CONCISE REPORT

Association of sister chromatid exchange frequencies in patients with ankylosing spondylitis with and without HLA-B27

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Ann Rheum Dis 2003;62:775-777

Background: The analysis of sister chromatid exchange (SCE) is a cytogenetic technique used to show DNA damage due to an exchange of DNA fragments between sister chromatids.

Objective: To determine whether HLA-B27 positive patients with ankylosing spondylitis (AS) were associated with higher SCE frequencies than patients without B27.

Methods: Lymphocytes from 38 patients with AS (15 women, 23 men) and 34 control subjects were examined. Peripheral lymphocytes were cultured in darkness for 72 hours in BrdU added culture. Metaphase chromosomes were stained with a fluorescence and a Giemsa stain after a standard harvest procedure.

Results: The frequency of SCE was significantly increased in patients with AS compared with controls ($p < 0.001$). Furthermore, the SCE frequencies in patients with positive HLA-B27 was much higher than in patients with negative HLA-B27 ($p < 0.001$). The difference between SCE frequencies in the control groups with and without HLA-B27 was not significant.

Conclusion: There is a strong association between HLA-B27 and the frequencies of SCE in patients with AS.

Spondyloarthropathies are complex genetic diseases whose development is influenced by environmental factors. Genetic, immunological, endocrinological, metabolic, and various environmental factors, including infections, may play a part in the aetiology.¹ The influence of genetic factors in the spondyloarthropathies has been clearly established. Ankylosing spondylitis (AS) is considered to be the prototype of a group of HLA-B27 associated rheumatological disorders called spondyloarthropathies. HLA-B27 is strongly associated with AS, uveitis, psoriasis, reactive arthritis, amyloidosis, and aortitis, and there is strong evidence that it is directly involved in the pathogenesis of these diseases.² Among the various hypotheses proposed to explain the mechanism of HLA-B27 association with disease, one suggests that HLA-B27 is the restriction element for an "arthritogenic" peptide that would be the target antigen of T cells activated on external challenge, such as a bacterial infection.³⁻⁵

Sister chromatid exchange (SCE) is known to result from reciprocal DNA interchange in homologous loci of sister chromatids in the replication process and it occurs spontaneously at certain rates in all cells.⁶ Therefore, SCE analysis has come into use as a sensitive means of monitoring DNA damage. Most chemical and physical agents causing DNA damage, such as various chemotherapeutic, antineoplastic drugs, and ultraviolet, have an influence on SCE frequency.⁷ However, variations in SCE frequency have also been found in some chronic diseases, viral and bacterial infections. Some reports have measured the SCE rates in connective tissue disease such

as AS, scleroderma, systemic lupus erythematosus, juvenile chronic arthritis, Behçet's syndrome, and polyarteritis nodosa.⁸⁻¹⁰

This study aimed at determining whether there is any correlation between HLA-B27 and SCE frequency in patients with AS.

PATIENTS AND METHODS

Patients

A total of 38 patients (15 women, 23 men) were chosen from among first time subjects with AS diagnosed according to the 1984 the modified New York criteria.¹¹ Patients included in the study had no history of any metabolic and inflammatory diseases such as diabetes mellitus, psoriasis, bowel disease, and all of them were serologically tested for HLA-B27 and divided into two groups: group A was HLA-B27 negative (8 women, 11 men; mean (SE) age 39.6 (1.5)), group B was HLA-B27 positive (7 women, 12 men; mean (SE) age 37.6 (2.0)). The control group were also divided into two: one group comprised 15 HLA-B27 positive subjects (6 women, 9 men; mean (SE) age 47.9 (4.8)), seven of the 15 were first degree relatives of the patient group. The other group comprised 19 healthy subjects (8 women, 11 men; mean (SE) age 44.0 (2.0)) without HLA-B27. The exclusion criteria for patients and control groups were prior chemotherapy or radiotherapy, current drug use, and smoking, which could increase the SCE frequency, in the previous 6 months. Heparinised peripheral blood samples were obtained from patients with AS and controls. The age of the patient and control groups ranged from 22 to 67 years.

