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Original article

Association of *IL-2RA* and *IL-2RB* genes with erosive status in early rheumatoid arthritis patients (ESPOIR and RMP cohorts)

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ABSTRACT

Objectives: To assess the impact of single nucleotide polymorphisms (SNPs) in *IL-2RA* (rs2104286) and *IL-2RB* (rs743777 and rs3218253) genes on the risk of erosions in rheumatoid arthritis (RA) patients.
Methods: This work is derived from 2 prospective cohorts of early RA: ESPOIR ($n = 439$) and RMP ($n = 180$). The proportions of patients with erosions at baseline and 1 year according to the genotypes of *IL2RA* (rs2104286) or the haplotypes constructed with the 2 SNPs of *IL2RB* were compared in the whole population and in ACPA positive patients. A meta-analysis assessing the risk of erosion depending on the haplotypes of the 2 SNPs of *IL-2RB* was performed using the Mantel-Haenszel method. A multivariate model was used to assess the independent effect of the haplotypes of *IL-2RB* on the risk of erosions.
Results: The AC haplotype of *IL-2RB* carriage was significantly associated with the rate of erosions in ACPA positive patients in ESPOIR cohort (rate of erosions: AC/AC: 78% versus GC or GT/GC or GT: 44%, $p = 0.001$). A meta-analysis of ESPOIR and RMP cohorts confirmed that the carriage of AC haplotype was significantly associated with the rate of erosions at 1 year in the whole sample (OR[95%CI] = 1.92[1.14–3.22], $p = 0.01$) and in ACPA positive patients (OR[95%CI] = 3.34[1.68–6.67], $p = 0.0006$). A multivariate model in ESPOIR cohort demonstrated the independent effect of the carriage of the AC haplotype (6.03[1.94–18.69], $p = 0.002$) on the risk of erosions in ACPA+ patients.
Conclusion: A haplotype constructed with 2 SNPs located on *IL-2RB* gene was associated with erosive status in early RA.

Rheumatoid arthritis (RA) is one of the most common systemic autoimmune disorders, characterized by peripheral synovial joint inflammation, which ultimately leads to joint destruction and increases mortality [1]. The etiology of RA is complex and multifactorial, but family aggregation and twin concordance studies indicate a significant causal role for genetic factors, with heritability being estimated at 60% [2,3]. The association between the human leukocyte antigen (HLA) region and RA susceptibility and severity has been established for many years and, more recently, specific interactions of *HLA–DRB1 shared epitope* (*HLA-DRB1*SE*) alleles with environmental factors, such as cigarette smoking, have been described [4]. Over the last few years, several new susceptibility factors have been identified, and their associations with RA have been independently replicated: *PTPN22*, *TRAF1/C5*, *OLIG3/TNFAIP3*, and *STAT4* [5,6]. In almost all cases, such susceptibility genes were found to be associated with anti-citrullinated protein antibodies (ACPA) positive RA but not ACPA negative RA. The presence of ACPA has become an important diagnostic and prognostic criterion for RA, and it has been suggested that ACPA positivity or negativity may define different disease enti-

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ties. Indeed, clinical studies show that RA patients with ACPA differ from the so-called autoantibody negative patients in terms of disease activity, structural severity and response to disease-modifying anti-rheumatic drugs [7,8]. Thus, the association between genetic polymorphisms and disease severity often depends on ACPA presence [9,10].

Many signaling pathways and molecules are involved in the development, homeostasis and function of this T-cell subset. Interleukin 2 (IL-2), a cytokine previously considered to be primarily involved in the activation and proliferation of T-cells, is one of these molecules.

The high affinity IL-2 receptor (IL-2R) is a heterotrimer consisting of the α chain (IL-2RA, CD25), the β chain (IL-2RB, CD122) and the common cytokine receptor γ chain (γc, CD132) [11,12]. As a result, targeted disruption of the *IL2*, *IL2RA* or *IL2RB* genes in mice causes systemic, multi-organ inflammation [13–15], as does spontaneous mutation of the IL-2 receptor in humans [16]. This phenotype is not due to impaired activation-induced cell death of activated T-cells, a process in which IL-2 also plays an important role, but rather to severe depletion of the peripheral CD4 + CD25+ Treg pool [17]. Numerous works have reported the importance of T-cells CD4 + CD25+ (expressing IL2Rα) in the rupture of the tolerance implicated in the RA pathogenesis [18].

