Rheumatoid-susceptible alleles of HLA-DRB1 are genetically recessive to non-susceptible alleles in the progression of bone destruction in the wrists and fingers of patients with RA

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Abstract

***Objective*—To assess the relationship between HLA-DRB1 genotypes and the progression of bone destruction in Japanese patients with RA.**

***Methods*—The HLA-DRB1 alleles were determined by polymerase chain reaction and allele specific oligonucleotide probe techniques in 160 Japanese patients with RA. HLA-DR 0101, 0401, 0404, 0405, 1001 and 1402 were regarded as susceptible alleles of RA according to previous reports. Patients were classified into three groups (S/S, S/N and N/N group), based on the possession of two, one or no susceptible factor. The grading of radiographic changes in the wrists and fingers were evaluated by Larsen's criteria. The radiographic grades were first compared with the results of genotyping in the 160 cross sectional cases. A retrospective study was then conducted on a subgroup consisting of 57 cases taken from the 160 cases used for the cross sectional study.**

***Results*—In the scatter diagram of the 160 cross sectional cases expressing the relationship between the stage of bone destruction and duration of RA, the regression line and the 95% confidence intervals separated the S/S group from the S/N and N/N groups in the early phase of development of bone destruction. In the retrospective study on the 57 cases the median years taken to development to stage V in the wrists after the onset of symptoms were 13·1 in the patients in the S/S group, 22·7 in the S/N group and 23·0 in the N/N group. The difference observed between the S/S and S/N group, and between the S/S and N/N group were statistically significant ($p < 0·01$), but that between the S/N and N/N groups was not. Thus the bone destruction in the wrists and fingers progressed more rapidly in the S/S group than in the S/N and N/N groups; and the rheumatoid susceptible alleles of HLA-DRB1 can be considered to be genetically recessive to the non-susceptible alleles in the progression of bone destructions in the wrists and fingers.**

***Conclusion*—Genotyping of HLA-DRB1 can be a useful prognostic marker in the early phase of RA.**

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The clinical courses of bone destruction in rheumatoid arthritis (RA) vary widely. Even when the patients are treated in similar ways, in some cases the bone destruction may progress slowly to the end stage in the course of several decades, whereas in others it may rapidly advance to bone ankylosis or resorption change in a few years. It seems very likely that genetic factors are involved in the development of bone destruction in RA.

Recently, Weyand *et al* reported that patients with RA with two disease linked sequences (that is, susceptible factors) in both HLA-DRB1 alleles were affected more severely than other groups in terms of the frequency of nodular disease, major organ failure and requirement of joint surgery.[3] However, they did not discuss the releationship between HLA-DRB1 genotypes and rapidity with which the disease progresses.

It has been reported that in most patients with RA the bone destruction of wrists and fingers progresses within a decade after onset of RA.[4] We were interested in the agents that affect the rapidity of the bone destruction in the early phase of RA. We proposed that if genotypes of the HLA-DRB1 alleles are associated with the clinical courses of bone destruction in RA, then genotyping the HLA-DRB1 alleles during the early phase of the disease might allow prediction of the progression of bone destruction.

In this study HLA-DRB1 allele genotyping was performed in Japanese patients with RA and the results were compared with the data for healthy Japanese subjects. The association of the number of susceptible factors with the clinical course of bone destruction in the wrists and fingers was also studied. Only radiographic changes in the wrists and fingers were assessed, because the bone destruction changes in these sites is a common and typical feature of RA, so that past records were more frequently available.

Materials and methods

One hundred and sixty Japanese outpatients and hospital patients in the Kansai Medical University Hospital with a rheumatoid factor level over 20 U/dl at present or past analysis (that is, seropositive RA), and diagnosed with RA based on the 1987 ACR criteria were enrolled in the study.[5] For all patients the relationship between the present state of

radiographic bone destruction of the wrists and fingers and the duration after the onset of symptoms was assessed.

Of the 160 patients, 57 had radiographic records of progressive change in bone destruction of the wrists and fingers for more than five years and they were reviewed for a retrospective study.

