Published in final edited form as:

Acta Paediatr. 2011 June ; 100(6): 819–823. doi:10.1111/j.1651-2227.2011.02185.x.

Klinefelter's syndrome (47,XXY) among men with systemic lupus erythematosus

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Abstract

Aim: To determine the rate of Klinefelter's syndrome among men with systemic lupus erythematosus, and to determine whether the manifestations of SLE in these men are different from that seen in 46,XY men.

Methods: A total of 276 men with SLE underwent a real-time PCR procedure to screen for more than one X chromosome. Those with results consistent with two X chromosomes were further characterized by karyotype and FISH. Clinical manifestations of SLE were determined by interview, questionnaire and medical chart review. Each man with Klinefelter's and SLE was matched to four 46,XY men with SLE. Rates of SLE manifestations were compared with chi square analyses.

Results: We found 7 of the 286 men with SLE had Klinefelter's syndrome. Four of these seven were non-mosaic 47,XXY, while two were mosaic 46,XY/47,XXY and one was 46,XX/47,XXY. The men with 47,XXY did not have severe manifestations of SLE including no proliferative renal disease, neurological disease, thrombocytopenia, autoimmune hemolytic anemia, discoid skin disease, or anti-RNP/Sm.

Conclusion: 47,XXY is found in excess among men with SLE. Men commonly have SLE that is more severe than that found among women, but the 47,XXY men had less severe SLE than other men.

Keywords

systemic lupus erythematosus; Klinefelter's syndrome; X chromosome; severity of disease

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a myriad of clinical and serological manifestations. SLE is the result of a complex interplay of genetic risk and environmental exposure (1). The disease is not uncommon, being found in about 1 in a 1000 women of European background and up to 1 in 200 African-American women (2). However, SLE is about 10 times more common in women than men. Despite the decreased risk, SLE is generally more severe in men compared to women with an increased incidence of renal disease, serositis, neurological disease and severe hematological manifestations such as autoimmune hemolytic anemia and thrombocytopenia as well more anti-RNP and anti-Sm (reviewed in 3).

Klinefelter's syndrome is found in about 1 in 500 live male births, and is characterized genetically by a 47,XXY complement of chromosomes. About two-thirds of Klinefelter men have two different X chromosomes, while the other third has two identical X chromosomes as the result of a second meiosis non-disjunction. Clinically, 47,XXY men have poor secondary sexual characteristic development as well as infertility (4). The condition is commonly diagnosed in the teenage years when gynecomastia is present, or during a work-up for infertility. However, given the wide range of human sexuality, only about 20-25% of men with 47,XXY are diagnosed (5).

We have proposed that the dimorphic risk of SLE between men and women is not related directly to sex, but instead is associated with the presence of either one or two X chromosomes. Based on heterozygosity of X chromosome genetic markers, we previously reported that about 1 of 40 men with SLE had 47,XXY (6). In addition, Turner's syndrome (female 45,XO) was not present among almost 2000 SLE women (6,7). Based in the incidence of SLE among men, Klinefelter's syndrome in the population, and Klinefelter's in SLE, we calculated that the risk of SLE among men with Klinefelter's syndrome is equal to the risk among women (6).

Our previous effort relied upon heterozygous X chromosomes, and therefore may have underestimated the true rate of Klinefelter's syndrome among men with SLE since those 47,XXY men with two identical X chromosomes would not have been identified. We undertook the present study to determine the presence of Klinefelter's syndrome in SLE men using methods designed to identify all men with 47,XXY. In addition, we investigated whether 47,XXY men have clinical SLE manifestations more similar to 46,XX women or 46,XY men.

Methods

Patients.

All SLE patients were from the Lupus Family Registry and Repository (LFRR), which is housed at Oklahoma Medical Research Foundation. Methods involved in developing the LFRR have been described previously (8,9). Briefly, all patients met the 1982 American College of Rheumatology classification criteria for systemic lupus erythematosus (10), as revised (11). Putative SLE patients completed an extensive questionnaire, were interviewed by study personnel and had medical records reviewed in order to determine classification criteria. Clinical manifestations of SLE were recorded as present or absent. Antibodies against extractable nuclear antigens were determined by immunodiffusion, and antibodies binding double stranded DNA (dsDNA) were determined by crithidia assay, in the Oklahoma Medical Research Foundation Clinical Immunology Laboratory as previously described (9).