In 2007, the first genome-wide association study in RA carried out by the Wellcome Trust Case Control Consortium (WTCCC) identified a number of loci reaching genome-wide significance, including one single nucleotide polymorphism (SNP) in the *IL-2RA* gene (rs2104286) and one SNP in the *IL-2RB* gene (rs743777) [19]. These results have been replicated in two independent populations [20,21]. A validation study of the initial WTCCC study identified another SNP in the *IL-2RB* gene (rs3218253) associated with RA susceptibility [22] and a recent study performed in Asian population identified an association between rs2104286 and rs3218253 polymorphisms and RA susceptibility [23].

In the present study, we investigated the association between these 3 *IL-2RA* and *IL-2RB* SNPs and the erosive status in 2 cohorts of early RA patients (ESPOIR and RMP cohorts), taking into account ACPA status.

1. Methods

1.1. Study population

1.1.1. ESPOIR cohort

This work is derived from a large national, multicenter, longitudinal, prospective cohort of 813 early arthritis patients, the ESPOIR cohort, aiming to investigate diagnosis, prognostic markers, epidemiology, pathogenesis and medico-economic factors in RA. The characteristics of the cohort have previously been described elsewhere [24]. Briefly, 813 early arthritis patients with arthritis duration < 6 months and no prior treatment with disease-modifying anti-rheumatic drugs (DMARDs) or glucocorticoids were included between 2002 and 2005 and prospectively followed every 6 months for the 2 first years, then annually. Patients had clinical, biological and radiological assessments at baseline and at each visit.

For the present study, we selected the patients who fulfilled at baseline the 1987 American College of Rheumatology (ACR) criteria [25] for RA, with available baseline and 1-year follow-up hand and foot radiographs, and with available genotyping data for the 3 *IL-2RA* and *IL-2RB* SNPs of interest.

Local institutional review boards approved the study, and written informed consent was obtained from all subjects in the study [24].

1.1.2. RMP cohort

An independent sample of 250 early arthritis patients, fulfilling the 1987 ACR Criteria for RA, included in a regional, longitudinal prospective cohort of early arthritis French patients, recruited from 1992 to 2001, the RMP (Rangueil Midi-Pyrénées) cohort, was used to validate the results of the association study conducted on the ESPOIR cohort [26–28]. All the patients had disease duration of < 1 year.

1.2. Clinical data

1.2.1. ESPOIR cohort

All patients had a clinical examination at baseline and at 1 year. Demographic characteristics including age, gender, symptom duration, current DMARD therapy and mean dose of glucocorticoids were collected, as well as disease activity established on the Disease Activity Score on 28 joints (DAS 28) [29]. Patients were classified as "smokers" or "never smokers" according to their reported smoking habits at baseline.

1.2.2. RMP cohort

All patients included in the study had a yearly clinical examination. Demographic characteristics, disease duration, DAS 28 were collected.

1.3. Immunologic variables

1.3.1. ESPOIR cohort

Anti-CCP2 antibodies (ACPA, ELISA, DiaSorin, France, Positive > 50 U/mL) were analyzed at baseline in a central lab using the same technique.

1.3.2. RMP cohort

Anti-CCP2 antibodies were detected by ELISA according to the instructions of the manufacturer (IMMUNOSCAN RA; Euro-Diagnostica, Arnhem, The Netherlands)

1.4. SNP genotyping

The 3 SNPs of interest located in *IL-2RA* (rs2104286) and *IL-2RB* (rs743777 and rs3218253) genes [19–22] were genotyped using allele-specific kinetic polymerase chain reaction analysis, by KBiosciences (Herts, UK) using the KASPar method both in ESPOIR and RMP cohorts.

The success rate was 97.9% for rs2104286, 98.03% for rs743777 and 96.9% for rs3218253 in ESPOIR cohort and 99.2% for rs2104286, 98.4% for rs743777 and 98.8% for rs3218253 in RMP cohort.

Rs743777 and rs3218253 of IL-2RB were in high linkage disequilibrium (D = 0.18, correlation coefficient R2 = 0.78), explained by the proximity of the locus (6800pb). Thus haplotypes of both SNPs of *IL2RB* were built with Plink software, using the expectation-maximization (EM) algorithm.

1.5. Radiographs

1.5.1. ESPOIR cohort

RA patients included in the ESPOIR cohort had hand and foot radiographs at baseline and at the 1-year visit. The radiographs were centralized and scored by a single experienced rheumatologist (CL) with blinding as to clinical and genetic data, according to the modified van der Heijde Sharp score [30]. The results were expressed in total Sharp score (mTSS), and erosive status (patients with a score > the smallest detectable change, with at least an Erosion Sharp Score ≥ 1). Intraclass correlation coefficient was calculated from a random sample of 30 radiographs scored twice and

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was about 0.99 [31]. The smallest detectable change was calculated at 1.0 mTSS unit.

