Diagnostic value of anti-cyclic citrullinated peptides and association with HLA-DRB1 shared epitope alleles in African rheumatoid arthritis patients

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Abstract

Introduction: The purpose of this study was to examine the diagnostic performance of autoantibodies against citrullinated peptides/proteins (ACPA) and to determine the prevalence of HLA-DRB1 shared epitope alleles (SE) in African patients with rheumatoid arthritis (RA).

Methods: Serum levels of anti-cyclic citrullinated peptides antibodies (anti-CCP2, anti-CCP3), IgM and IgA rheumatoid factors (RF) were measured by enzyme-linked immunosorbent assay in the serum of 56 consecutive RA patients regularly followed in the Rheumatology Unit of the School of Medicine, University of Yaoundé, Yaoundé, Cameroon. Genotyping of HLA-DRB1 alleles was performed by polymerase chain reaction and hybridization with sequence-specific oligonucleotide probes on microbeads arrays. Fifty-one patients with other inflammatory rheumatic diseases and 50 healthy individuals were included as controls.

Results: An anti-CCP2 assay showed the best diagnosis sensitivity (82%) and specificity (98%) with high positive predictive (PPV) (96%) and negative predictive values (NPV) (91%). Thirty percent of RA patients were carrying at least one copy of the HLA-DRB1 shared epitope (SE) compared to 10% and 14% of patients with other inflammatory rheumatic diseases and healthy individuals, respectively. The presence of the SE was associated with the production of ACPA.

Conclusions: Anti-CCP2 antibodies are useful markers of RA in African patients. In this cohort, the prevalence of the SE is higher in RA patients than in controls but lower than that reported in patient cohorts of European ancestry. The discrepancy between the high prevalence of ACPA-positive patients and the relatively low number of SE-positive cases suggest that, in addition to SE, other genetic factors control the development of ACPA in African RA patients.

Introduction

Rheumatoid arthritis (RA) is characterized by inflammation of the synovial membrane of diarthrodial joints leading to tissue destruction and severe disability. The cause of RA is unknown but genetic susceptibility and environmental factors appear to be involved. RA is the most frequent systemic autoimmune inflammatory disease with a prevalence of approximately 0.5 to 1% in populations of European ancestry. However, it appears to have a relatively lower prevalence among African populations, particularly those living in rural settings [1-3].

Two important autoantibody systems have been described in RA, including rheumatoid factors (RF) directed to the Fc fragment of IgG and autoantibodies against citrullinated peptides/proteins (ACPA). RFs are well-known autoantibodies associated with RA and are present in approximately 70 to 80% of RA patients, but because they are also detected in patients with other autoimmune diseases as well as in chronic infections and in lymphoma or other tumoral processes, they have
limited specificity. ACPA such as anti-cyclic citrullinated peptides (anti-CCP) are directed to antigens that contain arginyl converted to citrullyl residues by peptidylarginyl deiminase enzymes [4,5]. Several studies have shown that these antibodies are present in 60% to 80% of Caucasian RA patients with a high specificity of more than 95% [6]. However, there are no data regarding the presence of these antibodies in African patients with RA.

The genetic component of RA has been determined with heritability estimates of 50% to 60% [7]. The major susceptibility loci associated with susceptibility to RA were identified approximately 30 years ago and consist of the human leukocyte antigen (HLA) class II molecules. There is extensive evidence that some HLA-DRB1 alleles, including HLA-DRB1*0101, HLA-DRB1*0102, HLA-DRB1*0401, HLA-DRB1*0404, HLA-DRB1*0405, HLA-DRB1*0408, HLA-DRB1*0410, HLA-DRB1*1001, HLA-DRB1*1402 are associated with susceptibility to RA. These alleles share a common amino acid sequence (QKRAA, QRRAA, or RRRAA), also termed shared epitope (SE), located at positions 70 to 74 within the third hypervariable region of DRBI, forming part of the antigen-binding site. The shared epitope accounts for at least 30% of the total genetic susceptibility [8]. In addition, the associations between the SE and other genetic markers including PTPN22, CTLA4, CD40 genes, the TRAF1/C5 region and SNPs between OLIG3 and TNFAIP3 genes, and anti-CCP positivity have been reported in different populations (reviewed in [9]).

The objective of this study was to examine the prevalence of ACPA detected by anti-CCP2 and anti-CCP3 enzyme-linked immunosorbent assays (ELISAs), and that of HLA-DRB1 alleles in African RA patients in order to examine first the diagnostic performance of these serological tests as compared to RF, and then the distribution of the SE alleles and their association with ACPA.

