ORIGINAL ARTICLE

RS35705950 polymorphism of MUC5B Gene: Association with Rheumatoid Arthritis and Interstitial lung disease in Tunisian Population

Le polymorphisme RS35705950 du gène MUC5B: Une association avec la Polyarthrite Rhumatoïde et la pneumopathie interstitielle diffuse dans la population tunisienne

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Abstract

Introduction: Interstitial lung disease (ILD) is the most common extra-articular manifestation in rheumatoid arthritis (RA). Studies have concluded that there is an association between rs35705950 polymorphism of the MUC5B gene and RA-ILD.
Aim: To explore this polymorphism in a cohort of Tunisian patients suffering from RA with or without ILD and stufdy its association to ILD during RA.
Methods: A case-control study involving 61 patients followed for RA, 26 with ILD and 35 without pulmonary involvement and 62 healthy controls. This was an association study between genetic marker and RA-ILD by genotyping the rs35705950 polymorphism using PCR-RFLP.
Results: No association was found between rs35705950 polymorphism and RA. However, the comparison of RA-ILD patients with controls showed a significant association with the allele frequencies of rs35705950 polymorphism (p=0.008; OR=2.61; CI [1.2-5.66]). Indeed, the minor T allele increased the risk of developing ILD by 2.61 for RA patients compared to the controls. Comparison of allele frequencies in RA-ILD patients and RA patients without ILD showed a significant association between the minor T allele of the studied polymorphism and RA-ILD (p= 0.02; OR= 2.66; CI [1.09-6.5]). In the adjusted model, this risk increased in case of smoking (p=0.025; OR=3,84; CI [1,13-13,08]) and/or female gender (p=0.013; OR = 4,63; CI [1,33-16,17]).
Conclusion: Our work has confirmed the role of the polymorphism of MUC5B promoter in the appearance of ILD during RA in Tunisian patients. This variant could be used to early detect preclinical ILD in patients with RA.

Key words: rheumatoid arthritis-interstitial lung disease-genetic polymorphism-mucin 5b

Résumé

Introduction: La pneumopathie interstitielle diffuse (PID) est la manifestation extra-articulaire la plus fréquente au cours de la polyarthrite rhumatoïde (PR). Des études ont conclu à l'existence d'une association entre le polymorphisme rs35705950 du gène MUC5B et RA-ILD.
But: L'objectif de cette étude était d'explorer ce polymorphisme dans une cohorte de patients tunisiens atteints de PR avec ou sans PID et d'étudier son association à l'ILD au cours de la PR.
Méthodes: Une étude cas-témoins portant sur 61 patients suivis pour PR, 26 avec PID et 35 sans atteinte pulmonaire et 62 témoins sains. Il s'agit d'une étude d'association entre le marqueur génétique et la PR-PID par génotypage du polymorphisme rs35705950 à l'aide de la PCR-RFLP.
Résultats: Aucune association n'a été trouvée entre le polymorphisme rs35705950 et la PR. Cependant, la comparaison des patients atteints de PR-PID avec des témoins a montré une association significative avec les fréquences alléliques du polymorphisme rs35705950 (p=0,008 ; OR=2,61 ; IC [1,2-5,66]). En effet, l'allèle mineur T augmentait le risque de développer une PID de 2,61 pour les patients atteints de PR par rapport aux témoins. La comparaison des fréquences alléliques chez les patients PR-PID et chez les patients PR sans PID a montré une association significative entre l'allèle mineur T du polymorphisme étudié et la PR-PID (p=0,02 ; OR=2,66 ; IC [1,09-6,5]). Dans le modèle ajusté, ce risque augmentait en cas de tabagisme (p=0,025 ; OR=3,84 ; IC [1,13-13,08]) et/ou de sexe féminin (p=0,013 ; OR = 4,63 ; IC [1,33-16,17]).
Conclusion: Notre travail a confirmé le rôle du polymorphisme du promoteur MUC5B dans l'apparition de la PID au cours de la PR chez les patients tunisiens. Cette variante pourrait être utilisée pour détecter précocement une PID préclinique chez les patients atteints de PR.