The genomic DNA was isolated from mononuclear cells in peripheral blood according to the method of Erlich and Bugawan; HLA-DRB1 DNA was amplified with the primer sets by polymerase chain reaction (PCR).[6] Each HLA-DRB1 allele was genotyped by reverse dot blotting by hybridisation with a sequence-specific oligonucleotide. If the HLA-DRB1 alleles were from patients who appeared to be homozygous, justification of homozygosity was evaluated by HLA-DQA1 and DQB1 genotyping.[7] The genotyping of HLA-DRB1 alleles was undertaken by Shionogi Biomedical Company.

DISTRIBUTION OF HLA-DRB1

The gene frequencies of HLA-DRB1 alleles in our patients with RA were compared with the data for healthy Japanese subjects.[8]

According to the previous reports on the association between HLA-DR alleles and RA, HLA-DRB1 0401, 0404, 0405, 0101, 1001 and 1402 were regarded as susceptible alleles of RA in the study.[1 2] The patients were classified into three groups. The S/S group consisted of those with susceptible factors in both of the HLA-DRB1 alleles. The S/N group were made up of those having one susceptible factor and one non-susceptible factor. The N/N group consisted of those possessing two non-susceptible factors.

RADIOGRAPHIC CHANGE

Radiographs were assessed by five orthopaedic surgeons before they were informed of the results of genotyping. The stage of bone destruction in the wrist and finger joints was categorised into six radiographic groups according to the standard films of Larsen.[9] The staging of fingers was based on the joint which was affected most severely.

RELATIONSHIP BETWEEN BONE DESTRUCTION AND TYPES OF HLA-DRB1

In the cross sectional study on the 160 cases, the relationship between the duration of RA and the stage of bone destruction in the wrists and fingers in the S/S, S/N and N/N groups is illustrated on the scatter diagrams (fig 1). Regression lines and their 95% confidence intervals were also represented in the scatter diagrams of the three groups, taking the stage numbers as numerical values. As we can not be certain that the intervals between stages should be equal in a truly quantitative way, we added