**Materials and methods**

**Patients**

This study was carried out on 56 RA patients recruited consecutively from the outpatient Rheumatology Clinic of Yaoundé Central Hospital in Cameroon. These RA patients fulfilled the American College of Rheumatology 1987 criteria for RA [10]. Fifty-one patients (20 females) with other rheumatic conditions and ages ranging from 16 to 65 (median 28), and 50 healthy individuals (33 females) with ages ranging from 22 to 55 (median 34) were included as controls. Patients with other inflammatory rheumatic conditions were consecutively recruited from the same outpatient clinic, while healthy controls were recruited among medical students and hospital workers in Yaoundé. Patients with RA were treated with disease modifying antirheumatic drugs (DMARDs), including methotrexate, hydroxychloroquine, sulphasalazine, leflunomide, combinations of methotrexate, hydroxychloroquine and sulphasalazine and/or oral prednisone. RA patients were assessed for demographic characteristics, disease duration, duration of morning stiffness, pain by visual analogue scale, number of tender joints, number of swollen joints, the presence or absence of nodules, extra-articular manifestations, and co-morbidities. The disease activity score (DAS28) was calculated as previously described [11]. Hand radiographs were obtained for each RA patient. Approval of the Cameroon National Ethical Committee was obtained prior to the study and an informed consent was obtained from all patients and controls included in this study.

**IgM and IgA RF determinations by enzyme immunoassays**

Commercially available ELISA kits, purchased from Inova Diagnostics (Ruwag, Zurich, Switzerland), were used to detect IgM and IgA RF. The assays and calculations were performed according to the manufacturer’s instruction. Each kit included its own RF standard and the results were calculated as arbitrary units/ml. The diagnostic performance of these kits has been previously evaluated in a Swiss population of RA patients and controls [12].

**Anti-cyclic Citrullinated Peptide antibody determination by enzyme immunoassay**

The ELISA kits detecting the IgG anti-CCP2 antibodies (Immunoscan RA: regular, second generation of CCP antigen), were purchased from Euro-Diagnostica (Pharma Consulting, Burgdorf, Switzerland), and those detecting the IgG anti-CCP3 (Quanta Lite CCP3: third generation of CCP antigen), were purchased from Inova Diagnostics (Ruwag, Zurich, Switzerland). The assays and calculations were performed according to the manufacturers’ protocols. Each manufacturer uses its own anti-CCP calibrator and the results were calculated as arbitrary units/ml. In addition, to avoid false positive results for anti-CCP2 antibody determination, reactivity to non-citrullinated peptides containing arginyl instead of citrullyl residues was also tested. Diagnosis sensitivity and specificity of anti-CCP2 antibody determination have been previously determined in our laboratory in Swiss and in French patients with RA and in controls [13,14]. The sensitivity and specificity of anti-CCP3 were compared to those of anti-CCP2 in two studies on different populations [15,16].

**HLA-DRB1 genotyping**

Genomic DNA was extracted from 350 μl- aliquots of frozen blood samples by using the GenoM6 magnetic bead-based workstation. HLA-DRB1 generic typing was performed by PCR-SSOP (sequence-specific
oligonucleotide probes) reverse hybridization using the Luminex technology after locus-specific exon 2 amplification. The method is based on fluorescent microbeads coated with oligonucleotide probes specific for the polymorphic positions of DRB1 exon 2 sequences (LabType RSS02B HD, OneLambda). Automated reading (Labscan TM100, Luminex, Austin, Texas USA) and interpretation led to HLA-DRB1 high resolution (four-digit) typing as previously described [17].

**Statistical Analysis**

The disease characteristics of RA patients (Table 1) were described using standard non-parametric statistics (median and interquartile ranges) for continuous outcomes and percentages for dichotomous outcomes.

The diagnostic performances and cut off of all the serological assays used in this study have been initially validated in Caucasians. Thus, we decided to identify the most discriminant cut-off values of the different tests for this particular population, which were operationally defined as the cut-off values leading to the highest percentage of correctly classified patients. The most discriminant cut-offs to be considered as positive were with values $\geq 22$ units/ml for IgM RF, $\geq 1$ unit/ml for IgA RF, $\geq 32$ units/ml for anti-CCP2, $\geq 17$ units/ml for anti-CCP3, respectively. Using these established cut-offs, we computed the sensitivity, the specificity, the positive predictive value (PPV) and the negative predictive value (NPV) of the various biologic tests in this population. We used the area under the curve (AUC) of the receiver operating curves (ROC) to compare the diagnostic performance of the various biologic tests in this population.

