Hormonal replacement therapy may reduce the risk for RA in women with early arthritis who carry HLA-DRB1 *01 and/or *04 alleles by protecting against the production of anti-CCP: results from the ESPOIR cohort

Carine Salliot,[1,2] Claire Bombardier,[2,3] Alain Saraux,[4] Bernard Combe,[5] Maxime Dougados[1]

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[1]Paris Descartes University, Medicine Faculty UPRES-EA 4058 APHP, Rheumatology B Department, Cochin Hospital, Paris, France
[2]Division of Rheumatology, Mount Sinai Hospital, University of Toronto, Canada
[3]Department of Medicine at University of Toronto, Canada
[4]Department of Rheumatology, University Hospital, Brest, France
[5]Department of Rheumatology, Lapeyronnie University Hospital, Montpellier, France

Correspondence to
Dr Carine Salliot, Paris Descartes University, Medicine Faculty UPRES-EA 4058 APHP, Hôpital Cochin, Service de Rhumatologie B, rue du faubourg Saint-Jacques, 75014 Paris, France; carinesalliot@cch.aphp.fr

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ABSTRACT

Objective To assess the effect of reproductive factors, especially hormone replacement therapy (HRT) and its interaction with HLA-DRB1 *01 and/or *04 alleles on the diagnosis of rheumatoid arthritis (RA) and the presence of anti-cyclic citrullinated peptide (CCP) antibodies in women included in the ESPOIR cohort (early arthritis cohort).

Methods 568 patients were included in the analyses, which were performed using logistic regression.

Results HRT reduced the risk of RA due to the HLA-DRB1 *01 and/or *04 alleles from OR 1.88 (95% CI 1.32 to 2.68, p<0.000) for HLA-DRB1 *01 and/or *04 alleles alone to OR 1.07 (95% CI 0.51 to 2.26, p=0.85) in women with HLA-DRB1 *01 and/or *04 alleles who received HRT. One explanation might be the protective effect of HRT on the presence of anti-CCP antibodies (OR 0.43, 95% CI 0.24 to 0.77, p<0.006). Other reproductive factors such as the number of pregnancies, menopause and age at menopause, age at menarche and a history of pregnancy with poor outcome were not associated with the diagnosis of RA and the presence of anti-CCP antibodies.

Conclusion HRT may reduce the risk of RA due to HLA-DRB1 *01 and/or *04 alleles by protecting against the production of anti-CCP antibodies.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex multifactorial disease involving both genetic and environmental factors. Some alleles of HLA-DRB1 called the shared epitope (SE) (ie, *0101, 0104, 0404, 0102, 0408 alleles) are associated with the development of a subset of RA with rheumatoid factor (RF) or anti-cyclic citrullinated peptide (anti-CCP) antibodies.[1–3] The involvement of female hormones in the pathogenesis of RA has been suggested by numerous observations, and many studies have evaluated hormonal treatments and other reproductive factors with conflicting results.[4–14]

The ESPOIR cohort (French acronym for Study and Follow-up of Undifferentiated Early Arthritis) is a large national multicentre cohort of 813 adults with early arthritis.[15 16] Data regarding demographic and socioeconomic factors, comorbidities, reproductive factors in women, autoimmunity and HLA-DRB1 (*01 and *04 alleles) were collected at baseline (more details are given in the online supplement).

The objective of this study was to assess the effect of reproductive factors on the risk of the production of anti-CCP antibodies and the development of RA in women with early arthritis, especially the influence of hormone replacement therapy (HRT) and its interaction with HLA-DRB1 *01 and/or *04 alleles.

METHODS

Study population

A total of 568 women with early arthritis were included in the ESPOIR cohort and the study population (see figure 1).

Identification of the outcomes

The two outcomes were the presence of anti-CCP2 at baseline and the diagnosis of RA at 1 year of follow-up. The American College of Rheumatology (ACR) 1987 criteria for the diagnosis of RA have a limited value in the early stage of the disease but, when combined with the opinion of a rheumatologist for the diagnosis of RA, they are sensitive (87%) and highly specific (99% compared with 75%).[17] The Scientific Committee of the ESPOIR cohort therefore defined RA at 1 year of follow-up by the fulfilment of the ACR 1987 criteria (either at baseline or at 6- or 12-month follow-up visit) plus the rheumatologist's global assessment for the diagnosis of RA of at least 75 on a scale of 0–100 at 12 months.

