The X in sex: how autoimmune diseases revolve around sex chromosomes

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Recent estimates suggest that autoimmune diseases cumulatively affect 5–10% of the general population worldwide. Although the etiology and pathogenesis remain poorly understood in most cases, similarities between diseases outnumber differences in the initiation and perpetuation of the autoimmune injury. One major example is the predominance of affected women, and perhaps its most intriguing putative mechanism is related to sex chromosomes, based on the recent observation that women with autoimmune diseases manifest a higher rate of circulating leukocytes with a single X chromosome. In a complementary fashion, there have been several reports on a role of X chromosome gene dosage through inactivation or duplication in autoimmunity. It is important not to overlook men with autoimmune diseases, who might manifest a more frequent loss of the Y chromosome in circulating leukocytes. Taken together, sex chromosome changes might constitute the common trait of autoimmunity.

Key words: DNA methylation; female predominance; X chromosome inactivation; X monosomy.

AUTOIMMUNITY AT A GLANCE

Autoimmune diseases are believed to affect 5–10% of the general population and are a significant cause of morbidity and mortality worldwide. An estimate from the US National Institutes of Health suggests over 10 million cases of autoimmune diseases in the US, which results in direct economic costs almost twice those of cancer. There are currently tens of conditions in which some autoimmune features (most commonly serum autoantibodies) are encountered, although only for very few is an
autoimmune pathogenesis established. Specific age at onset, target tissue, and patterns of epidemiology characterize each autoimmune disease, yet several features are common throughout the majority of the autoimmunity spectrum. Among these, the recognized role for a permissive genetic background in determining individual susceptibility and the presence of a significant sex imbalance are common to most conditions, as illustrated in Table 1, in which sex ratios and genetically identical twin concordance rates are reported.

**SHOULD WE SAY ‘WHY WOMEN?’**

The fact that approximately 80% of patients with autoimmune diseases are women has been known for a long time, and the causes and mechanisms for this sex imbalance are based on several hypotheses proposed over the past years. These include sex hormones and reproductive history, environmental factors, fetal microchimerism, a skewing in X-chromosome inactivation patterns, and major defects in the sex chromosomes.

<table>
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<tr>
<th>Table 1. Female to male ratio and concordance rates in monozygotic twins for autoimmune diseases subdivided according to their sex predominance.</th>
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<tbody>
<tr>
<td><strong>Sex ratio</strong></td>
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<tr>
<td><strong>Female predominance</strong></td>
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<tr>
<td>Addison’s disease</td>
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<td>Antiphospholipid antibody syndrome</td>
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<td>Autoimmune chronic hepatitis</td>
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<td>Giant cell arteritis</td>
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<td>Graves’ disease</td>
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<td>Hashimoto’s disease</td>
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<td>Idiopathic thrombocytopenic purpura</td>
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<td>Multiple sclerosis</td>
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<td>Myasthenia gravis</td>
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<td>Myositis</td>
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<td>Pernicious anemia</td>
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<td>Primary biliary cirrhosis</td>
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<td>Rheumatoid arthritis</td>
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<td>Sjogren’s syndrome</td>
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<td>Systemic lupus erythematosus</td>
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<td>Systemic sclerosis</td>
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<td><strong>Sex equivalence</strong></td>
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<tr>
<td>Acute disseminated encephalomyelitis</td>
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<td>Bullous pemphigoid</td>
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<td>Celiac disease</td>
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<tr>
<td>Pemphigus vulgaris</td>
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<tr>
<td>Primary systemic vasculitis</td>
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<tr>
<td>Rheumatic fever</td>
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<tr>
<td>Type 1 diabetes mellitus</td>
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<td><strong>Male predominance</strong></td>
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<tr>
<td>Ankylosing spondylitis</td>
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<td>Guillain–Barré syndrome</td>
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</table>

Only diseases for which an autoimmune pathogenesis is widely accepted are represented.
Estrogens can influence lymphocyte maturation, activation, and synthesis of antibodies and cytokines\textsuperscript{7–10}; estrogen receptor ligands modulate antigen presentation.\textsuperscript{11} These observations were used as a rationale for the comparison of sex-hormone profiles, parity, or the use of hormonal treatments between women with autoimmune disease and controls, but reported data were negative or inconclusive. The onset at different ages (and different times in the reproductive history) and the lack of consistent effects of estrogen replacement therapies on disease course have further weakened the sex hormone theory.