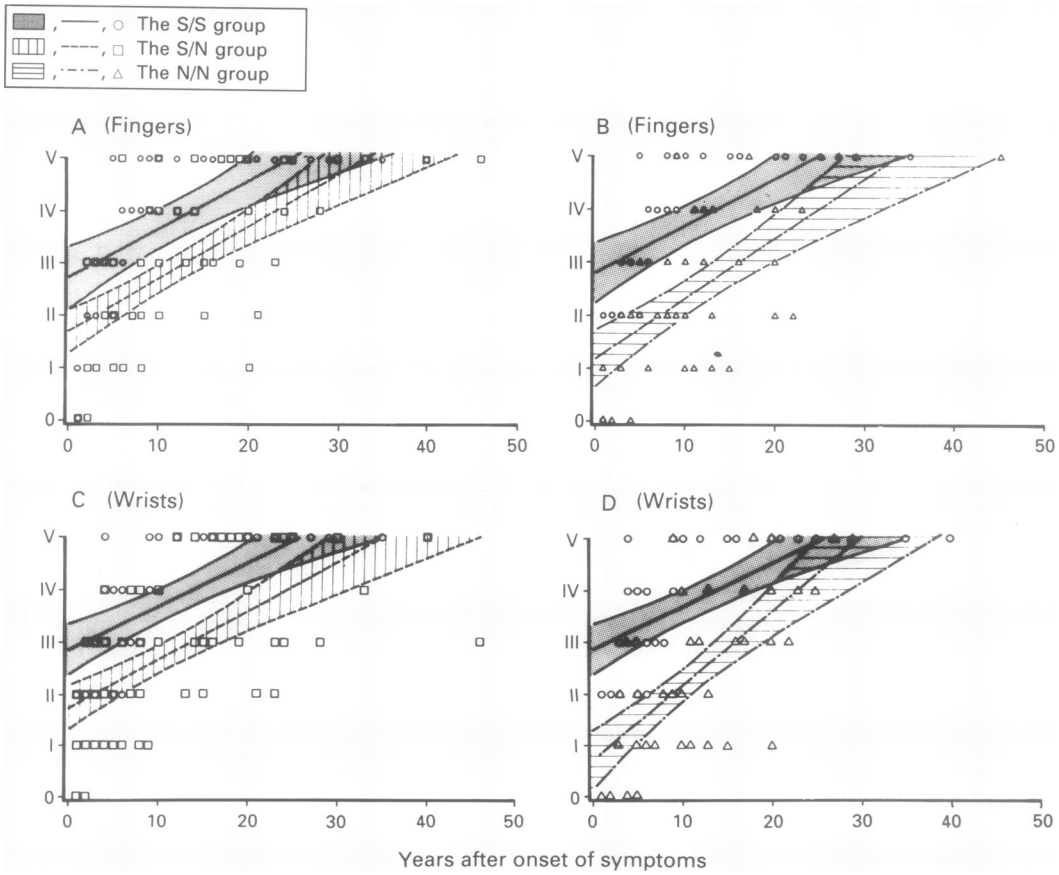


Figure 1 Scatter diagrams showing the relationship between the duration of RA and the stage of bone destruction in the wrists and fingers, with the regression lines and 95% confidence intervals. A) The S/S and S/N groups of the fingers; B) The S/S and N/N groups of the fingers; C) The S/S and S/N groups of the wrists; D) The S/S and N/N groups of the wrists. Each dot represents an individual, but if the dots of the same group overlap, only one dot is represented on the diagram.

Table 1 Patient characteristics of the 160 cases examined, and the 57 reviewed cases

	160 cases examined					*57 reviewed cases*			
	All (n = 160)	*S/S group (n = 32)*	*S/N group (n = 78)*	*N/N group (n = 50)*		*All (n = 57)*	*S/S group (n = 10)*	*S/N group (n = 27)*	*N/N group (n = 20)*
1 Sex					1 Sex				
Female	139	30	64	45	Female	50	9	23	17
Male	21	2	14	5	Male	7	1	4	3
2 Age					2 Age				
< 39	12	2	7	3	< 39	3	1	1	1
40–49	28	4	17	7	40–49	9	1	6	2
50–59	60	16	28	16	50–59	22	6	9	7
60–69	32	5	12	15	60–69	12	1	6	5
70–79	24	4	12	8	70–79	10	1	4	5
> 80	4	1	2	1	> 80	1	0	1	0
median	56	55	54	58	median	56	55	57	59
3 Disease duration					3 Disease duration				
0–4	43	8	25	10					
5–9	44	12	17	15	5–9	21	4	10	7
10–14	23	2	10	11	10–14	10	1	4	5
15–19	18	3	10	5	15–19	10	2	4	4
20–24	18	2	10	6	20–24	10	2	5	3
< 25	14	5	6	3	< 25	6	1	4	1
median	8·5	8·0	8·0	9·5	median	13·0	12·5	14·0	11·5

the assessment of the contingency tables showing the relationship between the stages of bone destruction and the different groups as a statistical support data.

In the retrospective study on the 57 reviewed cases, the changes in stage of bone destruction with years were studied and compared among the S/S, S/N and N/N groups. Reversion of stage of bone destruction was not observed; for example, stage III never recovered to stage I or II. The end stage was stage V which is analogous to 'death' on the statistical life table. We showed the 'life table' of development to stage V.

STATISTICAL ANALYSIS

The Chi-square test was used to determine the statistical significance of the differences in the sex, age and disease duration between the 160 cases examined in the cross sectional survey on the one hand, and the 57 cases selected for the retrospective study on the other; the differences between the RA and healthy subjects in gene frequencies of HLA-DRB1 alleles; and the differences in stage of bone destruction among the S/S, S/N and N/N groups in the contingency tables. The Kaplan-Meier method was used to assess the 'survival' probability that the bone destruction did not progress to stage V,[10] and the significance of the differences in the probability among the three groups was assessed by the log-rank test of Peto.[11]

Table 2 Distribution of genes in both HLA-DRB1 alleles in RA

The S/S group (n = 32)

	1402	*1001*	*0405*	*0404*	*0401*	*0101*
0101	0	0	8	0	1	0
0401	1	0	1	0	1	
0404	0	0	0	0		
0405	0	2	18			
1001	0	0				
1402	0					

The S/N group (n = 78)

	0402/–3/–6/–7 /–9/–10	*0802/3*	*0901*	*1101/2*	*1201/2*	*1301/2*	*1401/3/5*	*1501/2*	*1602*
0101	3	4	5	0	0	0	0	5	0
0401	0	1	1	1	0	1	3	0	
0404	0	0	0	0	0	0	0	0	
0405	3	1	11	2	3	1	8	21	1
1001	0	0	1	0	0	0	0	0	
1402	0	0	1	0	0	0	0	0	

The N/N group (n = 50)

