Influence of peptidylarginine deiminase type 4 genotype and shared epitope on clinical characteristics and autoantibody profile of rheumatoid arthritis

B Hoppe,[1] T Häupl,[2] K Egerer,[2] R Gruber,[3] H Kiesewetter,[2] A Salama,[2] G R Burmester,[2] T Dörner[2]

[1] Berthold Hoppe, Central Institute of Laboratory Medicine and Pathobiochemistry, Charité – Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany; [2] Charité – Universitätsmedizin Berlin, Berlin, Germany; [3] Ludwig-Maximilians-Universität, München, Germany

Correspondence to:
Dr B Hoppe, Central Institute of Laboratory Medicine and Pathobiochemistry, Charité – Universitätsmedizin Berlin, Campus Virchow-Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany; berthold.hoppe@charite.de

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ABSTRACT

Background: Recent evidence suggests that distinction of subsets of rheumatoid arthritis (RA) depending on anti-cyclic citrullinated peptide antibody (anti-CCP) status may be helpful in distinguishing distinct aetiopathologies and in predicting the course of disease. HLA-DRB1 shared epitope (SE) and peptidylarginine deiminase type 4 (PADI4) genotype, both of which have been implicated in anti-CCP generation, are assumed to be associated with RA.

Objectives: To elucidate whether PADI4 affects the clinical characteristics of RA, and whether it would modulate the effect of anti-CCPs on clinical course. The combined effect of SE and PADI4 on autoantibody profile was also analysed.

Methods: 373 patients with RA were studied. SE, padi4_94C>T, rheumatoid factor, anti-CCPs and anti-nuclear antibodies (ANAs) were determined. Disease severity was characterised by cumulative therapy intensity classified into ordinal categories (CTI-1 to CTI-3) and by Steinbrocker score.

Results: CTI was significantly associated with disease duration, erosive disease, disease activity score (DAS) 28 and anti-CCPs. The association of anti-CCPs with CTI was considerably influenced by padi4_94C>T genotype (C/C: $OR_{adj} = 0.93$, $p_{adj} = 0.92$; C/T: $OR_{adj} = 2.92$, $p_{adj} = 0.093$; T/T: $OR_{adj} = 15.3$, $p_{adj} = 0.002$). Carriage of padi4_94T exhibited a significant trend towards higher Steinbrocker scores in univariate and multivariate analyses. An association of padi4_94C>T with ANAs was observed, with noteworthy differences depending on SE status (SE−: $OR_{adj} = 6.20$, $p_{adj} < 0.04$; SE+: $OR_{adj} = 0.36$, $p_{adj} = 0.02$) and significant heterogeneity between the two SE strata ($p = 0.006$).

Conclusions: PADI4 genotype in combination with anti-CCPs and SE modulates clinical and serological characteristics of RA.

Rheumatoid arthritis (RA) is a common chronic autoimmune disease of unknown aetiology.[1] A central challenge in clinical practice is the identification of patients who need more intensive treatment at an early stage, as many patients develop joint damage during the first few months after disease onset.[2 3] Rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPAs) have been implicated to be associated with more severe joint destruction and a more aggressive disease course.[1 4 5] The major function of HLA-DRB1 shared epitope (SE) in the pathogenesis of RA is thought to primarily consist of giving the appropriate immunological context to respond to environmental challenges with production of ACPAs, which themselves influence disease.[6–9] The association of SE with ACPA-positive RA and its influence on ACPA concentration is well established.[5] Another genetic trait that presumably influences susceptibility to RA is the peptidylarginine deiminase type 4 (PADI4, MIM: 605347) genotype reflected by padi4_94C>T (rs2240340).[10–12] PADI4 post-translationally converts peptide-bound arginine residues into citrulline, and this process generates citrullinated epitopes, which are the essential targets of the RA-specific ACPAs.[5]

In this retrospective study, we analysed the association of padi4_94C>T and anti-citrullinated peptide antibodies (anti-CCPs), a prime example of ACPAs, with disease severity as reflected by cumulative therapy intensity (CTI), which was a priori classified into three ordinal categories.[13] Erosive joint status assessed by Steinbrocker score was used as an additional indicator of disease severity. To reveal possible modulating properties of the PADI4 genotype on the effect of anti-CCPs on disease severity, we stratified analyses by padi4_94C>T. Finally, we analysed the interactive effect of SE and PADI4 genotype on the autoantibody profile.

