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Genetic Susceptibility to Rheumatoid Arthritis in West African Patients: Correlation with Rheumatoid Factor, Anticitrullinated Protein Antibody and Radiological Damage

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Abstract

Background: Rheumatoïd arthritis (RA) is characterized by a multifactorial aetiology and a complex genetic background, with the major histocompatibility complex (MHC) region playing a major role.

Aim of the work: To detect the genetic susceptibility to RA in Benin population.

Patients and Methods: A prospective case-control study was conducted from December 2015 to December 2016. The selected unrelated patients suffered from RA according to the American College of Rheumatology (ACR) criteria. We genotyped *HLA-DRB1*01*, *HLADRB1*04* and *HLA-B* locus between 52 west african patients with RA and 48 unrelated controls to study the susceptibility of the genes to RA among Patients. Low-resolution genotyping for HLA B and H*LADRB1* alleles was performed using Polymerase Chain Reaction and Sequence-Specific Primers (PCR-SSP). The data were analysed using epidata software.

Results: Firty-two patients were recruited in the RA group and forty-eight in the control group. The mean age of the patients in the RA group was 44.6 ± 14.1 (15-69) and the sex ratio (male/female) was 0.14. The anticitrullinated protein antibody (ACPA) was present at 69.20 % of them. Among 3 genes studied, only *HLADRB1*01* (OR=1.60, 95% CI=0.70–3.66) and *HLADRB1*04* (OR=1.66, 95% CI=0.69–4.02) genes have shown a significant association with RA. There's no association with RA and *HLA-B* genes.

Conclusion: This work has allowed us to show the involvement of *HLADRB1*01* and *HLADRB1*04* genes in RA and know the immunogenetic profile of some RA West African patients.

Keywords: Rheumatoid arthritis; *HLADRB1*01*; *HLADRB1*04*; West africa

Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory rheumatic disease which affects several organs and tissue, predominantly the synovial joints. This disease leads to progressive destruction of articular cartilage and ankylosis of the joints [1,2]. In the last years, several epidemiological studies developed in countries from the North Europe and North America have shown variations in the incidence and prevalence of RA across populations estimating prevalences of 0.5–1.1% [3]. In Africa, Its prevalence is estimated between 0.8% and 2.3% [4,5]. There are great variations depending on countries and ethnic groups. Like many autoimmune diseases, the etiology of RA is multifactorial. Several factors are involved in the predisposition to the disease. These include psychological, hormonal, environmental and genetic factors [2]. RA genetic risk factors can be classified into two groups: major histocompatibility complex (MHC) genes especially Human Leucocyt Antigen (HLA) and non-MHC regions [6]. It is known that RA is associated with certain *HLA-DR* alleles in different populations [7-9]. Apart from the work carried out in the Maghreb countries such as Egypt and Morocco [10,11], few countries in subsaharan africa study an association between RA and the *HLA-BRB1* alleles [12,13]. Viatte et al. showed in their study that The alleles of non-HLA Caucasian suffered from RA were different between Caucasians and Africans and several polymorphisms were barely detectable in West/Central Africa [14]. The aim of this work was to study the genetic susceptibility of RA in Benin population, one of the less developed country in west Africa.

Patients and Methods

This was a prospective case-control study conducted from December 2015 to December 2016 in the rheumatology unit of the National Hospital University Hubert Koutoukou Maga of Cotonou and Laboratory of Cytogenetics and Molecular Biology of the Institute of Applied Biomedical Sciences of Cotonou. The patients who took part of the study have been consulted in the hospital rheumatology unit during the study period, have suffered from RA according to the 2010 EULAR/ACR criteria and have made all samples for HLA typing tests, have given consent.

The study was approved by the Scientific Ethical Committee of the National Hospital Univeristy Hubert Koutoukou Maga of Cotonou. All participants in this study provided their written informed consent.

Morever, The *HLA* frequencies of patients were compared with the ones of a control group consisting of unrelated individuals from the same geographic origin without disease and matched with sex and age.

