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Rare X chromosome abnormalities in systemic lupus erythematosus and Sjögren's syndrome

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Abstract

Background. Sjögren's syndrome and systemic lupus erythematosus (SLE) are related by clinical and serological manifestations as well as genetic risks. Both diseases are more commonly found in women compared to men at a ratio of about 10 to 1. Common X chromosome aneuploidies, 47,XXY and 47,XXX, are enriched among men and women, respectively, in either disease suggesting a dose effect on the X chromosome.

Methods. We examined cohorts of Sjögren's syndrome or SLE patients with intensity plots of X chromosome single nucleotide polymorphism (SNP) alleles along with karyotype of selected subjects.

Results. Among ~2500 women with SLE we found three patients with a triple mosaic consisting of 45,X/46,XX/47,XXX. Among ~2100 women with Sjögren's syndrome, one patient had 45,X/46,XX/47,XXX with a triplication of the distal p arm of the X chromosome in the 47,XXX cells. Neither the triple mosaic nor the partial triplication were found among controls. In another Sjögren's cohort, we found a mother-daughter pair with partial triplication of this same region of the X chromosome. The triple mosaic occurs in approximately 1 in 25,000 to 50,000 live female births, while partial triplications such are even rarer.

Conclusions. Very rare X chromosome abnormalities are present among patients with either Sjögren's or SLE, and may inform the location of a gene(s) that mediate an X dose effect as well as critical cell types in which such effect is operative.

Introduction

Systemic lupus erythematosus (SLE) and Sjögren's syndrome are related by common autoimmune serology, clinical manifestations, and genetics, as well as a strong bias towards the female sex. The sex bias for SLE is about 10-to-1^{1,2}, while among those with Sjögren's, women are over-represented between 10 and 15-fold.³

Aneuploidies of the X chromosome are common in the human population. Klinefelter's syndrome (male 47,XXY) occurs in about 1 in 500 live male births but 80% of these men are undiagnosed.⁴ Meanwhile, 47,XXX is found in about 1 in 1000 live born girls with only about 1% identified.^{5,6} We have found excess 47,XXY among men with either SLE^{7,8} or Sjögren's syndrome⁹ as well as excess 47,XXX among women with these diseases.¹⁰ On this basis, we have proposed an X chromosome dose effect for the sex bias of SLE and Sjögren's syndrome. Such a bias might be mediated by specific genes that escape X inactivation, global escape of X inactivation in specific cell types¹¹ or interference with immune tolerance by increased production of X chromosome gene products¹², or an effect on the immune system by the mosaicism of random X inactivation.¹³

We have previously reported a 46,XX man among 316 men with SLE.¹⁴ This X chromosome abnormality occurs in as few as 1 in 25,000 to 1 in 50,000 live born boys. We undertook the present study to examine women with either SLE or Sjögren's syndrome for rare X chromosome aneuploidies.

Methods

Subjects. We studied a large cohort of SLE patients with about 60% being of European heritage and 40% of African heritage, gathered for genetic and other studies, which has been described in detail¹⁵ as well as a large cohort of primary Sjögren's syndrome patients, where all subjects meeting criteria for other

autoimmune rheumatic disease (that is, those with secondary Sjögren's were excluded).^{16,17} Subjects met the respective research classification criteria for each of the diseases.^{18,19} Women demonstrated to not have SLE or Sjögren's served as controls. As previously described¹⁰, controls were screened for autoimmune rheumatic disease, and no such disease was identified.

X chromosome Analyses: The subjects were screened for X chromosome abnormalities by examination of intensity plots of X chromosome single nucleotide polymorphism (SNP) alleles, as we have previously described.^{8,10} In these displays (so-called, b plots) the fluorescent intensity of the b allele is divided by the intensity of the a and b alleles; thus, giving values of 1.0 for homozygous b SNPs, 0.5 for ab heterozygous SNPs and 0.0 for homozygous a SNPs. However, a person with three X chromosomes will have b plot results at 0.33 for aab SNP alleles and 0.67 for abb alleles. Subjects with abnormal b plots were then studied by karyotype of peripheral blood mononuclear cells, or florescent *in situ* hybridization (FISH), as previous described.⁸ Some subjects were studied directly with karyotype using standard methods in a Clinical Laboratory Improvements Amendments (CLIA)-approved laboratory.

