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CLINICAL SCIENCE

Determining in which pre-arthritis stage HLA-shared epitope alleles and smoking exert their effect on the development of rheumatoid arthritis

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Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2021-220546).

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Received 12 April 2021
Accepted 7 July 2021
Published Online First 20 July 2021

To cite: Wouters F, Maurits MP, van Boheemen L, *et al*. *Ann Rheum Dis* 2022;**81**:48–55.

ABSTRACT

Objectives The human leukocyte antigen-shared epitope (HLA-SE) alleles and smoking are the most prominent genetic and environmental risk factors for rheumatoid arthritis (RA). However, at which pre-arthritis stage (asymptomatic/symptomatic) they exert their effect is unknown. We aimed to determine whether HLA-SE and smoking are involved in the onset of autoantibody positivity, symptoms (clinically suspect arthralgia (CSA)) and/or progression to clinical arthritis.

Methods We performed meta-analyses on results from the literature on associations of HLA-SE and smoking with anti-citrullinated protein antibodies (ACPAs) in the asymptomatic population. Next, we studied associations of HLA-SE and smoking with autoantibody positivity at CSA onset and with progression to clinical inflammatory arthritis (IA) during follow-up. Associations in ACPA-positive patients with CSA were validated in meta-analyses with other arthralgia cohorts. Analyses were repeated for rheumatoid factor (RF), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA).

Results Meta-analyses showed that HLA-SE is not associated with ACPA positivity in the asymptomatic population (OR 1.06 (95% CI:0.69 to 1.64)), whereas smoking was associated (OR 1.37 (95% CI: 1.15 to 1.63)). At CSA onset, both HLA-SE and smoking associated with ACPA positivity (OR 2.08 (95% CI: 1.24 to 3.49), OR 2.41 (95% CI: 1.31 to 4.43)). During follow-up, HLA-SE associated with IA development (HR 1.86 (95% CI: 1.23 to 2.82)), in contrast to smoking. This was confirmed in meta-analyses in ACPA-positive arthralgia (HR 1.52 (95% CI: 1.08 to 2.15)). HLA-SE and smoking were not associated with RF, anti-CarP or AAPA-positivity at CSA onset. Longitudinally, AAPA associated with IA development independent from ACPA and RF (HR 1.79 (95% CI: 1.02 to 3.16)), anti-CarP did not.

Conclusions HLA-SE and smoking act at different stages: smoking confers risk for ACPA and symptom development, whereas HLA-SE mediates symptom and IA development. These data enhance the understanding of the timing of the key risk factors in the development of RA.

Key messages

What is already known about this subject?

- The HLA-shared epitope (HLA-SE) and smoking are the most important genetic and environmental risk factors for rheumatoid arthritis (RA), particularly for anti-citrullinated protein antibody (ACPA)-positive RA. It is unknown at which pre-arthritis stage HLA-SE and smoking exert their effect.

What does this study add?

- HLA-SE and smoking act at different pre-RA stages.
- Smoking confers risk for the development of ACPA and symptoms, whereas HLA-SE mediates symptom and arthritis development.

How might this impact on clinical practice or future developments?

- This study enhances the understanding of the timing of HLA-SE and smoking in the development of RA. This knowledge can guide pathophysiological studies seeking to determine the mechanisms in the trajectories leading to RA.
- The results could guide health-promoting behaviours: current results imply that smoking cessation can be helpful in preventing RA development especially in the asymptomatic phase, while this might be less effective in preventing RA in the symptomatic phase.

INTRODUCTION

The human leukocyte antigen-shared epitope (HLA-SE) is the most well-known and strongest genetic risk factor for the development of rheumatoid arthritis (RA), especially for anti-citrullinated protein antibody (ACPA)-positive RA.[1–14] Similarly, smoking is the strongest environmental risk factor for autoantibody-positive RA[2 9 10 12 15]; multiple studies have shown this effect is mostly present in people carrying HLA-SE alleles.[1 3 5 6 8 14 16] This knowledge is mostly obtained from case–control studies comparing patients with RA and healthy

controls. During the last decade, research attention has shifted to the stages that precede clinical arthritis and RA and several pre-RA stages have been discerned. However, so far it remains undetermined at which stage(s) HLA-SE alleles and smoking exert their effect.

The following stages are distinguished. An asymptomatic stage in which autoimmune responses can develop, resulting in autoantibody positivity. Then, autoimmune responses can mature and a symptomatic stage develops. The pattern of symptoms that is considered specific for an increased risk of RA is called clinically suspect arthralgia (CSA). Patients with CSA can progress to clinically apparent inflammatory arthritis (IA), the stage when RA is generally diagnosed.[17] This model suggests that genetic factors exert their influence first, followed by smoking with subsequent autoantibody development.[17 18] However, this time order has never been shown.