Data collection was done initially, using a survey form which identified the general characteristics (age, sex, occupation, address) of the two groups.

HLA genotyping:

It was performed by the following steps [15]:

Peripheral blood samples (10 mL) were collected in EDTA. Genomic DNA was obtained from proteinase-K–treated peripheral blood leukocytes by the phenol-chloroform technique.

Low-resolution genotyping for *HLA-B* and *HLA-DRB1* alleles (*DRB1 01, DRB1 04*) was performed using polymerase chain reaction and sequence-specific primers (PCR-SSP).

For HLAB, forward primer: 5' GGG AGG AGC GAG GGG ACC GCA 3'and reverse primer 5' ATC TCG GAC CCG GAG ACT CG 3'

For HLADRB1 01, forward primer: 5'GGC AGC TTA AGT TTG AAT G 3' and reverse primer: 5' TCG CCG CTG CAC TGT GAA G 3'

For HLADRB1*04, forward primer: TCG CCG CTG CAC TGT GAA G and reverse primer: GTT TCT TGG AGC AGG TTA AAC

The PCR was carried out according to the following protocol:

For *HLA-DRB1* alleles, the PCR reaction mixture contained: 3 µL of 1 X Buffer, 1.8 µL $MgCl_2$ (25 mM), 0.6 µL NTPs (10 mM), 0.9 µL, *HLA-DRB* allele (10 mM) forward primer, 9 µL *HLA-DRB* allele (10 mM) reverse primer, 6 µL DNA, 0.25 µL AmpliTaq (250U), 16.55 µL wáter.

For *HLA-B*, the PCR reaction mixture contained: 3 µL 1 X Buffer, 1.8 µL $MgCl_2$ (25 mM), 0.6 µL NTPs (10 mM), 1.2 µL *HLA*-B (10 mM) primer, 1.2 µL *HLA-B* (10 mM) reverse primer, 6 µL DNA, 0.25 µL AmpliTaq (250U), 15.95 µL wáter.

The reaction was carried out with following cycles:

Initial denaturation at 95°C for 5 min.

37 cycles of denaturation at 95°C for 30s.

Annealing at 57°C for 30s.

Extension at 72°C for 30s.

Final extension at 72°C for 7 min.

After cooling, the PCR products were deposited in the wells of the gel in a well-established order and subjected to electrophoretic migration in the T10E1 buffer solution at 50 MVOLT for 30 min. PCR-SSP products were visualized by the amplification product after electrophoretic migration. After 20 min of migration in the electric field, the gel was then read by means of a UV lamp transilluminator (Vulbert Lourmat brand) equipped with a camera and an LCD screen.

The reading was made according to the presence or not of the genes after comparing the bands obtained for the samples with respect to the bands obtained for the positive controls.

Otherwise, the detection of Rheumatoid Factor (RF) and ACPA (Anticitrullinated Protein Antibody) have been determined by Enzyme-Linked Immunosorbent Assay (ELISA) with a positivity threshold of 20 IU/ml for RF and 5 IU/ml for ACPA

Statistical analysis

Data was analyzed using EpiData and SPSS17.0 software. Student's test was used to compare the differences between both groups. Chi square and Z tests were used to test the significance for frequency and the corrected Chi square test for frequencies 2 groups respectively. Odds ratio (OR) with 95% confidence interval (95% CI) values were also calculated. Statistical significance was set at $p<0.05$.

Results

Fifty-two patients were recruited in the RA group and forty-eight in the control group. Among The RA patients, 10 come from the west African counties (Togo: 3 patients, Nigeria: 4 patients, Senegal: 1 patient, Burkina Faso: 1 patient and Mali: 1 patient). The mean age of the patients in the RA group was 44.6 ± 14.1 (15-69) and the sex ratio (male/female) was 0.15. The mean age at disease onset was 31.7 ± 7.03 (15–61) The mean Disease activities score for 28 joints (DAS 28) was 5.71 ± 2.04 and More than half of the patients had a Larsen score between 21 and 80. The demographic features, disease activity, laboratory parameters and treatment of the RA patients and the control group are summarized in Table 1.