Statistics. We calculated binomial 95% confidence intervals for ratios. To calculate the prevalence of SLE among individuals with X triple mosaic, we used Bayes' theorem as previously described.⁸ Briefly, $P(B/A) = P(A/B) \times P(B/PA)$, where $P(B/A)$ = prevalence of SLE among those with the triple mosaic, $P(A/B)$ = prevalence of the triple mosaic among SLE patients, $P(B)$ = prevalence of SLE in the population, and $P(A)$ = prevalence of the X triple mosaic in the population.

Results

Among 2,426 women with SLE, we found three with the rare triple mosaic of 45,X/46,XX/47,XXX based on the b plots; these results were confirmed by G-band karyotyping of 50 cells (Table 1). These SLE patients had approximately the same ratio of cells with the abnormal X chromosome number. Namely, only 3% of cells were either 45,X or 47,XXX, while a large majority (~94%) carried 46,XX. In examination of their clinical records, there was no diagnosis nor clinical evidence of Turner's syndrome, abnormal sexual development, or infertility. Binomial 95% confidence intervals for the ratio 3 in 2,426 (0.0012 or 1 in 809) were 0.0003 to 0.0036 (or 1 in 3333 to 1 in 278). And, therefore, did not include the estimated birth rate of 45,X/46,XX/47,XXX of 1 in 25,000.^{5,20,21} Thus, we concluded that there is a statistical increase in the prevalence of the X chromosome triple mosaic among SLE patients.

We used Bayes' theorem to calculate the prevalence of SLE among women carrying the X triple mosaic. For this calculation we assumed a prevalence for SLE of 1 in 1000 along with a prevalence of the X triple mosaic of 1 in 25,000. The calculation predicted that 1 in 32 individuals with 45,X/46,XX/47,XXX will have SLE.

Among 2138 women with Sjögren's syndrome we did not find a subject with the X chromosome triple mosaic described above. Instead, when examining X chromosome b plots, we noted a single subject with a partial triplication of the X chromosome p arm. That is, distal Xp showed 4 intensity bands at 0.0, 0.3, 0.6 and 1.0, indicating three copies of this portion of the X chromosome, but other areas of the X chromosome showed only three bands at 0.0, 0.5 and 1.0. The latter is the pattern for two X chromosomes (Figure 1A). We obtained a blood sample from this subject and performed karyotyping, which showed a triple X chromosome mosaic with 2 of 20 (10%) cells examined carrying 45,X and 13 of 20 (65%) cells carrying 46,XX, while 5 of 20 (25%) of cells had a triplication of the distal portion of the p arm of X (see Figure 1B). This additional, triplicated portion of X consisted of a non-reciprocal

translocation on to one of the X chromosomes, that is, 47,XX^{+Xp}. By karyotype this abnormality was designated 45,X/46,XX/46,X,der(X)(pter>q28::p11.4>pter) (Figure 1).

We have identified another Sjögren's patient with a rare X chromosome abnormality in a United Kingdom Sjögren's cohort. The patient was studied by karyotype as a child based on the presence of epilepsy, oligomenorrhea, and thyroid dysfunction. This study showed one normal X chromosome and one isochromosome Xp, that is, an X chromosome with 2 p arms (Figure 1C). Her mother had an inverted segment of Xq (Figure 1C). Both the mother and daughter have Sjögren's syndrome. This entire cohort of 940 Sjögren's patients has not been systematically studied for X chromosome abnormalities as approximate 700 are included among the 2138 studied above. So, while the identification of this partial triplication of Xp must be considered anecdotal, the triplicated region overlaps with the one found among the systematically studied patients (Figure 1C). Thus, this incidentally found X chromosome abnormality supports the idea that a gene or genes within distal Xp mediate the X chromosome dose effect.

We found no control subject (n=2712) with the X chromosome triple mosaic, or any variant thereof (Table 1).