Independent variables

The following data were assessed as potential associated factors with RA and production of anti-CCP antibodies: menopausal status, age at menopause, age at menarche, use of HRT, use of oral contraceptives, number of pregnancies, delivery within 6 months of onset and poor pregnancy outcomes (spontaneous abortion, therapeutic abortion and preterm delivery). We also evaluated the relationship between HLADRB1*01 and/or *04 alleles and hormonal treatments.

Statistical analyses

Univariate analysis and binary logistic regression were performed. More details are given in the online supplement.

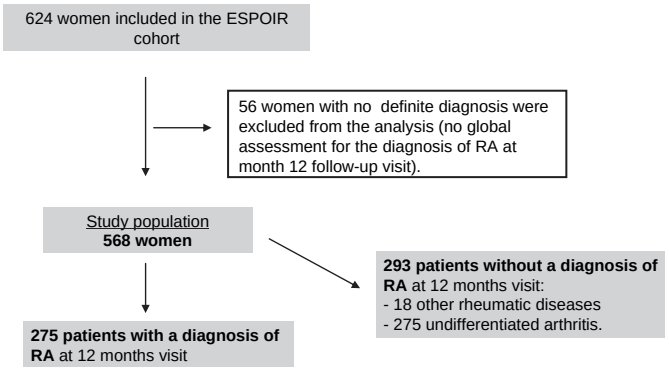

Figure 1 Selection process of the study population of women included in the ESPOIR cohort according to the definition of the diagnosis of rheumatoid arthritis (RA) at 12 months of follow-up.

RESULTS

Characteristics of study population at baseline

Among the 568 women, 275 had a diagnosis of RA and 293 had no RA (275 with undifferentiated arthritis, 18 patients with another rheumatic disease) (figure 1).

Table 1 shows the main baseline characteristics of the study patients. Two hundred and sixty-one women (45.9%) were postmenopausal and the mean±SD age at menopause was 49.4±4.8 years (range 22–61). A total of 160 patients (61.3%) had taken HRT (mean±SD duration 6.3±4.6 years), of whom 76 were still taking it at baseline. Among the patients who received HRT, 75 (46.8%) had used oral contraceptives. The mean±SD age at menarche was 13.0±1.6 years. A total of 109 women were nulliparous (19.2%); the mean±SD number of pregnancies per woman was 2.1±1.7 and 17 women (3.0%) had a delivery within 6 months of the onset of rheumatic disease. A total of 141 women (24.8%) experienced at least one pregnancy with a poor outcome: 101 had at least one spontaneous abortion, 43 a preterm delivery and 13 women had a therapeutic abortion (see table 1w in online supplement).

The results of univariate analysis for both outcomes are shown in table 1w in the online supplement. No significant difference was seen in menopausal status, age at menopause, age at menarche, delivery during the 6 months before onset of rheumatic disease, the number of pregnancies and a history of poor pregnancy outcome. A history of at least one pregnancy and the use of oral contraceptives were significantly more frequent in the anti-CCP negative group (see table 1w in online supplement). Nevertheless, after adjustment for several confounding factors including age, ethnicity, marital status, work status, obesity, hypertension and smoking, we could not confirm these associations.

Influence of HLADRB1 *01 and/or *04 alleles, HRT and a combination of the two on the diagnosis of RA at 1 year

Logistic regression suggested that HRT may reduce the risk of RA due to the SE (table 2). Indeed, in women with HLADRB1 *01 and/or *04 alleles, taking HRT reduced the OR from 1.88 (95% CI 1.32 to 2.68, p<0.000) for HLADRB1 *01 and/or *04 alleles alone to 1.07 (95% CI 0.51 to 2.26, p=0.85). Nevertheless, HRT alone was not significantly associated with RA (OR 0.61, 95% CI 0.36 to 1.04, p=0.07). In patients with HLADRB1 *01 and/or *04 alleles, the frequency of RA was significantly lower if they had ever received HRT than if they had not (8.1% vs 22%, p=0.002 for interaction, likelihood ratio test). This result suggested an interaction between HLADRB1 *01 and/or *04 alleles and HRT for the development of RA.