Sex Chromosomes.

All men with SLE were screened for the number of X chromosomes by a real-time polymerase chain reaction (rt-PCR) technique designed to count the number of X chromosomes, following the method of Ottesen, et al (12). In brief, each sample had reference genes on chromosome 12 (GAPDH), Y (SRY), and X (androgen receptor) amplified by RT-PCR. Due to the exponential nature of PCR, the number of X chromosomes in each sample could be calculated from the difference in number of cycles needed for each reference gene product to reach a threshold amount using a ratio of the concentration of these genes. With two X chromosomes, the ratio of the androgen receptor to GAPDH concentration is approximately one, while with one X chromosome the ratio is approximately 0.5. In order to confirm these results, all men underwent karyotype carried out on peripheral blood cells using standard techniques. All samples were evaluated in duplicate. After the RT-PCR, male samples with results indicating two X chromosomes were confirmed by fluorescent in situ hybridization (FISH) using methods designed for high throughput and small sample sizes (13,14). Cryopreserved Epstein Barr virus transformed peripheral blood mononuclear cells were used. Nuclei were swollen by the addition of 20µl of water to 5µl of cell suspension in a PCR tube and incubating at 37°C for 20 min in a thermocycler. 25µl of fixative (56% acetic acid, 44% ethanol) was added to the PCR tube and gently mixed with a pipette. All 50µl of suspension was placed onto a saline coated slide and allowed to air dry for 45 min at room temperature. DNA was dehydrated via ethanol series and then dried on a warm surface (~60°C) for 5 min.

Commercial FISH probes for the centromeres of chromosomes X and Y were used to confirm Klinefelter's syndrome (alpha-satellite repeats DXZ1 & DYZ3, PID# KI-20030, Veridex, LLC, Raritan, NJ, USA). Probes were diluted in hybridization buffer (50% formamide, 2 x SSC, 10% dextran sulfate (w/v), 50mM sodium phosphate pH 7) and 9µl was used under an 18x18mm coverslip sealed with rubber cement. Probes and DNA were co-denatured on a hot plate at 75-80°C for 5 min. Hybridization was performed at 37°C for 2 hours. Slides were washed three times for 5 min each in 50% formamide 2 x SSC at 37°C and then three times in 0.4 x SSC at ~70-72°C. DNA was counterstained with a drop of

DAPI (200ng/ml in 2 x SSC) for 5 min and then briefly rinsed with water. After drying, slides were mounted in antifade solution (2.33% DABCO, 20mM Tris pH 8, 90% glycerol (v/v)) and viewed under a Zeiss Axiovert fluorescent microscope using the appropriate filters. Fifty nuclei were scored for each subject.

Karyotype was carried out using standard techniques in the Cytogenetics Laboratory of the University of Oklahoma Health Sciences Center.

Statistics.

Each man with SLE and Klinefelter's syndrome was matched to four men with SLE and a normal set of sex chromosomes. Matching was for race, duration of SLE and age at entry into the study. Manifestations of SLE found in the Klinefelter men and the matched controls, that is, 46,XY men, were compared using a Chi square analysis with Yates correction. Adjusted Wald 95% confidence intervals (CIs) were calculated for the rate of Klinefelter's syndrome among the SLE men.

Results

A total of 286 men entered the LFRR and were confirmed to meet at least four of eleven American College of Rheumatology classification criteria for systemic lupus erythematosus. This is approximately 10% of all patients who have entered the LFRR. Seven of the 286 had a ratio of the X chromosome and autosome PCR products of 1, suggesting the presence of two X chromosomes. In order to confirm these results, we performed karyotype and/or FISH on these seven individuals. The results showed that all seven had 47,XXY. Thus, 7 of 286 men with SLE had Klinefelter's syndrome (95% CIs 0.0099-0.0498, that is 990 to 4980 per 100,000). As mentioned above, the expected rate of Klinefelter's syndrome in the population is 17 per 100,000 or about 1 in 500 (4,15).

Of the seven SLE men with Klinefelter's syndrome, four had all cells studied by karyotype or FISH carry a 47,XXY chromosomal complement. Two of seven men were mosaic with both 47,XXY and 46,XY cells. Such mosaics are present in about 10% of men with Klinefelter's syndrome (16). One man, however, had 47,XXY/46,XX, a much more unusual mosaicism (17,18). This finding was confirmed by both FISH and karyotype.