Discussion

SLE and Sjögren's syndrome, similar to many other autoimmune diseases, have a marked sex bias with women affected about 10 times more often than men. A number of hypotheses have been proposed, and some of these tested, to explain the marked sex bias in these diseases. An obvious difference is sex hormones, which might include not only estrogen, androgen and progesterone but also prolactin.²²

Several estrogenic effects, including differentiation of T helper cells, induction of interferon, and survival of B cells, may predispose to autoimmune disease²³ as well as recently described effects on the AIRE gene.²⁴ A counterargument to the hormone hypothesis is that sex hormones are within normal limits at the time of SLE diagnosis^{25, 26} and are not different between men with SLE and men with other chronic, non-autoimmune disease.²⁷ However, men with untreated hypogonadism are reported to be at high risk of SLE.²⁸ There is no evidence of acquired X monosomy among women with SLE^{29, 30}, as there is among those with autoimmune thyroid disease³¹ or primary biliary cirrhosis.³²

Failure to inactivate an X chromosome in activated CD4+ T cells is found in SLE patients³³⁻³⁵ and lupus-prone mice³⁶ with failure to methylate; and, thus silence, X-linked immune genes.³⁷ A recent study has shown a global difference in X chromosome inactivation in resting lymphocytes, which was associated with bi-allelic expression of genes from lymphocytes of SLE patients.¹¹ Others have proposed that an overall effect of X reactivation might perturb immune tolerance.¹² Each of these proposed mechanism might underlie a gene dose effect on the X chromosome, which might involve one or more immune-related proteins encoded on the X chromosome.

We have found very rare X chromosome abnormalities among patients with either SLE or Sjögren's syndrome. Based on karyotypes of consecutive live births,^{5, 20, 38} the 45,X/46,X/47,XXX triple mosaic is found in about 1 in 25,000 live born girls, but was present in about 1 in 800 women with SLE. We did not find and did not expect to find the rare triple mosaic among our controls, which number only just above 2000. But, past studies of more than 25,000 live female births^{5, 21, 38} serve as an external validation of the incidence and prevalence of the 45,X/46,XX/47,XXX mosaic. Meanwhile, the 45,X/46,XX/47,XX^{+Xp} found in one of our Sjögren's syndrome patients is nearly unprecedented.³⁹

Finding these X chromosome rarities has several implications. For instance, only a small percentage of cells carried the additional X or portion of the X chromosome in these patients. The data from Wang, et al. are consistent with this finding as the unusual maintenance of X chromosome inactivation in lymphocytes leads to bi-allelic expression of X-linked genes in only a few cells.¹¹ In addition, similar to those with 100% of cells carrying 47,XXX¹⁰, the 4 patients we described have no evidence of abnormal sex hormones or sexual development. Likewise, hormonal imbalance due to Turner's syndrome, which is likely under-represented among SLE patients⁷, is not expected with the low proportion of 45,X cells in our patients. Thus, estrogen, progesterone, or androgen differences cannot be evoked as an explanation of the apparent association of the X triple mosaic with SLE and Sjögren's syndrome.

Despite the associations we find, increased prevalence of these rare X chromosome aneuploidies does not prove causality. Nonetheless, given the rarity of these X chromosome abnormalities, association by chance alone seems unlikely to us. Determining mechanism by which additional X chromosomes or distal Xp fragments, increase the risk of these diseases remains to be determined, but will provide evidence of causality. Perhaps the findings reported herein give important clues, which can be used to direct and narrow the candidates for genes impacting the X chromosome dose effect. The distal p arm of the X chromosome contains multiple genes that escape X inactivation.¹¹ Thus, the finding of a partial trisomy of this portion of the X chromosome in these patients suggests, but does not prove, that the gene or genes mediating this effect are found in this region. There are a number of past examples in which identification of an abnormality on karyotype in an affected individual led to much more rapid identification of the genetic cause of a particular disease. These include Duchenne's and Becker's muscular dystrophy, Angelman's syndrome, and fragile X syndrome. But, perhaps relevant to the findings herein, was the identification of a chromosome X-21 balanced translocation in a girl with

Duchenne's muscular dystrophy. This finding gave the location of the gene on the X chromosome long before identification was possible through reverse genetics studies.⁴⁰

In summary, we suspect that these SLE and Sjögren's with X chromosome abnormalities inform greatly about the common situation, that is, the marked sex bias between 46,XX women and 46,XY men. Future studies, using the localization provided by the very rare patients reported herein, may be able to concentrate on genes within this shared triplicated region of Xp as the mediators of the X chromosome dose effect for sex bias in SLE and Sjögren's syndrome.

