RHEUMATOLOGY

Original article

PTPN22 R620W genotype–phenotype correlation analysis and gene–environment interaction study in early rheumatoid arthritis: results from the ESPOIR cohort

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Abstract

Objectives. To investigate genotype–phenotype correlation and gene–environment interaction between *PTPN22* R620W environmental factors such as tobacco/hormonal treatments in an inception cohort of RA patients.

Methods. An intra-cohort study including 532 Caucasian RA patients genotyped for the *PTPN22* rs2476601 polymorphism was performed. Anti-CCP and RF status at baseline, presence of bone erosions at 1 year, HLADR1 and/or DR4 status, demography, comorbidities, exposure to tobacco with the cumulative dose in pack-years, hormonal treatments and treatments received for RA were collected. Logistic regression was performed to estimate the ORs and multiplicative interaction with adjustment for confounding factors. Gene–environment interaction was estimated by the relative excess risk due to interaction (RERI), attributable proportion (AP) and synergy index (SI).

Results. *PTPN22* 620W risk allele was associated with ACPA production [odds ratio (OR) = 2.21, 95% CI 1.4, 3.4, $P < 0.0001$]. Hormonal treatment exposition and smoking were found to act with a protective effect against ACPA production (OR = 0.44, 95% CI 0.3, 0.7, $P = 0.001$) and early bone erosion (OR = 0.56, 95% CI 0.4–0.8, $P = 0.003$), respectively, and independently of HLADR and *PTPN22* status. No evidence for a gene–environment interaction was detected.

Conclusion. These data provide new insights into the pathogenesis of RA, underlying the pivotal key role of environmental factors in the typical heterogeneity of RA.

Key words: Early RA, Cohort, PTPN22 620W, Smoking, Hormonal treatments.

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Introduction

Gene–gene and gene–environment interactions are key features in the development of RA and other complex diseases. To date, the main pathogenesis hypothesis would be an interaction between the *HLA-DRB1*-shared epitope alleles (*HLA-DRB1*SE*) and tobacco smoking as risk factor for the production of anti-citrullinated protein/peptide antibodies (ACPAs), and then the development of RA [1–2].

Other genetic and environmental factors could play a role in the pathogenesis of RA. The protein tyrosine phosphatase non-receptor 22 (*PTPN22*) rs2476601 single nucleotide polymorphism (SNP) has been identified as a

susceptibility genetic factor for various autoimmune diseases including RA [3]. This association would be restricted to the rheumatoid factor (RF) and/or the ACPA positive subset. [4, 5].

Regarding others environmental risk factors, two meta-analyses of case–control and cohort studies suggested a protective effect of oral contraception (OC) regarding RA susceptibility [6, 7]. More recently, we have reported that hormonal replacement therapy (HRT) may reduce the risk of anti-CCP antibody production in RA women carrying HLADRB1*01 and/or HLADRB1*04 alleles [8]. Therefore, our objectives were (i) to perform a *PTPN22* rs2476601 genotype–phenotype correlation analysis; (ii) to test for interactions between *PTPN22* and environmental factors (tobacco smoking and global hormonal exposure) as influencing both ACPA and erosive status in RA, using intra-cohort analyses.

Methods

Study population and design

The Evaluation et Suivi des POlyarthrites Indifférenciées Récentes (ESPOIR) cohort has been previously described (French cohort of early arthritis) [8, 9]. The study was approved by the Institutional Review Board of the Montpellier University Hospital, which was the coordinating centre for this cohort. Before inclusion, all patients gave their written informed consent to participate in this prospective follow-up study.

From the 813 patients recruited from November 2002 to April 2005 with early polyarthritis, 532 were Caucasian with a diagnosis of RA by Year 1 and an available *PTPN22* rs2476601 genotype. RA was defined by fulfilment of the ACR 1987 criteria either at baseline, 6- or 12-month follow-up visit.

All the subjects of the ESPOIR sample were genotyped for the *PTPN22* rs2476601 SNP using a competitive allele-specific PCR system (Kaspar genotyping, Kbioscience, Hoddeston, UK) and Taqman SNP genotyping assay allelic discrimination method (Applied Biosystems, Foster City, CA, USA) as previously described [10]. The average genotype completeness was 98%. The accuracy was >99%, according to duplicate genotyping of 10% of all samples. HLADRB1 genotypes (*01 and/or*04) were determined in each centre.

