Skewed X-chromosome Inactivation in Scleroderma

Elif Uz[1], Laurence S Loubiere[2], Vijayakrishna K. Gadi[2,3] , Zeynep Ozbalkan[4], Jeffrey Stewart[5], J. Lee Nelson[2,3], Tayfun Ozcelik[1,6]

1. Department of Molecular Biology and Genetics, Faculty of Science, Bilkent University, Ankara, Turkey
2. Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
3. Department of Medicine, University of Washington, Seattle, WA, USA
4. Rheumatology Department, Ankara Numune Education and Research Hospital, Ankara, Turkey
5. Caldera Pharmaceuticals, Los Alamos, NM, USA
6. Institute for Materials Science and Nanotechnology (UNAM), Bilkent University, Ankara, Turkey

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Address for correspondence:

Tayfun Ozcelik
Bilkent University
Department of Molecular Biology and Genetics
Bilkent – Ankara 06800
Turkey
Tel: +90-312-290 2139; Fax: +90-312-266 5097; e-mail: tozcelik@fen.bilkent.edu.tr

Abstract

Scleroderma is a female-prevalent autoimmune disease of unclear etiology. Two fundamental gender differences, skewed X-chromosome inactivation (XCI) and pregnancy-related microchimerism have been implicated in scleroderma. We investigated the XCI patterns of female scleroderma patients and the parental origin of the inactive X chromosome in those patients having skewed XCI patterns (>80%). In addition, we investigated whether a correlation exists between XCI patterns and microchimerism in a well-characterized cohort. 195 female scleroderma patients and 160 female controls were analyzed for the androgen receptor locus to assess XCI patterns in the DNA extracted from peripheral blood cells. Skewed XCI was observed in 67 (44.9%) of 149 informative patients and in 10 of 124 healthy controls (8.0%) [odds ratio (OR) = 9.3 (95% confidence interval (CI) 4.3-20.6, P<0.0001)]. Extremely skewed XCI (>90%) was present in 44 of 149 patients (29.5%) but only in 3 of 124 controls (2.4%) (OR=16.9; 95% CI 4.8-70.4, P<0.0001). Parental origin of the inactive X chromosome was investigated for 10 patients for whom maternal DNA was informative, and the inactive X chromosome was of maternal origin in 8 patients and of paternal origin in 2 patients. Skewed XCI mosaicism could be considered as an important risk factor in scleroderma.

Introduction

Scleroderma (systemic sclerosis, SSc) is an autoimmune disease characterized by fibrosis, and alterations in the microvasculature. *(1)* Scleroderma is 3-10 times more prevalent in women than in men. *(2)* Most autoimmune diseases are more prevalent in females than in males, *(3)* and fundamental differences between male and female biology, such as hormone status, *(4)* pregnancy, *(5)* and X-chromosome inactivation *(6,7)* have been proposed as the underlying pathophysiological mechanisms leading to the female prevalence of autoimmune diseases. Indeed, both pregnancy-related microchimerism, *(8,9,10,11)* and skewed (*i.e*., not the expected 50:50 balance) X-inactivation mosaicism *(12)* have been shown to be associated with scleroderma.

This study was performed to investigate the X-inactivation patterns of female scleroderma patients along with the parental origin of the inactive X chromosome in those patients with extremely skewed X-inactivation. In addition, the correlation between skewed X-inactivation and both maternal and fetal microchimerism was examined in a well-characterized cohort of scleroderma patients.

Patients and Method

DNA samples were obtained from 195 scleroderma patients and 160 control women. Clinical characteristics of the patients and controls have been published elsewhere. *(12,13)* The ethics review boards at the participating institutions approved the study protocol. Informed consent was obtained from all subjects. The X-chromosome inactivation status of the patients and controls was determined by genotyping a highly polymorphic CAG repeat in the first intron of the androgen receptor as previously described. *(12,14)* Depending on the definition and quantitative accuracy of the

measurement method, a few percent to nearly one fifth of apparently healthy women display skewed (*i.e.*, non-random) patterns of X-inactivation. Whereas, ratios in the range of 50-79 percent is usually regarded as normal variation, deviation from this range in 80-89 percent of cells is defined as skewed, and above 90 percent of cells as extremely skewed X-inactivation. *(15,16,17)* Fisher's Exact test was used for statistical analyses.