METHODS

Study population and clinical data

In this study, 373 consecutive patients with RA presenting for regular visits were enrolled from the Department of Rheumatology and Clinical Immunology, Charité Berlin. All patients fulfilled the American College of Rheumatology criteria for the classification of RA.[14] The patients are under continuous medical attendance in our outpatient unit. Individual therapy courses of all enrolled patients were recorded. The CTI was a priori classified into three ordinal categories (CTI-1: methylprednisone, hydroxychloroquine, sulfasalazine; CTI-2: methotrexate, gold, leflunomide, ciclosporin A, azathioprine; CTI-3: cyclophosphamide, tumour necrosis factor α blockers). Patients were classified on the basis of the antirheumatic drug corresponding to the highest CTI category they had ever received. This classification is a local adaptation of an approach described previously for the assessment of RA severity.[13] Radiographic data from the time of enrolment were available in 290

cases. Classification into non-erosive (Steinbrocker score ⩽I) and erosive (Steinbrocker scores II–IV) disease was performed.[15]

The genotype frequencies of padi4_94C>T in a control group consisting of healthy people and non-RA patients from our geographic region were determined in 282 consecutive caucasian blood donors and 335 consecutive patients with no history of autoimmune diseases presenting at our clinic for thrombophilia work-up.[16]

The study was approved by the local ethics committee. All participants were included in the study after they had provided informed consent.

Autoantibody detection

Anti-CCP IgG antibodies were quantified in 346 patients at the time of enrolment with a second-generation ELISA using the Immunoscan RA kit (Euro-Diagnostica, Dahlewitz, Germany). A cut-off value of 25 arbitrary units/ml was used.

ANAs were determined in 354 patients using an indirect immunofluorescence assay on HEp-2 cells with an IgG-specific secondary antibody (Generic Assays, Dahlewitz, Germany). Patients with a titre of ⩾320 were considered ANA positive.

RFs were quantified in 359 patients using an ELISA-based IgM-specific technique and a cut-off value of 24 IU/ml (DLD Diagnostika, Hamburg, Germany).

Genotyping of PADI4 and SE

PADI4 genotypes were discriminated for all patients and controls by padi4_94C>T (rs2240340) using allele-specific primer pairs. Specificities of the primer pairs were evaluated on 30 sequenced DNA samples.[17 18]

SE was defined by HLA-DRB1 alleles with the following constellation at the corresponding DRβ1 chain residues: 67Leu–69Glu–71Lys or Arg–74Ala–86Gly or Val.[19] SE status was available for 215 patients with RA and 282 healthy controls. High-resolution HLA-DRB1 typing was performed using standard techniques (Dynal, Oslo, Norway; GenoVision, Vienna, Austria; Protrans, Ketsch, Germany).[18]

Table 1 Characteristics of the study population (n = 373)

Characteristic	Patients with RA
Age (years)	51 (38–61)
Female	76.1
Disease duration (years)	5 (1–11.3)
Rheumatoid factor (⩾25 IU/ml)	75.8
Titre (IU/ml)	97 (25–277)
Anti-CCPs (>25 AU/ml)	61.3
Titre (AU/ml)	91 (1–568)
Antinuclear antibodies (⩾320)	29.4
Titre	80 (80–320)
Shared epitope	49.3 (SE/se), 13.5 (SE/SE)
padi4_94C>T	49.3 (C/T), 19.8 (T/T)
Steinbrocker score	36.2 (⩽I), 41.4 (II), 14.1 (III), 8.3 (IV)
Disease activity score 28	5.0 (4.03–6.19)
<3.2	15.7
3.2–5.1	32.2
>5.1	52.2
CTI	38.6 (CTI-1), 46.9 (CTI-2), 14.5 (CTI-3)

Values are either percentage or median (interquartile range).
Anti-CCP, anti-cyclic citrullinated peptide; CTI, cumulative therapy intensity; SE/se, 1 copy of shared epitope; SE/SE, 2 copies of shared epitope; C/T, padi4_94C/T (variant heterozygous); T/T, padi4_94T/T (variant homozygous).