As outcomes, we chose the presence of ACPA at baseline and the presence of at least one erosion at 1 year (on hand and foot X-rays). As potential risk factors, we assessed the presence of the *PTPN22* 620W risk allele (as none/any), cigarette smoking as never/ever and categorized as <10 or ⩾10 pack-years and hormonal treatments (HRT and/or OC, never/ever). The main characteristics of the study population are summarized in Table 1.

Statistical analyses

Means and standard deviation (SD) were used to summarize continuous data and categorical data are summarized by counts and percentages. Student's *t*-test was used to compare most continuous variables between groups formed by anti-CCP or the presence of erosions by Year 1. For highly skewed data, the Mann–Whitney U test was used. For categorical variables, comparisons were made using Pearson's chi-square or Fisher's exact test.

To investigate the relationship between the *PTPN22* 620W risk allele and the two environmental factors in RA patients, we used two methods:

(i) Association measures: the dependence of each outcome on potential predictors was assessed using logistic regression and adjusting for potential confounding factors which were selected using the data in the literature and the results of the univariate analysis ($P < 0.20$) [11, 12]. For the model-building process of logistic regressions, we avoided assessing in the same model highly correlated covariates. The goodness of fit of the models was analysed as described by Hosmer and Lemeshow [13]. The results are presented as odd ratios (ORs) and 95% Confidence Interval (95% CI).

(ii) Biological interaction between two risk factors A and B means that A and B are not independent in causing a disease. If the combined effect of A and B is larger (or smaller) than (a) the sum of the individual effects, there is interaction on an additive scale, and (b) the product of the individual effects, there is interaction on a multiplicative scale.

For the assessment of gene–environment interactions on an additive scale, we estimated the relative excess risk due to interaction (RERI), attributable proportion (AP) and the synergy index (SI). RERI and AP of 0, and SI of 1 indicate no interaction. Multiplicative interaction was assessed by adding an interaction variable (product of the gene and environmental factor individual effects) to the logistic regression models [14]. Analyses were performed using Statistical Package for the Social Sciences software for windows version 16.0 (SPSS Inc., Chicago, IL, USA). A *P*-value of <0.05 was taken to indicate statistical significance. For estimations of RERI, AP and SI, we used the Microsoft Excel spreadsheet provided by G.Y. Zou [15].

Results

Effects of *PTPN22* 620W risk allele, smoking and the combination of both on the presence of ACPA at baseline or erosions at 1 year

Effect on the ACPA status

Univariate analysis found the PTPN22 620W risk allele significantly associated with the presence of ACPA (OR = 2.21, 95% CI 1.4, 3.4, *P* < 0.0001). Conversely, no association with tobacco smoking was detected (OR = 1.19, 95% CI 0.8, 1.7, *P* = 0.30). RA ACPA-positive (ACPA$^+$) patients who carried the *PTPN22* 620W risk allele and who have ever smoked, had an OR of 3.09 (95% CI 1.7, 5.7); *P* < 0.0001. After adjustment for several confounding factors using logistic regression, we confirmed these results