In addition to a nested case–control study,[19] several longitudinal studies assessed genetic factors and/or smoking and provided data either from healthy to IA but not the intermediate stages or from mixed populations of asymptomatic and symptomatic people.[20–24] These approaches do not allow determination of stage-dependent effects. As for the asymptomatic stage, contrasting findings are reported on associations between HLA-SE alleles and smoking and the presence of ACPA in the general population.[2 14 25–28] To the best of our knowledge, only one study evaluated the effect of smoking on the progression from ACPA positivity to CSA.[29] Furthermore, longitudinal studies within arthralgia are scarce and their findings varied.[30 31] The mentioned studies focused on ACPA; however, HLA-SE and smoking might also interact with other autoantibodies such as rheumatoid factor (RF), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA), the time effects of which have not yet been studied.

We aimed to determine at which pre-RA stage HLA-SE and smoking exert their effect by studying both original and previously reported data. More specifically, we performed meta-analyses on the literature from the general population, analysed our own data at CSA onset and during progression to IA and finally performed meta-analyses using data from different longitudinal arthralgia cohorts. In doing this, we focused on fine staging the effects in the development of ACPA-positive RA. Analyses were repeated for ACPA-negative RA and associations of RF, anti-CarP and AAPA.

METHODS

Summarising the literature obtained from the general population

The literature was reviewed on studies reporting associations between HLA-SE and/or smoking with the presence of ACPA in the asymptomatic population, as described supplementary. Results were pooled in meta-analyses. Although these studies were cross-sectional in nature, observed findings were considered to reflect the influence of HLA-SE/smoking on ACPA development, as this is most likely the first event in the development of ACPA-positive RA.

The symptomatic phase

Associations of HLA-SE and smoking with autoantibodies at CSA onset were investigated in the Leiden CSA cohort, we did not identify large cohorts for validation since most arthralgia cohorts did not include autoantibody-negative patients. Additionally, the role of HLA-SE and smoking in progression from arthralgia to IA was investigated in the Leiden CSA cohort. Results obtained in the ACPA-positive subgroup were validated in ACPA-positive arthralgia/at-risk patients from two independent cohorts (Amsterdam, Leeds).

Measurements at CSA onset

Patients presenting with CSA to the Leiden rheumatology outpatient clinic between April 2012 and September 2019 were studied. As described in detail previously,[32] patients had recent-onset (<1 year) arthralgia of small joints and were, according to the clinical expertise and pattern recognition of the rheumatologist, at risk for progression to RA. Patients were excluded if clinical arthritis was already present or if a different explanation for the joint pain was more likely. At baseline smoking status (present/past/never) was obtained through questionnaires. The presence of IgM RF (in-house ELISA, cut-off >3.5 IU/mL) and IgG ACPA (anti-cyclic citrullinated peptide 2 (anti-CCP2), Phadia, Nieuwegein, the Netherlands, cut-off >7 IU/mL) was determined during routine laboratory measurements in all patients and the presence of IgG anti-CarP and IgG AAPA was determined with in-house ELISA in a subset of patients. Detailed methods are described in online supplemental material. The HLA-SE alleles were extracted from whole-genome sequencing data; the HLA region was isolated and imputed using the SNP2HLA software and T1DGC reference panel.[33] HLA-SE positivity was subsequently defined as the presence of one or two of the HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408 and *1001 (see online supplemental material).[34]

Measurements on the progression from CSA to IA

Patients in the Leiden CSA cohort were prospectively followed (median (IQR) 106 weeks (43–114)) for the development of IA, which was defined as ≥1 swollen joints at physical examination by a rheumatologist. Treatment with disease-modifying antirheumatic drugs (including systemic or intra-articular corticosteroids) was not allowed before IA development. Analyses evaluating progression to IA were stratified for ACPA status and results from the ACPA-positive subgroup were studied in meta-analyses with the results from ACPA-positive patients included in the Amsterdam and Leeds cohorts. The Amsterdam cohort included ACPA-positive and/or RF-positive patients; for this study, the data from patients with ACPA-positive arthralgia were obtained and studied.[31] Data on smoking history, presence of HLA-SE, RF, ACPA and anti-CarP were collected previously and are described in online supplemental material. In addition, IgG AAPA was determined in baseline serum samples simultaneous with Leiden CSA samples. Results on predictive value of HLA-SE and smoking in ACPA-positive patients from the Leeds cohort were obtained from Rakieh *et al*,[30] detailed methods are described in online supplemental material. Anti-CarP and AAPA were not determined in the Leeds cohort.

In subanalyses, the association of HLA-SE and smoking with RA development was studied using Leiden CSA data; RA was defined as the development of IA plus fulfilment of the 1987 and/or 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria at that time.[35 36]

Statistics

Results from the literature on associations of HLA-SE and smoking with ACPA in the asymptomatic population were pooled in inverse-variance weighted meta-analyses.

Associations of HLA-SE and smoking with autoantibody positivity at CSA onset were investigated with logistic regression analyses. Results of smoking were also stratified for HLA-SE.

