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**Short communication**

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# Distribution of HLA-B27 in Romanian spondyloarthritides patients

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## Summary

The analysis of 310 Romanian spondyloarthritides patients confirmed the association of the HLA-B27 marker with the susceptibility to different diseases of this group. For ankylosing spondylitis, the HLA-B27 frequency in Romanian patients (72.1%) was similar to that found in several regions in the Mediterranean area.

## Introduction

The association between HLA-B27 and spondyloarthritides (SpA) is one of the strongest among HLA-associated diseases. However, the association degree varies markedly among different diseases of this group and also between different populations. Ankylosing spondylitis (AS) is the most common subtype of SpA (Zochling *et al.*, 2005) and exhibits the strongest association with HLA-B27, while psoriatic arthritis (PsA) shows the lowest degree of association (Paladini *et al.*, 2005). Genetic association data are still missing for some ethnic groups, especially for SpA other than AS.

Romania is a South-Eastern European country with its genetic background resulting from admixture between Roman and ancient local populations and further influences (to a variable and questionable degree) from subsequent migrating populations (Slavonic, Uralic, Turkish, etc.), as discussed by Cavalli-Sforza *et al.* (1994) and Stefan *et al.* (2001). The aim of this study was to characterize for the first time a relatively large group of Romanian spondyloarthritides patients from the point of view of the HLA-B27 status.

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## Materials and methods

A group of 310 Romanian spondyloarthritides patients – ankylosing spondylitis ($n$ = 158), psoriatic arthritis ($n$ = 60), reactive arthritis, ReA ($n$ = 22) and undifferentiated spondyloarthritis, USpA ($n$ = 70) – were enrolled into the study, after diagnosis at the Division of Rheumatology, Galati County Hospital, and 'St. Maria' Hospital and 'I.C. Cantacuzino' Hospital, Bucharest, Romania. The European Spondyloarthropathy Study Group (ESSG) diagnostic criteria for SpA (Dougados *et al.*, 1991) and additional specific criteria for the different diseases (Moll & Wright, 1973; Willkens *et al.*, 1981; Van der Linden *et al.*, 1984) were used. AS patients with psoriasis or enteropathies were not included in this study. The age of SpA patients ranged between 16 and 86 years (median 39 years). Among the patients, 50% of the PsA, 55% of the ReA and 60% of the USpA were males. In the AS group, the male/female ratio was 4:1 (125 males/33 females). A group of 211 Romanian non-related potential bone marrow/organ donors served as a control population. Romanian ethnicity was based on surnames and anamnestical (subject history) details. The study was approved by the local ethics committee; the details were explained to both control subjects and patients and afterwards, consent for genetic screening was obtained.

HLA-B*27 was genotyped by PCR-SSP using the B27-SSP low resolution kit (Olerup, Sweden). A group of 55 randomly selected individuals (~10%), from the 521 healthy and diseased subjects investigated, were genotyped twice with the same method and concordant result was obtained in each case. Because we have typed only for HLA-B*27 allelic group, homozygosity status for HLA-B*27 could not be evaluated. For this reason we will hereafter use the term HLA-B27 to name the phenotype characterized by the presence of an HLA-B*27 allele, regardless of its homozygosity/heterozygosity status in a given individual.

The data were analysed using the OpenEpi Collection of Epidemiologic Calculators Version 2.3. The

95% confidence limits (CL) for the HLA-B27 proportions in the analysed populations were estimated using the Mid-*P* exact test. The significance of the association was determined using the Mid-*P* exact test. The odds ratios (OR) were calculated as conditional maximum likelihood estimates and the corresponding 95% confidence intervals (CI) were calculated by the Mid-*P* exact method (Martin & Austin, 1991). The statistical power ($\alpha = 0.05$, two tailed) was calculated based on normal approximation. The comparison between populations was performed with a two tailed *t*-test.

## Results and discussion

In the present study we have determined for the first time the distribution of HLA-B27 in SpA patients of Romanian ethnicity. For the whole group of SpA we have found 161 of 310 patients to be B27-positive (51.9%). The highest frequency of HLA-B27 (72.1%) was observed in AS patients, while in the undifferentiated spondyloarthritis group, HLA-B27 was present in 37.1% of cases. Psoriatic arthritis and ReA patients revealed the lowest frequencies of the investigated marker, 25% and 27.2% respectively. Among the psoriatic arthritis group, 18 patients (30%) exhibited the axial pattern of arthritis, but only four of them were B27 positive (22%). In the control group of healthy Romanian subjects, the HLA-B27 marker was detected in 22 of 211 individuals (10.4%).

