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Female hormonal factors and the development of anti-citrullinated protein antibodies in women at risk of rheumatoid arthritis

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Abstract

Objectives. To analyse the association between female hormonal factors and the development of systemic autoimmunity associated with RA in women at increased risk for RA, namely first-degree relatives of patients with RA (RA-FDRs).

Methods. In an ongoing cohort study of RA-FDRs, we analysed all women with available ACPA status. The primary outcome was ACPA positivity. The predictors of interest were female hormonal factors, such as oral contraceptives, breastfeeding, post-menopausal status, early post-menopausal period and total number of ovulatory years.

Results. A total of 768 female RA-FDRs were analysed, of which 42 (5%) had developed ACPA positivity. ACPA-positive women were older (52 *vs* 44 years, $P = 0.001$). Hormonal factors significantly and independently associated with the presence of ACPA were the post-menopausal ($P < 0.001$) and the early post-menopausal periods ($P = 0.040$).

Conclusions. In women at increased risk of RA, characteristic systemic autoimmunity was associated with menopause, suggesting that the acute decline in ovarian function might contribute to the development of autoimmunity associated with RA and potentially to the increased risk of RA in women.

Key words: rheumatoid arthritis, epidemiology, reproductive, biomarkers, autoantibodies, anticitrullinated protein antibodies, risk factors, women

CLINICAL SCIENCE

Rheumatology key messages

- In women at increased risk of RA, being post-menopausal is associated with ACPA positivity.
- The acute decline in ovarian function may contribute to the development of autoimmunity associated with RA.
- Post-menopausal women who are first-degree relatives of RA patients, are a high risk group warranting RA screening.

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Introduction

RA is more prevalent in women, with a female-to-male ratio of 2–4:1 [1, 2]. RA has a major impact on women's health, with an increased risk of osteoporosis, cardiovascular diseases, malignancies, various other comorbidities and an increased mortality [1–8]. However, the role of female hormonal factors in the development of the disease has not been clearly elucidated.

Having a first degree relative with RA (RA-FDRs) increases the risk of RA by ~3-fold [9]. Some studies suggested that the familial aggregation was preponderant in women [10, 11], but more recent cohort studies did not

confirm a sex difference in familial risk of RA [9, 12, 13]. The increased prevalence of RA in women suggests a potential role for female hormonal and reproductive factors. Studies have associated RA development with high oestrogen exposure, such as the use of hormonal replacement therapy or oral contraceptives (OCs) [14–16], while other studies have linked RA to reproductive factors related to low oestrogen exposure, such as an early age at menopause [17–19] or the use of anti-oestrogen agents [20]. In addition, conditions characterized by various endocrine changes, such as polycystic ovary syndrome [14], pre-eclampsia [21] or the postpartum period have been associated with RA onset as well [22]. Overall, female hormonal factor associations with RA development have been inconsistent in epidemiologic studies [23–25]. A possible explanation for these discrepancies is that their impact may differ between the stages of RA development and could be stronger on the immunological events during the pre-clinical stages of the disease [26]. The transition from genetic risk to the development of systemic autoimmunity associated with RA is considered a key step for the development of RA and is often referred to as the immune onset of the disease [27]. The autoimmunity associated with RA is characterized by the presence of autoantibodies, such as ACPAs, and precedes RA onset by a median duration of ~5 years [28, 29]. The presence of a positive ACPA test is strongly predictive of future RA in RA-FDRs, with a risk of developing RA within 5 years of 69% [28]. The aim of this study was to analyse the association between female hormonal factors and the development of systemic autoimmunity associated with RA, among women at increased risk for RA, but without clinical evidence of the disease.

Methods

Study design

This study involves an ongoing, multicentre cohort of RA-FDRs (SCREEN-RA) started in 2009, comprising subjects without a diagnosis of RA at enrolment [30]. The study was approved by the Ethics Committee of the University Hospitals of Geneva, and all individuals willing to participate signed an informed consent form before enrolment, in accordance with the Declaration of Helsinki.

Data collection

Participants completed a questionnaire regarding demographic data, various putative environmental factors and early rheumatic symptoms. Factors included smoking, alcohol consumption, nutritional habits, professional exposures, infectious diseases, oral health and female hormonal factors. A clinical examination, performed by a rheumatologist or a specialized nurse assessed the weight, height, tender and swollen joint counts [31] and ruled out the presence of RA or other autoimmune conditions. Serum samples were collected for genetic testing and auto-antibodies assessment. RA-FDRs were followed annually to assess for the development of signs and symptoms of arthritis. We included all SCREEN-RA participants with available ACPA status and information on female hormonal factors. In order to examine the role of female hormonal factors on the development of ACPA in healthy FDR-RA and to avoid overestimating ACPA positivity, we excluded women with symptoms suggestive of possible RA based on the Connective Tissue Disease Screening Questionnaire [32] and participants who developed RA within 2 years of enrolment.