Mots clés: polyarthrite rhumatoïde-pneumopathie interstitielle-polymorphisme génétique-mucine5b

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the world population (1) with a female predominance (2). It is characterized by progressive destruction of peripheral joints. Approximately one in two patients presents extra-articular manifestations in which lung damage occupies the first place (3).
Respiratory complication is responsible for significant morbidity and mortality (4), it represents the second cause of mortality after cardiovascular damage and interstitial lung disease (ILD) is the most common of these pulmonary disorders (5).
The etiopathogenesis of this respiratory attack remains poorly understood. Various factors have been found associated with the occurrence of ILD during RA. These factors included male gender, advanced age, smoking, and genetic factors (6). Studies have reported increased mutations in TERT, RTEL1, PARN, and SFPTC genes in patients with RA-ILD (7). It was also shown that the rs35705950 variant of the MUC5B promoter was associated with ILD and particularly with the usual interstitial lung disease (UIP) pattern (8).
Genetic overexpression of MUC5B could lead to elevated levels of the glycoprotein mucin 5B, which leads to dysfunction of mucociliary clearance (6).
The presence of the MUC5B promoter variant is also significantly linked to radiographic indices of undiagnosed lung involvement and could be an important genomic marker for early identification of ILD. Consequently, MUC5B genotyping could guide the management of RA-ILD as it helps provide more information on the prognosis and pathogenesis of this condition (9).
To the best of our knowledge, no genotype-phenotype correlation study has been carried out in RA-ILD in a Tunisian population. The main objective of our study was to evaluate a possible association between the rs35705950 polymorphism of the MUC5B gene and RA with or without ILD.

Methods

Population Study

A cross-sectional case-control study involving 61 patients suffering from RA, collected at the Rheumatology department and 62 unaffected controls that represent DNA samples from healthy subjects with all pathologies, present in the genetics department bank which were preserved for research work after the consent of all subjects. The control group had a median age of 35 years, consisting of 33 men and 29 women. The study was carried out with the approval of Institutional Ethics Committees. Informed consent was obtained from all the subjects of the study.

Inclusion criteria

Patients are included meeting the criteria of the American College of Rheumatology (ACR) 1987 (10) or the criteria of the ACR/European League Against Rheumatism (EULAR) 2010 classification (11). Two groups of patients were included: a first group (RA-ILD) including 26 patients presenting ILD in the context of RA, confirmed by a computed tomography (CT) scan that was performed before the inclusion and a second group (RA without ILD) made up of 35 RA patients without CT interstitial lung damage.

Non-inclusion criteria

Patients presenting another connective tissue disease apart from RA likely to induce interstitial lung damage, and patients who have not been explored by chest CT are excluded.

Clinical data collection

Socio-demographic characteristics, clinical, biological and therapeutic parameters for each patient were collected. The presence or absence of ILD was confirmed for each patient by chest CT.

Genetic study

A case/control study is made for an association analysis between MUC5B gene promoter polymorphism (7) and rheumatoid arthritis with or without ILD.
Genotyping of the rs35705950 G/T polymorphism was carried out by RFLP-PCR: after blood collection, DNA extraction and PCR amplification, restriction digestion was made using HhaI endonuclease which cleaves the PCR fragment in presence of the G allele of the SNP studied.

Terminology

Minor allele: For a given single nucleotide polymorphism (SNP), the least common (or rarest) allele is called the "minor allele." An allele is defined by each of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome.
Codominance form: refers to a type of inheritance in which two versions (alleles) of the same gene are expressed separately to yield different traits in an individual.
Dominance form: refers to a specific relationship between two variants (alleles) of a single gene, in which one allele masks the effect of the other in influencing some trait.

Statistical analyses

The data were analyzed using the (IBM® SPSS Statistics 22.0) software. The results were considered to be significant at p values of less than 0.05. The comparison of the allelic frequencies was made by the chi-square test ($\chi 2$) using the Epi-Info 7.0 software. The differences in the distribution of genotype frequencies were calculated using the $\chi 2$ test. Genotype frequencies were checked for deviation from Hardy–Weinberg equilibrium using the SNP Stat software.