Statistical analysis

For univariate analyses, odds ratios (ORs) and exact 95% CIs were calculated. For stratified univariate analyses, Breslow and Day's (BD) χ^2 test of homogeneity was used to test whether an association differed significantly across the two strata. For calculations on the effect of allele dose of padi4_94C>T or SE genotype, the corresponding wild-type was used as the reference group, and a test for linear trend of the log odds (trend test) was included.

For multivariate analyses, ORs and 95% CIs were calculated by logistic regression analyses with adjustment for sex, duration of disease, disease activity score (DAS)28 and erosive joint status or CTI. For analyses on autoantibody profile, sex, duration of disease, DAS28, CTI and presence of RFs, anti-CCPs and ANAs were included. Depending on the variable used for stratification (SE or padi4_94C>T), the logistic regression model contained padi4_94C>T or SE genotype, respectively. The Wald test was used to test whether an association differed significantly across the two stratified logistic regression models. The comparisons of autoantibody concentrations between PADI4 and SE genotypes were performed by Mann–Whitney U test or, in the case of testing for allele-dose-dependency, by the Cuzick non-parametric test for trend. All statistical analyses were performed using Stata Statistical Software for Macintosh, release 10.0.

RESULTS

Characteristics of the study population

Table 1 gives the characteristics of the RA population.

The controls consisted of healthy individuals and non-RA patients. In healthy individuals, 62.1% were carriers of padi4_94T (1 copy, 47.9%; 2 copies, 14.2%). SE was present in 35.8% of healthy controls (1 copy, 28.7%; 2 copies, 7.1%). In the non-RA patients, 61.8% were carriers of padi4_94T (1 copy, 47.2%; 2 copies, 14.6%). There was no heterogeneity in the distribution of PADI4 genotypes between either part of the control group (p = 0.98).

PADI4 genotype and RA

Carriage of padi4_94T was associated with susceptibility to RA (OR = 1.38, p = 0.02). This association was allele-dose-dependent (trend test, p = 0.005; 1 copy: OR = 1.28, p = 0.09; 2 copies: OR = 1.70, p = 0.006). As the padi4_94C>T genotype distribution in our patients with RA was dependent on CTI and Steinbrocker score, we tested whether association of padi4_94C>T with susceptibility to RA would be restricted to patients with more severe disease. In fact, whereas there were no significant differences in PADI4 genotype distributions in patients with CTI-1 or Steinbrocker scores <II compared with controls (trend test, p = 0.82 and p = 0.24, respectively), in patients with higher disease severity, ie, CTI-2/3 (CTI-2/3 versus controls: trend test, p = 0.0003; 1 copy: OR = 1.47, p = 0.03; 2 copies: OR = 2.26, p = 0.0002) or Steinbrocker score ⩾II (Steinbrocker score ⩾II versus controls: trend test, p = 0.0005; 1 copy: OR = 1.29, p = 0.20; 2 copies: OR = 2.34, p = 0.0003), padi4_94C>T was significantly associated with RA.

Factors influencing CTI

In univariate and multivariate analyses, duration of disease, presence of erosive disease, anti-CCP status and DAS28 were significantly associated with CTI (CTI-2/3 versus CTI-1) (table 2). An association of padi4_94C>T carrier status with CTI could be demonstrated only in univariate analysis (table 2).

Table 2 Factors influencing disease severity as reflected by cumulative therapy intensity (CTI-2/3 versus CTI-1)

	OR (p value)	Adjusted OR (p value)
Duration of disease (quartiles) (n = 356)	$<10^{-26}$	<0.001
Erosive disease (n = 290)	4.50 ($<10^{-8}$)	2.14 (0.02)
Disease activity score (DAS) 28 (n = 370)	($<10^{-5}$)	(0.003)
<3.2 (n = 58)	1 (referent)	1 (referent)
3.2–5.1 (n = 119)	0.46 (0.05)	0.29 (<0.04)
>5.1 (n = 193)	0.21 (10^{-5})	0.18 (0.003)
Anti-CCP (n = 346)	1.69 (<0.02)	2.32 (<0.02)
padi4_94C/C (n = 105)	1.10 (0.81)	0.93 (0.92)
padi4_94T (n = 241)	2.00 (0.01)	3.46 (0.007)
padi4_94C (n = 276)	1.31 (0.28)*	1.78 (0.18)†
padi4_94T/T (n = 70)	5.20 (0.002)*	15.3 (0.002)†
padi4_94T (n = 373)	1.57 (<0.05)	1.22 (0.57)

Odds ratios (ORs) and p values of univariate and multivariate analyses (adjusted for sex, age, duration of disease, DAS28 and erosive joint status if applicable) are given. Numbers of patients are indicated.
*BD χ^2 test of homogeneity: p = 0.02.
†Wald test: p<0.02.