The fact that environmental factors are more commonly associated with women has also been suggested to play a role in autoimmunity initiation. Both a permissive genetic background and an environmental trigger have been suggested as being necessary (but neither on its own being sufficient) to induce autoimmune diseases\textsuperscript{12}, possibly through additional mechanisms such as apoptosis.\textsuperscript{13} Factors such as specific xenobiotics (as in cosmetics) or bacteria (possibly related to female-predominant urinary tract infections) are ideal candidates in the environmental model but solid evidence of a causative role is awaited. In the paradigmatic case of primary biliary cirrhosis, an organ-specific autoimmune disease, both these factors have been suggested, among others, by epidemiological or experimental data\textsuperscript{14–16}, but no mechanistic relation can be assumed at the present status of knowledge, despite the most recent xenobiotic-induced murine models.\textsuperscript{17}

The role of sex chromosomes in autoimmune diseases has been widely studied in the past decade, first with the suggested model of fetal microchimerism, subsequently with X-inactivation patterns, and finally with X-chromosome monosomy and duplication. Fetal microchimerism was first suggested based on the observation that most autoimmune diseases have their peak of incidence following menopause. Indeed, maternal and fetal cells are exchanged during pregnancy, leading to fetal cell persistence (i.e. microchimerism) in the mother. Chimeric fetal cells are often hematopoietic and can differentiate into somatic cells in multiple organs, potentially acting as targets for autoimmunity and resembling graft-versus-host disease after stem-cell transplantation. Microchimeric cells were first detected in peripheral blood mononuclear cells from patients with systemic sclerosis\textsuperscript{18}, but other authors have failed to reproduce these findings.\textsuperscript{19} No significant difference was found in the frequency of male microchimerism between women with primary biliary cirrhosis and controls.\textsuperscript{20} Cumulatively, available data on the role of fetal microchimerism in autoimmunity are weak or inconclusive, as naturally acquired fetal and maternal microchimerism is not uncommon in healthy women.\textsuperscript{21}

The available data and proposed theories on sex chromosome biology and their putative role in autoimmune diseases will be discussed herein, along with the potential implications and limitations of such observations.

THE OLD AND NEW BIOLOGY OF SEX CHROMOSOMES

Female cells carry both parental X chromosomes (maternal and paternal), whereas male ones carry only the maternal X. In the majority of mammals, females are functional mosaics for X-linked genes, due to the phenomenon of X chromosome inactivation. Random inactivation patterns occur in somatic cells to achieve equivalent expression levels between sexes (i.e. dosage compensation), as an evolutionary compensatory mechanism. In nonhuman organisms, such as flies and worms, dosage compensation is achieved by up- or down-regulation of X-linked
expression, respectively, while others do not manifest dosage compensation. A nonrandom X chromosome inactivation can be encountered as a stochastic event in nonpathological conditions, but skewing might also be secondary to X-linked mutations that cause the mutated chromosome to be predominantly inactivated. Until the appearance of recent data (discussed below), X inactivation patterns were analyzed by means of the androgen receptor gene. The methylation of this gene (which was found not to escape inactivation) is considered representative of the inactive X and is commonly studied by taking advantage of a methylation-sensitive cleavage site. Using this approach, it was reported that 16% of healthy women over 50 years of age manifest a skewed (i.e. >75:25 ratio) or extremely skewed (i.e. >90:10) X-chromosome inactivation pattern, yet in the majority of cases this does not lead to a clinically relevant phenotype. One possible exception to this latter observation is that an extremely skewed pattern has the potential to unmask unfavorable X-linked alleles carrying mutations, thus leading to disease onset in heterozygous subjects.