Culture method for SCE

The cultures were performed according to standard procedure. Briefly, lymphocytes were cultured in darkness for 72 hours in culture tubes containing 10 ml RPMI-1640 medium, 15% fetal calf serum, 2% phytohaemagglutinin, 5000 µg/ml streptomycin, 5000 IU/ml penicillin, and 10 mg bromodeoxyuridine. Then 0.1 mg/ml colcemid was added 1.5 hours before harvesting to arrest the cells at metaphase. The tube was centrifuged at 900 *g* for 10 minutes, the supernatant was removed, the cells were mixed thoroughly, and 5 ml of prewarmed hypotonic solution (0.075 M KCl) was added. The cells were subsequently incubated at 37°C for 20 minutes. The tube was centrifuged again at 900 *g* for 10 minutes, and after the supernatant was removed, the pellet was mixed thoroughly and 5 ml fresh fixative (one part acetic acid to three parts methanol) was added drop by drop. This fixation procedure was repeated three times and the tube was centrifuged for the last time. The cell pellet was then resuspended in a small volume (1 ml) of

Abbreviations: AS, ankylosing spondylitis; SCE sister chromatid exchange

Table 1 SCE frequencies in the control group without HLA-B27

	Sex	Age	Mean
1	M	36	6.68
2	M	46	6.46
3	M	41	6.62
4	M	39	7.21
5	F	61	6.98
6	M	58	5.98
7	F	56	6.36
8	F	44	6.48
9	M	32	6.73
10	M	38	6.90
11	F	44	7.13
12	F	52	6.74
13	F	47	6.88
14	M	30	6.46
15	F	46	7.12
16	M	37	6.34
17	M	32	6.66
18	F	50	7.24
19	M	46	7.18
Mean(SE)		44.0 (2.0)	6.74 (0.08)

Table 3 SCE frequencies in the patient group without HLA-B27

	Sex	Age	Mean
1	M	45	8.64
2	F	51	7.56
3	F	30	8.24
4	M	36	8.12
5	M	44	8.12
6	M	35	8.48
7	M	48	8.41
8	M	38	7.68
9	F	37	7.46
10	F	39	7.51
11	F	36	8.26
12	M	54	7.69
13	F	41	7.86
14	M	35	8.41
15	F	33	7.56
16	M	39	7.48
17	M	43	7.26
18	F	33	7.61
19	M	36	8.26
Mean(SE)		39.6 (1.5)	7.93 (0.09)

Table 2 SCE frequencies in the control group with HLA-B27

	Sex	Age	Mean
1	F*	49	6.39
2	M*	61	6.75
3	M*	51	6.53
4	M*	46	6.98
5	M*	67	6.48
6	F*	18	6.02
7	F*	46	6.72
8	M	29	6.46
9	M	42	6.72
10	M	46	7.02
11	M	37	6.39
12	F	48	6.81
13	F	19	6.74
14	M	28	6.56
15	M	61	6.36
Mean(SE)		43.2 (3.8)	6.60 (0.07)

*First degree relatives of patients with AS.

Table 4 SCE frequencies in the patient group with HLA-B27

	Sex	Age	Mean
1	M	42	9.10
2	M	35	11.26
3	M	37	11.44
4	F	32	10.36
5	M	48	10.12
6	M	33	8.96
7	M	44	9.48
8	M	46	8.51
9	M	37	9.14
10	M	56	9.36
11	F	24	9.50
12	F	22	9.71
13	F	35	10.23
14	M	42	9.63
15	M	22	10.02
16	M	38	9.72
17	F	47	8.96
18	F	36	12.46
19	F	39	11.34
Mean(SE)		37.6 (2.0)	9.96 (0.23)

Table 5 The mean age and SCE frequencies of the patients with AS and the control group

	n	Age	SCE (SE)
Controls			
HLA-B27 negative	19	44.0 (2.0)	6.74 (0.08)
HLA-B27 positive	15	43.2 (3.8)	6.60 (0.07)
Patients			
HLA-B27 negative	19	39.6 (1.5)	7.93 (0.09)*
HLA-B27 positive	19	37.6 (2.0)	9.96 (0.23)*

*Significant at $p < 0.001$.

fresh fixative and dropped onto a clean microscope slide and dried at room temperature in the dark for 24 hours. Bromodeoxyuridine incorporated metaphase chromosomes were stained by the fluorescence plus Giemsa stain technique as described by Perry and Wolff.¹² One hundred satisfactory metaphases were selected, and the results of SCE were recorded on the evaluation table.