**Table 1 Baseline characteristics of RA patients**

| Characteristic     | Value |
|--------------------|-------|
| Age (yrs)          | 53.5 (39 to 61.5) |
| Female sex (%)     | 95    |
| Disease duration (yrs) | 3 (2 to 6) |
| Erosions (%)       | 44    |
| Subcutaneous nodules (%) | 7     |
| DAS28              | 4.72 (3.8 to 6.4) |
| Morning stiffness (minutes) | 30 (10 to 60) |
| VAS-pain (0 to 10) | 5 (3 to 7) |
| CRP (mg/L)         | 12 (6 to 35) |
| Tobacco, N (%)     | 1 (2) |
| Prednisone, N (%)  | 51 (91) |
| Dose (mg/day)      | 10    |
| Methotrexate, N (%)| 43 (77) |
| Dose (mg/day)      | 10    |
| Sulfasalazine, N (%)| 7 (12) |
| Azathioprine, N (%)| 2 (5)  |
| Leflunomide, N (%) | 2 (5)  |
| D-penicillamine, N (%)| 1 (2) |

Continuous values are presented as median (interquartile range 25 to 75); CRP, C-reactive protein; DAS, disease activity score; VAS, visual analogue score.
Table 2 Serological and immunogenetic characteristics in RA patients, controls with inflammatory rheumatic diseases, and healthy individuals

| Laboratory values | RA (n = 56) | IRD (n = 51) | HI (n = 51) | Sensitivity (n = 50) | Specificity | PPV | NPV | AUC (ROC) (95%) CI |
|-------------------|------------|-------------|-------------|---------------------|-------------|-----|-----|-------------------|
| RF IgM (%)        | 43 (6)     | 4 (8)       | 77          | 93                  | 86          | 88  | 0.85 (0.79 to 0.91)|
| RF IgA (%)        | 47 (16)    | 0 (0)       | 84          | 92                  | 85          | 91  | 0.88 (0.82 to 0.94)|
| Anti-CCP2 (%)     | 46 (2)     | 1 (2)       | 82          | 98                  | 96          | 91  | 0.90 (0.85 to 0.96)|
| Anti-CCP3 (%)     | 43 (8)     | 1 (2)       | 77          | 95                  | 90          | 88  | 0.86 (0.80 to 0.92)|
| SE 1 or 2 copies (%) | 17 (14)  | 5 (10)      | 30          | 88                  | 59          | 70  | 0.59 (0.52 to 0.66)|
| - SE 1 copy (%)   | 15 (14)    | 5 (10)      | 27          | 88                  | 56          | 68  | 0.57 (0.51 to 0.64)|
| - SE 2 copies (%) | 2 (0)      | 0 (0)       | 4           | 100                 | 100         | 65  | 0.52 (0.49 to 0.54)|

Anti-CCP, anti-cyclic citrullinated peptides; AUC, area under the curve in the ROC analysis; HI, healthy individuals; IRD, inflammatory rheumatic diseases; RA, rheumatoid arthritis; RF, rheumatoid factor; Sensitivity, the percentage of RA patients who would be identified as having RA by the laboratory tests (positive test results); Specificity, the percentage of control patients (IRD and HI together) who would be identified as not having RA by the laboratory tests (negative test results); PPV, positive predictive value or the proportion of RA patients with positive test results who are correctly diagnosed as having RA; NPV, negative predictive value or the proportion of control patients with negative test results who are correctly diagnosed as not having RA; SE, shared epitope.

Of the 157 DRB1-typed samples a total of 21 alleles were identified. The following two allele groups were not resolved because the differences are located in the third exon: DRB1*1201/06/10 and DRB1*1401/54. The allele frequency distribution in the healthy controls (n = 50) was very similar to that reported in a sample of Cameroonesse students [18]. The seven most frequent alleles (DRB1*0301, *0302, *0804, *1101, *1301, *1302, and *1503) accounted for 77.5% of the alleles in our control group, as compared to 71.5% in the published cohort [18]. In our study group the SE was represented by only four alleles: DRB1*0101, *0102, *0405, and *1001 (Table 3). One copy of the SE was detected in 17 RA patients (30%), 7 patients with other rheumatic diseases (14%), and 5 healthy individuals (10%) (P = 0.029, Chi2 test). Two copies were detected in two RA patients but in none of the controls (Table 3). HLA-DRB1*0102, *1001, *0405 were the most frequent SE-positive alleles in RA patients and in control patients.

