X Chromosome Inactivation: Investigating the Disproportionate Ratio of Autoimmunity in Women

Ashley He

Received June 22, 2025
Accepted October 31, 2025
Electronic access November 30, 2022

Autoimmunity is a condition that generates autoantibodies which attack the immune system, leading to sensitivity and severe inflammation. The causes remain largely unclear. Alarmingly, many autoimmune diseases disproportionately affect women over men. X chromosome inactivation (XCI) could significantly contribute to these sex discrepancies. This review serves as a comprehensive resource that reviews the multifaceted aspects of XCI and autoimmunity in women. To date, no single study has synthesized information on *XIST* RNA function, miRNA interference, pregnancy, birth control, and thyroid function in the context of XCI to identify their collective role in severe female autoimmunity. Drawing on global research from 1996 to 2025, this article utilizes a narrative literature synthesis approach to analyze the role of XCI in autoimmunity, including current findings on the molecular basis of *XIST* RNA. This review highlights XCI, *XIST* function, and exceptions to the process, such as gene escape and skewing, in the context of female autoimmunity. Various studies on patients with thyroid autoimmune diseases are used quantitatively to further the hypothesis. The thyroid itself exhibits a complex relationship between the immune and endocrine system. Overall, XCI may significantly impact the sex ratio in autoimmunity by increasing gene dosage, impacting female hormones, contributing to epigenetics, and altering thyroid function. Continued research is necessary to support the development of specialized treatments tailored to women suffering from autoimmune diseases.

Keywords: Autoimmunity, X chromosome inactivation, *XIST*, miRNA, X chromosome inactivation escape, X chromosome skewing, thyroid autoimmune diseases, thyroid hormone

Introduction

Introduction

The immune system is a complex set of cells, tissues, and organs that work together to protect against pathogens, cancers, and other foreign substances. Women are more likely than men to inherit a stronger, more sensitive immune system, which grants the female body a powerful line of defense against the natural world. However, the immune system can lose its ability to distinguish self from nonself, leading to autoimmunity—described as "*horror autotoxicus*" by German immunologist Paul Ehrlich, directly translating to "the horror of self-toxicity" [1]. Essentially, autoimmunity refers to the body's immune cells losing the ability to distinguish between self and non-self-antigens, mistaking the body's own cells for foreign invaders and thus destroying them through autoantibody production. Autoantibodies, like the name suggests, target body cells even if they are harmless. Simply put, autoimmune disease can be interpreted as the body being allergic to itself, inducing severe immune system reactions. Autoimmunity is fairly uncommon and the exact cause of disease development is still not fully understood. The National Health and Nutrition Examination Survey (NHANES) began a program of studies in the 1960s to define the health and nutritional status of adults and children in the United States, including the prevalence of autoimmunity [2]. NHANES data have shown the prevalence of autoantibodies increasing in the past decades: 11% in 1988-1991, 11.4% in 1999-2004, and 16.1% in 2011-2012. This represents 22.3, 26.6, and 41.5 million individuals respectively [2].

An alarming pattern has been noticed among the rising cases of autoimmune disease, that being, there is a significantly greater number of women who are diagnosed with autoimmunity compared to men, with some diseases showing ratios as high as 10:1. XCI will increase the risk of autoimmunity in women as measurable through genetic analyses. This review aims to investigate how the mechanisms of XCI can contribute to the disproportionate ratio of autoimmune diseases in women compared to men, while also discussing other possible influences on the disproportionate ratio such as other epigenetic factors and nongenomic factors. This review also covers the concerning implications on autoimmunity that come with uniquely female processes—namely pregnancy and birth control—at the genomic and hormonal level.

One of the most notable distinctions between women and men is the sex chromosomes each inherits: XX for women and XY

for men. Indeed, the X chromosome boasts the most amount of immune related genes that code for different proteins, receptors, and regulators of the immune system in the entire human genome, suggesting a relationship between autoimmunity and the chromosome. Because women inherit two X-chromosomes, a phenomenon known as XCI occurs which randomly silences one of the X chromosomes in order to maintain genetic balance, leading to only one active X chromosome in each female cell. The active X chromosome can be either paternal or maternal and it differs between each individual cell. However, the process is not perfect and can incompletely silence the chromosome it attempts to, leading to some genes escaping the phenomenon, which may contribute to dysregulation in the immune system and the development of autoimmunity.

Although researchers are still investigating the many mysteries of XCI, the process likely contributes to the higher prevalence of autoimmune diseases in women by disrupting genetic balance, altering hormone signaling, influencing epigenetic mechanisms, and affecting thyroid function. While data is limited, studies examining XCI in thyroid autoimmune diseases suggest a strong correlation.

Significance and Purpose

Autoimmunity is a worldwide issue, but it is challenging to research in large part because of the complexity of the immune system, and because there is still debate among the scientific community on the very definition of what constitutes an autoimmune disease. Even the number of identified autoimmune diseases differs between sources, some claiming there are around 80 while others claim there are over 100. More importantly, patients who suffer from autoimmunity are often told there is no cure or cause to their disease, leaving many puzzled and even distressed by the idea they are stuck with an incurable illness.

With the rising number of women being diagnosed with autoimmune conditions, it is crucial to be aware of the impacts this can have on women in all communities. The financial burden and psychological distress associated with treating obscure health conditions underscore the need for continued support of autoimmune disease research. This research benefits not only women, but public health as a whole.