Results

Characteristic features of the patients

Patients with RA-ILD, compared to those without ILD, were more likely to be female, older, and more likely to have ever smoked (9 % vs. 2 %). After adjustment for sex, patients with RA-ILD and those with RA without ILD did not differ significantly with respect to positivity for rheumatoid factor or anti–citrullinated protein antibody (yes or no), erosive status of RA (erosions present or not), exposure to methotrexate (yes or no), the mean duration of RA from diagnosis to study inclusion, or the presence of extra articular manifestations. Overall, 69.23 % of patients with RA-ILD had a usual interstitial pneumonia (UIP) or possible UIP pattern on high-resolution CT.
The Comparison of the different parameters from the two groups is summarized in table 1.

Table 1. General characteristics of the studied groups

	RA-ILD	RA without ILD	p
Age (years)	63.08	58.34	0.196
Gender (female/male)	11.6	34.10	0.06
Smoker statut (%)	9	2	**0.004**
RA duration (years)	11.19	13.14	0.373
Erosive statut (%)	76.92	68.57	0.798
DAS28 score	5.82	4.91	**0.010**
Rheumatoid nodules	6	2	0.101
Dry syndrome	9	3	0.916
Renal manifestations	1	0	0.242
Osteoporosis	14	15	0.395
Mean ESR (mm h1)	50.26	33,74	**0.032**
Mean CRP (mg/l)	51,30	22,31	**0.006**
Anti-CCP (+)> 50UI, n (%)	14 (58,33)	14 (46,67%)	0.394
RF (+)>20 UI, n (%)	15 (62,5)	14 (43,75)	0.165
Methotrexate exposure, n (%)	18 (69,23)	26(74,28)	0,6

Genetic analysis

Genotypes are deduced from the agarose gel electrophoresis of the HhaI digest product of the amplified DNA fragment which contains the polymorphism analyzed (Figure 1).

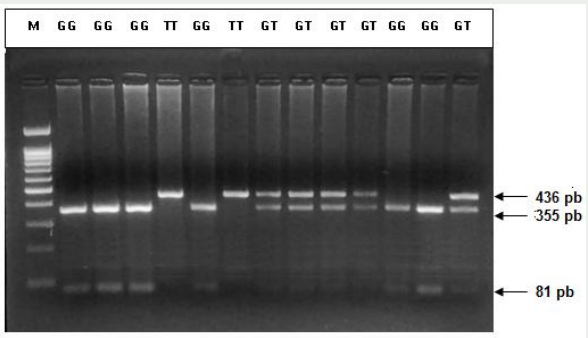

Figure 1. RFLP-PCR products of MUC5B promoter polymorphism.

GG genotypes are deduced in presence of two fragments (355 and 81 bp); **TT** genotypes are deduced with the unique 436 bp undigested fragment. **GT** genotypes are deduced in presence of the three fragments.

MUC5B promoter variant and risk of RA

The study of the association between the rs35705950 polymorphism of MUC5B gene and the risk of developing RA showed the absence of significant difference in allelic and genotype frequencies between the cohort of patients and that of controls, whether for the crude or the adjusted model by age, sex and smoking. This suggests that there is no association between rs35705950 polymorphism and the development of RA (Table 2).

Table 2. Research of association between the rs35705950 polymorphism of the MUC5B gene and the risk of developing RA

	Patients (n=61)	Controls (n=60)	Crude model	Adjusted model (Age+Sexe+Tabagisme)
			P	P
Allele				
G	96 (0,79)	106 (0,85)	0,18	
T	26 (0,21)	18 (0,15)		
Genotype				
GG	40 (0,66)	46 (0,74)	0,39	0,68
G/T	16 (0,26)	14 (0,23)		
T/T	5 (0,08)	2 (0,03)		

MUC5B promoter variant and risk of RA-ILD

The comparison of allele frequencies between RA patients with ILD and RA patients without ILD showed that the T minor allele of rs35705950 polymorphism of MUC5B was associated with the risk of ILD development in RA patients. The presence of this allele increases 2.66-fold the risk of disease susceptibility (P = 0.02; OR = 2.66; CI [1.09-6.5]).
By analyzing the genotype frequencies in the crude and adjusted models for age, sex and smoking status combined, the association was only observed for the adjusted model with the codominant and the dominant form (P = 0.034; OR = 3.79; CI [0.96-14.98] and P = 0.014; OR = 4.71; CI [1.31-17]) respectively (Table 3). This analysis showed that the TT genotype increased the risk of ILD complications without relation to age (p = 0.15). However, gender and smoking status intervene in this association: in the dominant model, the risk of developing ILD is increased in female gender (P = 0.025, OR = 3.84) and in individuals with the smoking status (P = 0,013; OR = 4.63) (table 4). These results are summarized in Tables 2 and 3.

