Sister chromatid exchange analysis in the lymphocytes of patients with ankylosing spondylitis

Sinan Sönmez, Kazım Şenel, Sıtkı Öztaş, Akın Erdal, Lale Cerrahoğlu

Abstract

***Objective*—This study investigated whether any genomic change occurs in DNA level in ankylosing spondylitis (AS) by measuring sister chromatid exchange (SCE) frequency.**

***Methods*—SCE frequency was detected on metaphase chromosomes obtained from peripheral blood lymphocyte cultures in 15 patients diagnosed as having AS. SCE values were also obtained from 15 healthy subjects as a control group. SCE frequencies were detected from metaphases obtained from standard blood cultures by using bromodeoxyuridine and staining by Giemsa.**

***Results*—SCE frequencies of AS were statistically higher than those of the control group ($p<0.005$).**

***Conclusion*—These results suggest that genetic factors may play a more important part than realised in the aetiology of AS.**

(*Ann Rheum Dis* 1997;**56:**275–277)

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. [1] 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.[2] However, variations in SCE frequency have also been emerged in some chronic diseases, viral and bacterial infections.[3]

Ankylosing spondylitis (AS) is considered to be the prototype of a group of human leucocyte antigen (HLA)-B27 associated rheumatological disorders called spondyloarthropathies. Although HLA-B27 has been directly implicated in the pathogenesis of AS, additional evidence favours the involvement of an additional genetic factor(s). It is probable that genetic, immunological, endocrinological, metabolic, and various environmental factors, including infections, may play a part in the aetiology of AS.[4 5]

Because SCE can be used as a sensitive indicator of genetic impairment, we measured SCE frequencies in patients with AS.

Departments of Medical Genetics
S Sönmez
S Öztaş

and Physical Medicine and Rehabilitation
K Şenel
A Erdal
LCerrahoğlu

Atatürk University Faculty of Medicine, Erzurum, Turkey

Correspondence to:
Dr S Sönmez, Atatürk Universitesi Tip Fakültesi Tibbi Genetik Anabilim Dali, 25240 Erzurum, Turkey.

Accepted for publication
9 December 1996

Patients and methods

Peripheral blood samples that were heparinised were obtained from 15 non-smoking patients (12 men) with AS and 15 healthy non-smoking controls (11 men). The age of patient and control groups ranged from 31 to 48 years. All the patients were diagnosed and then SCE analysis was carried out before treatment. The cultures were performed according to standard procedure.[6] Briefly, lymphocytes were cultured in darkness for 72 hours in culture tubes containing 5 ml RPMI-1640 medium, 20% fetal calf serum, 2% phytohaemagglutinin, and 10 mg bromodeoxyuridine. Then 0.1 mg/ml colcemid was added 1.5 hours before harvesting. Chromosome preparations were obtained after hypotonic treatment in 0.075 M KCl and fixation in a 1:3 dilution of acetic acid-methanol treatment. Bromodeoxyuridine incorporated metaphase chromosomes were stained by FPG (florescence plus Giemsa) technique as described by Wolff and Perry. [7] By selecting 20 satisfactory metaphases, the results of SCE were recorded on the evaluation table. The data were evaluated statistically by the Student *t* test.

Results

Table 1 and table 2 show the clinical and SCE data obtained from the patient and control groups. SCE count per metaphases of the patients were normally distributed. A statistically significant difference was found between SCE frequencies obtained from the patient and control groups (table 3).

Discussion

Even though the mechanism of SCE has not been completely recognised, DNA damage and repair mechanism defects may play an

Table 1 Clinical and SCE data obtained from patients with AS

Patient number	*Sex*	*Age (years)*	*Mean SCEs per mitosis*
1	M	33	7.98
2	M	46	6.76
3	F	48	7.23
4	M	39	8.56
5	M	44	5.87
6	M	56	8.54
7	F	41	6.99
8	M	31	9.24
9	F	47	7.42
10	M	42	7.25
11	M	36	7.49
12	M	39	8.21
13	M	40	8.76
14	M	42	7.85
15	M	45	8.26

Table 2 Clinical and SCE data obtained from control group

Control number	*Sex*	*Age (years)*	*Mean SCEs per mitosis*
1	F	35	6.76
2	M	43	8.11
3	M	42	7.64
4	M	37	5.80
5	M	44	6.30
6	F	43	4.70
7	M	40	5.45
8	M	35	8.10
9	F	44	6.12
10	M	41	5.61
11	M	39	7.12
12	M	36	6.73
13	M	43	6.87
14	M	45	5.98
15	F	43	6.87