Results

X-inactivation ratios

Our data show that skewed X-chromosome inactivation (>80%) was associated with disease. X-chromosome inactivation status was informative in 94 of the 125 scleroderma patients (75%) and in 124 of the 160 controls (78%). Only the individuals whose alleles resolved adequately were included in the subsequent densitometric analysis, thus some of the heterozygous individuals were considered as uninformative. Skewing in the range of 80-89% was observed in 15 of the 94 patients (16.0%) but in only 7 of the 124 controls (5.6%; P<0.0001; Table 1). Extremely skewed X-inactivation (>90%) was observed in 17 of the 94 patients (18.1%) but in only 3 of the 124 controls (2.4%; P<0.0001). When the data from the present and the previously published *(12)* studies were combined (Table 1), of the total of 195 patients, 149 were informative for the androgen receptor polymorphism. Skewed X-inactivation was observed in 67 of 149 patients (44.9%) but in only 10 of 124 healthy controls (8.0%). Expressed as a risk factor for scleroderma, the odds ratio (OR) was 9.3 (95% confidence interval (CI) 4.3-20.6; P<.0001). Numerous studies conducted in different control populations indicate that extremely skewed X-inactivation is a rare event not exceeding 3-5 percent. *(15,16,17)* For the current

study, extremely skewed X-inactivation was present in 44 of 149 patients (29.5%) but in only 3 controls (2.4%) and the OR was 16.9 (95% CI 4.8-70.4, P<0.0001).

Parental origin of the inactive X chromosome

19 patients with skewed X-inactivation for whom maternal DNA was available were further analyzed to determine the parental origin of the inactive X chromosome. For this analysis, DNA samples from mothers of patients were analyzed for the androgen receptor gene polymorphism, and informative results were obtained for ten patient-mother pairs. For the remaining 9 pairs, both the mother and the daughter were heterozygous for the androgen receptor polymorphism, thus providing no difference in allele sizes. Therefore, it is not possible to determine the parental origin of the alleles in the absence of information regarding the paternal genotype. The inactive X chromosome was of maternal origin in 8 patients and of paternal origin in 2 patients. In 3 mothers of patients studied, X-inactivation pattern was also skewed (Table 2). Interestingly, the same allele was skewed in both the mothers and the patients, and one mother had been diagnosed with an autoimmune condition, namely temporal arteritis.

Evaluation of skewed X-inactivation for correlation with microchimerism

Maternal and fetal microchimerism was investigated as described previously. *(18)* We examined X-inactivation ratios for correlation with microchimerism in a limited number of patients for whom microchimerism data was also available. We observed that among patients with more than 80 percent skewing, 7 of 12 (58.3%) tested positive for maternal microchimerism and 8 of 14 (57.1%) for fetal microchimerism (Table 3). In patients with random patterns of X-inactivation, 10 of 15 (66.7%) were positive for

maternal microchimerism, and 14 of 25 patients were positive for fetal microchimerism (56%). Neither maternal (P=0.7) nor fetal microchimerism (P=1.0) correlated with skewed X-inactivation.

Discussion

Autoimmune disorders affect more than 5% of the population, and a high female prevalence is characteristic of most autoimmune diseases including scleroderma, autoimmune thyroid diseases, systemic lupus erythematosus, and Sjögren's syndrome. *(3)* Pregnancy related microchimerism, *(10)* and skewed X-inactivation *(12)* have been proposed as potential contributors to the pathophysiology of scleroderma. In this study we observed skewed X-inactivation patterns in a significant proportion (34%) of females with scleroderma. Of these females, approximately 18% displayed extreme skewing (>90:10). This result is consistent with our previous study, *(12)* and indicates that skewed X-inactivation could be a common finding in different population groups.