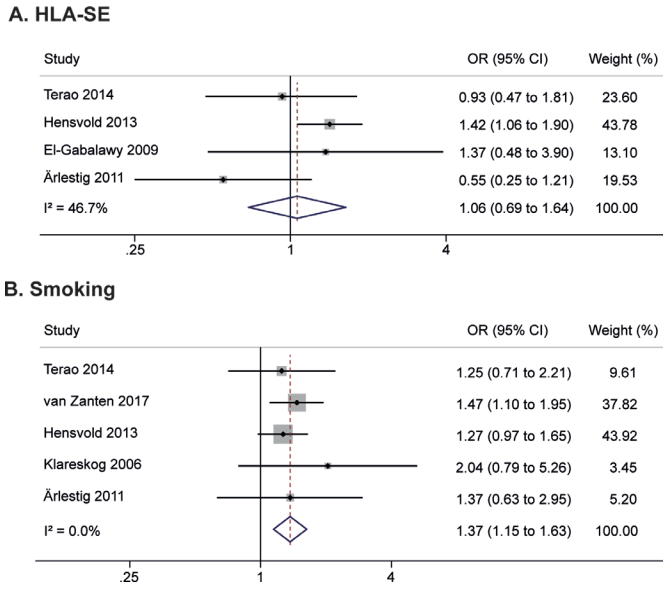

Figure 1 Meta-analyses on HLA-SE (A) and smoking (B) in asymptomatic healthy individuals and first-degree relatives, showing associations with the presence of ACPA for smoking but not for HLA-SE. ACPA, anti-citrullinated protein antibody; HLA-SE, human leukocyte antigen-shared epitope.

Associations of HLA-SE and smoking with ACPA level in ACPA-positive patients were evaluated with Mann-Whitney U tests and logistic regression.

Associations with IA development were studied with Cox regression, also stratified for ACPA. Results in ACPA-positive arthralgia were summarised in inverse-variance weighted meta-analyses.

Associations of anti-CarP and AAPA with IA development were corrected for concomitant ACPA and RF positivity in multivariable analyses with the autoantibody-negative group as reference in the Leiden data (the Amsterdam cohort did not include autoantibody-negative patients). The additional value of anti-CarP and AAPA to ACPA and RF positivity for prediction of IA development was determined in the ACPA+RF+ subgroup from the Leiden and Amsterdam cohorts.

P values<0.05 were considered statistically significant. IBM SPSS Statistics (V.25) and STATA (V.16) were used.

RESULTS

Summarising the literature obtained from the asymptomatic stage

Four studies were identified on the association of HLA-SE with ACPA and five on smoking (online supplemental file 1). Meta-analyses revealed that HLA-SE was not associated with ACPA positivity (OR 1.06 (95% CI: 0.69 to 1.64)), whereas smoking was associated (OR 1.37 (95% CI: 1.15 to 1.63)), figure 1. This suggests that smoking, but not HLA-SE, conferred risk for ACPA development in the asymptomatic stage.

Associations with ACPA at CSA onset

Characteristics of patients presenting with CSA (n=577) are provided in the online supplemental materials. HLA-SE-positive patients with CSA were more often ACPA positive (OR 2.08 (95% CI: 1.24 to 3.49), this relation was dependent on the number of alleles (table 1). Patients who smoked were also more often ACPA positive (OR 2.41 (95% CI: 1.31 to 4.43)), which was also dose dependent with a higher OR for current smokers than ex-smokers (table 1). In addition, within smokers, it was dependent on number of packyears, because the odds for being ACPA positive increased per increase in packyear (OR 1.03 (95% CI: 1.00 to 1.06)). As it has been reported in RA that the association of smoking is dependent on HLA-SE status, we stratified the analyses of smoking (ever vs never) for HLA-SE; smoking was associated with ACPA status in both HLA-SE-negative and HLA-SE-positive patients with CSA (table 1). The association of HLA-SE and smoking with ACPA positivity was present for both ACPA double positivity (ACPA+RF+) and single positivity (ACPA+RF−), and thus independent from RF (online supplemental table 2). Studying the levels of ACPA within ACPA-positive patients at CSA onset revealed that HLA-SE-positive patients tended to have higher levels than HLA-SE-negative patients (median (IQR) 236 (72–340) vs 144 (32–340), p=0.12), while no effect on ACPA levels was present for smoking (229 (64–340) vs 222 (52–340), p=0.89), see online supplemental table 3 for results from regression analyses.