The classic view of X-chromosome inactivation was recently undermined by the report of new data on additional players such as small, noncoding RNA; antisense transcription; and chromosome-wide chromatin changes. Yet DNA methylation remains the most studied phenomenon of gene silencing and X-chromosome inactivation. In particular, a recent study demonstrated that the inactive chromosome manifests the lower degree of DNA methylation, somehow in conflict with the previous data on X inactivation and reversing the established relationship between overall methylation and expression potential as such enhanced methylation is concentrated in the gene body, rather than the promoter. More importantly, it is now established that these mechanisms do not cause an entire X chromosome to be silenced, as 10–15% of X-linked genes escape silencing and are expressed from both X chromosomes in a variable proportion of healthy women. Taken altogether, these findings have radically changed our understanding of the biology of X chromosomes and allow specific genes to achieve double or a null expression in physiological conditions. The existence for some of these genes of a Y chromosome homologues makes the hypothesis ideal to explain the occurrence of autoimmune diseases also in men, as discussed below.

SEX CHROMOSOMES AND (AUTO)IMMUNITY

Clinical and experimental evidence has strengthened the link between sex chromosomes and immunity. From a clinical standpoint, we have now established that the immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and the X-linked recessive severe combined immunodeficiency (XSCID) syndromes are caused by mutations of the transcription factor fork head box P3 (FoxP3) on Xp11.23,28 and by interleukin 2 receptor gamma chain on Xq13.129, respectively. Similarly, the hyper-IgM syndrome is caused by defects of CD40 ligand on Xq26.30 This latter condition also provides a link between the X chromosome and autoimmunity, because it is often accompanied by autoimmune features, e.g., in Wiskott–Aldrich syndrome, an inherited X-linked, recessive disease due to mutations in the WAS gene (Xp11.4-p11.21), sharing immune dysregulation and autoimmune manifestations. Furthermore, it is of particular interest that conditions characterized by major abnormalities of the X chromosome, such as Turner syndrome or premature ovarian failure are characterized by autoimmune comorbidities in a significant proportion of cases, and might thus provide some helpful hints in female-predominant autoimmunity such as autoimmune cholangitis.


**X-chromosome silencing in autoimmunity**

Based on the illustrations presented thus far, can we hypothesize that a skewed X-chromosome inactivation pattern causes tolerance breakdown and autoimmunity development? Can X-chromosome inactivation explain the somehow disappointing concordance rates for all autoimmune diseases in monozygotic twin sisters? As in other fields of autoimmunity, systemic lupus erythematosus (SLE) was the first condition to be tested for de novo hypotheses. Indeed, the inactivation issue was suggested several years ago but data were not conclusive in SLE\(^{35,36}\) or in other autoimmune diseases\(^{37}\), although in the latter case not all included conditions were female predominant. The same case-control study design and androgen-receptor-based molecular approach has also been utilized in multiple sclerosis but failed to demonstrate significant differences between patients and controls.\(^{38}\)

In an elegant study, the X-linked CD40 ligand gene was found to be hypomethylated in women with SLE\(^{39}\), in contrast with previous reports of overexpression in peripheral lymphocytes.\(^{40}\) Through a broader approach, we note that the pharmacological inhibition of DNA methylation leads to the development of lupus-like disease in animal models\(^{41}\), and that DNA overall methylation is reduced in peripheral leukocytes from patients with SLE\(^{42}\), although the significance of these findings in disease etiology remains uncertain.\(^{43}\)

In recent years, four female-predominant, late-onset, often co-existing autoimmune diseases characterized by different tissue specificity have been studied for X-chromosome inactivation skewed patterns. First, it was reported that a significant (30–49%) proportion of women with systemic sclerosis manifest an extremely skewed X inactivation\(^{44,45}\), yet it should be noted that the authors utilized only the androgen receptor as a marker for X inactivation, and that additional familial samples have determined that the maternal X is more frequently silenced.\(^{45}\)

