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X Chromosome Inactivation and Female Predisposition to Autoimmunity

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Introduction

The human X chromosome is the favorite chromosome of many geneticists because of its peculiar inheritance pattern and unique biology. The peculiar inheritance pattern stems from its hemizygosity in males and gives rise to a disproportionately high number of Mendelian diseases associated with a human chromosome. As high as 70% of the genes with a known function on the X are associated with disease phenotypes. The unique biology, termed X-inactivation, describes the transcriptional silencing of one of its copies in females. A substantial deal of sex differences in health issues has its origins in the genes on the X chromosomes and the X-inactivation process [1]. For example, a single copy of X chromosome in males leads to their vulnerability to X-linked diseases, such as X-linked immunological diseases (Bruton agammaglobulinemia and Wiskott–Aldrich syndrome), Duchenne muscular dystrophy, or a variety of X-linked mental retardation syndromes, such as fragile X syndrome.

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X Chromosome and Autoimmune Diseases

The X chromosome is increasingly being more implicated in the function of the immune system and the development of autoimmune diseases. For example, forkhead box 3 on Xp11.23 is a member of the forkhead–winged-helix family of transcriptional regulators. It has a central role in T-cell biology, and its germ-line mutations are associated with X-linked autoimmunity–allergic dysregulation syndrome also known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome [2, 3]. X chromosome is home to 32 genes, which have a melanoma antigen (MAGE) domain. Only four other *MAGE* genes have been reported in the rest of the genome [4]. The *MAGE* gene products are members of the cancer–testis antigen group, and they are potential targets for tumor immunotherapy [5]. Structural and numerical abnormalities of the X chromosome have been implicated in autoimmunity. For example, X autosome translocations and deletions of the X are associated with premature ovarian failure [6]. Patients with monosomy X (Turner's syndrome) often manifest autoimmune features [7].

Female Preponderance in Autoimmune Diseases

It has been recognized more than a century ago that women are more affected by autoimmune diseases than men [8]. The most striking sex differences are observed in scleroderma, autoimmune thyroid disease (Hashimoto's thyroiditis and Graves' disease), systemic lupus erythematosus, and Sjögren syndrome, in which the patient population is greater than 80% women. Other diseases such as rheumatoid arthritis, multiple sclerosis, and myasthenia gravis are

relatively more common in women. And finally, in some diseases, such as insulin-dependent diabetes mellitus, subgroups characterized by distinct clinical manifestations may have female preponderance.

Kast–Stewart Hypothesis

Paths to understanding the genetic basis of autoimmune diseases have shown that simple Mendelian traits arising from single-gene mutations and complex traits resulting from interactions between multiple genotypes and the environment contribute to the breakdown of self-tolerance. Important environmental factors include pathogen exposure, pregnancy, and lifestyle. Despite significant advances, our present day understanding of the mechanisms of self-tolerance and its breakdown is not complete, and novel mechanisms including those other than classical patterns of inheritance may play a critical role. One such mechanism, first hypothesized by Kast [9] and further developed by Stewart [10], is disturbances in the X chromosome inactivation process. The "Kast–Stewart hypothesis" has its roots in two simple observations; first, an overwhelming majority of autoimmune diseases are exceedingly more prevalent in females and, second, X-inactivation is a fundamental biological regulation that is in effect only in women. X chromosome inactivation is an epigenetic event in early development that leads to the transcriptional silencing of one of the pair of X chromosomes [11]. Therefore, X chromosome inherited by either parent is silenced at random, and normal women are thus a mosaic of two cell populations [1]. Although the attractive Kast–Stewart hypothesis caught the attention of investigators working on autoimmune diseases, X-inactivation patterns comparable to those of control women were observed in peripheral blood from female patients with systemic lupus erythematosus, juvenile diabetes, multiple sclerosis, and juvenile rheumatoid arthritis [12].

X-Inactivation in Scleroderma

While working on a male patient with classic Rett syndrome [13] and reviewing related literature on mosaicism [14], the author of this article suspected that extremely skewed X-inactivation, especially in hematopoietic stem cells, could be involved in the pathogenesis of scleroderma (systemic sclerosis). The focus on scleroderma has its roots on reports [15, 16] which raise the possibility that localized scleroderma, including the linear and frontal or frontoparietal (en coup de sabre) forms, may follow the lines of Blaschko [17]. Localized scleroderma is an autoimmune disorder, [18–20] and interestingly Blaschko's lines define dermatome areas which are thought to derive from a single X-inactivation derivative [21–23]. To test the potential involvement of skewed X-inactivation in the pathogenesis of scleroderma, we analyzed the methylation status of a highly polymorphic CAG repeat in the androgen receptor gene and demonstrated that X-inactivation mosaicism is extremely skewed in the blood but not in the skin lesions of nearly half of a group of 55 females with scleroderma. This was the first study that established an association between X-inactivation and female predisposition to autoimmunity [24]. An extension of this study, which investigates the parental origin of the inactive X chromosome, is presented in the accompanying manuscript [25].

X-Inactivation in other Female Prevalent Autoimmune Diseases

Following the publication of the scleroderma study, several studies that analyze X-inactivation patterns in female-prevalent autoimmune diseases have been initiated by our group and others. According to published results, significantly increased proportions of patients with autoimmune thyroid diseases [26, 27], preeclampsia [28], and premature ovarian failure [29] display extremely skewed X chromosome inactivation, when compared to controls (Table 1). However, skewing in blood cells was not observed in individuals with primary biliary cirrhosis [30] and Sjögren's syndrome (unpublished observations), which may indicate that this is probably not a factor in all autoimmune diseases.