Table 1 Associations of HLA-SE and smoking with the presence of ACPA in patients newly presenting with CSA

	ACPA positive, n (%)	ACPA negative, n (%)	OR (95% CI)	P value
All patients				
HLA-SE				
Absent	27 (39)	259 (57)	Reference	
Present	42 (61)	194 (43)	2.08 (1.24 to 3.49)	0.006
HLA-SE				
0	27 (39)	259 (57)	Reference	–
1	31 (45)	161 (36)	1.85 (1.06 to 3.21)	0.029
2	11 (16)	33 (7)	3.20 (1.45 to 7.04)	0.004
Smoking				
Never	15 (23)	185 (42)	Reference	–
Ever	49 (77)	251 (58)	2.41 (1.31 to 4.43)	0.005
Smoking				
Never	15 (23)	185 (42)	Reference	–
Ex-smoker	28 (44)	161 (37)	2.15 (1.12 to 4.16)	0.024
Current smoker	21 (33)	90 (21)	2.88 (1.42 to 5.85)	0.003
HLA-SE-positive subgroup				
Smoking				
Never	10 (27)	77 (45)	Reference	–
Ever	27 (73)	95 (55)	2.19 (1.00 to 4.80)	0.051
Smoking				
Never	10 (27)	77 (45)	Reference	–
Ex-smoker	13 (35)	57 (33)	1.76 (0.72 to 4.29)	0.22
Current smoker	14 (38)	38 (22)	2.84 (1.15 to 6.98)	0.023
HLA-SE-negative subgroup				
Smoking				
Never	4 (18)	99 (43)	Reference	–
Ever	18 (82)	130 (57)	3.43 (1.12 to 10.45)	0.030
Smoking				
Never	4 (18)	99 (43)	Reference	–
Ex-smoker	11 (50)	89 (39)	3.06 (0.94 to 9.95)	0.063
Current smoker	7 (32)	41 (18)	4.23 (1.17 to 15.22)	0.027

Numbers on smoking in HLA-SE strata do not add up to numbers in the total CSA group as some patients with data on smoking have missing data on HLA-SE.
ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

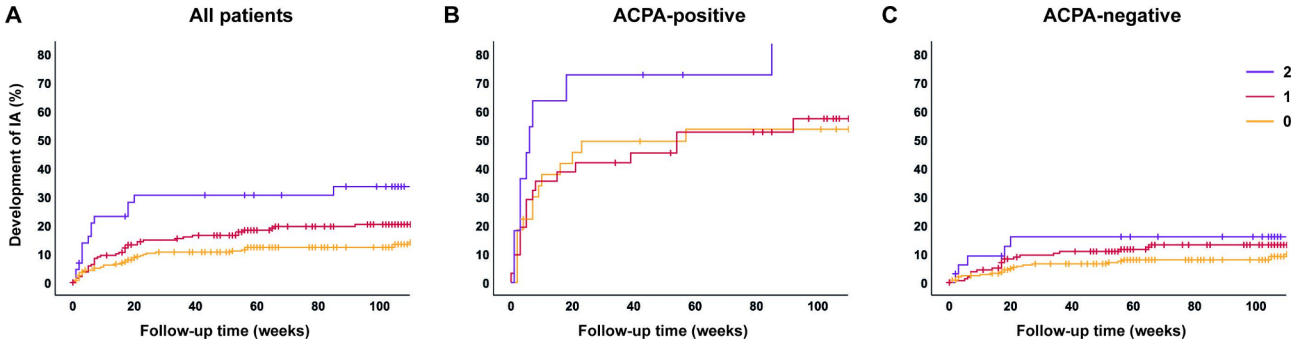

Figure 2 Associations of number of HLA-SE alleles (0/1/2 alleles present) with progression from CSA to inflammatory arthritis (IA). Corresponding HRs, with 0 HLA-SE alleles as reference category were: (A) HR 1.65 (95% CI: 1.06 to 2.56) and HR 3.03 (95% CI: 1.64 to 5.61) for 1 and 2 HLA-SE alleles, respectively, (B) HR 1.05 (95% CI: 0.52 to 2.13) and HR 2.32 (95% CI: 1.00 to 5.41) and (C) HR 1.66 (95% CI: 0.94 to 2.94) and HR 2.00 (95% CI: 0.76 to 5.28), see online supplemental table 4. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

Progression to IA in ACPA-positive CSA

Patients were followed for the development of IA; median time till IA was 16 weeks (IQR 3–36), non-progressors were followed for median 109 (62–116) weeks. The presence of HLA-SE was significantly associated with IA development in all patients with CSA (HR 1.86 (95% CI: 1.23 to 2.82)), also here a dose–response relation was present (figure 2A,(online supplemental table 4). Within the ACPA-positive subgroup the HR was 1.29 (95% CI: 0.67 to 2.47, figure 2B, online supplemental table 4). Because of the small sample size after stratification and risk of type II error, we performed meta-analysis including ACPA-positive patients from two other arthralgia cohorts. This showed that HLA-SE significantly associated with IA development in ACPA-positive patients (HR 1.52 (95% CI: 1.08 to 2.15), figure 4A).

Smoking was not associated with IA development, neither in the total CSA population (HR 1.40 (95% CI: 0.90 to 2.18), figure 3A, online supplemental table 5) nor in the ACPA-positive subgroup (HR 0.59 (95% CI: 0.29 to 1.18), figure 3B, online supplemental table 5) and nor in meta-analysis including ACPA-positive patients from three cohorts (HR 0.94 (95% CI: 0.67 to 1.33), figure 4B).

Thus, HLA-SE, but not smoking, influenced the risk to progress from ACPA-positive CSA to RA.