Table 3. Association between rs35705950 polymorphism of the MUC5B gene and the risk of developing ILD in patients with RA in crude and adjusted models (age, sex and smoking combined).

	ILD (+) (n=26)	ILD (-) (n= 35)	Crude model		Adjusted model (Age+Sexe+Tabagisme)	
			P	OR	P	OR
Allele						
G	36 (0,69)	60 (0,86)	**0,02**	**2,66 (1,09-6,5)**		
T	16 (0,31)	10 (0,14)				
Genotype						
GG	14 (0,54)	26 (0,74)		REF		
G/T	8 (0,31)	8 (0,23)		1,86 (0,57-6,02)		**3,79 (0,96-14,98)**
T/T	4 (0,15)	1 (0,03)	0,12	7,43 (0,76-73,04)	**0,034**	**10,8 (0,95-123,24)**
Dominant (G/T+T/T vs G/G)			0,097	2,48 (0,84-7,3)	**0,014**	**4,71 (1,31-17)**
Recessif (TT vs G/G+G/T)			0,074	6,18 (0,65-59,01)	0,081	6,71 (0,63-71,06)

Table 4. Association between MUC5B gene rs35705950 polymorphism and risk of developing ILD in RA patients in crude and adjusted models (age, sex and smoking separately).

	ILD (+) (n=26)	ILD (-) (n= 35)	Crude Model		Adjusted model					
					Age		Gender		Smoking statut	
			P	OR (IC)	P	OR (IC)	P	OR (IC)	P	OR (IC)
Allele										
G	36 (0,69)	60 (0,86)	**0,02**	**2,66 (1,09-6,5)**						
T	16 (0,31)	10 (0,14)								
Genotype										
GG	14 (0,54)	26 (0,74)		REF		REF		REF		REF
G/T	8 (0,31)	8 (0,23)	**0,12**	1,86 (0,57-6,02)	0,15	1,91 (0,57-6,36)	**0,055**	3,08 (0,82-11,52)	**0,026**	3,62 (0,95-13,77)
T/T	4 (0,15)	1 (0,03)		7,43 (0,76-73,04)		6,44 (0,65-63,78)		9,13 (0,82-101,67)		12,51 (1,12-139,77)
Dominant form (G/T+T/T vs G/G)			0,097	2,48 (0,84-7,3)	0,1	2,47 (0,82-7,49)	**0,025**	**3,84 (1,13-13,08)**	**0,013**	**4,63 (1,33-16,17)**
Recessive form (TT vs G/G+G/T)			0,074	6,18 (0,65-59,01)	0,1	5,33 (0,55-51,27)	0,088	6,21 (0,61-63,73)	0,057	7,69 (0,75-78,95)

MUC5B promoter variant and clinical data

No association was observed for RA-ILD patients, in the limit of the study sample, between the rs35705950 polymorphism and the pattern UIP or PINS of ILD (p = 0.5), the rheumatoid factor and the anti-CCP antibodies (p = 0.5 and p = 0.28 respectively) and the RA activity, according to DAS28 score (p =0.5).

Discussion

The MUC5B promoter variant rs35705950 is the strongest genetic risk factor for idiopathic pulmonary fibrosis (IPF). In this study, we found that it was also a strong risk factor for RA-ILD. However, the MUC5B promoter this variant does not appear to be a risk factor for the development of RA. This has been demonstrated by previous genome-wide association studies (12). In the study by Juge et al (7), the comparison of RA-ILD patients and controls revealed that none of the case series showed a significant difference in the frequency of the MUC5B promoter variant, results which suggest a lack of association between the variant of this gene and RA.