Study outcomes definitions

The primary outcome of the study was systemic autoimmunity associated with RA, defined by ACPA positivity, which was operationally characterized by a positive result to any of the anti-CCP antibodies tests (2.0, 3.0 or 3.1) performed at any time during follow-up. Autoantibodies were measured using standard, commercially available ELISA kits [anti-CCP 2 (CCPlus Immunoscan, Eurodiagnostica), anti-CCP 3.1 (QUANTA Lite CCP3.1 IgG/IgA, Inova Diagnostics) or anti-CCP 3 (QUANTA Lite CCP3 IgG)]. RF was measured using the QUANTA Lite IgM and IgA ELISAs and QUANTA Flash IgM and IgA chemiluminescent immunoassays (research use only, Inova Diagnostics). ACPA and RF positivities were defined according to the manufacturers' cut-off values (Anti-CCP2 $\geqslant$25 U/ml, anti-CCP3.1 and 3 $\geqslant$ 20 U/ml, RF QUANTA Lite $\geqslant$ 6 U/ml and RF QUANTA Flash $\geqslant$ 10344 RLU for IgM and $\geqslant$7425 for IgA) [33].

Assessment of exposure

All female hormonal factors were self-reported by the participants. Female hormonal data included the number of pregnancies, number of live births, ever breastfeeding and average duration of breastfeeding in months by child, age at menarche, regularity of cycles between 20 and 35 years of age, ever use of OCs and duration of use in years. Parous women were defined as having had $\geqslant$1 pregnancy. Post-menopausal status was defined using the following question: are you post-menopausal? If the answer was affirmative, age at menopause was requested with the following question: at what age have your menses stopped permanently? Women in their early post-menopausal period were defined as women within <6 years after menopause [34, 35]. Early menopause was defined as that occurring at <45 years of age [17–19, 36]. We further asked post-menopausal women about any history of hysterectomy with or without oophorectomy, the use of hormonal replacement therapy and the duration of use in years. Natural menopause was defined as menopause without history of hysterectomy [37]. To attempt to summarize the time of oestrogen exposure among post-menopausal women in a single measure, we used the total ovulatory years calculated as the reproductive span minus the number of childbirths (12 months each) and minus years of OCs use, as previously described [38, 39]. The reproductive span was defined as the number of years between the age at menopause and the age at menarche [40].

Other variables examined in the analysis were age, ethnicity, tobacco smoking, current alcohol consumption and

overweight/obesity using the WHO definition (BMI $\geqslant$ 25 kg/m^2). We defined ever tobacco smoking as the presence of current or past smoking. Heavy smoking was defined by >10 pack-years ever. More than one FDR with RA and the presence of shared epitope (SE $\geqslant$ 1 copy) were also analysed.

Statistical analysis

General characteristics were examined for their association with systemic autoimmunity associated with RA using descriptive statistics: chi-squared or Fisher's exact test for categorical variables, Student's *t*-test or Mann–Whitney U test for continuous variables. We computed odds ratios (ORs) with 95% CIs to examine the association between ACPA positivity and female hormonal factors, using logistic regression analyses. We analysed univariable and multivariable associations for each hormonal factor, adjusting for potential confounders, such as age, tobacco smoking and SE. We performed a stratified analysis in pre- and post-menopausal women to explore potential effect modification by menopausal status.

We performed a sensitivity analysis excluding only participants with missing ACPA status, without excluding FDRs with joint symptoms or participants who subsequently developed RA. We further carried out another sensitivity analysis aimed at verifying the robustness of our findings, including all measures of ACPAs per participant and taking into account that some of them had more than one ACPA test available. For that purpose we performed a general estimation equations analysis with a logit link and robust variance estimates to assess the OR of ACPA positivity associated with the predictors of interest. We analysed the association between female hormonal factors and seropositivity, a broader definition of autoimmunity associated with RA, defined by the presence of RF positivity and/or ACPA positivity. We also performed an exploratory analysis in men to study the association between general characteristics, such as ever tobacco smoking, alcohol consumption, BMI and age with ACPA positivity.

Cut-offs for categories of continuous variables (i.e. age, breastfeeding time, total ovulatory time) were based on quartiles or median values. Sporadically missing data were managed using multiple imputations. $P < 0.05$ were considered statistically significant. All analyses were performed with STATA 14.2 (Stata Corp LP, College Station, TX, USA).