Statistical analysis confirmed the positive association of HLA-B27 with susceptibility to each of the investigated SpA disease groups and to SpA as a whole (Table 1). The study reports adequate levels of statistical power ranging from 78.7% for PsA to 100% for AS and SpA group as a whole and a lower statistical power for the ReA disease (60.3%).

We compared the frequency of the HLA-B27 marker in our Romanian population samples with the frequency of the same marker in several other European populations. In the general population, the HLA-B27 frequency in Romania was significantly higher than that reported from Italy (5%, $P = 0.03$, Ferri *et al.*, 1982), but not different from those reported from countries in the surrounding geographic area – Greece (6%, Alamanos *et al.*, 2004), Bulgaria (10.8%, Minev *et al.*, 1979), Serbia (12.3%, Jajić, 1979), Hungary (12.8%, Gömör *et al.*, 1977) and also from Spain (9.3%, Fernández-Sueiro *et al.*, 2004), United Kingdom (9.5%, Brown *et al.*, 1996), Norway (15.9%, Gran *et al.*, 1984) and Finland (10.4%, Jaakkola *et al.*, 2006).

In the AS group, the HLA-B27 frequency in our Romanian sample was significantly lower than that reported from different European countries (Table 2) in which this marker has a high frequency among AS patients. In contrast, the HLA-B27 frequency in Romania was not significantly different from that found in several regions in the Mediterranean area: Italy (68–76%, Ferri *et al.*, 1982; Paladini *et al.*, 2005), Greece (80.5%, Alamanos *et al.*, 2004), Turkey (70%, Gunal *et al.*, 2008), Tunisia (62%, Kchir *et al.*, 2009).

In the PsA group, the frequency of the HLA-B27 marker in the investigated Romanian sample was significantly higher than that reported from continental Italy (12.4%, $P = 0.0403$, Paladini *et al.*, 2005) and Serbia (7%, $P = 0.0164$, Pavlica *et al.*, 2005), but not different from the frequencies reported from United Kingdom (26%, Woodrow & Ilchysyn, 1985) and Spain (22%, Collantes *et al.*, 2007). In Italy, there are two other studies reporting frequencies of HLA-B27 that are not statistically different from our study (13% in Sardinia, Paladini *et al.*, 2005; 32.6% in Scarpa *et al.*, 1992). In our PsA group, the pattern of arthritis appears to have no influence on the association of this disease with HLA-B27 as reported for some populations (Lopez-Larrea *et al.*, 1990; Queiro *et al.*, 2006).

For ReA, the HLA-B27 frequency in our group was 27.2%, while between 50% and 80% in some studies (Olivieri *et al.*, 2002; Kataria & Brent, 2004), and between 30% and 50% in others (Carter, 2006). However, as the number of our ReA patients was small, the HLA-B27 frequency we found may not reflect the real situation, as implied also by the suboptimal statistical power of this part of the study. Several authors reported that the HLA-B27 association in ReA is influenced by the causative agent of the disease (Kwiatkowska & Filipowicz-Sosnowska, 2009). We were not able to associate the HLA-B27 status with the initial infection event, because of the lack of data regarding the triggering bacteria in our Romanian ReA patients.

For undifferentiated SpA patients, there are few genetic studies and the reported frequency of HLA-B27 varies from 70% to 84% in different populations

**Table 1.** Association between HLA-B27 and susceptibility to SpA in Romania

| Disease | HLA-B27 positive % (95% CL) | *P*-value, Mid-*P* exact test | OR | CI |
|---|---|---|---|---|
| Spondyloarthritides (*n* = 310) | 51.9 (46.4–57.5) | <0.0000001 | 9.24 | 5.7–15.5 |
| Ankylosing spondylitis (*n* = 158) | 72.1 (64.8–78.7) | <0.0000001 | 22.00 | 12.7–39.3 |
| Psoriatic arthritis (*n* = 60) | 25 (15.3–37.1) | <0.01 | 2.85 | 1.3–5.9 |
| Reactive arthritis (*n* = 22) | 27.2 (11.9–48.3) | 0.02 | 3.20 | 1.1–8.9 |
| Undifferentiated spondyloarthritis (*n* = 70) | 37.1 (26.5–48.9) | <0.00001 | 5.04 | 2.6–9.8 |
| Controls (*n* = 211) | 10.4 (6.8–15.1) | | | |

*P*-value for the disease association with HLA-B27; OR, odds ratio; CI, confidence interval for OR.

