family according to sibship size, among the 512 families included in the study, is displayed in Figure 1. The numbers of affected and unaf-

fected siblings shown include all siblings reported by the proband, regardless of whether they participated in the study. As the total sibship size increased from 2 to 16, the mean number of affected siblings remained remarkably consistent (between 2 and 3). The mean total sibship size for the entire set of 512 families was 5.1. We did not find evidence that the clustering of RA cases within these families was an artifact of large sibship size.

DISCUSSION

These results provide strong evidence of familial clustering of several disease features in RA, including seropositivity, nodules, and age at diagnosis. Although we cannot determine based on the current study alone whether this clustering relates primarily to shared genetic or nongenetic factors, these findings highlight the importance of considering specific disease characteristics in future genetic and epidemiologic studies of RA.

Although previous research examining clustering of disease features within families has been limited by the difficulty in obtaining large numbers of families suitable for study, it is of interest to compare our results with those available in the literature. Based on an examination of 33 multicase families, Silman et al (14) did not find evidence of familial clustering of disease features, including age at disease onset, calendar year of onset, pattern of joint involvement, and presence of nodules, Sjögren's syndrome, or antinuclear antibodies. In contrast, MacGregor and colleagues (15) examined disease features among 14 RA-concordant monozygotic twin pairs and found similarity within twin pairs for age at disease onset, presence of erosions, and presence of IgM-RF; however, other disease features, including pattern of joint involvement, presence of extraarticular manifestations, adverse drug reactions, disease course, and reported disability levels, were not strikingly similar. Although the results of these 2 studies differ somewhat from those of the present study, the investigations are not directly comparable because of differences in sample characteristics (e.g., the inclusion of monozygotic twins) and analytic methods. In addition, the power of the previous studies was limited by small sample sizes.

It has been argued that familial aggregation of RA is more commonly observed in large sibships and that familial clustering of RA might therefore be an artifact of sibship size (4–6). The mean sibship size among the 512 families in the present study was 5.1. Although differences in methods of ascertainment prevent us from directly comparing sibship size across studies, we did not observe an increase in the number of affected siblings as the total sibship size increased (Figure 1). Thus, our results suggest that familial clustering of RA cases is not simply an artifact of large sibships. The striking difference in our results compared with those reported by investigators in The Netherlands (5,6) indicates the need for further study of this issue.

There are a number of limitations of the current study that warrant discussion. First, the group of families studied does not represent a population-based sample. Although this would have been a desirable design feature, the assembly of such a sample in the US would have been prohibitively expensive. Nonetheless, the representativeness of the current collection is enhanced by virtue of its large size and the fact that families were identified through a variety of recruitment strategies. Second, the requirement for erosive disease among at least 1 affected member of each family certainly influenced the composition of the sample. However, we viewed this entry criterion as an important means of ensuring a study sample that was optimal for RA gene mapping studies. Specifically, this enhanced our confidence about the accuracy of diagnoses and should have eliminated undesirable degrees of genetic heterogeneity. Nonetheless, the characteristics of this sample exhibit substantial variation in disease features, which was the focus of the study. Finally, the power to evaluate clustering of rare manifestations, such as other extraarticular manifestations, was limited by the infrequency of these outcomes.

In summary, the creation of a large and well-characterized collection of multicase RA families by the NARAC provides a unique opportunity to study the aggregation of disease features in familial RA. Analysis of these families demonstrates striking familial clustering of certain disease features, including seropositivity, nodules, and age at diagnosis. Future efforts will be needed to define the specific genetic and/or nongenetic causes of these disease features.

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