The effect of the MUC5B promoter variant on the development of ILD in patients with RA was similar in magnitude and direction to that observed in patients with IPF. In the same study of Juge et al (7), the rs35705950 promoter variant, which is the most important risk factor for the development of IPF, could also explain the risk of ILD in RA patients. In this study, the authors tested the association of the rs35705950 polymorphism of MUC5B gene in RA patients with and without ILD. They concluded on an association between the minor allele of the MUC5B promoter with RA-ILD patients compared to the control group (OR = 3.8; P = 9.7×10−17).

The relationship between the MUC5B promoter variant and RA-ILD appears to be specific to the UIP pattern (7). But this was not demonstrated in our study. The UIP pattern is most associated with the rs35705950 polymorphism (13). It was found in 63.2% of patients carrying this polymorphism (6). In another study, the presence of the MUC5B rs35705950 variant was found to increase fivefold the risk of UIP-like RA-ILD (14).

The best-known pathophysiological explanation is that the MUC5B gene encodes the airway mucin glycoprotein 5B, which plays a role in mucociliary clearance. Genetic overexpression of MUC5B could result in elevated levels of this protein, impairing mucociliary clearance and disrupting normal lung repair mechanisms. It may be hypothesized that the possible mechanism for the effects of MUC5B variants in pulmonary involvement in RA might be explained in two other ways. First, increased levels of mucin-5B may be expressed in the metaplastic epithelium lining the reticular fibrosis cysts in RA-ILD. Second, the exon variants may affect the charge of mucin-5B in which the low-charge glycated form of mucin-5B leads to the structural anomalies and functional changes in the lung in RA-ILD (6).

In our study, there was no association between the MUC5B polymorphism and age in developing ILD in RA patients. Our results are consistent with those of previous studies focusing on the rs35705950 polymorphism in RA-ILD patients that did not find its association with age (6).

Although tobacco represents an important risk factor for the development of ILD in RA (15), no interaction of tobacco smoke exposure with the MUC5B promoter variant was observed in Kadura et al study (16). On the contrary, in our work, the adjusted analysis showed that genotypes carrying the T allele increase the risk of ILD complications in RA when the individual are smokers.

On another hand, male gender is known to be a risk factor for the appearance of ILD during RA, but a significant association between the rs35705950 polymorphism of the MUC5B gene and the gender of the individual has not been described in the literature. However, the study carried out in China (6) showed that 63.2 % of RA-ILD patients presenting the rs35705950 polymorphism of the MUC5B gene are female, but without being statistically significant. The results in our study support those of the Chinese team and show significant association: RA women had 3.84 times the risk of developing ILD (P = 0.025) in the dominant model.

Through the results of our study, the MUC5B promoter variant could be used to detect preclinical ILD in patients with RA. However, we should increase the number of RA patients analyzed in order to confirm our findings. We should also consider systematically requesting a chest CT in all patients at the time of diagnosis of RA and during their follow-ups in order to detect this pulmonary involvement and monitor its evolution.

Finally, our work was the First in Tunisia to study the association between ILD in RA patients and polymorphism rs35705950 of MUC5B gene. This study also evaluated the association between this polymorphism and the appearance of RA and it has studied the factors influencing its expression.

The small number of patients is the main drawback. A selection bias is present. The patients selected are those who have RA and who have already had a chest CT scan. We were unable to perform the chest CT scan after the selection of RA patients due to lack of resources and time.

Conclusion

Based on our study and those in the literature, the minor allele of the rs35705950 variant of the MUC5B promoter could be identified as a risk factor for RA-ILD Tunisian patients and more precisely for the UIP pattern. This risk increases in case of smoking and/or female gender. The MUC5B promoter variant could be used to early detect preclinical ILD in RA patients. The estimates of the association of the MUC5B promoter variant with RA-ILD are equivalent to those observed with IPF. Therefore, drugs known to be effective in treating patients with IPF may be evaluated in the treatment of RA-ILD. The pathogenesis of RA-ILD is complex and multifactorial, but the involvement of the MUC5B gene and its protein product, mucin 5B, has been implicated in the development of this pathology. Further research is needed to fully understand the underlying mechanisms and develop targeted therapies for this condition.

Abbreviations
CT: Computed Tomography
ILD: Interstitial Lung Disease
IPF: Idiopathic Pulmonary Fibrosis
RA: Rheumatoid Arthritis
UIP: Usual Interstitial Pneumonia
RF: Rheumatoid Factor

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