**Table 2.** Comparison of HLA-B27 distribution in AS patients from Romania versus other European regions

| Country | HLA-B27 positive (%) | *P*-value | Reference |
|---|---|---|---|
| Romania | 72.1 | | |
| Bulgaria | 88 | 0.0005 | Minev *et al.* (1979) |
| Hungary | 92.7 | 0.0001 | Gömör *et al.* (1977) |
| Spain | 84.14 | 0.0018 | Collantes *et al.* (2007) |
| Spain-Galicia | 94.3 | <0.0001 | Fernández-Sueiro *et al.* (2004) |
| Germany | 82.2 | 0.029 | Rudwaleit *et al.* (2009) |
| Norway (north) | 93 | <0.0001 | Bakland *et al.* (2005) |
| United Kingdom | 90.2–94 | <0.0002 | Brown *et al.* (1996), Freeston *et al.* (2007) |
| Finland | 93 | <0.0001 | Jaakkola *et al.* (2006) |

(Zochling *et al.*, 2005). Considering these as expected values, the HLA-B27 frequency in our Romanian USpA sample is significantly lower ($P < 0.0001$). The HLA-B27 frequency in USpA is influenced by the prospective course of the disease, because at least some of the patients – 59% according to a German study (Mau *et al.*, 1988) – will develop AS.

HLA-B27 represents a group of over 60 allelic variants and about 50 proteins (http://www.anthonynolan.org.uk/research/hlainformaticsgroup), but only a few are common and have shown association with SpA. Among these, two major alleles, B*2702 and B*2705 show a positive association with the whole group of SpA (Khan, 2002). In this context, the limitation of our study is the inability of determining HLA-B*27 alleles by high resolution typing. In the future, more detailed molecular studies of Romanian spondyloarthritides patients can bring interesting and valuable information about this association.

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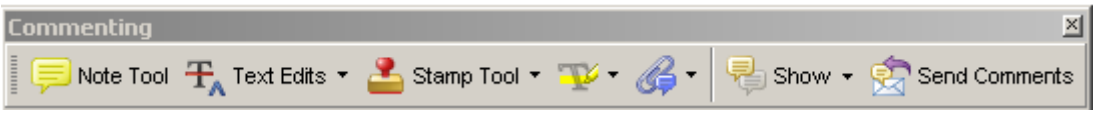


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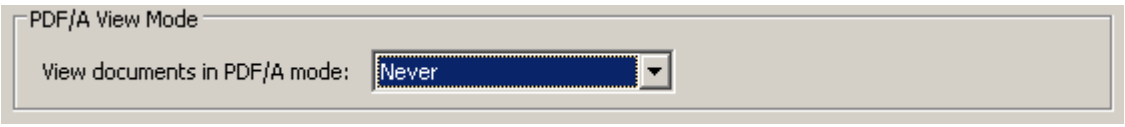


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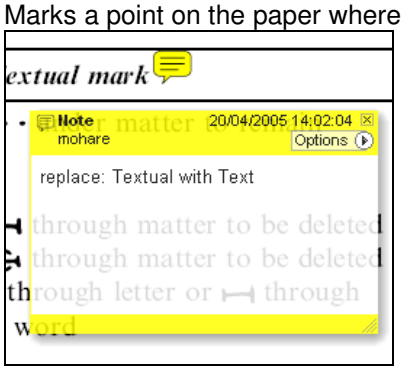


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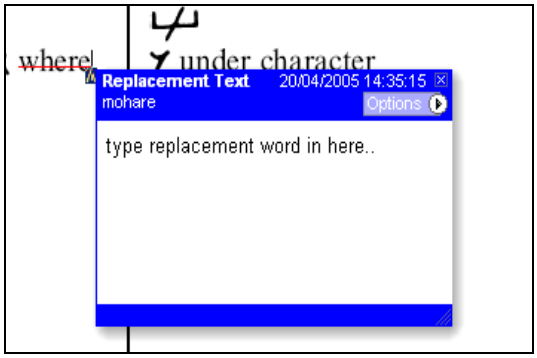