Features	RA patients (n=52)	Controls group (n=48)
	mean ± SD (range) or n (%)	mean ± SD (range) or n (%)
Age (years)	44.6 ± 14.1 (17-69)	41.2 ± 13.4 (17-66)
Sex-ratio (M/F)	0.15	0.33
Disease duration (years)	10.3 ± 7.1 (0.5-25)	-
Age at disease onset (years)	31.7 ± 7.03 (15–61)	-
VAS (mm)	56.7 ± 18.3	-
Number of painful joints	8.67 ± 6.34	-
Number of swollen joints	2.51 ± 4.27	-

Deformations	37/52 (71.1)	-
Laboratory parameters		-
High ESR (mm/1st H)	38/52 (73,07)	
CRP positivity (mg/L)	32/52 (61.5)	
RFpositivity (UI/L)	46/52 (88,4)	
ACPA positivity (UI/L)	36/52 (69.2)	
DAS 28	5.71 ± 2.04	-
Radiological (Larsen score)		-
0	5 (9.6)	
120	7 (13.4)	
21-40	10 (19.2)	
41-60	11 (21.15)	
61-80	9 (17.45)	
>80	10 (19.2)	
Treatment		-
Prednisone (31/52) (mg/day)	9.75 ± 7,64 (2,5–20)	-
Methotrexate (45/52) (mg/week)	12.62 ± 6.83 (5-25)	-
Salazopyrine (3/52) (mg/day)	1500 ± 500 (1000-2000)	-
Hydrohychloquine (1/52) (mg/day)	400 ± 00	-
Rituximab (2/52) (mg/year)	2000	-
No DMARDs (2/52)	-	-
VAS: Visual Analogue Scale; ESR: Erythrocyte Sedimentation Rate; CRP: C Reactive Protein; RF: Rheumatoid Factor; ACPA: Anticitrullinated Protein Antibody; DAS28: Disease Activities Score for 28 joints.		

Table 1: Characteristics of the populations.

Among 3 genes studied, only *HLA DRB1*01* and *HLA DRB1*04* genes have shown a significant association with RA (OR=1.60 and 95% CI=0.70–3.66 for *HLADRB1*01*, OR=1.66 and 95% CI=0.69–4.02 for *HLADRB1*04*). Otherwise, Allele 04 appears to be more common in RA patients than the allele 01. There's no association with RA and *HLA-B* genes. The Table 2 summarized the association between *HLA* genes and RA.

	RA (n=52)	Controls (n=48)	p	OR (95% CI)
*HLA-DRB1*01*	36 (69.2%)	28 (58.3%)	0.0002	1.60 (0.70-3.66)
*HLA-DRB1*04*	40 (76.9%)	32 (66.6%)	0.0002	1.66 (0.69-4.0)
HLA-B	32 (61.5%)	32 (66.6%)	0.59	0.8 (0.35-1.81)
HLA: Human Leukocyte Antigen; RA: Rheumatoid Arthritis; Bold value is significant at p<0.0				

Table 2: Association between *HLA B, HLADRB1*01*, *HLADRB1*04* and RA.

Frequency of *HLA-DRB1* genotypes among ACPA and RF positive and negative RA patients is presented in Table 3. The both two alleles were higher significantly associated with RF positive (p<0.0001). Therefore, positive *DRB1*04* allele was the only higher significantly associated with ACPA positivity (p=0.002).

	ACPA				RF	
Allele	**Positive**	**negative**	**p**	**positive**	**negative**	**P**
*DRB1*01*	26	10	0.045	34	2	<0,0001
*DRB1*04*	30	10	0.002	37	3	<0,0001
NONE	2	5		4	9	
RF: Rheumatoid Factor; ACPA: Anticitrullinated Protein Antibody.						

Table 3: Correlation of ACPA and RF with the HLA DRB1 genotypes alleles.