1.5.2. RMP cohort

Radiographs of the hands and feet of RA patients were obtained at the start of the study and at 1 year. All radiographs were scored by the same investigator (AC), according to the Sharp/van der Heijde method. To determine intraobserver reliability, 30 randomly chosen pairs of radiographs of the hands and feet were scored twice. The intraclass correlation coefficient was 0.98 for mTSS.

1.6. Statistical analysis

Tests for deviation from Hardy–Weinberg equilibrium (HWE) were performed using a standard χ^2 test (1 d.f.).

To take advantage of the prospective character of the data from the ESPOIR and RMP cohorts, which consisted of repeated measurements, and to avoid multiple testing by performing statistical tests at each time point, a generalized linear mixed model for the longitudinal data was used to compare the erosive status according to the genotype distribution for IL-2RA rs2104286 and *IL-2RB* haplotypes of rs743777 and rs3218253 SNPs at baseline and at 1 year, in ESPOIR and RMP cohorts.

Because of the non-normal distribution of the mTSS, the logarithms of mTSS were used and compared between each genotype using a linear model for longitudinal data, at baseline and at 1 year in ESPOIR and RMP cohorts.

The same analyses were repeated on the ACPA positive population of RA patients (ESPOIR: $n = 210$, RMP: $n = 133$).

Since 1 SNP and 1 haplotype of 2 SNPs were evaluated in 2 different populations (whole sample size and ACPA positive patients) with 2 different outcomes (log(mTSS) and erosive status) a Bonferroni correction was applied; the p value for significance was set at 0.004.

The ESPOIR and RMP cohorts were pooled and a meta-analysis was performed to assess the association between the carriage of the risk allele of rs21042286 or the risk haplotype of IL-2RB and the risk of erosion using a fixed effects model with the Mantel-Haenszel method. Statistical heterogeneity among studies was assessed with the I^2-statistics. I^2-values of 25, 50 and 75% were assigned as low, moderate and high estimates, respectively. Heterogeneity was considered significant for $p < 0.10$.

Finally a multivariate analysis was performed to assess the risk of erosion depending on the locus of risk carriage of *IL2-RB* risk haplotype through a generalized linear mixed model for longitudinal data in patients with ACPA presence in ESPOIR cohort. The following factors were firstly tested for the association with erosive status at one year: age, gender, smoking habits, disease duration, DAS28, presence of *HLA-DR Shared Epitope* (including the following alleles of *HLA-DRB1*: *01:01, *01:02,* 04:01, *04:04, *04:05, *04:08 *14:02,*1001) at the inclusion, DMARDs use that proved a structural benefit (methotrexate, leflunomide and biologic agents) and glucosteroids use at one year. The factors associated with erosive status at one year with a p value < 0.1 were then included in the model. A backward procedure was used to exclude variables with no significant association.

All the analyses were performed using the SAS 9.2 software package.

2. Results

2.1. Patients

Among the 813 early arthritis patients included in the ESPOIR cohort, 579 fulfilled the 1987 ACR criteria for RA at baseline; 456 had available sets of hand and foot radiographs at baseline and at 1 year follow-up. Finally, genotyping data for both *IL-2RA* (rs2104286) and *IL-2RB* (rs743777 and rs3218253) were available in 439 RA patients who constituted the final sample of the study. Among the 250 patients included in the RMP cohort, 185 had radiographs at baseline and 1 year and 180 patients had all the 3 SNPs genotyped. The main baseline and one-year demographic and disease characteristics of the 439 early RA patients of ESPOIR cohort and the 180 RA patients of RMP cohort, are presented in Table 1.

Table 1
Demographic and disease characteristics at baseline and at 1 year in ESPOIR and RMP cohorts.

	ESPOIR cohort ($n = 439$)	RMP cohort ($n = 180$)
Baseline characteristics		
Age, years, med [IQR]	51.8 [41.0–57.9]	49.5 [39.3–58.3]
Gender, female, *n* (%)	335 (76)	137 (76)
Smoking status, *n* (%)	216 (49)	NA
Symptom duration, months, median [IQR]	4.9 [3.1–7.4]	6.8 [4.0–10.8]
DAS 28, med [IQR]	5.3 [4.6–6.1]	5.0 [4.0–6.2]
Rheumatoid factor positive, *n* (%)	233 (53)	130 (72)
ACPA positive, *n* (%)	210 (48)	133 (74)
Total Sharp Score, med [IQR]	3 [1–8]	0 [0–4]
Erosive patients, *n* (%)	268 (61)	74 (41)
One-year characteristics		
DAS 28, med [IQR]	3.1 [2.2–4.1]	3.6 [2.3–5.0]
Total Sharp Score, med [IQR]	4 [1–9]	5 [0–14]
Erosive patients, number (%)	287 (65)	123 (68)
Steroids, *n* (%)	177 (40)	99 (58)
DMARDs[a], *n* (%)	284 (65)	73 (49)
Biologics, *n* (%)	45 (10)	0