Table 3 Comparison of the mean SCE frequencies in patient and control groups

Group	*No of subjects*	*SCEs per cell (mean (SEM))*
Patient	15	7.761 (0.227)
Control	15	6.544 (0.251)
	t: 3.66	p<0.005

important part. For this reason, SCE frequency is a more sensitive marker of mutagenesis than chromosomal abnormalities.[8] Although some reports have measured the SCE rates in connective tissue disease such as scleroderma, systemic lupus erythematosus, juvenile chronic arthritis, Behçet's syndrome, and polyarteritis nodosa, there are no adequate studies that determine SCE frequency in AS.[9 10] Therefore, we conclude that SCE may be an important indicator showing a relation between AS and genetic impairment resulting from DNA damage and repair mechanism errors.

AS is known to be strongly associated with the class I major histocompatibility complex antigen HLA-B27. The prevalence of AS in B27 positive unrelated people is about 1% to 6.7%, compared with 0.1% in the general population. In B27 positive relatives of such patients, the prevalence is additionally 10 times greater.[11] On the other hand, the predisposition of some B27 positive people and not others to develop AS remains unexplained.

Certain theories have been proposed to explain the association between B27 and AS. According to the one gene theory, B27 represents a dominantly inherited disease causing gene with low penetrance such as 20%, and this AS gene contributes directly to the pathogenesis of this disorder.[12] The second theory proposes that B27 is the major gene but in fact, AS is a multifactorial polygenic disease and other genetic and environmental factors also contribute to the expression of this disease.[13] Lastly, perhaps as a third theory, B27 induces the disease but the expressed phenotype is determined by non-B27 genes.

Our results may confirm the second hypothesis that AS largely seems to be a multifactorial polygenic disease. To support this hypothesis, there are several studies describing different genetic factors other than B27 contributing to the aetiology of AS.[14–16] Some reports demonstrated additional HLA markers such as HLA-A2, Cw2, DR2 V49, B51, and B60 related to genetic susceptibility for developing AS.[15 16] In family studies, important genetic differences other than B27, between B27 positive diseased relatives and B27 positive healthy subjects have been shown.[17] Sibling pair studies have also shown that, while the genetic factors seem to be more important in influencing prognosis by disability and pain index, environmental factors have a greater importance in the timing of onset.[18] In another study, the pairwase concordance rate was found to be 50% in HLA-B27 positive monozygotic twins and 20% in HLA-B27 positive dizygotic twins.[19] This result also suggests that AS is not a monogenic disease, genes other than B27 probably play an important part.

Sex ratio differences in AS can be an additional proof that B27 is not sufficient alone. A model that multifactorial inheritance with liability varying by sex have been used to explain the unusual sex ratio.[20] Calin *et al* recently proposed some susceptibility genes for explaining the greater prevalence of AS in at risk families, and severity genes in the patients with sporadic AS showing more severe symptoms than those in familial type. [21]

In this study, the significant increase in the SCE frequencies of the patient group compared with the control group suggests that AS is a largely impaired genetic based disease. Our results may also suggest the hypothesis that other genetic and environmental factors might participate together with the B27 allele in the susceptibility to AS. The increased SCE rates may be interpreted as an indicator providing a genetic predisposition to AS rather than increased rate of somatic mutation. As shown before, the impaired DNA repairing mechanism may play a part in pathogenesis of some connective tissue diseases such as rheumatoid arthritis, it can also be supposed that, DNA damage and DNA repair mechanism defects may contribute to the AS process.[22 23]

In conclusion, although one gene theory suggests that the HLA-B27 antigen itself is directly involved in the pathogenesis of the disease, our results suggest that other genetic or perhaps environmental triggers causing DNA damage may play an important part in the onset of AS.