We examined the association between the presence of the SE and that of ACPA. We observed a positive trend between the presence of SE and anti-CCP2 and anti-CCP3 (Table 4). In addition, a univariate logistic regression, the association between the SE and the diagnosis of RA disappeared when the presence of ACPA was taken into account in the model, thus further supporting the relationship between SE and ACPA-positive RA.

All the immunological tests were significantly correlated to each other and the agreement ranged between 70% and 90% (kappa test). In a multivariate logistic regression analysis, IgM RF, IgA RF, anti-CCP2, and anti-CCP3 were independently associated with RA, which suggests that all these autoantibodies provide complementary information for the diagnosis of RA.

Discussion

Our study of African RA patients confirmed previous studies in patients of European ancestry showing that anti-CCP2 and anti-CCP3 antibodies exhibit high diagnostic specificity for RA with anti-CCP2 antibodies having the highest PPV and NPV. However, the number of patients and controls included in our study limits the interpretation of these results, and future studies including a larger number of individuals should be carried out in the African population to confirm these findings. Interestingly, a recent report including Dutch patients with undifferentiated arthritis and comparing anti-CCP2, anti-CCP3, anti-citrullinated vimentin, and RF showed that anti-CCP2 tended to achieve the highest PPV for RA development [19].

Table 3 Shared epitope related HLA-DR distribution in RA patients and controls

| DRB1* | RA N = 56 | IRD N = 51 | HI N = 50 |
|-------|-----------|------------|-----------|
| 0101  | 1         | 0          | 0         |
| 0102  | 5         | 4          | 3         |
| 1001  | 5         | 1          | 2         |
| 0405  | 4         | 2          | 0         |
| 0102/0405 | 1   | 0          | 0         |
| 0102/1001 | 1   | 0          | 0         |
| Total: | 17        | 7          | 5         |

HI, healthy individuals; IRD, inflammatory rheumatic diseases; RA, rheumatoid arthritis.
IgM RF is the only serological marker for RA currently available in Cameroon. The validation of the assay for the Cameroonian population led to a marked increase of cut-off values, as compared to those recommended by the vendor, in order to improve the specificity of the test. The presence of elevated background levels of IgM RF in African controls (16% of IgM RF-positive healthy controls when using the recommended cut-off values) is probably caused by non-specific activation of the immune system by different infectious and parasitic diseases. Interestingly, the results of ACPA tests, in particular anti-CCP2 positivity, were not influenced in a similar manner as IgM RF.

Only a few studies have been conducted to determine the association between HLA-DRB1 and RA in Africa. These studies included a limited number of patients and mainly referred to HLA-DR antigens detected by serological typing or by low resolution DNA typing. One study on Zimbabweans showed a higher prevalence of HLA-DR4 in RA patients, [20]. A study performed in Senegal showed that the relative risk (RR) of developing RA was significantly associated with HLA-DR10 (RR 32), but not HLA-DR4 (RR 0.8) [21]. The frequency of SE-containing HLA-DRB1 alleles was 25.2% in African Americans with RA as compared to 13.6% in healthy subjects. Thus, the SE was significantly associated with susceptibility to RA, but the percentage of SE positivity was globally lower than that reported in RA patients of European ancestry, which ranges between 50 to 70% [22,23]. The frequency of HLA-DRB1*0401, 0404, 0405, and 1001 alleles were higher among African American RA patients than in healthy controls. Of note, a higher level of European admixture was associated with a higher likelihood of carrying the SE among African Americans. More specifically, HLA-DRB1*0401 but not the other alleles encoding the SE, was significantly associated with the presence of European ancestry [24]. In another study, HLA-DRB1*0102 and HLA-DRB1*0405 were significantly more frequent among African American RA than European RA patients with odds ratio (OR) of 8.66 and 2.75, respectively. HLA-DRB1*1001 tended also to be more frequent in African American RA than European RA patients (OR 2.11). In contrast, an opposite result was found regarding HLA-DRB1*0401 with an odds ratio of 0.15 [25]. Thus, our results as well as recent reports on African American patients indicate that, although the presence of the SE is associated with RA, its frequency is much lower than that observed in patients of European ancestry, with also a distinct SE allele profile characterized in particular by a lower HLA-DRB1*0401 frequency.