TABLE 1 Characteristics of the 532 Caucasian patients with RA

Demographic data	
Gender: female, *n* (%)	405 (76.1)
Age at inclusion, mean (S.D.), years	48.9 (12.0)
Diagnosis of RA	
Symptom duration in days,[a] mean (S.D.); median (range)	96.3 (42.6); 90 (7–180)
Fulfilled ACR criteria at baseline, *n* (%)	488 (91.7)
Medical history and comorbidities at baseline	
Body mass index (BMI), mean (S.D.) (range)	25.1 (4.5) (16.3–43.5)
Cigarette smoking ever, *n* (%)	263 (49.4)
Cumulative dose of tobacco consumption, mean (S.D.) (range), pack-years	9.4 (14.9) (0–92)
Cumulative dose of tobacco, *n* (%)	
<10 pack-years	354/526 (67.3)
⩾10 pack-years	172/526 (32.7)
Duration of tobacco consumption, mean (S.D.) (range), years	10.2 (13.1) (0–50)
History of CVD, *n* (%)	89 (16.7)
History of diabetes, *n* (%)	21 (4.0)
Hypercholesterolaemia, *n* (%)	76 (14.3)
Hypertriglyceridaemia, *n* (%)	18 (3.4)
History of malignancy, *n* (%)	18 (3.4)
Hormonal treatments in women, *n* = 405	
OC, *n* pts (%)	170/405 (42.0)
Menopause, *n* (%)	185/405 (45.7)
HRT, *n* (%)	114/405 (28.1)
Hormonal treatment,[b] *n* (%)	242/405 (59.7)
Rheumatic disease treatment received from baseline to 1 year of follow-up	
Ever DMARDs, *n* (%)	465/517 (90.0)
Ever MTX, *n* (%)	351/517 (67.9)
Ever anti-TNF, *n* (%)	40/517 (7.7)
Number of DMARDs, mean (S.D.) (range)	1.3 (0.8) (0–5)
Ever oral steroids, *n* (%)	299/509 (58.7)
Ever NSAID, *n* (%)	504 (94.7)
Blood tests and genetics at baseline	
CRP, mean (S.D.) (range), mg/l	21.9 (34.9) (0–384)
Immunoglobulin (Ig) M RF, mean (S.D.) (range), UI/ml	147.5 (645.8) (2–9924)
IgM RF + (ELISA, positive if >9 UI/ml), *n* (%)	285 (53.6)
Anti-CCP2 + (ELISA, positive if >50 U/ml), *n* (%)	247 (46.4)
Anti-CCP2, mean (S.D.) (range), U/ml	528.1 (1302.7) (0–13 428)
Presence of HLA-DRB1 alleles (*01 and/or *04), *n* (%)	293/506 (58)
Presence of *PTPN22* 620w alleles (GA or AA), *n* (%)	115 (21.6)
Structural damage (baseline 1 year)	
Total modified Sharp score at 1 year, mean (S.D.) (range)	7.9 (11.3) (0–83)
Presence of erosion at 1 year (⩾1), *n* (%)	301/470 (64)
Erosion at 1 year, mean (S.D.) (range)	4.5 (7.9) (0–66)

[a]Delay in days between the first permanent swollen joint and inclusion in the ESPOIR cohort. [b]Hormonal treatment means OC and/or HRT. CVD: cardiovascular diseases (at least one cardiovascular event among hypertension, myocardial infarction or stroke).

(Table 2). Smoking alone was not associated with ACPA[+] status, whatever the cumulative dose. However, in patients who carried the *PTPN22* 620W risk allele, smoking appeared as a risk factor for the presence of ACPA with a smoking dose effect as suggested by the increase in the ORs associated with cumulative dose of tobacco (<10 pack-years and ⩾10 pack-years). These findings suggested an interaction of a combined effect of the *PTPN22* 620W risk allele and cigarette smoking (OR = 4.28, 95% CI 2.0, 9.2, $P < 0.0001$) was larger than the sum and the product of individual effects. Nevertheless, this was not confirmed using interaction measures: RERI and AP were not significantly different

from 0 and SI from 1, even in heavy smokers (at least 10 pack-years).

Effect on the early erosive status

Regarding the progress of erosions at 1 year, univariate analysis found no association with the *PTPN22* 620W risk allele (OR = 1.12, 95% CI 0.7, 1.8, $P = 0.63$). Of most interest, tobacco smoking was found to be associated with RA erosive status, acting with a protective effect (OR = 0.56, 95% CI 0.4, 0.8, $P = 0.003$). Multivariate logistic regression after adjustment for several confounding factors confirmed that smoking reduces significantly the risk of bone erosion (OR = 0.25, 95% CI 0.1, 0.6,

TABLE 2 Effect of *PTPN22* 620W, smoking and the combination of both on the presence of anti-CCP and erosions in RA patients (logistic regressions and interaction measures)

PTPN22 620w	Smoking	Anti-CCP$^+$ (n = 247)	Anti-CCP$^-$ (n = 285)	OR^a (95% CI)	P-value
None	Never	88	124	1.0	
None	Ever	88	117	1.26 (0.8-1.8)	0.25
Any	Never	31	26	2.59 (1.6-4.1)	<0.0001
Any	Ever	40	18	4.28 (2.0-9.2)	<0.0001
RERI (95% CI)		1.39 (−0.43, 3.97)			
AP (95% CI)		0.44 (−0.34, 0.70)			
SI (95% CI)		2.88 (0.57, 14.37)			
p multi^b		0.138			
None	<10 pack-years	121	162	1.0	
None	≥10 pack-years	53	76	0.97 (0.6, 1.6)	0.91
Any	<10 pack-years	39	32	1.90 (1.0, 3.4)	0.03
Any	≥10 pack-years	31	12	4.05 (1.8, 9.1)	0.001
RERI (95% CI)		1.89 (−0.09, 5.37)			
AP (95% CI)		0.54 (−0.23, 0.75)			
SI (95% CI)		4.38 (0.65, 28.83)			
P multi^b		0.078			