Statistics

Kruskal-Wallis variance analysis was used to compare the frequencies of SCE in control and patient groups.

RESULTS

In the control groups, the total mean (SE) SCE per cell was 6.74 (0.08) and 6.60 (0.07) in HLA-B27 negative and positive groups, respectively (tables 1 and 2). Tables 3 and 4 show the effect of AS on the induction of SCE frequencies in both group A (without HLA-B27) and group B (with HLA-B27). The SCE frequencies of patients with AS was 7.93 (0.09) and 9.96 (0.23) in groups A and B, respectively. Table 5 gives a statistical comparison of the ages and SCE frequencies of the patients

and the control groups. According to these results, there was no significant difference in the SCE frequencies between HLA-B27 positive and negative control group subjects ($p > 0.05$). The SCE frequencies of both group A and group B

patients with AS were increased significantly compared with the control groups ($p < 0.001$). However, the SCE frequencies in group B (HLA-B27 positive) was much higher than group A (HLA-B27 negative) ($p < 0.001$).

DISCUSSION

AS is a chronic inflammatory disorder that mainly affects the lumbar spine and sacroiliac joints, although it can also affect the peripheral joints and eye structures such as the uvea. The inflammation can lead to fibrosis and ossification.¹³ In addition, spondyloarthropathies represent complex genetic diseases whose development is also influenced by environmental factors. Although the cause of AS remains unclear, chromosomal instability and HLA type seem to be strongly associated with the disorder. Estimates suggest that three to nine loci may be responsible for most genetic susceptibility to AS.¹⁴ The only susceptibility locus identified to date in multiple populations is HLA-B, where HLA-B27 is strongly associated with disease.^{15,16} The prevalence of AS in HLA-B27 positive unrelated people is about 1–6.7%, compared with 0.1% in the general population. In HLA-B27 positive relatives of such patients, the prevalence is additionally 10 times greater. Despite considerable efforts to define how HLA-B27 contributes to the disease, its role remains enigmatic.¹⁷ However, two fundamental theories have been put forward to explain the association of HLA-B27 with AS. One is the receptor theory, which explains that T cell receptors recognise foreign or self peptides, 8–20 amino acids in length, in association with a major histocompatibility complex molecule, forming an HLA and receptor complex.^{18,19} The other theory is the molecular mimicry theory, which suggests that disease is caused by antigenic components of micro-organisms which partially resemble or cross react with HLA molecules. Once antibacterial antibodies have been produced as a result of infection they will bind to HLA molecules on lymphocytes, fibroblasts, and chondrocytes. Activation of the complement cascade will lead to inflammation and clinical symptoms of disease.²⁰

Although the mechanism of SCE is not well understood, DNA damage and repair mechanism defects may play an important part. SCE frequency is, therefore, a more sensitive marker of mutagenesis than chromosomal abnormalities.^{6,7}

SCE frequencies of the control groups were compared with those of patient groups, and SCE frequencies were also assessed according to the presence or absence of HLA-B27 in both patient and control groups. Significantly raised SCE frequencies were found in the patient group compared with controls, results similar to those of Sonmez *et al.*¹⁰ Additionally, higher SCE frequencies were found in the HLA-B27 positive patient group than in the HLA-B27 negative patient group. The difference between SCE frequencies in the control groups with and without HLA-B27 was not significant. The increased frequency of SCE in patients with AS with the HLA-B27 allele might be due to molecular mimicry. As a result of micro-organisms, antibacterial antibodies are produced that cross react with HLA molecules and at the same time the infection itself might have an effect on SCE. The possible explanation of why HLA-B27 positive controls did not have raised SCE might be hidden in the molecular structure of the subgroup of B27 and the effect of micro-organisms.

In this study we investigated whether HLA-B27 positive patients with AS were associated with higher SCE frequencies than patients without HLA-B27. HLA-B27 could be associated with susceptibility to higher SCE frequencies in AS. Our findings may help in understanding the mechanism of higher SCE frequencies or increased susceptibility to chromosomal instabilities in patients with AS.

As far as we know this is the first demonstration of increased SCE frequencies in HLA-B27 positive patients with AS. More detailed studies might elucidate this association.

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Accepted 22 November 2002

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