RA is a clinically heterogeneous disease and there has been some speculation recently that it may comprise at least two distinct subgroups characterized by the presence/absence of ACPA. For example, the carriage of the SE appears particularly confined to anti-CCP positive RA cases [26,27]. In addition, a significant association between SE and the presence of anti-CCP2 antibodies was demonstrated in African American RA patients [24]. In our study, there was also an association between ACPA and SE. However, this association was relatively weak, probably due to the limited number of patients included in our study. The fact that ACPA are present in a similar percentage of African patients as previously reported in European patients despite a major difference in the proportion of SE-positive patients, suggests that other non-HLA genetic factors contribute to the development of RA and of these autoantibodies in African RA patients. Of note, an African specific allele of CTLA4 has recently been shown to confer protection against RA in African Americans [28].

Tobacco use was shown to be associated with the development of the disease, in particular in anti-CCP2-positive, SE-positive RA patients [26]. In one study, the conjunction of tobacco use and HLA-DRB1*0101 or *0102 was the strongest factor for the development of these antibodies [29]. With the exception of one case, none of our patients smoked, which is in line with general living habits of African women. This finding suggests that other environmental factor may be involved in the development of RA.

To our knowledge this study is the first report on combined HLA-DRB1 SE and ACPA status in an African patient cohort without known European admixture. It has, however, limitations due to the limited number of patients and controls included. In addition, the patient population is highly selected as we had only access to outpatients followed in a university hospital, representing patients from an urban setting with moderate to severe disease. However, the Rheumatology Unit is the only specialized center available for the population of Yaoundé and its suburbs and we reduce this bias by including all the RA patients attending the clinic without any further selection.

Conclusions
Our study showed that anti-CCP2 antibodies are sensitive and specific diagnostic markers of RA also in African patients. The discrepancy between the high prevalence of ACPA-positive patients and the relatively low number of SE-positive cases as well as the relative lack of tobacco smokers suggest that other genetic and environmental factors control the development of ACPA in African RA patients.

Abbreviations
ACPA: anti-citrullinated peptides/proteins antibodies; AUC: area under the curve; CCP: cyclic citrullinated peptides; DMARDs: disease modifying...
antirheumatic drugs; IRD: inflammatory rheumatic diseases; HI: healthy individuals; PPV: positive predictive value; NPV: negative predictive value; RA: rheumatoid arthritis; RF: rheumatoid factor; ROC: receiver operating curve; RR: relative risk; SE: shared epitope.