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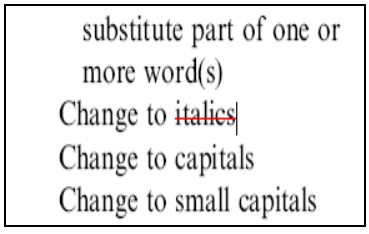


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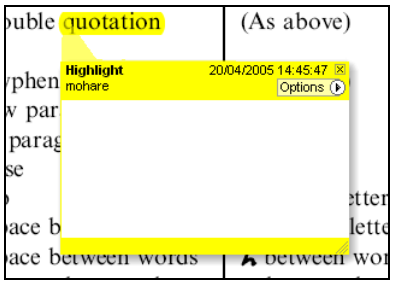


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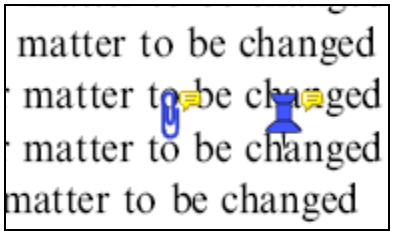


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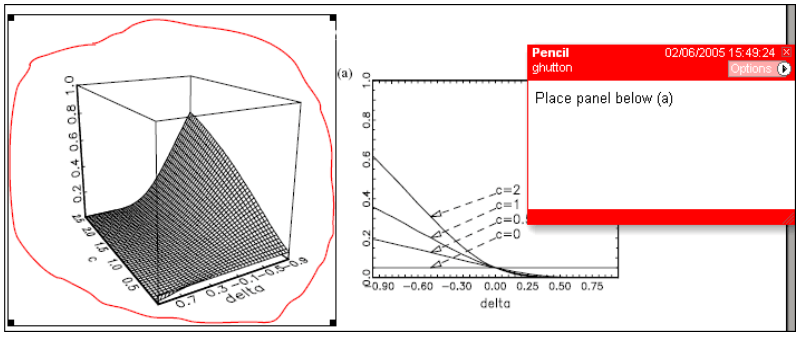


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## Help

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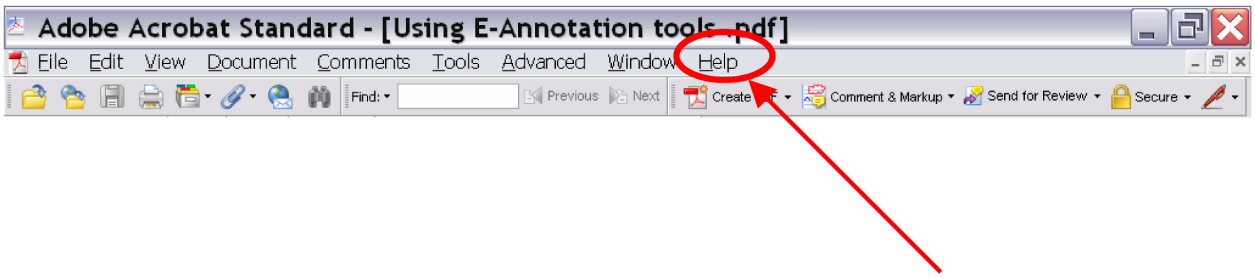
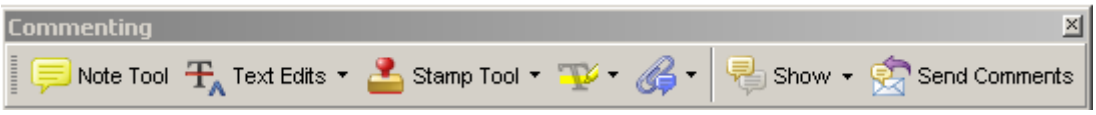


# USING E-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

## Required Software

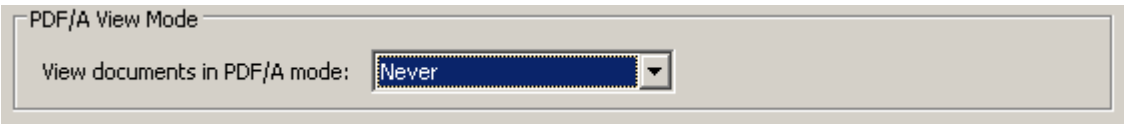
Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: http://www.adobe.com/products/acrobat/readstep2.html