Accepted Article

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Figure 1. A. B plot of the X chromosome of a Sjögren's syndrome patient. The distal aspect of the p arm demonstrates a 4-band pattern consistent with three copies of this portion of the X chromosome while the centromeric half of Xp and Xq demonstrate a 3-band pattern consistent with two copies of these portions of the X chromosome. B. A karyotype of the X chromosome of all three cells types found in the Sjögren's patient. One normal X chromosome was found in 10% of cells (B, left), two normal X chromosomes were found in 65% of cells (B, middle), and one normal X chromosome along with an X chromosome with a non-reciprocal translocation of distal Xp was found in 25% of cells (B, right). The arrow shows the junction of Xq with the translocated distal Xp. Cytogenetic designation is given for this patient's X chromosome complement. C. Diagram of the X chromosome abnormalities found. A normal X is shown on the far left, the pSS-affected mother

Table 1. Patients with an X chromosome triple mosaic were identified in SLE and Sjögren's syndrome cohorts. The estimated birth rate for the triple mosaic (45,X/46,XX/47,XXX) is 1 in 25,000 to 50,000 live female births.

	Total	X triple mosaic	Binomial 95% CI
SLE	2426	3	1 in 278 to 1 in 3333*
Sjögren's	2138	1	1 in 385 to 1 in 100,000
Combined	4564	4	1 in 1111 to 1 in 5000*
Controls	2712	0	NA

* Neither 95% CI cross the known birth rate of 45,X/46,XX/47,XXX; and, thus, demonstrate a statistically significant result. NA- not applicable

References

1. Borchers AT, Naguwa SM, Shoenfeld Y and Gershwin ME. The geoepidemiology of systemic lupus erythematosus. *Autoimmunity Reviews*. 2010; 9: A277-87.
2. Rees F, Doherty M, Grainge M, Davenport G, Lanyon P and Zhang W. The incidence and prevalence of systemic lupus erythematosus in the UK, 1999-2012. *Ann Rheum Dis*. 2016; 75: 136-41.
3. Qin B, Wang J, Yang Z, et al. Epidemiology of primary Sjogren's syndrome: a systematic review and meta-analysis. *Ann Rheum Dis*. 2015; 74: 1983-9.
4. Bojesen A, Juul S and Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab*. 2003; 88: 622-6.
5. Nielsen J and Wohler M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet*. 1991; 87: 81-3.
6. Otter M, Schrander-Stumpel C and Curfs LMG. Triple X syndrome: a review of the literature. *Eur J Hum Genet*. 2010; 18: 265-71.
7. Cooney CM, Bruner GR, Aberle T, et al. 46,X,del(X)(q13) Turner's syndrome women with systemic lupus erythematosus in a pedigree multiplex for SLE. *Genes Immun*. 2009; 10: 478-81.
8. Scofield RH, Bruner GR, Namjou B, et al. Klinefelter's syndrome (47,XXY) in male systemic lupus erythematosus patients: support for the notion of a gene-dose effect from the X chromosome. *Arthritis Rheum*. 2008; 58: 2511-7.
9. Harris VM, Sharma R, Cavett J, et al. Klinefelter's syndrome (47,XXY) is in excess among men with Sjogren's syndrome. *Clin Immunol*. 2016.
10. Liu K, Kurien BT, Zimmerman SL, et al. X Chromosome Dose and Sex Bias in Autoimmune Diseases: Increased Prevalence of 47,XXX in Systemic Lupus Erythematosus and Sjogren's Syndrome. *Arthritis & rheumatology (Hoboken, NJ)*. 2016; 68: 1290-300.
11. Wang J, Syrett CM, Kramer MC, Basu A, Atchison ML and Anguera MC. Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X. *Proc Natl Acad Sci U S A*. 2016.
12. Forsdyke DR. X chromosome reactivation perturbs intracellular self/not-self discrimination. *Immunol Cell Biol*. 2009; 87: 525-8.
13. Migeon BR. *Females are mosaics: X inactivation and sex differences in disease*. Oxford: Oxford University Press, 2014.
14. Dillon SP, Kurien BT, Li S, et al. Sex chromosome aneuploidies among men with systemic lupus erythematosus. *J Autoimmun*. 2012; 38: J129-34.
15. Rasmussen A, Sevier S, Kelly JA, et al. The lupus family registry and repository. *Rheumatology (Oxford)*. 2011; 50: 47-59.
16. Rasmussen A, Ice JA, Li H, et al. Comparison of the American-European Consensus Group Sjogren's syndrome classification criteria to newly proposed American College of Rheumatology criteria in a large, carefully characterised sicca cohort. *Ann Rheum Dis*. 2013; 73: 31-8.
17. Lessard CJ, Li H, Adrianto I, et al. Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nat Genet*. 2013; 45: 1284-92.
18. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982; 25: 1271-7.
19. Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis*. 2002; 61: 554-8.
20. Hamerton JL, Canning N, Ray M and Smith S. A cytogenetic survey of 14,069 newborn infants. I. Incidence of chromosome abnormalities. *Clin Genet*. 1975; 8: 223-43.