We do not know the cause of skewed X inactivation in scleroderma, and probable mechanisms have been discussed in the accompanying manuscript. *(19)* Amongst them, X-linked lethal mutations, which would be compatible with life in females because of X-inactivation mosaicism, is an appealing causative mechanism. If the cause of skewing is indeed X-linked mutations, these should be inherited from the maternal lineage unless they occur *de novo* during gametogenesis. We therefore analyzed the parental origin of the inactive X chromosome in 19 patient-mother pairs, and of the 10 pairs with informative genotyping results, inactive X was found to be of maternal origin in eight pairs. This result is on the border of statistical significance

(P=0.055). Assuming this result holds, we will be left with an interesting puzzle. Why are maternally inherited X chromosomes more likely to be inactivated in highly skewed patients? One possibility is that some X chromosomes confer a selective disadvantage to both the organism itself and the peripheral cells within the organism. This precise situation has been documented in X-linked immunodeficiency, where heterozygous (female) carriers have high X-inactivation skew (because of natural selection within the organism), while affected males have a survival disadvantage. *(20)* When an X-encoded genotype is selectively disadvantageous to cells, skew is an outcome. When an X-encoded genotype is selectively disadvantageous to an organism, preferential maternal inheritance is an outcome. Together, this leads us to the hypothesis that some highly skewed patients have an X chromosome that would confer a selective disadvantage on homozygotic women and on males.

Because scleroderma is the first disease in which pregnancy related microchimerism has been documented, we investigated a subset of patients for whom microchimerism data was also available for correlation of skewed X-inactivation with microchimerism. Neither maternal nor fetal microchimerism were found to be correlated with skewed X-inactivation. In conclusion, the two types of female mosaicism—skewed X-inactivation and maternal/fetal microchimerism—appear to be independent risk factors in scleroderma. One possible explanation for these data is that mosaicism itself is an underlying cause of scleroderma and, by extension, female-prevalent autoimmune disease in general.

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Table 1. Proportions of scleroderma patients and controls with skewed X-inactivation.

	No. (%) observed with skewing			
Degree of skewing (%)	**Present study (n=94)**	**Previous study[11] (n=55)**	**Combined (n=149)**	**Control (n=124)**
+90	17 (18.1%)	27 (49.1%)	44 (29.5%)	3 (2.4%)
80-89	15 (16.0%)	8 (14.5%)	23 (15.4%)	7 (5.6%)
70-79	15 (16.0%)	0 (0.0%)	15 (10.1%)	22 (17.7%)
60-69	24 (25.5%)	5 (9.1%)	29 (19.5%)	29 (23.4%)
50-59	23 (24.5%)	15 (27.3%)	38 (25.5%)	63 (50.8%)
For comparison by χ^2, P<0.0001(both >80% skewing and >90% skewing)				

Table 2. Parental origin of the inactive X chromosome in scleroderma patients with skewed X-inactivation.

Sample	Degree of skewing	Parental origin of the inactive X chromosome
Patient 1	95	Maternal
Mother 1	95	
Patient 2	95	Maternal
Mother 2	95	
Patient 3	86	Maternal
Mother 3	88	
Patient 4	100	Maternal
Mother 4	Not informative	
Patient 5	90	Maternal
Mother 5	Not informative	
Patient 6	90	Maternal
Mother 6	Not informative	
Patient 7	85	Maternal
Mother 7	67	
Patient 8	85	Maternal
Mother 8	58	
Patient 9	85	Paternal
Mother 9	70	
Patient 10	80	Paternal
Mother 10	70	

Table 3. Proportion of maternal (MMc) and fetal (FMc) microchimerism in scleroderma patients.

	MMc			FMc	
Skewed (>80 %)	12 tested	58.3% positive for MMc	**Skewed (>80 %)**	14 tested	57.1% positive for FMc
	7 positive 5 negative			8 positive 6 negative	
Random (<80 %)	15 tested	66.7% positive for MMc	**Random (<80 %)**	25 tested	56.0% positive for FMc
	10 positive 5 negative			14 positive 11 negative	