RA: Rheumatoid arthritis, med: median, IQR: interquartile range, n: number of patients, DMARDs: Disease-Modifying anti-Rheumatic Drugs.
[a] DMARDs that proved a structural benefit (Methotrexate, Leflunomide, Biologic agents).

In RMP cohort, baseline disease duration was slightly higher; the proportion of ACPA positive patients was higher, while baseline radiographic score was lower than in ESPOIR cohort. Furthermore the rate of progression of the radiographic score in RMP cohort within 1 year was higher.

2.2. Association between IL-2RA genotypes or IL-2RB haplotypes and mTSS

The genotype frequencies fit the HWE expectations for both *IL-2RA* (rs 2104286) and *IL-2RB* (rs743777 and rs3218253) SNPs in ESPOIR and RMP cohorts (Table 2). The haplotype frequencies of *IL-2RB* were: AC = 605 (69%), GC: 44 (5%), GT: 229 (26%).

Neither *IL-2RA* genotypes nor *IL-2RB* haplotypes were significantly associated with mTSS in ESPOIR cohort in the whole sample size or in the subgroup of ACPA positive RA.

Neither *IL-2RA* genotypes nor the *IL-2RB* haplotypes were significantly associated with ACPA production (data not shown).

Neither *IL-2RA* genotypes nor *IL-2RB* haplotypes were significantly associated with mTSS in RMP cohort (data not shown).

2.3. Association between IL-2RA genotypes or IL-2RB haplotypes and the risk of erosion

2.3.1. ESPOIR cohort

Neither *IL2RA* genotypes nor *IL-2RB* haplotypes were significantly associated with the presence of erosion after a Bonferroni correction in the whole sample size of the ESPOIR cohort (Table 3). A post-hoc power calculation was performed and the power to detect an association between *IL2RA* genotypes and presence of erosions in the whole sample size was about 8.3% whereas the power to

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Table 2
Association between *IL-2RA* genotypes or *IL-2RB* haplotypes and Total Sharp Score modified by van der Heijde in 439 RA patients in ESPOIR cohort.

Genotypes	Baseline mTSS, med [IQR]	1 year mTSS, med [IQR]	*p* value
IL2RA (rs 2104286)			
ESPOIR			
Whole sample size			
AA (*n* = 252/*57%*)	3 [5–8]	4 [1–8]	0.8
AG (*n* = 158/*36%*)	4 [1–9]	4 [1–10]	
GG (*n* = 29/*7%*)	4 [1–8]	4 [1–8]	
ACPA+ patients			
AA (*n* = 113/*54%*)	3 [1–11]	5 [2–13]	0.8
AG (*n* = 84/*40%*)	5 [1–10]	6 [1–14]	
GG (*n* = 13/*6%*)	5 [1–8]	8 [2–8]	
RMP			
Whole sample size			
AA (*n* = 109/*61%*)	1 [0–4]	8 [3–22]	0.6
AG (*n* = 63/*35%*)	0 [0–3]	7 [1–23]	
GG (*n* = 8/*4%*)	0 [0–1]	5 [0–17]	
ACPA+ patients			
AA (*n* = 82/*62%*)	2 [0–5]	12 [4–24]	0.6
AG (*n* = 46/*35%*)	0 [0–3]	11 [2–28]	
GG (*n* = 5/*4%*)	0 [0–0]	9 [1–9]	
Haplotypes of *IL2RB* (rs743777 and rs3218253)			
ESPOIR			
Whole sample size			
AC/AC (*n* = 213/*49%*)	4 [1–7]	4 [1–9]	0.2
AC/GC or GT (*n* = 179/*41%*)	4 [0–9]	5 [1–10]	
GC or GT/GC or GT (*n* = 47/*11%*)	2 [0–6]	2 [0–7]	
ACPA+ patients			
AC/AC (*n* = 96/*46%*)	5 [2–10]	7 [2–14]	0.6
AC/GC or GT (*n* = 89/*42%*)	5 [1–11]	6 [2–14]	
GC or GT/GC or GT (*n* = 25/*12%*)	3 [0–5]	4 [0–6]	
RMP			
Whole sample size			
AC/AC (*n* = 78/*43%*)	1 [0–4]	9 [3–23]	0.9
AC/GC or GT (*n* = 84/*47%*)	0 [0–4]	7 [1–21]	
GC or GT/GC or GT (*n* = 18/*10%*)	0 [0–3]	5 [0–27]	
ACPA+ patients			
AC/AC (*n* = 60/*45%*)	1 [0–5]	10 [4–26]	0.9
AC/GC or GT (*n* = 60/*45%*)	1 [0–5]	11 [4–24]	
GC or GT/GC or GT (*n* = 13/*10%*)	0 [0–3]	12 [0–27]	