Associations of HLA-SE and smoking in ACPA-negative CSA

The presence of HLA-SE was associated with IA development in ACPA-negative patients (HR 1.71 (95% CI: 0.99 to 2.96)), although the CI just included 1 (figure 2C, online supplemental table 4). Within ACPA−/RF− and ACPA−/RF+ CSA patients associations of HLA-SE with IA development were HR 1.64 (95% CI: 0.90 to 2.99) and HR 2.07 (95% CI: 0.55 to 7.75), respectively.

The tendency of HLA-SE to associate with IA development in ACPA-negative patients disappeared in sensitivity analyses with the outcome RA, in contrast to the effect that remained within ACPA-positive patients (online supplemental figure 3). Hence, HLA-SE was not convincingly associated with progression from symptoms to IA in ACPA-negative patients.

Smoking did also not associate with progression to IA in ACPA-negative patients (HR 1.30 (95% CI: 0.73 to 2.33)), figure 3C, online supplemental table 5.

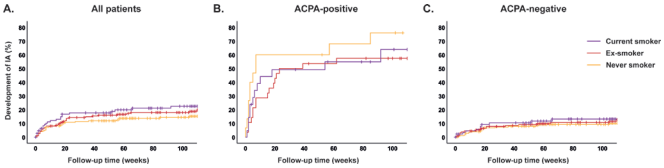

Figure 3 Associations of smoking with progression from CSA to inflammatory arthritis (IA). Corresponding HRs with never smoker as reference category were: (A) HR 1.25 (95% CI: 0.76 to 2.06) and HR 1.66 (95% CI: 0.97 to 2.83) for ex-smoker and current smoker, respectively, (B) HR 0.55 (95% CI: 0.26 to 1.19) and HR 0.64 (95% CI: 0.28 to 1.45) and (C) HR 1.17 (95% CI: 0.61 to 2.24) and HR 1.56 (95% CI: 0.76 to 3.18), see online supplemental table 5. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia.

Associations of HLA-SE and smoking with anti-CarP and AAPA at CSA onset

Neither HLA-SE positivity nor smoking was associated with a higher frequency of RF, anti-CarP or AAPA at presentation with CSA, both in univariable analyses and after correction for concomitant presence of ACPA (online supplemental table 6).

Associations of anti-CarP and AAPA with IA development

In univariable analyses, anti-CarP and AAPA were associated with IA development (table 2). Correcting for ACPA and RF in the Leiden cohort revealed that AAPA was significantly associated with RA development, but anti-CarP was not. Similar multivariable analyses were not possible in the Amsterdam cohort because of the lack of an autoantibody-negative reference group. Instead, we studied the association of both AMPA's in the ACPA+/RF+ subgroups. Meta-analyses of data from the two cohorts revealed a significant association for AAPA (HR 1.53 (95% CI: 1.02 to 2.28)), but not for anti-CarP (HR 1.29 (95% CI: 0.85 to 1.97), figure 5).

DISCUSSION

Although it has been extensively shown that HLA-SE and smoking are risk factors for RA, it was thus far unclear in which pre-arthritis stage these factors exert their effect. We aimed to fine stage the effects of HLA-SE and smoking, taking advantage of our own cohort data, as well as published data. Results from meta-analyses in people in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in the development of ACPA. At CSA onset, both HLA-SE and smoking were associated with the presence of ACPA, although only HLA-SE associated with progression towards arthritis and RA. Presuming that autoantibody development as a proxy for the emerging autoimmune response is the first event, these results imply that smoking is involved in autoantibody development and possibly symptom