Table 1 Characteristics of the study population: 568 women from the ESPOIR cohort

Demographic data	
Age at inclusion	47.3±12.6
Caucasian	525 (92.4%)
Diagnosis of RA	
Symptom duration (days)*	100.9±53.3
Fulfilled cumulative ACR criteria †	446 (78.5%)
Fulfilled ACR criteria at 12-month follow-up visit	231/562‡ (41.1%)
Fulfilled ACR criteria at baseline	403 (71.0%)
Rheumatic disease onset and activity at baseline	
Acute ons et	292 (51.4%)
Monoarthritis	103 (18.1%)
Oligoarthritis	171 (30.1%)
Polyarthritis	294 (51.7%)
Symmetrical involvement	339 (59.7%)
Additive involvement	465 (81.8%)
General symptoms§	134 (23.6%)
Swollen joints/28	7.1±5.4
Tender joints/28	8.7±7.1
Physician global assessment/100	50.4±22.7
Patient global assessment/100	60.4±25.4
Ritchie index/159	18.5±18.3
DAS28 score	5.1±1.3 (1.4–7.6)
HAQ score	1.0±0.7 (0–2.8)
Rheumatic disease treatment at baseline	
DMARDs ever	32 (5.6%)
MTX ever	16 (2.8%)
MTX dose	10.9±1.7
Oral steroids ever	72 (12.7%)
NSAID ever	509 (89.6%)
Blood tests at baseline	
CRP (mg/l)	18.8±49.6 (0–384)
IgM RF+ (ELISA, positive if >9 IU/ml)	261 (46.0%)
IgA RF+ (ELISA, positive if >9 IU/ml)	239 (42.1%)
Anti-CCP2+ (ELISA, positive if >50 IU/ml)	215 (37.8%)
HLA-DRB1 alleles (*01 and/or *04), n (%) patients¶	291 (53.4%)

Results are mean±SD with ranges for continuous variables or number (%) of patients for dichotomous variables.
*Delay in days between the first permanent swollen joint and inclusion in the ESPOIR cohort.
†Fulfilment of American College of Rheumatology (ACR) criteria either at baseline or at 6- or 12-month follow-up visit.
‡Six patients already classified with other rheumatic disease at 6-month visit.
§Defined as fever or appetite loss, weight loss and fatigue.
¶23 missing data.
anti-CCP2, anti-cyclic citrullinated peptide antibodies; DAS28, 28-joint Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire; MTX, methotrexate; NSAID, non-steroid anti-inflammatory drug; RF, rheumatoid factor.

Influence of HLADRB1 (*01 and/or *04 alleles), HRT and a combination of the two on the presence of anti-CCP antibodies at baseline

Univariate analysis showed that patients without anti-CCP antibodies were more likely to have ever taken HRT (33% vs 20.4%, p=0.001) (see table 1w in online supplement). After adjustment for confounding factors (shown in the footnote to table 2), HRT may protect against the production of anti-CCP antibodies (OR 0.43, 95% CI 0.24 to 0.77, p=0.005). With regard to the relationship between HRT and anti-CCP+, the duration of HRT may also have a role; the OR for patients who had taken HRT for 6–9 years was 0.42 (95% CI 0.14 to 0.84, p=0.028) compared with 0.35 (95% CI 0.16 to 0.75, p=0.007) when HRT has been taken for at least 10 years while, in those who had taken HRT for <6 years, the results were not significant (using patients who never received HRT as reference and after adjustment for the confounding factors listed in table 2). Moreover, HRT

Table 2 Assessment of HLA-DRB1 (*01 and/or *04), HRT and a combination of the two as independent risk factors for RA and the production of anti-CCP antibodies (multivariate analysis binary logistic regression, results are in odd ratios, 95%CI, p values)

	Model 1	p Value	Model 2
Potential determinants of RA			
HRT and SE			
No HLADRB1 (*01 and/or *04) and never HRT	1.0 (ref)	–	1.0 (ref)
HLADRB1 (*01 and/or *04) alone	1.89 (1.34 to 2.66)	0.0001	1.88 (1.32 to 2.68), p<0.0001
HRT alone	0.82 (0.55 to 1.20)	0.31	0.61 (0.36 to 1.04), p=0.07
HLADRB1 (*01 and/or *04) and HRT	1.48 (0.88 to 2.48)	0.13	1.07 (0.51 to 2.26), p=0.85
Potential determinants of production of anti-CCP2			
HRT and SE			
No HLADRB1 (*01 and/or *04) and never HRT	1.0 (ref)	–	1.0 (ref)
HLADRB1 (*01 and/or *04) alone	4.04 (2.75 to 5.94)	0.0001	4.21 (2.82 to 6.27), p<0.0001
HRT alone	0.39 (0.25 to 0.61)	0.0001	0.43 (0.24 to 0.77), p=0.006
HLADRB1 (*01 and/or *04) and HRT	1.60 (0.92 to 2.75)	0.09	1.51 (0.67 to 3.43), p=0.32