The presence of neither RFs, ANAs nor SEs directly influenced CTI. With respect to the relation between anti-CCPs and CTI, a diverging effect was found after stratification for padi4_94C>T, with restriction of the association of anti-CCPs with CTI to the padi4_94T positive stratum (OR = 2.00, p = 0.01) (table 2). The difference in the effect of anti-CCPs on CTI between the two padi4_94C>T strata in univariate analysis did not reach statistical significance (BD χ^2 test of homogeneity, p = 0.21). However, in strata defined by padi4_94C, ie, carriage of at least one padi4_94C (wild-type) allele, and padi4_94T/T (two padi4_94T copies), anti-CCPs were significantly associated with CTI only in the padi4_94T/T stratum (OR = 5.2, p = 0.002) with significant heterogeneity between the two strata (BD χ^2 test of homogeneity, p = 0.02) (table 2). Logistic regression analyses confirmed that anti-CCPs were independently associated with CTI only in padi4_94T-positive patients (padi4_94T: $OR_{adj} = 3.46$, $p_{adj} = 0.007$; padi4_94T/T: $OR_{adj} = 15.3$, $p_{adj} = 0.002$) (table 2).

On comparison of padi4_94T carrier status in all CTI categories, among anti-CCP-positive patients the frequency of padi4_94T carriers increased significantly from 63.0% in CTI-1 to 73.8% and 88.9% for CTI-2 and CTI-3, respectively (trend test, p = 0.004; CTI-2: OR = 1.65, p = 0.13; CTI-3: OR = 4.70, p = 0.005 (reference: CTI-1)) (fig 1A). In anti-CCP-negative patients, the frequency of padi4_94T carriers was essentially the same as that of the control group for all CTI categories (CTI-1: 63.5%; CTI-2: 67.2%; CTI-3: 61.5%) (fig 1B).