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:



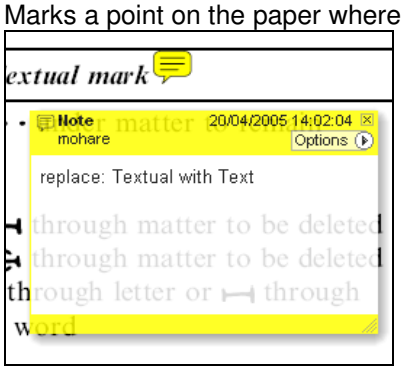

If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the "Documents" category under "Edit – Preferences", please select the category 'Documents' and change the setting "PDF/A mode:" to "Never".




## Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.

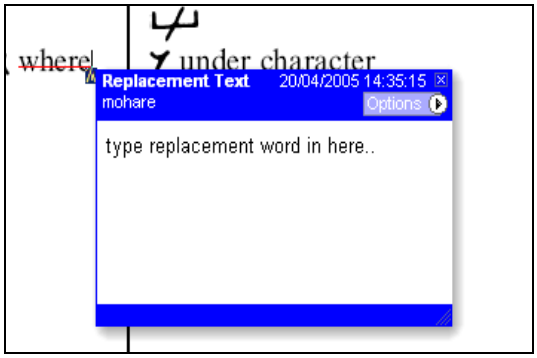



How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

## Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.

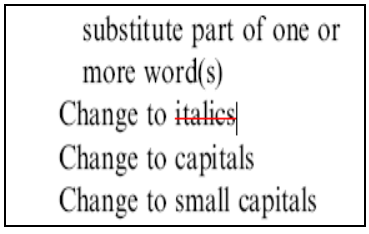



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

## Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.




How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

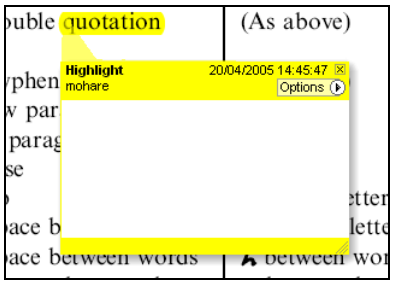
## Approved tool — For approving a proof and that no corrections at all are required.


How to use it:

1. Click on the Stamp Tool in the toolbar
2. Select the Approved rubber stamp from the 'standard business' selection
3. Click on the text where you want to rubber stamp to appear (usually first page)

## Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.

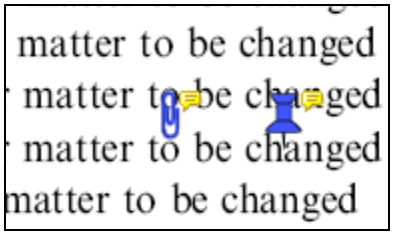



How to use it:

1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

## Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.

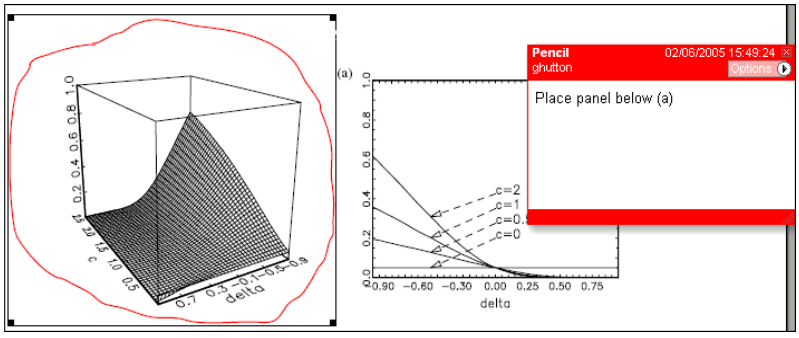



How to use it:

1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
3. Select the saved file from your PC/network
4. Select appearance of icon (paperclip, graph, attachment or tag) and close

## Pencil tool — For circling parts of figures or making freeform marks

Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.




How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
6. Click the X in the top right hand corner of the note box to close.

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