Similar findings were obtained in a Danish cohort of control women and monozygotic twins with autoimmune thyroid disease.\(^{46}\) Whereas a skewed X-chromosome inactivation was significantly more common in twins than in unrelated women, we note that patterns were associated with the risk of disease in discordant twins. Similar associations were later independently recapitulated in smaller case-control studies in autoimmune thyroid disease\(^{47,48}\) and Sjögren syndrome\(^{49}\), but not in primary biliary cirrhosis.\(^{50}\) We should note that these negative data were obtained in a large series of patients and matched controls and utilized the androgen receptor and three additional markers to map the X chromosome inactivation for the entire chromosome.

**More gene dosage in autoimmunity**

Further evidence on the role of X-chromosome gene dosage in the development of autoimmunity was provided with the study of X monosomy (i.e. loss of one X chromosome) and subjects with Klinefelter syndrome (i.e. with a XXY karyotype). We recently reported (Table 2) that women with organ-specific and systemic female-predominant, late-onset autoimmune diseases, such as primary biliary cirrhosis, systemic sclerosis, and autoimmune thyroid disease, manifest higher frequencies of peripheral blood cells with X monosomy than age-matched women.\(^{51,52}\) Interestingly, this observation was not replicated in SLE, as a paradigmatic disease characterized by earlier age of onset\(^{53}\) and was not secondary to circulating male cells (i.e. fetal microchimerism).\(^{51,52}\)

Based on these intriguing observations, we first proposed\(^{54,55}\) that the enhanced X-chromosome monosomy might cause haploinsufficiency of X-linked genes escaping
silencing. We note that an age-dependent X-chromosome loss is not pathogenic per se and commonly accompanies immunosenescence, the well-known state of dysregulated immune function of the elderly responsible for the increased susceptibility to infections and, possibly, to the appearance of autoantibodies. The autoimmunity-associated haploinsufficiency is more common in cells of the adaptive rather than innate immune system and might thus enhance the transition between immunosenescence and overt autoimmunity in three possible ways. In the first model, X-encoded genes (as in the case of FoxP3 for T regulatory cells) are involved in immune-system homeostasis; their dysregulation would have a direct effect on B- and T-cell tolerance. In the second, alternative but not mutually exclusive model, immature T cells might fail to develop tolerance to self-antigens encoded by one of the two X chromosomes. In the third model, autoreactive CD4\(^+\) T cells in target tissues might stimulate B cells expressing the target X-encoded antigen, thus resulting in an autoimmune injury. We further hypothesize that the hemizygous status resulting from X monosomy unmaskps peculiar haplotypes responsible for susceptibility to autoimmunity, as supported by the data that support a preferential X chromosome loss in peripheral blood cells. Such loss is progressively acquired with age and parallels the incidence of autoimmune diseases, while taking place more frequently in high-mitotic-activity cell populations, such as B and T lymphocytes.

Additional supporting evidence has been obtained from Klinefelter’s syndrome, a condition resulting from the 47,XXY karyotype observed in 17 of 10,000 live male births. Following earlier sporadic reports of the occurrence of SLE in Klinefelter’s syndrome, there were attempts, in small case series, to prove this association by cytogenetic tests of patients with lupus and autoantibody testing in subjects with Klinefelter’s syndrome; however, neither approach provided encouraging data. When Klinefelter’s syndrome was recently tested in 213 men with SLE (representative of approximately 2300 patients) its presence was observed in a subgroup of cases with a frequency significantly higher compared to the general population. Albeit preliminary, these data are complementary to our X-monosomy observations and support the view that X-chromosome gene dosage is critical to autoimmunity development.