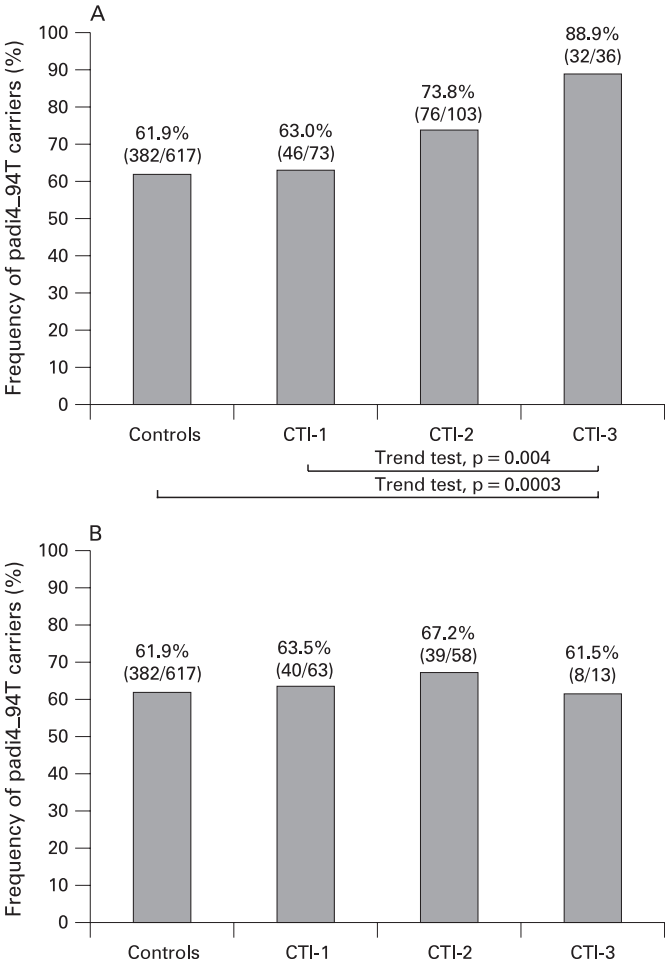

Figure 1 Influence of padi4_94C>T on cumulative therapy intensity (CTI) dependent on the antibody to cyclic citrullinated peptide (anti-CCP) status. Carrier frequencies of padi4_94T in controls and in (A) anti-CCP-positive and (B) anti-CCP-negative patients with rheumatoid arthritis ordinally categorised by CTI (CTI-1 to CTI-3) are shown.

Factors influencing erosive joint status

Duration of disease ($p<10^{-9}$, $p_{adj}<0.0001$) and CTI (OR = 4.50, $p<10^{-8}$; $OR_{adj} = 2.05$, $p_{adj} = 0.03$) influenced directly erosive joint status (Steinbrocker score ⩾II) in univariate and multivariate analyses. The presence of neither anti-CCPs (OR = 1.45, p = 0.15), RFs, ANAs, SE, DAS28, nor padi4_94T were significantly associated with the presence of erosive disease (Steinbrocker score ⩾II). When padi4_94T carrier status was analysed in ordinal categories defined by Steinbrocker scores, a trend towards higher padi4_94T carrier frequencies with increasing Steinbrocker scores could be detected in our study population (trend test, p = 0.06 (reference: Steinbrocker score ⩽I) and p<0.004 (reference: controls)) (fig 2A). When carriage of two padi4_94T copies (padi4_94T/T) was considered, the increasing frequency of carriers with increasing Steinbrocker scores was even more pronounced (trend test, p<0.003 (reference: Steinbrocker score ⩽I) and $p<10^{-5}$ (reference: controls)) (fig 2B). After adjustment for possible confounding factors (sex, age, duration of disease, DAS28, CTI, serological status), the trend for higher padi4_94T and padi4_94T/T frequencies with increasing Steinbrocker scores continued to be detectable at comparable statistical significance levels (padi4_94T: $p_{adj}<0.004$; padi4_94T/T: $p_{adj} = 0.008$ (reference: Steinbrocker score ⩽I)) (fig 2).

Factors influencing autoantibody profile

The combined influence of SE and padi4_94C>T on autoantibody profile was analysed in 215 patients, who had been completely characterised for both genotypes. Presence of anti-CCPs and the anti-CCP concentration were clearly associated with the presence of SE (OR = 2.93; p = 0.0002, Mann–Whitney U test: p<0.0001) and number of SE alleles (trend test: p = 0.0001). Presence of anti-CCPs was significantly influenced by neither carriage of padi4_94T (OR = 1.43, p = 0.13) nor padi4_94T allele dose (1 copy: OR = 1.32, p = 0.27; 2 copies: OR = 1.77, p = 0.08; trend test: p = 0.07 (reference: padi4_94C/C)). In univariate analyses, no further association between presence of anti-CCPs, RFs or ANAs and a single genotype (SE or