PTPN22 620w	Smoking	EROSION$^+$ (n = 301)	EROSION$^-$ (n = 169)	OR^a (95%CI)	P-value
None	Never	130	56	1.0	
Any	Never	38	14	1.02 (0.6, 1.7)	0.95
None	Ever	103	78	0.44 (0.3, 0.7)	<0.0001
Any	Ever	30	21	0.25 (0.1, 0.6)	0.002
RERI (95% CI)		−0.12 (−1.36, 0.61)			
AP (95% CI)		−0.199 (−2.88, 0.89)			
SI (95% CI)		–			
P multi^b		0.665			

^{a}Adjusted ORs (see supplementary table 1 for univariate analysis, available as supplementary data at *Rheumatology* Online): for presence of anti-CCP: gender, BMI, age at baseline, HLADR1 and/or DR4, CVD, malignancy, diabetes, hypercholesterolaemia and hypertriglyceridaemia; and for the presence of erosions: gender, age at baseline, HLADR1 and/or DR4, anti-TNF ever, MTX ever, number of DMARDs received, oral steroids ever, presence of anti-CCP at baseline, HAQ score and DAS-28 score at baseline. ^{b}P-value for multiplicative interaction term between *PTPN22* risk allele*smoking.

P = 0.002). No interaction between the *PTPN22* 620W risk allele and tobacco was detected (Table 2).

Effects of PTPN22 620W risk allele, hormonal treatments in women and the combination of both on the presence of ACPA at baseline or erosions at 1 year

Effect on the ACPA status
After adjustment for several confounding factors, multivariate logistic regression analysis confirmed the results of univariate analysis (data not shown): the *PTPN22* 620W risk allele was associated with ACPA$^+$ status (OR = 2.13, 95% CI 1.3, 3.5, P = 0.003). Interestingly, global exposure to hormonal therapy was found to decrease the risk of ACPA production (OR = 0.44, 95% CI 0.3, 0.7, P = 0.001), independently of HLADR status.

When both factors were combined, no influence of the combined factors was observed (OR = 0.91, 95% CI 0.4, 1.8, P = 0.78), suggesting an antagonistic gene-environment interaction. However, interaction measures did not confirm such interaction (Table 3).

Effect on erosion status
Neither carrying *PTPN22* 620W risk allele, hormonal treatments nor the combination of both were associated with the presence of bone erosions at 1 year (Table 3).

Discussion

The results of the study reported herein provide evidence for a contribution of the *PTPN22* 620W risk allele to ACPA production, but not to early bone erosion. Further case–control association studies have reported such association between *PTPN22* and ACPA-positive RA [4, 5]. However, only three have focused on intra-cohort analysis, which can provide an estimate of the true contribution of a genetic susceptibility factor to a specific phenotype regarding a given disease [16–18]. Regarding structural damage, two previous intra-cohort analyses have been published with opposite results: an association was found with PTPN22 in the European Research on Incapacitating DIsease and Social Supports (EURIDISS) cohort but not in the Leiden Early Arthritis Cohort (EAC) cohort [19, 20]. Hence, *PTNP22* appears to be a RA

TABLE 3 Effect of *PTPN22 620W*, hormonal treatments and the combination of both in the presence of anti-CCP and erosions in RA women (logistic regressions and interaction measures)

PTPN22 620w	Hormonal treatments	Anti-CCP$^+$ (*n* = 179)	Anti-CCP$^-$ (*n* = 226)	OR^a (95%CI)	*P*-value
None	Never	60	52	1.0	
Any	Never	23	10	2.40 (1.3, 4.4)	0.005
None	Ever	75	144	0.44 (0.3, 0.7)	0.001
Any	Ever	21	20	1.41 (0.6, 3.3)	0.43
RERI (95% CI)		−0.53 (−3.13, 0.77)			
AP (95% CI)		−0.58 (−4.23, 0.62)			
SI (95% CI)		–			
P multi^b			0.744		