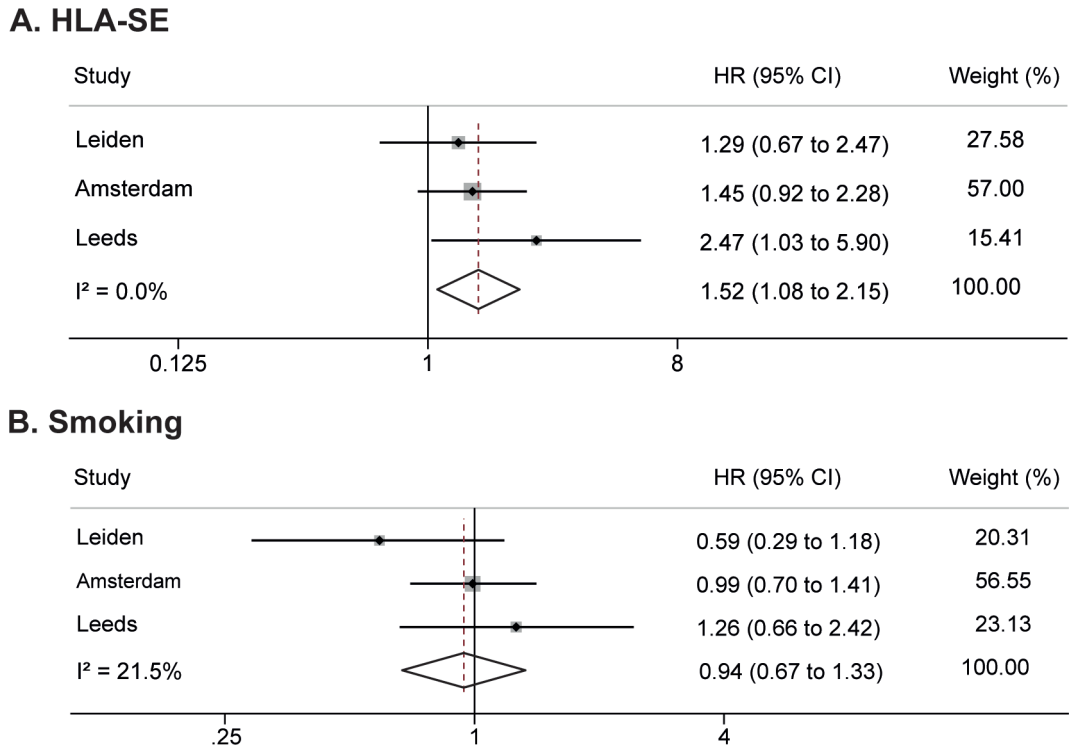

Figure 4 Meta-analyses on HLA-SE (A) and smoking (B) in three cohorts of patients with ACPA-positive arthralgia, showing an association with clinical arthritis development for HLA-SE but not for smoking. Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt *et al* were obtained and analysed. Results from the Leeds cohort were obtained from Rakieh *et al* (table 2 from reference 30). ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

development, but not with further IA development. In contrast, HLA-SE is not involved in initial autoantibody development, but rather associated with autoantibody maturation and symptom development as implied by results found at CSA onset. Furthermore, it associates with further progression to clinical disease (figure 6).

To evaluate the role of HLA-SE and smoking in the asymptomatic phase, we reviewed the literature following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic literature reviews as much as possible (online supplemental file 1).[37] The results of identified studies performed in asymptomatic populations were combined in meta-analyses. These revealed an effect for smoking and absence of an association of HLA-SE with ACPA positivity. Recent data in patients with RA indicated that smoking does not associate with ACPA as such, but rather with RF or autoantibodies in general.[6 15 16 38 39] Although not all of the studies included in the meta-analyses contained data on RF, pooled analysis did not identify an association between smoking and RF in the asymptomatic population (online supplemental material). Also in patients with CSA no association between RF and smoking was found. All included studies were cross-sectionally performed in the general population. As we presumed that ACPA positivity is the first event in the development of ACPA-positive RA, we believe the observed findings reflect effects of HLA-SE and smoking on autoantibody development.

For smoking an association with ACPA was found at the asymptomatic stage and at CSA onset. Our analyses at CSA onset were cross-sectional in nature; therefore, we cannot definitely conclude whether smoking truly associates with progression from autoantibody positivity to symptom development (alternatively, the association found at CSA onset could be reflective of the association with ACPA development). However, one longitudinal study evaluated ACPA-positive individuals from the general population until the development of CSA and showed a significant association of smoking with CSA development.[29] Together with our data this suggests that smoking plays a role in the development of ACPA, further maturation and symptom development.

The absence of an association of HLA-SE with ACPA in the asymptomatic population, the presence of this association at CSA onset and the finding that ACPA levels tended to be higher

Table 2 Associations of autoantibodies with the development of inflammatory arthritis in patients newly presenting with arthralgia

	Univariable Cox regression		Multivariable Cox regression		Multivariable Cox regression	
CSA cohort	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
ACPA IgG	3.29 (2.11 to 5.13)	<0.001	2.55 (1.44 to 4.53)	0.001	2.97 (1.73 to 5.10)	<0.001
RF IgM	1.72 (1.11 to 2.67)	0.015	1.01 (0.61 to 1.69)	0.96	0.98 (0.58 to 1.67)	0.95
AAPA IgG	3.07 (1.90 to 4.98)	<0.001	1.79 (1.02 to 3.16)	0.043	–	–
Anti-CarP IgG	2.85 (1.59 to 5.11)	<0.001	–	–	1.47 (0.75 to 2.87)	0.26

AAPA, anti-acetylated protein antibody; ACPA, anti-citrullinated protein antibody; anti-CarP, anti-carbamylated protein antibody; CSA, clinically suspect arthralgia; RF, rheumatoid factor.