Results

Study population

In total, 1194 subjects were enrolled in the SCREEN-RA cohort up to September 2016, of which 900 (75%) were women. In order to study the presence of ACPA in healthy women, we excluded six participants who developed RA during follow-up, 58 who had symptoms related to possible RA and 68 with missing ACPA status. A total of 768 RA-FDR women were analysed, with a median age of 45 years [interquartile range (IQR): 34–55]. ACPA positivity was found in 42 (5%) women, 25 (3%) had high ACPA positivity and 168 (22%) were classified as seropositive.

General characteristics of RA-FDRs by ACPA

The characteristics of the women participating in SCREEN-RA cohort are summarized in Table 1. Women with ACPA positivity were older ($P < 0.001$), and being in the age group 45–55 years was significantly associated with ACPA positivity ($P = 0.002$). We found a trend towards an association between heavy tobacco smoking and ACPA positivity, which did however not reach statistical significance.

Female hormonal factors and ACPA positivity

Being post-menopausal was significantly associated with ACPA positivity (P < 0.001), particularly being in the early post-menopausal period (within 6 years after menopause) ($P = 0.040$). We analysed the impact of natural menopause, excluding women with a history of hysterectomy ($n = 68$) and the association between ACPA and menopause remained (OR = 3.5, 95% CI: 1.8, 6.9; $P < 0.001$). In the analysis adjusted by age, ACPA positivity and postmenopause remained associated (OR = 2.4, 95% CI: 0.9, 6.6), and the association between being at the early postmenopausal period and ACPA positivity was stronger (OR = 3.0, 95% CI: 1.0, 8.9). In the multivariable analysis, the

Table 1 Characteristics of female first-degree relatives of patients with RA by ACPA status ($n = 768$)

	ACPA negative, $n =$ 726 (95%)	ACPA positive, $n =$ 42 (5%)
Age at baseline, median (IQR)	44 (33–54)	52 (47–58)**
Age groups		
<35	207 (29)	5 (12)
35–45	176 (24)	5 (12)
45–55	175 (24)	20 (47)*
$\geqslant$55	168 (23)	12 (29)
White European	678 (93)	39 (93)
Ever tobacco smoking[a]	336 (46)	24 (57)
Heavy tobacco smoking	44 (6)	5 (12)
Alcohol consumption[a]	588 (81)	35 (83)
BMI, median (IQR),[a] kg/m^2	23 (21–26)	23 (21–26)
Overweight/obese, BMI $\geqslant$ 25	212 (29)	13 (31)
RF[b]	126 (17)	7 (17)
HLA-SE ($\geqslant$1 copy)[c]	377 (52)	24 (57)
First-degree relatives with RA > 1	115 (16)	7 (17)

Values are *n* (%) unless otherwise stated. *P* values were calculated using Fisher's exact test for categorical variables or the Mann–Whitney U test for continuous variables. [a]1–2% of observations were missing and imputed. [b]3–4% of observations were missing and imputed. [c]5–7% of observations were missing and imputed. $^{**}P < 0.001$. $^{*}P < 0.05$. SE: Shared epitope.

association between ACPA positivity and post-menopausal status persisted (OR = 2.3, 95% CI: 0.9, 6.2; $P = 0.1$), as well as its association with the early post-menopausal period (OR = 3.1, 95% CI: 1.0, 9.4; $P = 0.041$), adjusted for the presence of the SE, tobacco smoking and age as continuous. Other menopausal factors, such as age at menopause or history of hysterectomy were not associated with ACPA development. ACPA-positive women RA-FDRs did not differ in age at menarche, irregularity of menstrual cycles, OCs use or breastfeeding characteristics. No significant association was found between ACPA positivity and the total number of ovulatory years, nor with the reproductive span (Table 2).

In a sensitivity analysis, including participants who later developed RA or had symptoms related to possible RA during follow-up and excluding only 77 participants with missing ACPA status (9 with symptoms related to possible RA), we analysed 823 women, and the significant association of being post-menopause and ACPA positivity remained (supplementary Table S1, available at *Rheumatology* Online). We also performed an analysis including all the ACPA tests obtained during follow-up (a total of 904 tests from 768 subjects, with a median of 1 (IQR: 1–2) test per participant) using general estimation equation analyses and found qualitatively similar results (supplementary Table S2, available at *Rheumatology* Online). In a sensitivity analysis using complete cases only, without imputation of hormonal factors information ($n = 737$), we found similar results regarding the association between menopause and ACPA positivity (OR = 3.0, 95% CI: 1.6, 5.9, in univariable analysis; OR = 2.0, 95% CI: 0.7, 5.8, adjusted by age and OR = 1.9, 95% CI: 0.7, 5.5, in multivariable analysis). In an analysis examining the association between female hormonal factors and seropositivity ($n = 168$, 22%), instead of ACPA positivity, we found a similar association with menopause (supplementary Table S3, available at *Rheumatology* Online).