ACPA: anti-citrullinated peptide antibodies mTSS: total Sharp Score modified by Van der Heijde, IQR: interquartile range, *p* value: obtained with a linar model for longitudinal data.

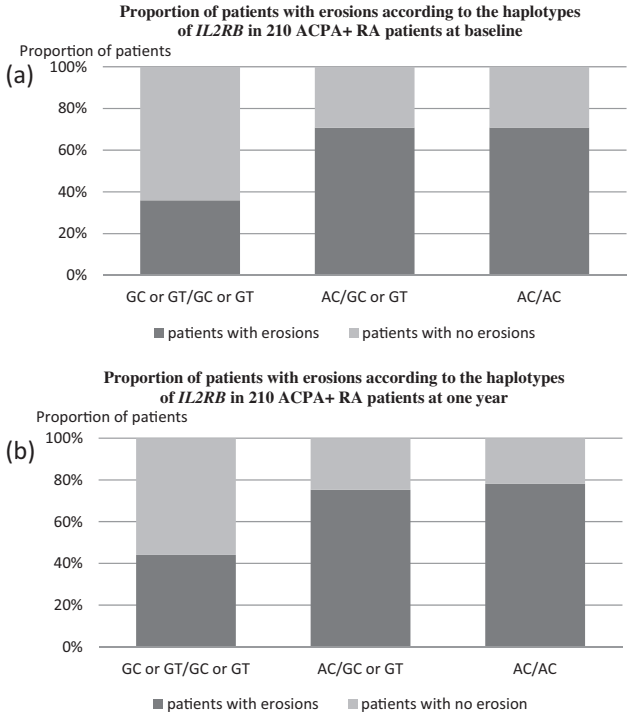

Fig. 1. Proportion of patients with erosions at baseline or at one year according to the *IL2RB* haplotypes in the ACPA+ population (*n* = 210) of ESPOIR cohort at baseline and one year. ACPA: anti-citrullinated peptide antibodies, RA: rheumatoid arthritis.

detect an association between *IL2RB* haplotype and the presence of erosions was about 22%.

In the subgroup of ACPA positive RA patients, the haplotype AC of *IL-2RB* was associated with a higher rate of erosions at baseline and at 1 year follow-up in comparison to other haplotypes (by genotype analysis: proportion of patients with erosions at baseline: AC/AC: 71%, AC/GC or GT: 71%, GC or GT/GC or GT: 36%, at 1 year: AC/AC: 78%, AC/GC or GT: 75%, GC or GT/GC or GT: 44%, $p = 0.001$ for comparison of AC/AC to GC or GT/GC or GT and $p = 0.002$ for comparison of AC/GC or GT to GC or GT/GC or GT).

The proportion of ACPA positive RA patients with erosions at baseline or at one year according to *IL2RB* haplotypes is presented in Fig. 1.

No association was found between the genotypes of *IL2RA* genotypes and the risk of erosion in the subgroup of ACPA positive RA patients. The power to detect an association between *IL2RA* genotypes and presence of erosions in the ACPA+ sample was about 2.8% whereas the power to detect an association between *IL2RB* haplotype and the presence of erosions was about 55%.

2.3.2. RMP cohort

In RMP cohort, the rate of erosion was higher in AC haplotype carriers at baseline and 1 year (by genotype analysis: proportion of patients with erosions at baseline: AC/AC: 46%, AC/GC or GT: 39%, GC or GT/GC or GT: 28%, at one year: AC/AC: 71%, AC/GC or GT: 70%, GC or GT/GC or GT: 50%) but we failed to show a statistical significance probably because a lack of power in this sample (AC haplotype carriage: $p = 0.1$). However, there was a significant trend towards higher rates of progression among AC haplotype carriers (Cochran-Armitage trend test: $p = 0.04$).

