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Skewed X chromosome inactivation in scleroderma: comment on the article by Özbalkan et al

To the Editor:

Özbalkan et al (Ozbalkan Z, Bagislar S, Kiraz S, Akeyerli CB, Ozer HTE, Yavuz S, et al. Skewed X chromosome inactivation in blood cells of women with scleroderma. Arthritis Rheum 2005;52:1564–70), using a polymerase chain reaction (PCR) involving the androgen receptor locus for the assessment of methylation status, have determined that there is skewing of X chromosome inactivation in blood cells from women with scleroderma. Based on recent disclosures following the sequencing of 99% of the human X chromosome, their conclusion may be unwarranted; it may be the androgen receptor locus and not the inactivated X chromosome that is skewed.

One or the other of the 2 X chromosomes is suppressed in each cell of a woman's body. However, sequencing of 99% of the human X chromosome (Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 2005;434:400–4) revealed that ~15% of genes escape this X inactivation, and those genes that escape vary from woman to woman. The genes that escape X inactivation are expressed from both X chromosomes. Therefore, the level of expression of such genes is higher than that of genes expressed from only one X chromosome. If the androgen receptor gene was among those that escape and are expressed from both chromosomes, the PCR would detect increased expression of the androgen receptor gene in that chromosome. Rather than skewing X chromosome inactivation, this would reflect skewed expression of the androgen receptor gene locus.

Although skewed expression of the androgen receptor gene could, in some instances, account for the apparent skew of X chromosome inactivation, that happenstance might remain significant or become even more significant. The increased expression of the androgen receptor gene, if observed primarily in patients with scleroderma, may mean that the androgen receptor gene has a role in the disease etiology.

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Reply

To the Editor:

Dr. McGrath questions the conclusion of our study of X chromosome inactivation in women with scleroderma in light of a recent study that revealed extensive variability in X-linked gene expression in females (1). He raises 2 issues: first, that the androgen receptor locus and not the inactivated X chromosome could be skewed, and second, that the androgen receptor locus could escape from X chromosome inactivation, and biallelic expression of this gene could be involved in disease pathogenesis. Indeed, ~15% of X-linked genes escape inactivation to some degree, and an additional 10% of X-linked genes show variable patterns of inactivation. However, androgen receptor is not one of them. The report by Carrel and Willard (1; supplementary Table 3) and numerous previously published studies firmly establish that at least 214 X-linked genes, including the androgen receptor locus, are subject to X chromosome inactivation. With respect to the isolated skewing of the androgen receptor locus, this is also highly unlikely. It is well known that inactivation occurs early in development, leading to silencing that is mitotically stable, so that females are mosaics for cell populations in which either the paternal or the maternal X is silenced (2). It is also well-established that DNA methylation is involved in the maintenance of the inactive X chromosome silencing, and the well-established human androgen receptor assay used in our study determines the methylation status of the androgen receptor (3). In conclusion, we could not identify any experimental data that support the points raised by Dr. McGrath.

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Does oral glucosamine prevent the loss of proteoglycans in a rabbit model of osteoarthritis? Comment on the article by Tiraloche et al

To the Editor:

A number of studies have been performed to investigate the effects of glucosamine on cartilage metabolism and clinical trials have been performed to test its potential for the treatment of osteoarthritis. The clinical studies have mostly been based on evaluations of radiographic progression of the disease and Western Ontario and McMaster Universities Osteoarthitis Index analyses (1) while only a few reports showing histologic or biochemical data have been published. Thus, the report by Tiraloche et al (2), presents important data on the effects of glucosamine on articular cartilage in an experimental osteoarthritis model. One of the major conclusions drawn from that study is that oral administration of glucosamine prevented the loss of proteoglycans observed in the femoral condylar cartilage of the placebo group, compared with the unoperated contralateral joint cartilage.

A different interpretation can also be made on the basis of data from biochemical analysis of glycosaminoglycans (GAGs) that are presented in Figure 4 of their report. In the placebo group, the average concentration of GAGs in the femoral condyle of the operated joint was lower than that in the controls, while in the contralateral unoperated joint, the concentration was higher than that in the control group. As a result, the operated and the contralateral joints showed a significant difference in their GAG concentrations. This phenomenon has been observed previously, using a similar model in dogs (3) and a model in which 1 leg was immobilized by splinting (4). In terms of absolute concentrations of GAGs the same observation could also be made for the glucosamine-treated, anterior cruciate ligament (ACL)–transected animals, although the difference between the operated and the contralateral joints was not significant. This was interpreted to show that glucosamine treatment prevented the loss of proteoglycans in the operated joint.

However, Figure 4 in their report (2) also shows that, in femoral condylar cartilage, the lowest average value for GAG concentration is in the glucosamine-treated, operated group. Therefore, another way to interpret the data would be to state that glucosamine treatment did not restore GAG content in the operated knee joint, but prevented the contralateral joint from benefiting from the increased load subjected to it, due to the presence of a more instable joint in the ACL-transected knee. In the tibial condyle, the absolute level of GAGs in the glucosamine-treated, operated joint was lower than that in the corresponding placebo group as well.

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Reply

To the Editor:

We appreciate Dr. Lammi's interest in and critical comments on our report. Our study primarily entailed a detailed, histologic assessment of disease modification by glucosamine in articular cartilage in a rabbit ACL transection model of osteoarthritis. These analyses revealed that the loss of proteoglycan, associated with the development of joint disease (based on Safranin O staining), was significantly reduced in the lateral tibial plateau cartilage in the operated joints of the glucosamine-treated group. Likewise, on macroscopic analysis of the cartilage, the lateral tibial plateau exhibited significantly less change in the glucosamine-treated group, consistent with the histologic assessment. Despite these interesting and statistically significant observations, we noted that these effects were modest and that the administration of glucosamine did not prevent fibrillation and/or erosions of the articular cartilage.

The biochemical analyses of articular cartilage were used to determine whether glucosamine administration altered changes in matrix composition and collagen damage. There was a significant reduction ($P = 0.02$) in GAG content in the femoral condyles of the placebo-treated, ACL-transected joints compared with their respective contralateral unoperated joints, but this was not seen when glucosamine was administered ($P = 0.56$). In Figure 1 (which also shows median values), the results of the Wilcoxon signed rank analyses are shown.

Furthermore, based on a Kruskal-Wallis test to evaluate differences in cartilage GAG levels among control unoperated and contralateral unoperated limbs in both the placebo and glucosamine-treated groups, no significant differences ($P = 0.62$) were detected between the median GAG levels in the contralateral unoperated limbs and the limbs from control