Analysis by menopausal status

We stratified the analysis by pre-menopausal, ($n = 498$, 65%) and post-menopausal status ($n = 270$, 35%). Among post-menopausal women, being in the early post-menopausal period was associated with ACPA positivity ($P = 0.04$) (supplementary Table S4, available at *Rheumatology* Online).

Table 2 Female hormone factors in female first-degree relatives of patients with RA by ACPA status ($n = 768$)

Female hormonal factors	ACPA negative, $n = 726$ (95%)	ACPA positive, $n = 42$ (5%)	Univariable analysis OR (95% CI)	Adjusted by age OR (95% CI)
Age at menarche, median (IQR),[a] years	13 (12–14)	13 (11–14)	0.9 (0.8, 1.2)	0.9 (0.8, 1.2)
Irregular menstrual cycles[b,c]	154 (22)	13 (31)	0.6 (0.3, 1.2)	0.6 (0.3, 1.3)
Oral contraceptives use (ever)[d]	549 (76)	31 (74)	0.9 (0.4, 1.8)	0.9 (0.5, 1.9)
Hormonal contraceptive time, median (IQR),[e] years	9 (4, 15)	10 (3, 18)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
Parous women	400 (55)	27 (64)	1.5 (0.8, 2.8)	0.9 (0.5, 2.0)
Number live-born children, median (IQR)[d,f]	2 (1–2)	2 (1–2)	0.6 (0.4, 0.9)	0.6 (0.3, 0.9)
Ever breastfeeding[f]	330 (82)	22 (81)	0.9 (0.3, 2.5)	1.1 (0.4, 3.2)
Breastfeeding time >6 months[f]	134 (34)	7 (26)	0.7 (0.3, 1.7)	0.8 (0.3, 1.9)
Post-menopausal women[d]	244 (34)	26 (62)	3.2 (1.7, 6.1)**	2.4 (0.9, 6.6)
Early post-menopausal period[g]	81 (33)	14 (54)	2.3 (1.0, 5.3)*	3.0 (1.0, 8.9)*
Age at menopause, median (IQR)[g]	50 (48–53)	51 (48–53)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
Early age at menopause (<45)[g]	34 (14)	3 (12)	0.8 (0.2, 2.8)	0.7 (0.2, 2.6)
History of hysterectomy[g]	63 (26)	5 (20)	0.7 (0.3, 1.9)	0.7 (0.3, 2.0)
Reproductive span, median (IQR),[g] years	38 (34–41)	38 (35–40)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
Total ovulatory time, median (IQR),[g] years	29 (21–36)	29 (20–37)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
<10	7 (3)	1 (4)	1	1
10–19	42 (17)	5 (19)	0.8 (0.1, 8.2)	0.9 (0.1, 8.7)
>19–29	74 (31)	7 (27)	0.7 (0.1, 6.2)	0.7 (0.1, 6.6)
>29	121 (50)	13 (50)	0.8 (0.1, 6.6)	0.8 (0.1, 7.4)
Hormonal replacement therapy (ever)[h]	86 (23)	10 (30)	1.4 (0.7, 3.1)	1.4 (0.6, 3.2)

Values are *n* (%) unless otherwise stated. For all analyses $n = 768$, unless otherwise stated. Odds ratio calculated using logistic regression analysis, unadjusted (univariable) and adjusted by age as a continuous variable. [a]Five to seven observations were missing and imputed. [b]A total of 3–4% of observations were missing and imputed. [c]Older than 20 years old, $n = 741$. [d]A total of 1–2% of observations were missing and imputed. [e]Women with oral contraceptive use, $n = 580$. [f]In parous women, $n = 427$. [g]In post-menopausal women, $n = 270$. [h]In post-menopausal women and pre-menopausal women >45 years old, $n = 402$. $^{**}P < 0.001$. $^{*}P < 0.05$.

Analyses in men

We performed an analysis in 263 men, who were excluded from the main analysis. Age was not significantly associated with ACPA positivity in men and we observed a strong effect of tobacco smoking, which did however not reach statistical significance due to the small number of ACPA-positive men ($n = 6$) (supplementary Table S5, available at *Rheumatology* Online).