No additional statistical significant association was found in RMP cohort after stratification on ACPA presence. The power to detect an association between *IL2RA* genotypes and presence of erosions in the whole sample was about 15% whereas the power to detect an association between *IL2RB* haplotype and the presence of erosions was about 25%. In the ACPA+ sample, the power to detect an association between *IL2RA* genotypes and presence of erosions in the whole sample was about 14% whereas the power to detect an association between *IL2RB* haplotype and the presence of erosions was about 17%.

2.4. Meta-analysis assessing the risk of erosions at 1 year in ESPOIR and RMP cohorts according to the carriage of AC haplotype of IL2RB

A fixed effects model with the Mantel-Haenszel method meta-analysis of ESPOIR and RMP data was then performed to assess the risk of erosion at 1 year according to the carriage of AC haplotype of *IL2-RB* (Figs. 2–3). No significant heterogeneity was found ($I^2 = 0\%$, $p > 0.1$). The analyses showed that AC haplotype carriage increased the risk of erosion at one year in the whole sample (AC haplotype carriage: OR[95%CI] = 1.92[1.14–3.22], $p = 0.01$) and the strength of this association was increased in ACPA positive patients (AC haplotype carriage: OR[95%CI] = 3.34[1.68–6.67], $p = 0.0006$).

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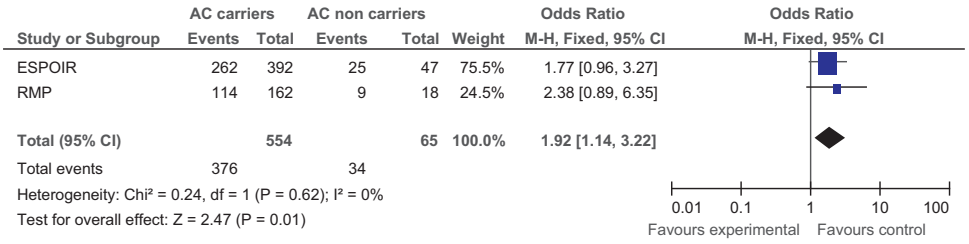

Fig. 2. Meta-analysis assessing the risk of erosion depending on carriage of AC haplotype of *IL-2RB* in all RA patients.

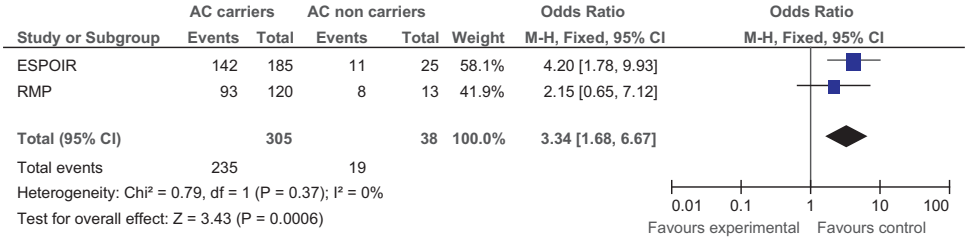

Fig. 3. Meta-analysis assessing the risk of erosion depending on carriage of AC haplotype of *IL-2RB* in ACPA+ RA patients. MH: Mantel-Haenszel; CI: 95% confidence interval.

2.5. Identification of independent risk factors of erosions in early RA in ESPOIR cohort

The multivariate analysis was only performed in ESPOIR cohort because some important data such as smoking habits were missing in RMP cohort and in the subgroup of ACPA positive patients since the association between the carriage of AC haplotype of *IL-2RB* and erosions was statistically significant only in this stratum in ESPOIR cohort.

Firstly, univariate analyses were performed to identify the variables associated with erosions in 210 ACPA positive RA patients of ESPOIR cohort. Age, gender, disease duration at the inclusion, smoking habits and DMARDs use that proved a structural benefit (including methotrexate, leflunomide and biologic agents) use were significantly associated with erosion with a p value >0.1 and included in the generalized linear mixed model. A backward procedure was used to exclude the variables not significantly associated with erosion with a p value >0.05.

AC haplotype carriage was independently associated with the risk of erosion in this model (OR [95%CI] = 6.15 [2.03–18.56], $p = 0.001$). The other variables significantly associated with the risk of erosions in both models were age and smoking habits (Table 4).