	1602	*1501/2*	*1401/3/5*	*1301/2*	*1201/2*	*1101/2*	*0901*	*0802/3*	*0402/–3/–6/ –7/–9/–10*
0402/–3/–6/ –7/–9/–10	0	3	1	0	1	0	1	2	0
0802/3	0	2	4	2	1	0	5	0	
0901	1	2	0	2	3	1	9		
1101/2	0	0	0	0	2	0			
1201/2	0	0	0	1	0				
1301/2	0	0	0	0					
1401/3/5	1	2	0						
1501/2	0	4							
1602	0								

Results

POPULATIONS OF SUBJECTS

One hundred and sixty patients, 139 women and 21 men, ranging from 23 to 86 years of age (median, 56), were studied for an initial cross sectional study. The duration of disease ranged from 0·5 to 46 years (median, 8·5). The 160 cases were composed of 32 cases in the S/S group, 78 cases in the S/N group and 50 cases in the N/N group (table 2).

Of the 160 cases, 57 cases for which past radiographic records for more than five years were available, were selected for further retrospective study. This group of 57 reviewed cases were not significantly different in the medians and ranges of age and disease duration from the initial group of 160 cases ($p < 0·05$), and consisted of 10 cases in the S/S group, 27 cases in the S/N group and 20 cases in the N/N group (table 1).

DISTRIBUTION OF HLA-DRB1 ALLELES

The gene frequencies of the HLA-DRB1 alleles in patients with RA and healthy Japanese subjects are shown in table 3.

The susceptible factor which was found in a high frequency in patients with RA in this study was HLA-DRB1 0405, although it is also a comparatively common allele in healthy Japanese subjects. The frequency of 0405 was very much higher in our patients with RA than in healthy Japanese subjects ($n = 493$), ($p < 0·01$). The frequencies of the other

Table 3 The gene frequencies of HLA-DRB1 alleles in the patients with RA and healthy Japanese subjects

	Susceptible alleles					
	0101	*0401*	*0404*	*0405*	*1001*	*1402*
RA patients (n = 160)	8·8%	3·1%	0%	31·3%	0·6%	0·6%
Healthy Japanese subjects (n = 493)	5·0%	1·8%	0·7%	12·5%	0·9%	0·5%

	Non-susceptible alleles									
	0402/3/6/7/9/10	*0802/3*	*0901*	*1101/2*	*1201/2*	*1301/2*	*1401/3/5*	*1501/2*	*1602*	*Others*
RA patients (n = 160)	4·7%	6·9%	16·3%	1·9%	3·7%	1·9%	5·4%	14·1%	0·9%	0%
Healthy Japanese subjects (n = 493)	7·5%	11·5%	13·7%	2·2%	5·9%	4·9%	6·9%	16·0%	0·7%	9·3%

susceptible factors (HLA-DRB1 0101,0401, 0404, 1001 and 1402) in the RA patients in this study were separately not high enough to allow significant comparison with healthy Japanese subjects, but when they were added together, the sum was significantly higher than the corresponding sum for healthy Japanese subjects ($p < 0·05$).