### THE Y CHROMOSOME IN MALE AUTOIMMUNITY

Compared with the X chromosome, the Y chromosome has received significantly less attention in experimental research. This omission was justified by the limited number

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<th>Table 2. X-monosomy rates in peripheral blood mononuclear cells from patients with female-predominant autoimmunity.</th>
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<tr>
<td><strong>X monosomy rate</strong></td>
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<tr>
<td>Primary biliary cirrhosis</td>
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<td>Autoimmune thyroid disease</td>
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<td>Systemic sclerosis</td>
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The data from systemic lupus erythematosus are not illustrated but differences did not reach statistical significance. Continuous variables are expressed as geometric mean \( \pm \) standard deviation.
of genes mapping on this chromosome. However, this assumption changed when the genetic bases of the long-known BXSB strain of mice were demonstrated; this model was first found to spontaneously develop a lupus-like autoimmune syndrome affecting male mice with a more aggressive phenotype than in females. Further studies found that such a manifestation was secondary to a genetic abnormality present in BXSB Y-chromosome known as Y-linked autoimmune acceleration (Yaa), and that the Yaa mutation is a translocation from the telomeric end of the X chromosome (containing the gene encoding the Toll-like receptor 7) onto the Y chromosome, causing the gene overexpression, although its duplication alone is not sufficient to cause Yaa-mediated acceleration of lupus-like disease. Putative mechanistic views are well represented by the higher induction of a type 1 cytokine response by Toll-like receptor 7 stimulation in female peripheral cells. These data cumulatively suggest that the translocation of more than one X-linked gene to the Y chromosome might contribute to the initiation and perpetuation of autoimmunity. In this scenario, additional candidates include the Toll-like receptor 8 gene, which contributes to the development of a drug-induced humoral autoimmune response mimicking human SLE. In the absence of translocation, the Y chromosome might play a protective role in the development of autoimmunity and explain the female predominance as well as the occurrence of autoimmune diseases in men.

**SHOULD WE THEN SAY ‘WHY NOT MEN?’**

Could observed differences be due to physicians overlooking autoimmunity in men and thus maximizing the female predominance? One cannot rule out the possibility that observer awareness plays a role in determining the probability of a possible autoimmunity diagnosis, as recently exemplified by the opposite case of male-predominant heart disease. In this case, female patients with identical symptoms are asked significantly fewer questions and are prescribed fewer diagnostic tests and less appropriate treatment. From a pathogenetic standpoint, could we possibly be missing the point and should we be looking for factors that protect men rather than predispose women? As illustrated in the previous chapters, this question remains unanswered.

**WHAT IS NEXT?**

Autoimmunity is the result of a multifactorial process in which environmental triggers, genomic background, and other factors might well coexist and all be necessary in different magnitudes, although none is sufficient per se. Based on previously illustrated data, sex chromosomes appear as ideal candidates to link all the major features of autoimmunity and should be the target of future high-density, chromosome-wide genomic association studies. Similarly, the new high-throughput tools should be utilized to map the methylation and histone code of the X chromosome in discordant siblings or twins. Indeed, the study of clinically discordant monozygotic twins might hold the key to nongenomic causes of autoimmunity and provide an ideal setting for the study of sex-chromosome biology. Other complementary approaches will include the collection of a large number of men with autoimmunity through a multicenter effort to investigate the Y chromosome. All of these future developments are necessary to define the role of sex chromosomes in autoimmune diseases and to confirm the current hypotheses.
Research agenda

- True population-based, case-finding studies should be performed to determine the exact sex ratio in autoimmunity.
- A multicenter, worldwide effort should be encouraged to collect a large number of patients (particularly with rare diseases); a sufficient number of male cases should be included in future studies.
- The data for an enhanced X-chromosome loss in three female-predominant autoimmunities should be replicated in other late-onset autoimmune diseases.
- Mapping the X-chromosome methylation and histone codes should be a priority, as it could explain the disease discordance between monozygotic twins.
- The study of the Y chromosome in male patients with autoimmunity should be initiated.

REFERENCES