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Authors’ contributions
MSN recruited the patients and collected the samples. MSN and CG designed the study. SB and J-MT performed the analysis. AF performed the statistical analysis. MSN and CG drafted the manuscript and all authors revised the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Beighton P, Solomon L, Valkenburg HA: Rheumatoid arthritis in a rural South African Negro population. Ann Rheum Dis 1975, 34:136-141.
2. Brightwell SW, de la Harpe AL, van Staden DJ, Badenhorst JH, Myers OL: The prevalence of rheumatoid arthritis in a rural African population. J Rheumatol 1988, 15:405-408.
3. Silman AJ, Ollier W, Holligan S, Birell F, Adelobo A, Asuzu MC, Thomson W, Pepper L: Absence of rheumatoid arthritis in a rural Nigerian population. J Rheumatol 1993, 20:618-622.
4. Gibal-Neuhauser E, Durieux JJ, Arnaud M, Dalbon P, Sebbag M, Vincent C, Simon M, Senshu T, Masson-Bessiere C, Jolivet-Reynaud C, Jolivet M, Serre G: The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of profilaggrin by deamination of arginine residues. J Immunol 1999, 162:585-594.
5. Schellekens GA, de Jong BA, Hoogen van der AH, Rutgeerts PJ, de Vries LS: Cricritline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998, 101:273-281.
6. Cruysen B, Bogner J, Van Laere K, Van der Perre J, Deforce D, Elewaut D, Serre G, De Keyser F: Do all anti-citrullinated protein/peptide antibody tests measure the same? Evaluation of discrepancy between anti-citrullinated protein/peptide antibody tests in patients with and without rheumatoid arthritis. Ann Rheum Dis 2008, 67:542-546.
7. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, Silman AJ: Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000, 43:30-37.
8. Deighton CM, Walker DJ, Griffiths ID, Roberts DF: The contribution of HLA to rheumatoid arthritis. Clin Genet 1989, 36:178-182.
9. Klareskog L, Catrina AI, Paget S: Rheumatoid arthritis. Lancet 2009, 373:659-672.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller J, Sharp JT, Wilder RL, Hunder GG: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988, 31:315-324.
11. Prevoo ML, van ’t Hof MA, Kuper HH, van Leeuwen MA, Putte van de LB, van Reil PL: Modified disease activity score that include twenty-eight-joint count. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995, 38:44-48.
12. Bas S, Pemeeger TV, Kunde E, van der Woude van der D, Ioan-Facsinay A, Levarht EW, Steenken-Rijssenbergen G, Huzinga TW, Toes RE, Helms-van Mil van der AH: The contribution of HLA-DRB1 shared epitope alleles for susceptibility to rheumatoid arthritis. Clin Rheumatol 2009, 28:153-158.
13. Rahal M, Kervaire B, Villard J, Tyrcy JM: DNA typing by microprobe arrays and PCR-SSP: apparent false-negative or -positive hybridization or amplification signals disclose new HL-A and -DRB1 alleles. Tissue Antigens 2008, 71:238-241.
14. Pimtanothai N, Hurey CK, Leke R,写字楼 W, Johnson AH: HLA-DR and -DQ polymorphism in Cameroon. Tissue Antigens 2001, 58:1-8.
15. Linden van der MP, Woude van der D, Ioan-Facsinay A, Levarht EW, Steenken-Rijssenbergen G, Huzinga TW, Toes RE, Helms-van Mil van der AH: Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum 2009, 60:2232-2241.
16. Martel RW, Stem M, Davis P, West G, Emmanuel J, du Toit ED: The association between HLA and rheumatoid arthritis in Zambian blacks. Tissue Antigens 1990, 36:125-126.
17. Deyie A, Diallo S, Diatta M, Thiama A, Ndiaye R, Bao O, Sanhour JL: Identification of HLA-DR alleles for susceptibility to rheumatoid polyarthritis in Senegal. Dakar Med 1997, 42:111-113.
18. Silman AJ, Pearson JE: Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002, 4(Suppl 2):S265-272.
19. Thomson W, Harrison B, Ollier W, Miles N, Peyton T, Barrett J, Symmons D, Silman A: Quantifying the exact role of HLA-DRB1 alleles in susceptibility to inflammatory polyarthritis: results from a large, population-based study. Arthritis Rheum 1995, 38:757-762.
20. Hughes LB, Morrison D, Kelley JM, Hughes LB, Faggard JD, Danila MI, Crawford MH, Edberg Y, Padilla MA, Vaughan LK, Westfall AO, Dwivedi H, Mikuls TR, Hokers VM, Farrish LA, Alarcon GS, Conn DL, Jonas BL, Callahan LF, Smith EA, Gilkeson GS, Howard G, Moreland LW, Patterson N, Reich D, Bridges SL Jr: The HLA-DRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. Arthritis Rheum 2008, 58:349-358.
21. Del Rincon I, Battadavalo DF, Arroyo RA, Murphy ET, Fischbach M, Escalante A: Ethnic variation in the clinical manifestations of rheumatoid arthritis: role of HLA-DRB1 alleles. Arthritis Rheum 2003, 49:200-208.
22. Klareskog L, Stolt P, Lundberg K, Karlberg H, Bengtsson C, Grunevold J, Ronnefeld J, Hansi HE, Ulfberg AK, Akerware-Dahlyquist S, Ekbloa E, Fadysud E, Alfredsson L: A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006, 54:38-46.
23. Helmi van Mil van der AH, Verpoort KN, Breedveld FC, Huijtinga TW, Toes RE, de Vries RR: The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006, 54:1117-1121.
24. Kelley JM, Hughes LB, Faggard JD, Danila MI, Crawford MH, Edberg Y, Padilla MA, Tiwari HK, Westfall AO, Alarcon GS, Conn DL, Jonas BL, Callahan LF, Smith EA, Brasidingon RD, Allison DB, Kimberly RP, Moreland LW, Edberg JC, Bridges SL Jr: An African ancestry-specific allele.
of CTLA4 confers protection against rheumatoid arthritis in African Americans. PLoS Genet 2009, 5:e1000424.

29. Helm-van Mil van der AH, Verpoort I, van de Vries RR, Toes RE. The HLA-DRB1 shared epitope alleles differ in the interaction with smoking and predisposition to antibodies to cyclic citrullinated peptide. Arthritis Rheum 2007, 56:425-432.

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