RELATIONSHIP BETWEEN BONE DESTRUCTION AND TYPES OF HLA-DRB1

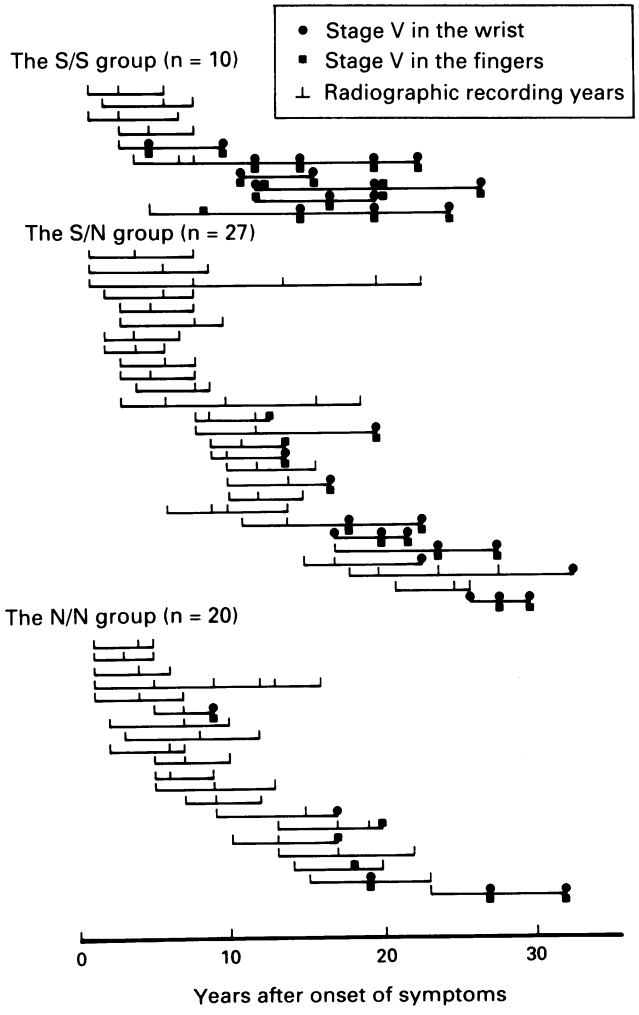
A The cross sectional study

In the scatter diagrams showing the relationship between the stage of bone destruction in the wrists and the duration of RA, the regression lines and their 95% confidence intervals of the three groups are represented in (fig 1).

Both in the wrists and fingers, the 95% confidence interval of the S/S group did not overlap with that of the S/N group in the early phase when bone destruction progressed most rapidly (fig 1-A, C). The 95% confidence intervals of the S/S and N/N groups also did not overlap in the early phase of progression of bone destruction (fig 1-B, D). However, the 95% confidence intervals of the S/N and N/N groups overlapped for the whole range of years after RA onset in both the fingers and the wrists.

As mentioned in the Introduction, bone destruction of wrists and fingers in RA progresses most rapidly within the first decade of the early phase. We therefore constructed the contingency tables of the cases with RA for less than a decade (data not shown). Significant differences in distribution of the stages were observed between the S/S and the S/N groups ($p = 0·0316$ in the wrists, 0·0086 in the fingers) and the S/S and N/N group ($p = 0·0226$ in the wrists, 0·00015 in the fingers), but not between the S/N and N/N group ($p = 0·627$ in the wrists, 0·189 in the fingers).

Table 4 The life tables indicating the observation period and development to stage V in the 57 reviewed cases



B The retrospective study

The life table of development to stage V of the 57 reviewed cases is illustrated (table 4). The observation periods were longer than five years, beginning at various times after onset of the disease, and at least three radiographs taken at different time points were assessed in all cases.

The progression of bone destruction in the fingers in the S/S group was more rapid than in the S/N and N/N groups as shown by the survival probability that the bone destruction did not progress to stage V (fig 2A). In this graph all cases of the S/S group progressed to stage V within 17 years after RA onset. The median years taken to develop to stage V after the onset of symptoms of the fingers determined by the log-rank test were 13·1 for the S/S group, 22·7 for the S/N group and 23·0 for the N/N group. The differences were statistically significant between the S/S and S/N groups and between the S/S and N/N groups (both $p < 0·01$), but not between the S/N and N/N groups ($p > 0·05$).

Similar statistical differences between the respective groups were observed in the bone destruction of the wrists (fig 2B). Thus the

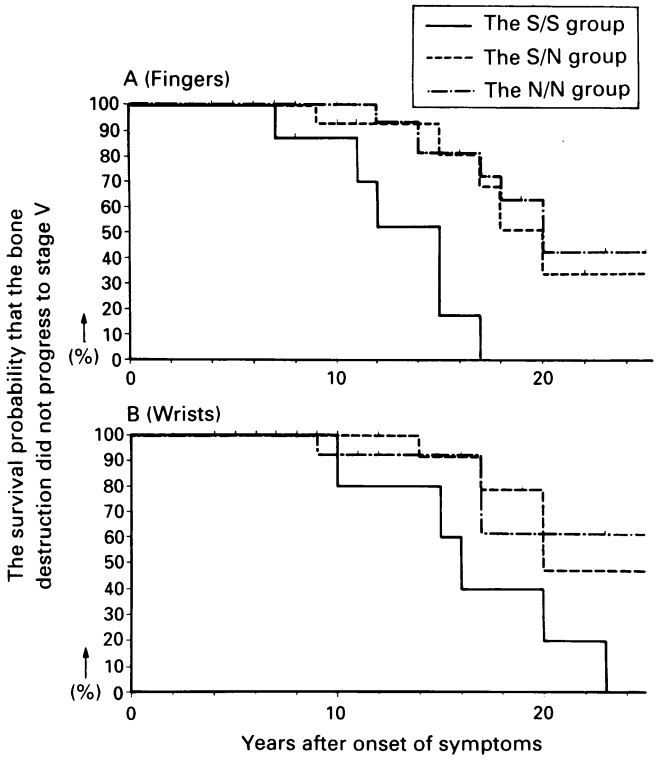


Figure 2 The 'survival' probability that the bone destruction did not progress to stage V based on table 4. A) In the fingers; B) In the wrists.