3. Discussion

The present study investigated the contribution of *IL-2RA* (locus 10p15) and *IL-2RB* (locus 22q12) on RA severity, assessed on the risk of erosions in early RA patients. Our results revealed an association between a haplotype of 2 SNPs of *IL-2RB* (rs743777 and rs3218253) and the risk of erosions in ACPA positive but not in ACPA negative RA patients while there was no association between the *IL-2RA* SNP (rs2104286) and structural damage. This association remained significant after multivariate analysis, suggesting that *IL-2RB* is an independent risk factor for erosions in ACPA positive early RA patients. The restriction of the association between the haplotype of *IL2RB* and erosions to the subgroup of ACPA positive patients illustrates that ACPA positive and negative RA are 2 different entities with different genetic background and determinants.

A GWAS undertaken in the British population comparing 2,000 RA patients and 3,000 controls, first reported a moderate evidence of association between SNP mapping close to both the alpha (rs2104286) and beta (rs743777) chains of IL-2R and RA susceptibility.[19] A validation study undertaken in subjects of European ancestry, which compared 4,106 RA patients and 11,238 controls, confirmed the previously reported association between the A allele of the SNP mapping 6 kb upstream (rs743777) of the transcription start site of *IL-2RB* and revealed an association between C allele of another SNP mapping within intron 1 of *IL-2RB* (rs3218253) and RA susceptibility [22]. The contribution of these 2 SNPs to the risk of RA was then assessed in an independent Dutch case-control study, which compared 616 RA patients and 545 healthy ethnically and geographically matched controls, and provided additional evidence for the association between *IL-2RA* and *IL-2RB* polymorphisms and RA susceptibility [20]. Recently, a GWAS meta-analysis of 5,539 autoantibody-positive individuals with RA and 20,169 controls of European descent, followed by replication in an independent set of 6,768 RA cases and 8,806 controls, established the association between G allele of *IL-2RA* (rs2104286) and C allele of *IL-2RB* (rs3218253) and RA susceptibility.[32] Finally, another validation study on 983 RA patients and 1007 healthy controls in North-India found a significant association between both rs2104286 and rs3218253 and RA susceptibility [23]. To our knowledge, an association between these polymorphisms and the severity of RA has never been demonstrated.

In the validation study on RMP cohort, the statistical analyses failed to demonstrate a significant association between the haplotype located on *IL-2RB* and the risk of erosions. However, the power of the test was only about 25% in the whole sample size and 17% in the ACPA+ population and we assume that the poor sample explain the failure of the test to detect a significant difference. We assume that this negative result may be due to a lack of power. Furthermore, after meta-analysis, the association remained significant with no heterogeneity suggesting that the association had the same direction in both cohorts. There was a difference between both cohorts in terms of rate of progression within one year. Indeed, RMP patients had a higher rate of progression than ESPOIR patients. These differences might be explained by the different inclusion criteria and the different dates of inclusion in both cohorts (between 2002 and 2005 for ESPOIR and between 1992 and 2001 for RMP) implicating differences in the treatment strategies and a worse prognosis in RMP patients. As these polymorphisms are newly identified and

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Table 3
Association between *IL-2RA* genotypes or *IL-2RB* haplotypes and erosive status in 439 RA patients in ESPOIR cohort.

Genotypes	Baseline	1 year	OR [95%CI]	*p* value
IL2RA (rs2104286) ESPOIR				
Whole sample size				
AA (*n* = 13252/*57%*)	156 (62)	166 (66)	1.46 [0.54–3.96]	0.5
AG (*n* = 13158/*36%*)	96 (61)	104 (66)	1.41 [0.50–3.94]	0.5
GG (*n* = 1329/*7%*)	16 (55)	17 (59)	1	–
ACPA+ patients				
AA (*n* = 1396/*46%*)	77 (68)	83 (73)	0.88 [0.19–4.06]	0.9
AG (*n* = 1389/*42%*)	54 (64)	60 (71)	0.74 [0.15–3.49]	0.7
GG (*n* = 1325/*12%*)	9 (69)	10 (77)	1	–
RMP				
Whole sample size				
AA (*n* = 13109/*61%*)	50 (46)	76 (70)	2.82 [0.57–13.77]	0.2
AG (*n* = 1363/*35%*)	22 (35)	43 (68)	1.37 [0.69–2.70]	0.4
GG (*n* = 138/*4%*)	2 (25)	4 (50)	1	–
ACPA+ patient				
AA (*n* = 1382/*62%*)	41 (50)	64 (78)	3.43 [0.48–24.20]	0.2
AG (*n* = 1346/*35%*)	20 (43)	34 (74)	1.33 [0.60–2.91]	0.3
GG (*n* = 135/*4%*)	1 (20)	3 (60)	1	–
Haplotypes of IL2RB (rs743777 and rs3218253) ESPOIR				
Whole sample size				
AC/AC (*n* = 13213/*49%*)	131 (62)	140 (66)	1.96 [0.86–4.42]	0.1
AC/GC or GT (*n* = 13179/*41%*)	114 (64)	122 (68)	2.23 [0.97–5.11]	0.06
GC or GT/GC or GT (*n* = 1347/*11%*)	23 (49)	25 (53)	1	–
ACPA+ patients				
AC/AC (*n* = 1396/*46%*)	68 (71)	75 (78)	6.45 [2.09–19.82]	0.001
AC/GC or GT (*n* = 1389/*42%*)	63 (71)	67 (75)	5.94 [1.92–18.42]	0.002
GC or GT/GC or GT (*n* = 1325/12%)	9 (36)	11 (44)	1	–
RMP				
Whole sample size				
AC/AC (*n* = 1378/*43%*)	36 (46)	55 (71)	2.70 [0.87–8.34]	0.08
AC/GC or GT (*n* = 1384/*47%*)	33 (39)	59 (70)	2.24 [0.73–6.85]	0.2
GC or GT/GC or GT (*n* = 1318/10%)	5 (28)	9 (50)	1	–
ACPA+ patients				
AC/AC (*n* = 1360/*45%*)	31 (52)	46 (77)	2.54 [0.69–9.26]	0.2
AC/GC or GT (*n* = 1360/*45%*)	27 (45)	47 (78)	2.16 [0.60–8.07]	0.2
GC or GT/GC or GT (*n* = 1313/*10%*)	4 (31)	8 (62)	1	