Men with SLE have more severe SLE than women (reviewed in 3). We hypothesized that Klinefelter men with SLE will have less severe SLE than 46,XY men with SLE. In order to test that hypothesis, we evaluated Klinefelter men and 46,XY men for the SLE manifestations reported to be more common in men compared to women. None of the seven 47,XXY SLE men had discoid lupus, serositis, neurological disease, autoimmune hemolytic anemia, or thrombocytopenia. In addition, there was no anti-RNP, anti-Sm or anti-dsDNA. All these manifestations of SLE are reported to be more common among men than women (3). The matched control SLE men with 46,XY had each of these clinical and serological manifestations at rates expected for men with SLE (Table 1). When all these manifestations were considered together, there was a statistical difference between Klinefelter men and 46,XY men ($\chi^2=5.7$, $p<0.003$). That is, there was a statistical increase in non-Klinefelter's men having at least one of these manifestations of SLE, compared to Klinefelter's men.

Of interest was renal disease, the most common severe manifestation of SLE. Of course, renal disease is highly variable among SLE patients. We found three of the seven Klinefelter men had renal disease but that each of these men had membranous glomerulonephritis. Thus, there was no crescentic or proliferative glomerulonephritis, which usually threatens kidney function and can lead to dialysis or renal transplant in SLE patients. Meanwhile, membranous glomerulonephritis is generally less severe and does not severely impair kidney function (19). So, there was no immune complex mediated, proliferative glomerulonephritis in the Klinefelter men with SLE, while proliferative renal disease was common among 46,XY SLE men.

Discussion

Typical of most autoimmune diseases, women are at higher risk of SLE than men. In SLE, this risk is present and largest among women of childbearing age, but the increased risk of SLE among females also is present among pre-pubertal girls and boys as well as at a post-menopausal age (20). While there are numerous abnormalities in sex hormones among SLE patients, both male and female, there are no differences when patients are studied at the onset of SLE (21).

Our data that men with 47,XXY are over-represented 15-fold in SLE support the notion that the sexually dimorphic risk of SLE is based on the number of X chromosomes, but these results do not preclude a role for sex hormones in the sex bias seen in SLE. However, our data that X chromosome dosage is related to risk of autoimmune disease are also supported by animal data in which the risk of experimental autoimmune disease is associated with X chromosome number in sex reversed (i.e., XX male and XY female) animals (22). We find SLE-affected men with 47,XXY have less severe SLE than 46,XY men, but these findings are based on a small number of patients who were referred for inclusion in the LFRR. Thus, these findings may not apply to a more general population with SLE.

Men with Klinefelter's syndrome are not known to have other autoimmune diseases at a rate higher than that expected for men, but there are only a few other studies (23). Women with Turner's syndrome have a high incidence of autoimmune thyroid disease, but this risk may be associated with a particular sex chromosome abnormality (24). Our previous data suggest the women with Turner's syndrome do not have SLE as commonly as 46,XX women (6,7). However, these data are certainly not definitive.

SLE is a disease that is widely variable, but typically men have worse disease than women. Serious manifestations such as hemolytic anemia, serositis, thrombocytopenia, renal disease, neurological disease, and discoid rash are all more common among men with SLE (3). We find that 47,XXY men with SLE have disease that is less severe than 46,XY men with SLE. In several 47,XXY men with SLE treatment with androgen replacement therapy has resulted in improvement (6,25). One possibility is the lower androgen and higher estrogen levels found in Klinefelter men results in SLE that is more similar to the disease in women.

In summary, men with SLE are much more likely to have Klinefelter's syndrome than the general male population. Despite the apparent increased risk of SLE among men with Klinefelter's syndrome, the SLE among these men is not as severe as among 46,XY men.

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Table 1.

Manifestations of SLE among 46,XY men and 47,XXY men (Klinefelter's syndrome).

Manifestation	46,XY (n=279)	47,XXY (n=7)
discoid rash	40	0
serositis	113	0
neurological	25	0
AIHA	25	0
thrombocytopenia	62	0
anti-RNP	33	0
anti-Sm	6	0

AIHA = autoimmune hemolytic anemia