1 Dean BJ, Danford N. Assays for the detection of chemically-induced chromosome damage in cultured mammalian cells. In: Venitt S, Parry JM, eds. *Mutagenicity testing. A practical approach*. Oxford: IRL Press, 1984: 187-232.
2 Gebhart E. Sister chromatid exchanges and structural chromosome aberration in mutagenicity testing. Hum Genet 1981; 58:235-54
3 Tezel A Atabey N, Emre S, Sakizli M. SCE frequency of herpes simplex virus type I infected cells. Mutat Res 1994; 306:81-3.
4 Ball GV. Ankylosing spondylitis. In: McCarty DJ and Koopman LJ, eds. *Arthritis and allied conditions.* Philadelphia: Lea and Febiger 1993: 1051-60.
5 Khan MA. An overview of clinical spectrum and heterogeneity of spondyloarthropathies. In: Khan MA, eds. *Rheumatic disease clinics of North America, Spondyloarthropathies.* Philadelphia: WB Saunders, 1992: 1-10.
6 Rooney DE, Czepulkowski BH. *Human cytogenetics: A practical approach*. Lancaster: IRL, 1992.

7 Wolff S, Perry P. Differential Giemsa staining of sister chromatids and the study of sister chromatid exchange without autoradiography. Chromosoma 1974; 48:341-53.
8 Carrano AV, Thompson LH, Lindl PA, Minkler JL. Sister chromatid exchange as indicator of mutagenesis. Nature 1978; 271:551-3
9 Palmer RG, Pereira RS, Dore CJ, Denman AM. Sister chromatid exchange frequencies in patients with scleroderma and their relatives. Ann Rheum Dis 1986; 45:409-11.
10 Palmer RG, Dore CJ, Denman AM. Sister-chromatid exchange frequencies in lymphocytes of controls and patients with connective tissue diseases. Mutat Res 1986; 162:113-20.
11 Feltkamp TE. Factors involved in the pathogenesis of HLA-B27 associated arthritis. Scand J Rheumatol Suppl 1995; 101: 213-7.
12 Rubin LA, Amos CI, Wade JA, Martin JR, Bale SJ, Little AH, *et al.* Investigating the genetic basis for ankylosing spondylitis. Linkage studies with the major histocompatibility complex region. Arthritis Rheum 1994; 37: 1212-20.
13 Moller P. HLA-B27 is necessary but not sufficient. Scand J Rheumatol Suppl 1990; 87: 131-3.
14 Reveille JD, Suarez Almazor ME, Russell AS, Go RC, Appleyard J, Barger BO, *et al.* HLA in ankylosing spondylitis: is HLA-B27 the only MHC gene involved in disease pathogenesis? Semin Arthritis Rheum 1994; 23: 295-309.
15 La Nasa G, Mathieu A, Mulargia M, Carcassi C, Vacca A, Ledda A, *et al.* Association of the HLA-A2, CW2, B27, S31, DR2 haplotype with ankylosing spondylitis. A possible role of non-B27 factors in the disease. Dis Markers 1993; 11: 191-203.
16 Vargas-Alarcon G, Garcia A, Bahena S, Melin-Aldana H, Andrade F, Iba–nez-de-Kasep G, *et al.* HLA-B alleles and complotypes in Mexican patients with seronegative spondyloarthropathies. Ann Rheum Dis.1994;53:755-8.
17 Calin A, Marder A, Becks E, Burns T. Genetic differences between B27 positive patients with ankylosing spondylitis and B27 positive healthy controls. Arthritis Rheum 1983; 26:1460-64.
18 Calin A, Elswood J. Relative role of genetic and environmental factors in disease expression: sib pair analysis in ankylosing spondylitis. Arthritis Rheum 1989; 32:77-81
19 Jarvinen P. Occurrence of ankylosing spondylitis in a nationwide series of twins. Arthritis Rheum 1995;38: 381-3.
20 James WH. The sex ratios of probands and of secondary cases in conditions of multifactorial inheritance where liability varies with sex. J Med Genet 1991; 28: 41-3.
21 Calin A, Kennedy GL, Edmunds L, Will R. Familial versus sporadic ankylosing spondylitis, two different diseases? Arthritis Rheum 1993; 36:676-81.
22 Tuschl H, Altmann H. Unscheduled DNA synthesis in lymphocytes of rheumatoid arthritis patients and spleen cells of rats with experimentally induced arthritis. Med Biol 1976; 54: 761223-9.
23 Frank O, Klein W, Kocsis F, Altmann H. DNA-"rejoining" in lymphocytes of patients with a manifestation of rheumatoid arthritis in older age. Aktuelle Gerontol 1977; 7: 12:629-31.