21. Grant WW and Hamerton JL. A cytogenetic survey of 14,069 new born infants. II. Preliminary clinical findings on children with sex chromosome anomalies. *Clin Genet.* 1976; 10: 285-302.
22. Peeva E, Venkatesh J, Michael D and Diamond B. Prolactin as a modulator of B cell function: implications for SLE. *Biomed Pharmacother.* 58: 310-9.
23. Hughes GC and Choubey D. Modulation of autoimmune rheumatic diseases by oestrogen and progesterone. *Nature Reviews Rheumatology.* 2014; 10: 740-51.
24. Dragin N, Bismuth J, Cizeron-Clairac G, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J Clin Invest.* 2016; 126: 1525-37.
25. Mok CC and Lau CS. Profile of sex hormones in male patients with systemic lupus erythematosus. *Lupus.* 2000; 9: 252-7.
26. Chang DM, Chang CC, Kuo SY, Chu SJ and Chang ML. Hormonal profiles and immunological studies of male lupus in Taiwan. *Clin Rheumatol.* 1999; 18: 158-62.
27. Mackworth-Young CG, Parke AL, Morley KD, Fotherby K and Hughes GR. Sex hormones in male patients with systemic lupus erythematosus: a comparison with other disease groups. *Eur J Rheumatol Inflamm.* 1983; 6: 228-32.
28. Jimenez-Balderas FJ, Tapia-Serrano R, Fonseca ME, et al. High frequency of association of rheumatic/autoimmune diseases and untreated male hypogonadism with severe testicular dysfunction. *Arthritis Research.* 2001; 3: 362-7.
29. Invernizzi P. The X chromosome in female-predominant autoimmune diseases. *Ann N Y Acad Sci.* 2007; 1110: 57-64.
30. Invernizzi P, Miozzo M, Oertelt-Prigione S, et al. X monosomy in female systemic lupus erythematosus. *Ann N Y Acad Sci.* 2007; 1110: 84-91.
31. Invernizzi P, Miozzo M, Selmi C, et al. X chromosome monosomy: a common mechanism for autoimmune diseases. *J Immunol.* 2005; 175: 575-8.
32. Invernizzi P, Miozzo M, Battezzati PM, et al. Frequency of monosomy X in women with primary biliary cirrhosis. *Lancet.* 2004; 363: 533-5.
33. Lu Q, Wu A, Tesmer L, Ray D, Yousif N and Richardson B. Demethylation of CD40LG on the inactive X in T cells from women with lupus. *J Immunol.* 2007; 179: 6352-8.
34. Zhao M, Liu S, Luo S, et al. DNA methylation and mRNA and microRNA expression of SLE CD4+ T cells correlate with disease phenotype. *J Autoimmun.* 2014; 54: 127-36.
35. Hewagama A, Gorelik G, Patel D, et al. Overexpression of X-linked genes in T cells from women with lupus. *J Autoimmun.* 2013; 41: 60-71.
36. Richardson B, Sawalha AH, Ray D and Yung R. Murine models of lupus induced by hypomethylated T cells (DNA hypomethylation and lupus...). *Methods Mol Biol.* 2012; 900: 169-80.
37. Sawalha AH, Wang L, Nadig A, et al. Sex-specific differences in the relationship between genetic susceptibility, T cell DNA demethylation and lupus flare severity. *J Autoimmun.* 2012; 38: J216-22.
38. Goad WB, Robinson A and Puck TT. Incidence of aneuploidy in a human population. *Am J Hum Genet.* 1976; 28: 62-8.
39. Rigola MA, Carrera M, Ribas I, et al. Identification of two de novo partial trisomies by comparative genomic hybridization. *Clin Genet.* 2001; 59: 106-10.
40. Verellen-Dumoulin C, Freund M, De Meyer R, et al. Expression of an X-linked muscular dystrophy in a female due to translocation involving Xp21 and non-random inactivation of the normal X chromosome. *Hum Genet.* 1984; 67: 115-9.