Presence of the two alleles of DRB1 were significantly correlated with the radiological severity scores (p=0.032 and 0.021 respectively with *DRB1*01* and *DRB1*04*) as showned in Table 4.

	*HLADRB1*01*			*HLADRB1*04*		
	Positive	**Negative**	**X2 (p)**	**Positive**	**Negative**	**X2(p)**
	(n=36)	**(n=16)**		**(n=40)**	**(n=12)**	
radiological (Larsen score)						
Mild	5	7	X2:3.218	3	4	X2=3.623
			P=0.15			P=0.27
Moderate	7	7	X2=1.796	8	7	X2=3.188
			P=0.63			P= 0.35
Severe	24	2	X2=5.2	29	1	X2=5.558
			P=0.032			P=0.021
Mild: Larsen score<20; Moderate: Larsen Score between 21-60; Severe: Lasen score >60.						

Table 4: Correlation of radiological lesions with the HLA DRB1 genotypes alleles.

Discussion

Rheumatoid arthritis is the most frequent inflammatory rheumatism disease. RA is common in Benin population such as the other west african population [5,12,13]. ACPA were present in 69% of RA populations. This is less than those describe by Dieye and adewale respectively in Senegalese and Nigeria populations. The difference lies in the size of the different study samples.

Several studies were aimed to understand its physiopathogenesis in particular the association between some *HLA-DR* alleles and rheumatoid arthritis. Among all factors identified on the sickness, genetic factors take an important place.

Susceptibility to and outcome for rheumatoid arthritis (RA) have been associated with particular *HLA-DR* alleles, but these alleles vary among ethnic groups and geographic areas [15-17]. The frequency of *HLA-DR1* (*HLA-DRB1*0101, DRB1*0102*) and *HLA-DR4* (*DRB1*0401*, *DRB1*0404)* alleles is elevated among Caucasian and magrebian patients with RA [10,11,16].

Through a systematic review of published works, some authors identified some common HLA class II alleles that contribute to susceptibility to AIDs like RA in Americans an European populations [16]. In our study we observed associations of different *HLADRB1* alleles with RA In Benin populations. In subsaharian Africa, this association is difficult to proove because of but the lack of publications and studies.

Our results reported a frequency of 58%, 66% and 66% for *HLA DRB1 01*, *04* and *HLA-B* respectively in the normal subjects. In Egypt, The frequency in the normal subjects was 25% and 30% respectively [10]. In Asian populations, the frequency was 6 and 5% and in the European populations it was 25.3% and 26.5% respectively [15]. These various studies reveal the very great variability of the *HLADRB01* gene in the different populations [10,11,16]. However, in both the Asian and European populations the reported frequency was limited to some alleles of *HLA DRB1 04* [16,17]. In SubSaharian africa, the high cost of HLA genotyping (which is often not realized on the spot but rather routed in France or Belgium) does not allow a mapping of the gene in the populations.

Therefore the identification of susceptible allele in RA patients may assist a physician to take early decision regarding starting of intensive therapy to prevent joint damage. The presence of the *HLA-DRB1*01* and *HLA-DRB1*04* genes determines the occurrence of the disease. If rheumatoid factor and anti-CCP antibodies are involved in the disease, there would be a strong correlation between these genes and the production of antibodies [10].

Conclusion

In Africa, research on all the functional *HLA-DRB* genes in systemic disease was lacking or limited by the high cost of genotyping. Although different alleles were associated with RA, this work has allowed us to show the involvement of HLADRB1*01 and *HLADRB1*04* genes in RA and know the immunogenetic profile of some RA patients in Benin.

Conflict of Interest

None.

Authors Contributions

Zavier Zomalheto: For selecting patients in rheumatology unit. For writing and finalisation of the paper.

Michee Assogba: For selecting patients and correction of the paper.

Germaine Avohou: For laboratory work.

Junior Azombakin: For laboratory work.

Anatole Laleye: For supervision of laboratory work and correction of the paper.

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