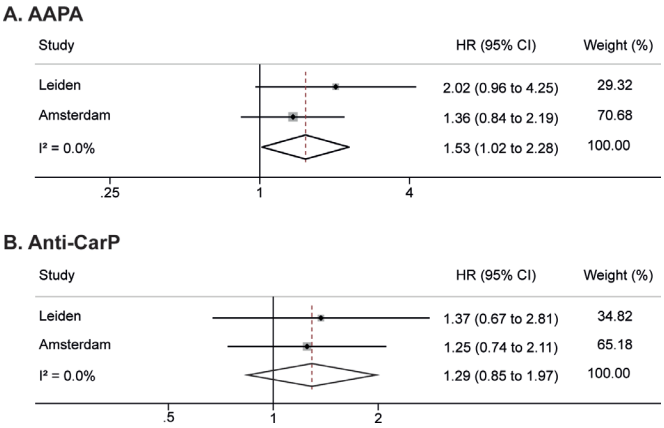

Figure 5 Meta-analyses on AAPA (A) and anti-CarP (B) in two cohorts of patients with ACPA-positive/RF-positive arthralgia, showing an association with IA development for AAPA but not for anti-CarP. Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt *et al* were obtained and analysed. AAPA, anti-acetylated protein antibody; ACPA, anti-citrullinated protein antibody; anti-CarP, anti-carbamylated protein antibody; RF, rheumatoid factor.

in HLA-SE-positive patients with CSA (which is in line with a previous study on ACPA levels in arthralgia[40]) suggest that HLA-SE associates with maturation of the ACPA response and/or symptom onset. However, the latter implication is based on deductions from cross-sectional data, longitudinal data from ACPA positivity to symptom onset would have been preferable.

Several nested case–control studies have shown that autoantibody development and the increase in levels can occur years before disease onset.[41–43] The current study and previous studies on CSA showed that the period between CSA onset and clinical arthritis development is on average 4–6 months.[44] We recently showed that the autoantibody response had already matured at CSA onset and did not mature further towards RA development.[45] Together these results indicate that autoantibody-response maturation took place before symptom onset and was influenced by smoking and HLA-SE. However, although case–control studies have found gene–environment interactions,[6 9 10 14] we found no statistically signification interaction between HLA-SE and smoking for the presence of ACPA at CSA onset (p=0.52). Interestingly, in the asymptomatic phase ACPA positivity can seroreverse to negativity, as is shown in symptom-free relatives of patients with RA.[23] This is in contrast to what is described in the symptomatic phases of CSA and clinical RA,[45–48] where autoantibody status and levels were shown to be stable and seroreversion was infrequent. Regarding timelines, this suggests that the autoimmune response is no longer reversible at symptom onset. However, disease chronicity is then not yet established; only a proportion of patients with CSA develop RA and both joint symptoms and subclinical inflammation can resolve spontaneously, also in ACPA-positive patients.[49] The final processes resulting in irreversible ACPA-positive RA remain to be elucidated. However, the current data also suggest that this final step is influenced by HLA-SE.

This is not the first longitudinal study on HLA-SE and smoking and the progression from arthralgia to clinical arthritis. We took advantage of existing data to strengthen the findings and show consistency in the ACPA-positive group. Furthermore, the fact that the Leiden CSA cohort included patients based on the clinical phenotype and not on autoantibody status ensured inclusion of also autoantibody-negative patients with CSA. This served to explore the role of HLA-SE and smoking in ACPA-negative RA. Although HLA-SE seemed to promote IA development in ACPA-negative patients, this effect was not present for RA development as outcome. Large case–control studies have suggested a role for HLA-SE also in ACPA-negative RA although with a smaller effect size than in ACPA-positive RA.[50] The present longitudinal data on ACPA-negative IA or RA development were insufficient to support a role for HLA-SE in the symptomatic pre-RA stage.

This study focused on associations of ACPA as measured with anti-CCP2, associations with other ACPA tests (eg, anti-CCP3) were not studied. However, in addition to ACPA, we did evaluate other AMPAs. Although different studies have shown cross-reactivity between ACPA and other AMPAs,[51 52] associations