OR[95%CI]: Odds ratios with 95% confidence interval obtained with a generalized linear mixed model for longitudinal data. ACPA: anti-citrullinated peptide antibodies

the sample size of the cohorts small compared to other studies, the association with severity might be overestimated in ESPOIR because of the bias of the winner's curse. This might also explain the lack of association in RMP cohort. Further studies should be necessary to confirm the association.

While SNP rs743777 maps 6 kb upstream of the transcription start site of *IL-2RB* and SNP rs3218253 maps within intron 1 of *IL-2RB*, the molecular mechanisms through which these SNPs affect RA susceptibility and severity remain speculative. Furthermore, a linkage disequilibrium was observed between both SNPs and thus we may assume that both SNPs could be markers of another polymorphism that could play a role in the expression or the conformation of IL2RB. IL-2 is a cytokine that exerts its pleiotropic biological activities by binding to its receptor on T-cells or other cell types. IL-2Rα (CD25) affords high-affinity to IL-2R, which is comprised of β (CD122) and γc (CD132) chains.[17] The signaling function of the high-affinity IL-2R is mediated through its IL-2Rβ and IL-2Rγc chains. Thus, SNPs affecting either *IL-2RB* transcriptional level or IL-2Rβ structural and/or functional properties could affect both immune and inflammatory responses, via IL-2/IL-2R.

Table 4
Multivariate analysis assessing erosive status at baseline and one year including carriage of AC haplotype of *IL-2RB*, age, sex, clinical center, symptoms duration, DAS 28, smoking habits as covariates in 210 ACPA positive RA patients from ESPOIR cohort.

	OR [95%CI]	*p* value
Age	1.08 [1.04–1.12]	< 0.0001
Female gender	0.49 [0.18–1.29]	0.1
Disease duration	1.05 [0.99–1.11]	0.09
Smoking habits	0.34 [0.14–0.76]	0.009
DMARDs[a] use	1.22 [0.50–2.96]	0.7
Time of visit	1.68 [0.95–2.93]	0.07
Carriage of AC haplotype of *IL2RB*	6.03 [1.94–18.69]	0.002

OR[95%CI]: Odds ratios with 95% confidence interval obtained with a generalized linear mixed model for longitudinal data; DMARDs: Disease-Modifying Anti-Rheumatic Drugs.

[a] DMARDs that proved a structural benefits (methotrexate, leflunomide and biologic agents).

In this study, the association between *IL2RB* haplotype and the presence of erosions was significant in the meta-analysis in the whole sample but was stronger in the ACPA+ subgroup. Most of studies assessing the association between SNPs and severity had identified such association in ACPA+ RA patients [9,10]. Such differences increase the hypothesis that ACPA+ and ACPA– RA are 2 distinct diseases supported by different genetic backgrounds.

This study provides additional evidence for the association of *IL-2RB* with RA by revealing an association between a haplotype of 2 *IL2-RB* SNPs and the risk of erosions in early French RA patients. The challenge will now be to identify and characterize the causal variants of the *IL2RB* gene and their functional consequences to better understand the role played by IL2RB in the structural damages which characterize the severity of RA.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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