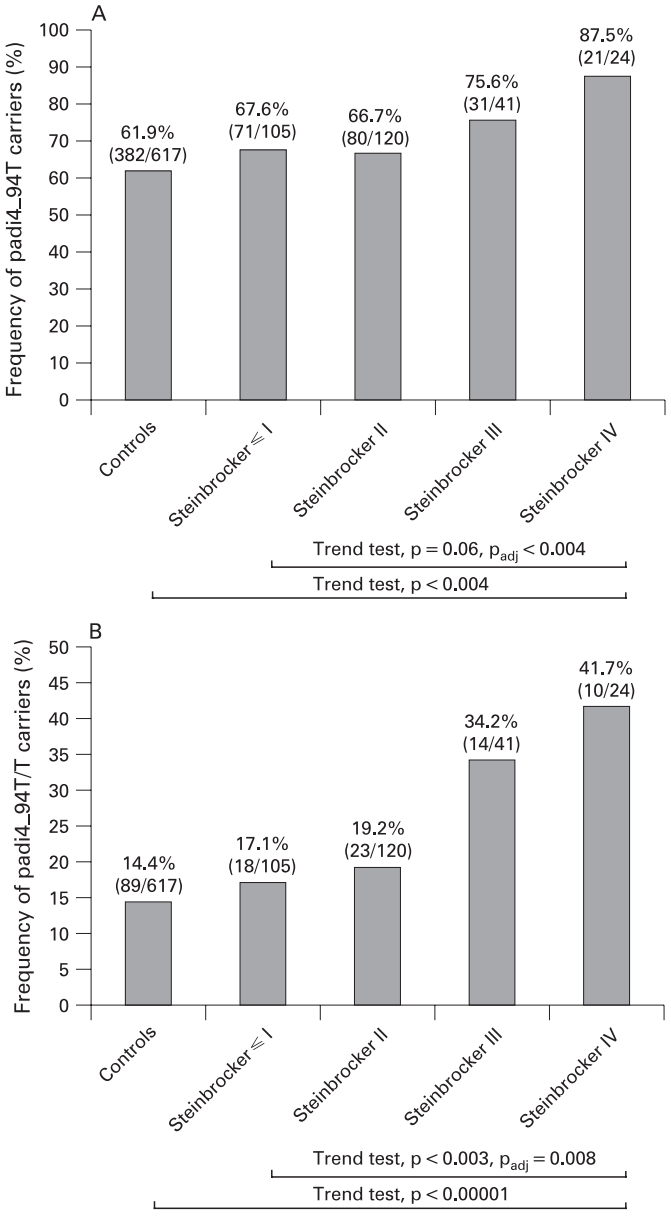

Figure 2 Influence of padi4_94C>T on erosive joint status. Frequencies of carriers of (A) one padi4_94T copy (padi4_94T) and (B) two padi4_94T copies (padi4_94T/T) in controls and patients with RA ordinally categorised by Steinbrocker score (⩽I, II, III, IV) are shown. For analyses based on Steinbrocker score ⩽I as reference group p values after adjustment for sex, age, duration of disease, disease activity score 28, cumulative therapy intensity and serological status are given.

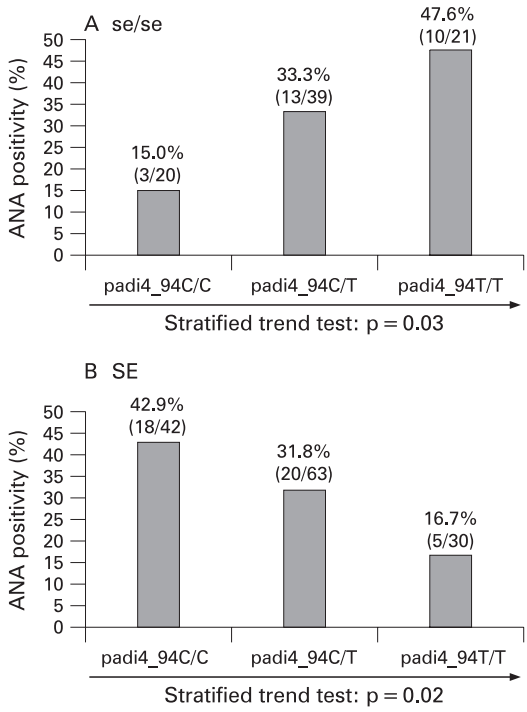

Figure 3 Presence of antinuclear antibodies (ANAs) is interactively dependent on padi4_94C>T genotype and shared epitope. Frequencies of ANA-positive (titre ⩾320) patients with RA in dependency on padi4_94C>T genotype and presence (SE) or absence (se/se) of shared epitope are given. There was significant (p = 0.001) heterogeneity between the two strata with respect to the relation between padi4_94C>T and ANA positivity.

padi4_94C>T) could be identified. However, after stratification for SE, the influence of padi4_94C>T genotype on autoantibody profile was notable (fig 3). In univariate and multivariate analyses, significant heterogeneity existed between SE-positive and SE-negative patients with respect to association of padi4_94C>T genotype with ANAs (test of homogeneity, $p<0.01$ and $p_{adj} = 0.006$, respectively), with positive association in the SE-negative stratum and negative association in the SE-positive stratum (table 3).