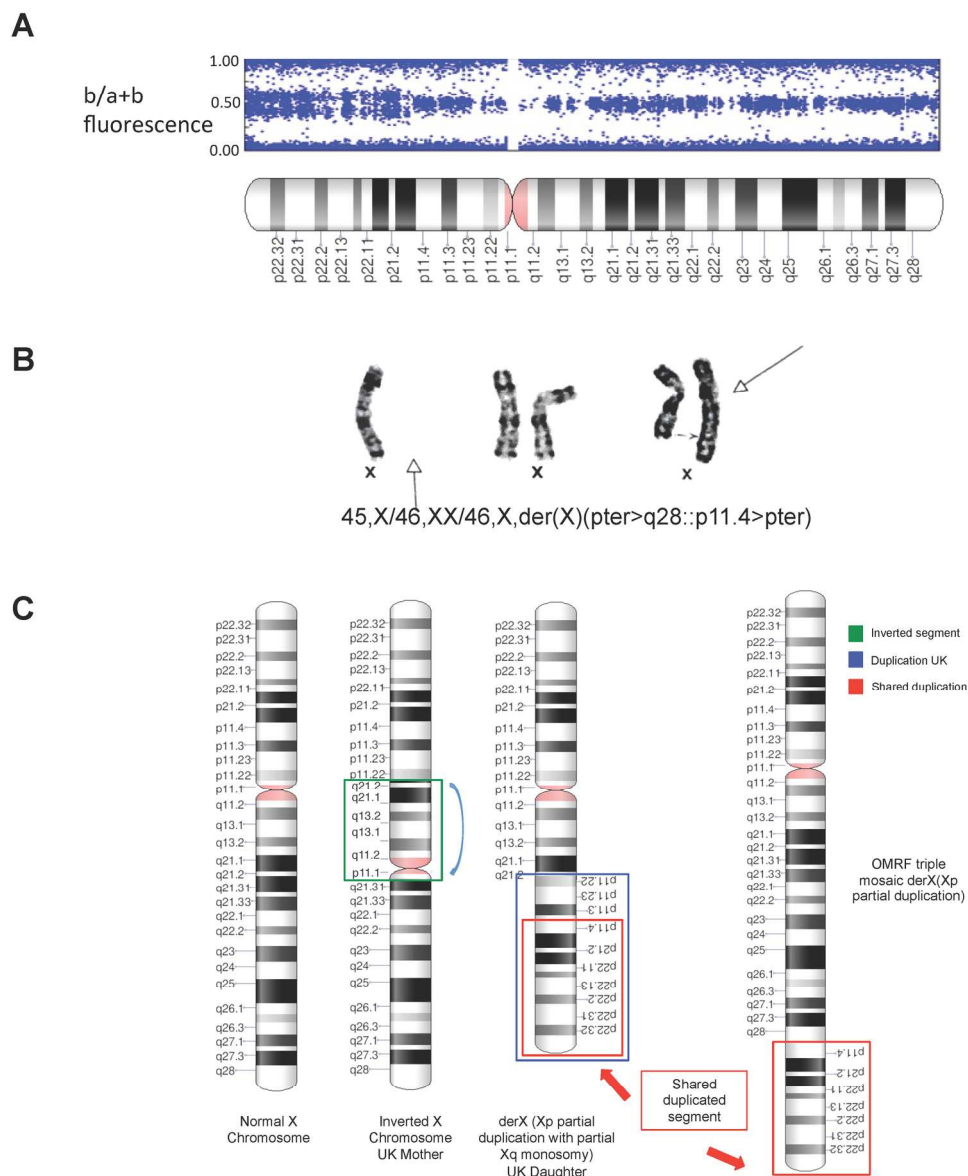


Figure 1. A. B plot of the X chromosome of a Sjögren's syndrome patient. The distal aspect of the p arm demonstrates a 4-band pattern consistent with three copies of this portion of the X chromosome while the centromeric half of Xp and Xq demonstrate a 3-band pattern consistent with two copies of these portions of the X chromosome. B. A karyotype of the X chromosome of all three cells types found in the Sjögren's patient. One normal X chromosome was found in 10% of cells (B, left), two normal X chromosomes were found in 65% of cells (B, middle), and one normal X chromosome along with an X chromosome with a non-reciprocal translocation of distal Xp was found in 25% of cells (B, right). The arrow shows the junction of Xp with the translocated distal Xp. Cytogenetic designation is given for this patient's X chromosome complement. C. Diagram of the X chromosome abnormalities found. A normal X is shown on the far left, the pSS-affected mother

181x215mm (300 x 300 DPI)