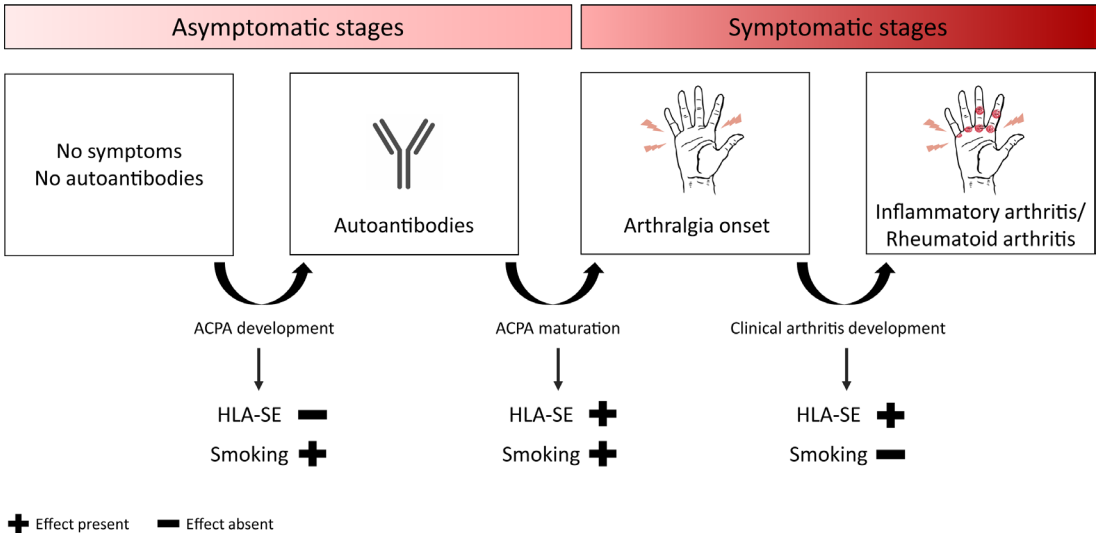

Figure 6 Summary of results on the role of HLA-SE and smoking in the asymptomatic and symptomatic phase of rheumatoid arthritis development. Meta-analyses in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in the development of ACPA. At CSA onset, both HLA-SE and smoking were associated with the presence of ACPA. Only HLA-SE further stimulated progression towards arthritis and ACPA-positive RA. Together these data imply that smoking is involved in autoantibody and symptom development, HLA-SE plays a role in autoantibody maturation, symptom development and progression to clinical disease. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

with HLA-SE and smoking at CSA onset seemed to be specific for ACPA as no such associations were found for AAPA and anti-CarP in our patient population. This is in line with findings in RA, where anti-CarP was also not associated with HLA-SE and smoking.[53]

We aimed to fine stage the effects of HLA-SE and smoking. Identification of predictive markers for IA or RA development in CSA was not our primary aim. Nonetheless, we included an exploration and observed that AAPA, but not anti-CarP, associated with IA, independent of ACPA and RF. Further research is needed to ascertain the diagnostic value of these autoantibodies, especially their relevance on top of ACPA and RF that are measured in daily practice.

This study has extended knowledge on the timing of HLA-SE and smoking in the different stages of RA development. Intriguingly, HLA-SE and smoking exert their effect in partly different phases. Although requiring further biological exploration, it is tempting to speculate that initial autoantibody development is stimulated by smoking, whereas further expansion of the autoimmune response is promoted differently, by an HLA-SE-restricted T-cell reaction that drives further ACPA-response maturation. As such, smoking may contribute to the development of autoantibodies in general.[6 15 16 38 39] This initial antibody development does, most likely, require T-cell help as the antibodies are of the IgG isotype and hence the antibody producing B cells have undergone isotype switching, a T-cell dependent process. However, as no association with the HLA system is observed at this stage, these T cells most likely act in an HLA-SE-independent manner. In contrast, the subsequent expansion of the ACPA response does associate with HLA-SE, indicating that another, second, T-cell response is involved in the further expansion of the ACPA response. These T cells are associated with HLA-SE and, conceivably, recognise other antigens than the ones involved in the T-cell response underlying the 'initial' ACPA response. Thereafter, ACPA-positive persons with HLA-SE are particularly prone for further progression towards RA. These insights in timing of environmental and genetic factors support a further refinement of the SE hypothesis; the HLA-SE-specific T-cell response may not promote the initial break of tolerance to citrullinated antigens, but rather promotes the expansion of the (already existing) ACPA response prior to disease onset. Conceptually, this would explain why ACPA-positive patients with HLA-SE develop RA more often than ACPA-positive patients without HLA-SE and why HLA-SE does not associate with the other autoantibodies.

The findings of our study can guide future prevention studies. Prevention often concentrates on health-promoting behaviours. Our results on smoking not only imply that cessation of smoking might be able to influence the risk of ACPA development and/or symptom onset but also imply that it may not be effective in reducing the risk of progression from CSA to clinical arthritis. This would mean that trials on smoking cessation might preferably assess the efficacy in disease prevention in the asymptomatic population (primary prevention), rather than in patients with arthralgia (secondary prevention).

To conclude, HLA-SE and smoking act in partly different pre-RA stages. Smoking confers risk for the development of ACPA and/or joint symptoms, but does not further associate with IA development. In contrast, HLA-SE does not associate with ACPA in the general population, but does mediate symptom development and progression to IA. Even though the underlying time-specific biological pathways need further exploration, these data enhance understanding of timing of key genetic and environmental risk factors in the development of RA.

Contributors FW and AvdH-vM were involved in study conception and design. FW, MPM, RK, LvB and ALD contributed to collection of the data. FW and MV performed the data analyses. FW, AvdH-vM and REMT interpreted the results and wrote the first version of the manuscript. All authors critically revised the manuscript and approved the final version.

Funding This work was supported by the European Research Council under the European Union's Horizon 2020 research and innovation programme (starting grant, agreement no. 714312) and the Dutch Arthritis Society.

Competing interests None declared.

Patient and public involvement statement Patient partners were involved in the design of the clinically suspect arthralgia cohort.

Patient consent for publication Not required.

Ethics approval Clinically suspect arthralgia cohort Leiden: Local Medical Ethics Committee, named 'Commissie MedischeEthiek', approval number NL38832.058.11. Amsterdam cohort: The Ethics Committee of the Slotervaart Hospital and Reade, Amsterdam.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data can be requested from the corresponding author.

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