median years taken to development to stage V in the wrists were 13·1 in the S/S group, 23·1 in the S/N group and 20·3 in the N/N group.

Discussion

Several studies using immunological and serological techniques have revealed that HLA-DR4 antigen is associated with more progressive radiographic bone destruction in patients with RA.[12-13] Recently, genotyping by PCR and oligonucleotide probe techniques has been applied for assessing this association.[3 14] Emery reported that early symmetrical (rheumatoid-like) arthritis patients with the conserved class II major HLA complex genes associated with RA (susceptible factors) were at a relatively high risk for the development of radiographic erosions prospectively.[14]

In our cross sectional and retrospective studies a classification by the number of susceptible factors present in the alleles was used to evaluate the association with progression of bone destruction in patients with RA. Two conclusions emerge from this study, which are closely related; one is that the results of genotyping using this classification can be a useful prognostic marker in the early phase of RA, and the other is that rheumatoid susceptible alleles of HLA-DRB1 are genetically recessive to non-susceptible alleles with respect to the progression of bone destruction in the wrists and fingers.

A HLA-DRB1 alleles as prognostic marker

In our cross sectional study the difference between the S/S group and the S/N group was significant, as was that between the S/S group and the N/N group, but the difference between the S/N group and the N/N group was not significant, in the early phase of development of bone destruction. In the retrospective study the median years taken to development to stage V after the onset of symptoms were significantly smaller in the patients in the S/S group than in the S/N group and in the N/N group. The conclusion from these data is that, when the early phase of seropositive RA is diagnosed in a patient who has two susceptible factors in both HLA-DRB1 alleles, the investigator can predict that bone destruction in the wrists and fingers will advance within a relatively shorter period after the onset of symptoms, than in a patient who has only one or no susceptible factor. He or she should plan the clinical management corresponding to the anticipated course.

The role of rheumatoid factor as a prognostic factor has been examined previously.[15-16] Burns and Calin reported that radiographic bone destruction in the wrists and hands of the seropositive group differed significantly from those of the seronegative group.[16] In this study only seropositive cases were selected to assess the bone destruction change. We are currently trying to assess the relationship between bone destruction and HLA-DRB1 alleles in seronegative cases. But the bone destruction of seronegative cases is not so easy to assess as in seropositive cases, as Burns and Calin have reported. Larsen *et al*[9] also reported that his criteria was very useful for seropositive rheumatoid arthritis, but not for seronegative arthritis.

The merits of genotyping as a means of predicting diagnosis are that the result remains unchanged throughout life and that it can be determined in the early phase of the disease. We believe that the genotyping test holds some promise as a prognostic marker in the early phase of RA.

B Recessiveness of susceptible alleles

The relationship between HLA-DRB1 alleles and the aetiology of RA is still unclear, although several hypotheses have been proposed.[17-18] It is relevant whether the susceptible alleles are dominant over or recessive to the non-susceptible alleles. Wayand *et al* noted that patients with HLA-DR4 homozygosity have a higher risk for more severe disease than the patients with heterozygosity for susceptible and a non-susceptible alleles. However, they did not discuss the dominance or recessiveness of susceptible alleles.[3] Our data clearly indicated that rheumatoid susceptible alleles of HLA-DRB1 are genetically recessive to non-susceptible alleles with respect to the progression of bone destruction in the wrists and fingers. In patients affected by RA for less than a decade, the distribution of the stages were significantly different between the S/S and the S/N group, and also between the S/S and N/N group, but not between the S/N and N/N group, both for the wrists and fingers. The median years taken to develop to stage V in the wrists and fingers were shorter in the S/S group than in the S/N and N/N group, whereas significant differences were not detected between the S/N amd N/N group.

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