Discussion

This study focused on the impact of female hormonal factors on the development of systemic autoimmunity associated with RA, among women at increased risk for RA. Our results demonstrate that post-menopausal women have an increased risk of developing ACPA positivity, in particular during the early post-menopausal period.

Factors related to menopause, in particular early age at menopause, have been previously associated with RA. Results from the Nurses' Health Study reported that being post-menopausal increased the risk of seronegative-RA (seropositive defined as presence of RF and/or ACPA) with a HR of 2.1, 95% CI: 1.4, 3.0, particularly with an early age at menopause (<44 years, HR = 2.4, 95% CI: 1.5, 4.0) [19, 36]. In a case–control study nested in a community-based health survey, early age at menopause (<45 years) was associated with subsequent development of RA with an OR of 2.4 (95% CI: 1.3, 4.4) [17] and confirmed in another study reporting that early age at menopause (<40 years) significantly increased the risk of RA with an OR of 2.5 (95% CI: 1.4, 4.5) [18]. In our FDR-RA cohort, being post-menopausal was associated with the development of ACPA positivity, but also with seropositivity. Furthermore, being in the immediate period following the drop of oestrogens, the early post-menopausal period [34, 35], seems to be particularly associated with ACPA positivity. We found an association with the age group 45–55 years that probably reflects the same phenomenon. We no longer found an effect of age in the stratified analysis by menopausal status (supplementary Table S4, available at *Rheumatology* Online), suggesting that ACPA positivity is associated with menopause and not with age *per se*, strengthening the hypothesis that the acute ovarian decline may constitute a risk factor for the development of systemic autoimmunity associated with RA. Finally, we found no significant effect of age on ACPA positivity in men; however, only a small number of men were ACPA positive (supplementary Table S5, available at *Rheumatology* Online).

The research on female hormonal factors as risk factors for RA has so far been inconsistent, partly because of varying definitions of exposures and outcomes. For example, one of the female hormonal factors that has been evaluated as a risk factor for RA is OCs treatment. OCs use has been reported to decrease the risk of RA [41], but also to increase the risk for ACPA-positive RA [16, 42]. Two meta-analyses did not find a significant association between OCs and development of RA, but showed strong heterogeneity between studies [43, 44]. The dose of OC oestrogens has significantly decreased over recent decades, limiting the interpretation of results. In this study, we analysed OCs use ever as in previous studies, but also the years of hormonal contraception as a more robust measure, and we did not find any significant association with ACPA positivity among FDRs.

Clinical research has focused on the effects of female hormonal factors on established RA [45]. To our knowledge, there are no studies about the effects of hormonal factors on the development of autoimmunity associated with RA. The study of female hormonal factors is challenging because they vary throughout a woman's lifetime and interact with environmental, genetic and other endocrine factors affecting the immune system. The mechanisms of how oestrogens may interact with the immune system are only partially understood. Oestrogens could have different effects on specific immune cells, depending on the serum concentration, the reproductive stage of the woman, the expression of oestrogen receptors or the intracellular metabolism [23, 45]. In particular, IL-6, TNF-α and IL-1β levels have been found to be high in peripheral blood mononuclear cell supernatants from post-menopausal healthy women [46], and are reduced after adding oestradiol in a dose-dependent manner; this phenomenon, however, is not observed in pre-menopausal women [47]. These findings underline the fact that the role of oestrogens on immune function is important and may change over time.

A limitation of our study is the self-reported assessment of female hormonal factors. We used widely accepted definitions of reproductive factors; however, summarizing lifetime oestrogen exposure in one single measure proved to be challenging because of the lack of well-validated scores [38, 39, 48]. Another limitation of this study is the still limited follow-up of this cohort, it being easily possible that over the years some of the ACPA-negative RA-FDRs will develop systemic autoimmunity or RA. This relatively short follow-up should not bias our results, but it may have limited statistical power. In order to study only people at risk of the disease, we have chosen *a priori* to restrict our analysis to healthy individuals without RA and attempted as much as possible to exclude participants with very early presentations of the disease, as this could lead to overestimation of prevalent ACPA positivity.

In summary, we demonstrated that, in women at increased risk of RA, the presence of ACPA is associated with menopause and with the early post-menopausal period. These results suggest that the acute decline in ovarian function and oestrogen bioavailability contributes to the development of autoimmunity associated with RA and potentially with an increased risk of RA in women. As preventive interventions for RA are being developed, we will need to define which group of individuals deserves screening [49]. Post-menopausal FDR-RA women, and particularly those women in the early post-menopausal period, could be one of the high-risk groups warranting screening for RA.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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