PTPN22 620w	Hormonal treatments	EROSION$^+$ (*n* = 226)	EROSION$^-$ (*n* = 135)	OR^a (95%CI)	*P*-value
None	Never	67	31	1.0	
Any	Never	23	7	0.91 (0.5, 1.7)	0.78
None	Ever	118	78	0.66 (0.4, 1.1)	0.13
Any	Ever	18	19	0.44 (0.1, 1.2)	0.12
RERI (95% CI)		−0.78 (−3.33, 0.23)			
AP (95% CI)		−1.78 (−9.03, 0.56)			
SI (95% CI)		–			
P multi^b			0.156		

Hormonal treatments include OC and/or HRT. ^{a}Adjusted ORs: for the presence of anti-CCP: smoking ever, BMI, age at baseline, HLADR1 and/or DR4, CVD, malignancy, diabetes, hypercholesterolaemia, hypertriglyceridaemia; and for the presence of erosions: smoking ever, age at baseline, HLADR1 and/or DR4, anti-TNF ever, MTX ever, number of DMARDs received, oral steroids ever, presence of anti-CCP at baseline, HAQ score and DAS-28 score at baseline. ^{b}*P*-value for multiplicative interaction term between *PTPN22* genotype and hormonal treatments.

susceptibility factor and also a genetic factor influencing the RA phenotype (i.e. ACPA production), but not structural damage.

Unlike the reported case–control studies comparing RA cases with controls [20] or with undifferentiated arthritis [21], no interaction between smoking and *PTPN22* on ACPA production was observed in our RA intra-cohort analysis. Nevertheless, this result was in accordance with Kallberg *et al.*'s [22] study performed in two RA cohorts. Other RA intra-cohort analyses are required to better understand the role of both *PTPN22* and smoking in ACPA production.

In good agreement with two large intra-cohort studies, cigarette smoking was detected as a protective environmental factor against bone erosion with a dose effect [23, 24]. Conversely, a worse radiographic outcome in RA patients who have ever smoked was detected in a very small sample size, possibly explaining such discrepancy [25].

We have previously reported protective effect on the production of anti-CCP of HRT in patients who carry HLA-DRB1*01 and/or HLA-DRB1*04 [8]. Interestingly, the present study provides evidence for a direct role of hormonal exposure by protecting against ACPA production, independently of both *PTPN22* and *HLA-DRB1*.

Methodological limitations of genetic and environmental studies must always be considered. Indeed, many studies that could not be replicated in independent groups appear statistically underpowered in the field of complex genetic

diseases and are often shown to be falsely positive. Appropriate sample sizes are critical for detecting slight differences between two populations, as expected in these diseases, and efforts to recruit large cohorts are mandatory to provide sufficient statistical power. For association measures, the number of patients included seems to be sufficient. Nevertheless, the lack of interaction between the *PTPN22* R620W variant and environmental factors could be due to the lack of power for interaction measures: calculations showed a power of ~55% for interaction measures [26]. Moreover, the genetic background of the studied population should be as homogeneous as possible, thereby limiting the possibility of result bias by population stratification. To avoid these biases, we have focused on European Caucasian individuals and recruited a large sample size through the French networks to detect any association that thus highly favoured the validity of the observed association. Nonetheless, independent RA intra-cohort analysis is mandatory to provide a reliable replication of our results, which could be interpreted as conflicting with those previously reported in case–control studies.

In conclusion, the current report confirmed the involvement of *PTPN22* in ACPA production and provides evidence for a direct role of both hormonal exposure and tobacco smoking as environmental factors acting with a protective effect against ACPA production and early bone erosion, respectively. Interestingly, such associations were found to be independent of both *PTPN22* and

HLA-DRB1 RA patient status. No evidence for a gene–environment interaction was detected, suggesting that the three genetic and environmental factors investigated contribute independently to modulation of the RA phenotype. These data provide new insights into RA pathogenesis, underlying the pivotal role of environmental factors in the typical heterogeneity of RA.

Rheumatology key messages

- Gene–phenotype correlation between *PTPN22* risk allele and ACPA-positive RA.
- In women, hormonal treatment appeared to be a protective factor against ACPA in RA.
- No evidence for a gene–environment interaction (between *PTPN22* risk allele, hormonal treatment and smoking) was detected.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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