Model 1: ORs for HRT alone (ie, with adjustment for HLADRB1 *01 and/or *04) and HLADRB1*01 and/or *04 alone (ie, with adjustment for HRT).
Model 2 corresponds to model 1 with adjustments for the confounding factors:
With RA diagnosis as outcome: age at inclusion, ethnicity, number of children, marital status (live alone vs no), diabetes, menopausal status, number of preterm deliveries, oral contraception (ever), cumulative dose of tobacco in pack-years.
With production of anti-CCP as outcome: age at inclusion, ethnicity, menopausal status, current obesity, history of hypertension, hypercholesterolaemia, marital status (live alone vs no), current work status, number of pregnancies and history of pregnancy with bad outcome, oral contraception (ever), cumulative dose of tobacco in pack-years.
CCP, cyclic citrullinated peptide; HRT, hormone replacement treatment; RA, rheumatoid arthritis; SE, shared epitope.

reduced the risk provided by HLADRB1 *01 and/or *04 alleles; the adjusted OR was 4.21 (p<0.0001) for women with *01 and/or *04 alleles and decreased to 1.92 (p=0.01) for women with *01 and/or *04 alleles who received HRT.

Among the patients with HLADRB1 *01 and/or *04 alleles who received HRT, 6.4% had anti-CCP antibodies compared with 21.5% of women who had never taken HRT (p<0.0001 for interaction, using the likelihood ratio test). These results also suggest an interaction between HLADRB1 *01 and/or *04 alleles and HRT on the risk for the presence of anti-CCP antibodies.

DISCUSSION

The SE (defined as the presence of HLADRB1 *01 and/or *04 alleles in some studies) is a well-known risk factor for RA, especially seropositive RA.[18] There is also evidence of an interaction between smoking and these HLADRB1 alleles as a risk factor for the presence of anti-CCP antibodies and a diagnosis of RA.[1–3]

A major finding of this study is the suggestion of a new gene–environment interaction. It is hypothesised that HRT interacts with HLADRB1 (*01 and/or *04 alleles) and reduces the risk of developing RA, probably by reducing the risk for the presence of anti-CCP antibodies. Some previous studies did not find any association between HRT and the development of RA[10–14] but, to our knowledge, none assessed the interaction between HRT and some HLADRB1 alleles.

The analyses were performed using a large sample of patients included in a nationwide cohort of women with early arthritis (n=568) with no missing data regarding outcomes, independent variables and covariates (except 23 missing data on HLADRB1 genotyping) which has the required power for the analyses (>80%). We used binary logistic regression to assess, after adjustment, the influence of HRT and its interaction with HLADRB1 *01 and/or *04 alleles on the diagnosis of RA and the presence of anti-CCP antibodies. Before and after adjustment for several confounding factors, the results expressed in ORs were stable, which provided strength to the findings.

Because tobacco smoking is a potential risk factor for RA and anti-CCP antibodies, the apparent protective effect of HRT on the presence of anti-CCP antibodies could be explained by fewer smokers among women who received HRT. Among the 160 women who used HRT, 62.5% never smoked and 37.5% ever smoked, but this difference was not significant (p=0.21). Moreover, in the logistic regression we included the cumulative dose of tobacco (from 0 for non-smokers to 60 pack-years) as a confounding factor (see table 2, model 2).

No excess risk for RA is observed during pregnancy, perhaps because of the immunosuppressive effects of oestrogen and progesterone (regulation of production or action of cytokines such as tumour necrosis factor α and interleukins 1, 6, 12 and 10). The increased risk of RA in postpartum breast feeding women might be explained by the high concentration of prolactin and the fall in oestrogen and progesterone levels after delivery.[19 20] Brennan *et al*[19] suggested that the polymorphisms of the prolactin gene would be in linkage disequilibrium with HLADR4, prolactin gene and the HLA region being located close on the short arm of chromosome 6. Postmenopausal women who carry HLADR4 may therefore have an increased risk for RA (mediated by an increased prolactin level and low concentrations of oestrogen and progesterone). In these women, taking HRT may reduce this risk through the immunosuppressive effect of oestrogen and progesterone.

These results indicate another potential gene–environment interaction associated with RA and anti-CCP antibodies. Further studies are needed to confirm these findings and to better understand the interaction between HLADR1 or DR4 and female hormones.

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Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Lapeyronnie University Hospital, Montpellier, France.

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