Table 1 X-inactivation patterns in patients with autoimmune diseases and controls

	Degree of skewing, No. (%) observed		
	90+	80–89	50–79
Disease			
SSc-TR (n=70) [24]	27 (49.1)	8 (14.5)	20 (36.4)
SSc-US (n=125) [25]	17 (18.1)	15 (16.0)	62 (66.0)
AITD-TR (n=110) [26]	16 (19.3)	12 (14.5)	55 (66.2)
AITD-Scand. (n=40) [27]	5 (15.6)	6 (18.8)	21 (65.6)
PEE-TR (n=67) [28]	10 (21.7)	6 (13.0)	30 (65.2)
POF-JP (n=43) [29]	5 (18.5)	9 (33.3)	13 (48.1)
Sjögren-TR (n=51)	1 (3.1)	3 (9.1)	29 (87.8)
Controls			
Children-TR (n=92)	2 (2.8)	6 (8.3)	64 (88.9)
Newborn-TR (n=91)	2 (3.8)	2 (3.8)	48 (92.3)
Newborn-US (n=590) [42]	4 (0.7)	29 (4.9)	557 (94.4)
Adult-TR (n=160) [24]	3 (2.4)	7 (5.6)	114 (91.9)
Adult-US (n=415) [42]	22 (5.3)	59 (14.2)	334 (80.5)

SSc Scleroderma; *AITD* autoimmune thyroid diseases; *PEE* pre-eclampsia; *POF* premature ovarian failure; *TR* Turkey; *US* United States; *Scand*. Scandinavia; *JP* Japan

In light of the known causes of primary biliary cirrhosis, lack of association is not an unexpected finding. Initiation of autoimmunity in this disease is most probably due to molecular mimicry [31].

The Cause of Skewed X-Inactivation

At present, we do not know the nature of the relationship between skewed patterns of X-inactivation and autoimmune diseases. However, two possibilities could be considered: skewing may arise as a result of breakdown in self-tolerance, or breakdown of self-tolerance could be the result of skewing. We believe the latter is more likely simply because the degree of skewing is at the extreme of 95:5 or 100:0 in the majority of the patients. If the skewing were to arise as a result of an autoimmune reaction in the body, then the ratios would more likely be in the milder ranges. Monosomy X has been reported as a common finding in autoimmune diseases including scleroderma and autoimmune thyroiditis [32]. Could this factor influence the interpretation of the genotyping results as skewed X-inactivation? We find this possibility highly unlikely because skewing is at the extreme range. Therefore, the attractive "haploinsufficiency hypothesis" [33] may not apply to scleroderma and autoimmune thyroiditis. It has been proposed that demethylation of the inactive X chromosome may explain the female predilection of lupus [34]. This is a very interesting proposal, and it could be extrapolated to a "diploid overexpression hypothesis," which has the potential to explain why autoimmune diseases are more prevalent in females. But we find it difficult to attribute our extremely skewed ratios to generalized methylation defects because then the demethylation process should be a widespread event on the X, affecting the expression levels of many genes including androgen receptor. However, expression profiling did not reveal a global increase of X-linked genes in scleroderma [35, 36]. Based on these considerations, we conclude that deleterious X-linked mutations or X chromosome rearrangements and their differential expression patterns could provide a disadvantage to affected blood cells and lead to skewed X-inactivation. A circumstantial evidence in support of this explanation could be the increased incidence of recurrent spontaneous abortions in the extremely skewed patients in both scleroderma [24] and autoimmune thyroiditis [26]. Recurrent spontaneous abortions are associated with skewed X-inactivation [37, 38], and an association between autoimmune diseases and recurrent spontaneous abortions has been reported [39].

A key point to consider at this point is the location of these putative X-linked mutations. We propose that they do not need to be at a certain gene or locus on the chromosome. On the contrary, any mutation that affects the viability of the cell could lead to skewed X-inactivation. In addition, X–autosomal translocations, which fall within and outside genes [40] or other X chromosome aberrations, could affect the X-inactivation ratios. Therefore, female predisposition to autoimmunity could be initiated by a variety of X chromosomal events including a single mutation in a very rich repertoire of genes. This may also explain why despite the extensive linkage genome scans, X chromosome is not clearly implicated in familial cases of autoimmune thyroid diseases, [41] or to that effect why a major locus for autoimmunity proves to be so difficult to find in the genome. It is probably time to consider "loss of mosaicism" for X-linked gene expression as the first step of the cellular events that lead to breakdown of self-tolerance in females.

Future Perspectives

Although extremely skewed X-inactivation is rare and it is associated with autoimmune diseases, it does not lead to the breakdown of self-tolerance in all women. We believe this point is of paramount importance in identifying the putative X-linked genes associated with autoimmune diseases and propose that breakdown of self-tolerance requires the co-inheritance of two distinct events on the X chromosome: first, an X-linked mutation leading to "loss of mosaicism" and second "heterozygosity for allelic variants of the putative critical genes". Developing research strategies in line with the above proposed mechanisms could prove to be very fruitful in understanding the molecular determinants of autoimmunity and identify potential targets for therapy.

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