The same phenomenon was observed when padi4_94T allele dose was considered. In SE-positive patients, the number of padi4_94T alleles was negatively associated with ANAs (trend test, $p<0.02$), and in SE-negative patients there was a positive association (trend test, $p<0.03$) when padi4_94C/C was used as the reference group (fig 3). The heterogeneity of this allele-dose-dependent effect between the two SE strata was highly significant in univariate ($p = 0.001$) and multivariate ($p_{adj} = 0.0002$) analyses (table 3).

DISCUSSION

The results of this study suggest that the PADI4 genotype critically modulates the effect of anti-CCPs on clinical characteristics of RA. The PADI4 genotype itself appears to influence joint destruction. Interactively, PADI4 genotype and SE influence the autoantibody profile of RA. Hence, it could be hypothesised that information on the two genetic traits characterise different RA subsets with distinct aetiopathogeneses. Finally, an association of padi4_94C>T with susceptibility for RA was confirmed, and, on the basis of the aforementioned associations of PADI4 genotype with clinical RA characteristics, possible explanations for the heterogeneity of previous association studies assessing PADI4 in RA may be delineated.

In this study, we tested the hypothesis that PADI4 genotype influences the clinical course of RA directly or dependent on the presence of anti-CCPs. As our study population consisted predominantly of patients with established disease, we primarily used CTI as a marker of disease severity as previously suggested.[13] CTI represents one major confounder of the erosive joint status itself, as the therapeutic intensity significantly influences joint destruction.[20] The strong and highly significant association of erosive disease with CTI in univariate and multivariate analyses corroborates the validity of CTI as an indicator of disease severity. Moreover, a highly significant negative association between CTI and DAS28 was observed (table 2). This association is in line with previous reports

Table 3 Interactive influence of shared epitope and padi4_94C>T genotype on presence of antinuclear antibodies

Shared epitope	padi4_94C>T	OR (p value)	p*	Adjusted OR (p value)	p*
se/se	C/C	1.0 (referent)	<0.01	1.0 (referent)	0.006
se/se	T	3.52 (0.05)		6.20 (<0.04)	
SE	C/C	1.0 (referent)		1.0 (referent)	
SE	T	0.49 (0.07)		0.36 (<0.02)	
se/se	C/C	1.0 (referent)	0.001	1.0 (referent)	0.0002
se/se	C/T	2.83 (0.14)		4.71 (0.09)	
se/se	T/T	5.15 (<0.03)		9.58 (<0.02)	
SE	C/C	1.0 (referent)		1.0 (referent)	
SE	C/T	0.62 (0.25)		0.50 (0.13)	
SE	T/T	0.27 (<0.02)		0.16 (0.004)	

Odds ratios (ORs) and p values of univariate and multivariate analyses (adjusted for sex, antibody to cyclic citrullinated peptide, rheumatoid factor, disease duration, disease activity score 28, cumulative therapy intensity) are given. se/se, shared epitope negative; SE, shared epitope positive.
*p values for test of homogeneity across SE strata.

describing significantly more pronounced DAS reductions with more intensive treatment strategies.[21 22] DAS28 reflects current disease activity and the underlying disease biology, but it is also influenced by the therapeutic approaches. The fact that CTI is associated with erosive disease and related to DAS28 underscores the value of this disease activity tool. Considering our primary hypothesis, we revealed a strong modulating effect of padi4_94C>T genotype on the anti-CCP-mediated influence on CTI (table 2). The association of anti-CCPs with CTI was restricted to carriers of padi4_94T. From a different perspective, in anti-CCP-negative patients, there was no significant association of padi4_94C>T genotype with CTI (fig 1B), but, in anti-CCP-positive patients, the proportion of padi4_94T carriers increased progressively with increasing CTI categories (trend test, $p = 0.004$) (fig 1A). When this phenomenon was analysed in relation to padi4_94T copy number, there was an increase in anti-CCP-positive patients with high therapy intensity (CTI-2/3) from 53.5% for padi4_94C/C (wild-type) to 65.1% and 81.3% for the presence of one and two padi4_94T alleles, respectively (trend test, $p<0.003$) (data not shown). The categorisation of CTI, which we defined a priori as a local adaptation of the one described previously, is of course arbitrary. Moreover, this measure of disease severity is difficult to standardise. However, post hoc analyses based on CTI categories, which exactly reflect those previously described, did not substantially alter the results.[13] A modulating effect of padi4_94C>T genotype on anti-CCP-mediated severity of disease can be explained in several ways. The stability of mRNA derived from PADI4 susceptibility genotypes is increased, and high concentrations of PADI4 in inflamed synovium are specific to patients with RA.[12 23] Thus, an increased amount of intra-articular immune complexes may increase disease severity in anti-CCP-positive carriers of padi4_94T. Differences in responsiveness to anti-rheumatic drugs may be another explanation for this finding.

The second major finding of this study is the significant trend towards higher padi4_94T carrier frequency with increasing Steinbrocker score (fig 2), although padi4_94C>T itself was not associated with erosive disease defined as Steinbrocker score ⩾II. Thus, PADI4 genotype was associated with more intense joint destruction corresponding to Steinbrocker scores III and IV. Interestingly, the padi4_94T carrier frequencies of patients with RA with the lowest CTI (CTI-1, 63.0%) or non-erosive disease (Steinbrocker score ⩽I, 67.6%) are nearly exactly the same as in our controls (61.9%) (figs 1 and 2). This finding may help us to understand discrepant results from different association studies performed on PADI4 genotype and susceptibility to RA[10 11] because the disease severity of the study population appears to influence significantly the padi4_94C>T genotype distribution. In the present study, padi4_94T carrier status and padi4_94T allele dose were significantly associated with susceptibility to RA when a case–control analysis was performed (OR = 1.38, $p = 0.02$ and trend test, $p = 0.005$, respectively). This association of padi4_94T with RA was restricted to patients with higher CTI (CTI-2/3) or erosive disease (Steinbrocker score ⩾II). Thus, study populations from tertiary care units consisting of patients with RA with longstanding disease and long therapeutic histories could be positively selected for so-called susceptibility genotypes of PADI4.

Anti-CCP presence is a well-established risk factor for erosive disease.[24 25] The lack of association of anti-CCP presence with erosive disease in our study population needs consideration. The predictive value of anti-CCPs with respect to joint destruction seems to depend on the treatment strategy, as, in a recent study, progressive disease could only be predicted by anti-CCPs in those patients treated with sequential monotherapy.[26] As a step-up combination therapy approach was predominantly chosen in our patients, the effect of anti-CCPs on joint destruction may have been attenuated. The current data mandate a prospective study, which should not only address the genetic and serological profile but also take advantage of more sensitive methods for evaluating radiological changes.

Finally, the interactive influence of padi4_94C>T and SE on ANAs should be addressed (table 3). The highly heterogeneous association of padi4_94C>T genotype with ANAs between the two SE strata is an unexpected finding. However, as this phenomenon was found for padi4_94T carrier status as well as in a marked padi4_94C>T allele-dose-dependent fashion, this finding appears to be conclusive. However, despite the high level of significance for the testing for heterogeneity between the two SE strata, further studies should be carried out to confirm this finding. It could be argued that SE contributes to susceptibility to RA primarily by mediating anti-CCP generation, and that, in SE-negative RA, alternative pathogenetic pathways are involved, which may be promoted by padi4_94T possibly by generating autoantibodies predominantly reactive with nuclear antigens.

Several limitations of our study should be discussed. The main findings are based on retrospective case-only analyses. Thus, biases due to the selection of an appropriate control group can be excluded.[27] Nevertheless, confounding due to possible selective referral of patients has to be kept in mind. The size of

the study population does not allow a detailed sub-stratification, which would help us to completely understand the interactive effects of SE and padi4_94C>T on autoantibody profile and the conjoint influence of all three variables on clinical course of RA. Moreover, data on environmental RA risk factors in our study population are missing, which may have helped to elucidate gene–environment interactions.

Nevertheless, the data presented suggest that combined information on PADI4 genotype and anti-CCP status may represent key data for defining signatures of RA subsets with different clinical courses. Moreover, the interactive influence of PADI4 genotype and SE on the generation of differentiated autoantibody profiles may provide further evidence for the existence of different pathogenetic pathways in RA.

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B Hoppe, T Häupl, K Egerer, et al.

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