The sex ratio of autoimmunity is staggering—around 80% of autoimmune disease patients being female—and this review serves as a call for advancements in medical research and treatment that specifically addresses autoimmunity in women[3]. This article reviews what is currently known about XCI and autoimmune diseases, including recent developments in the field, with the goal of identifying relationships between these areas to prompt new hypotheses and deepen understanding of this pressing issue.

Scope and Limitations

Data availability is limited, as intensive research on the molecular basis of XCI and *XIST* RNA is still ongoing, moreover, its connection to women's heightened susceptibility to autoimmunity. There are a broad range of autoimmune diseases that individuals can be diagnosed with and it is not feasible to investigate each in a single review. Therefore, this review relies on studies focused specifically on thyroid autoimmune diseases to be used for quantitative data.

Other autoimmune diseases that largely target women are systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis[1]. It is also important to consider that potential biases may e*XIST* in studies conducted by researchers of different genders and experiments may differ due to different geographical or cultural environments. However, the data itself remains highly informative and valuable to this analysis.

Methodology Overview

This article reviews literature on what is currently known about XCI, its link to autoimmunity in women, and the latest research developments addressing this connection. Sources were derived from a diverse range of researchers worldwide via Google Scholar. This review uses a narrative literature synthesis approach to analyze the role of XCI in female autoimmunity.

An in-depth description of XCI and its exceptions is provided with the goal of understanding how the process works and how it may contribute to autoimmunity. In addition, quantitative data from experiments and statistical analyses are incorporated, specifically from studies on females with thyroid autoimmune diseases. Two experiments, conducted in Japan and the United Kingdom, both used similar methods to examine the relationship between X chromosome skewing and thyroid autoimmunity, and their findings are used here to further investigate XCI's role in female autoimmunity.

X Chromosome Inactivation

The sheer size of the X chromosome causes females to inherit twice the amount of genetic information males receive, resulting in the need for epigenetic interference to maintain genetic balance. Humans are sensitive to chromosomal dosage, and in most cases, aneuploid humans, those with an extra or a missing chromosome, suffer damaging malformations in their lifetime. This dosage sensitivity is one of the driving factors behind the evolutionary development of XCI in females.

XCI is an epigenetic mechanism that occurs in every female and silences one X chromosome in each female cell to balance genetic expression. XCI occurs independently in each cell during the blastocyst stage of embryogenesis and either the paternal

or maternal X chromosome is silenced[4]. Because this silencing is, for the most part, random, every female becomes a genetic mosaic, with different cells expressing either the maternal or paternal X chromosome. This phenomenon is visually displayed in calico and tortoiseshell cats. This uniquely female cellular mosaicism is due to each cell expressing exclusively her mother's or father's X-linked genes[4].

In addition, the key driver of XCI that is responsible for triggering the inactivation of more than 1,000 genes on the X chromosome is X-inactive specific transcript, or *XIST* (pronounced "e*XIST*"), a long noncoding RNA (lncRNA) molecule[5]. Molecularly, almost half of the *XIST* transcript is composed of tandem repeats designated A through F; A, B, and F are the most highly conserved in copy number across species, suggesting their function for silencing[6]. The lncRNA initiates gene silencing, large scale chromatin remodeling, and formation of the inactive X chromosome through interacting with chromatin-modifying proteins, transcriptional silencers, and other RNA-binding proteins[7]. Interestingly, *XIST* does not code for a protein itself; it remains an RNA molecule that spreads from its transcription site and coats the X chromosome while remaining in the nucleus as the chromosome soon inactivates, suggesting a causative role for *XIST* in XCI[6]. Using a fluorescence in situ hybridization analysis, researchers observed that *XIST* appeared highly particulate and was noted to be located to a larger nuclear domain corresponding to the space occupied by the inactive X chromosome throughout the entire cell cycle[8]. *XIST* also enacts cis-localization to the chromosome from which it is expressed in addition to silencing genes, and these tasks are mediated by genetically independent domains of the RNA[6]. A model for explaining the marking of one X chromosome to be the active chromosome hypothesizes that a blocking factor, named since it functions to block XCI, is responsible for making this mark. It is hypothesized that *XIST* RNA decreases the affinity of a cis-linked counting element for blocking factors, negatively influencing the active X chromosome choice[6]. As studied in mammalian cells, *XIST* has been implicated as a "choice element" of XCI, meaning a chromosome which has *XIST* will likely be chosen to be inactivated[6]. When compared to a mouse model, the *XIST* gene structure is conserved, but sequences vary[6]. Again using a mouse model, *XIST* RNA was observed to be able to trigger the formation of facultative heterochromatin by recruiting different interactive factors, such as *spen* and *rbm15*, to impact nuclear localization and 3D chromosome architecture[6]. In humans, many *XIST* RNA isoforms are produced by extensive alternative splicing occurring within the 3' half of the gene, but the significance of this heterogeneity is unknown[6]. *XIST* is not expressed in male cells because it is only activated when more than one X chromosome is present, consistent with its function in females. However, this does not apply to men with Klinefelter's syndrome, who inherit two X chromosomes and one Y. These individuals also undergo XCI[9]. Notably, males with Klinefelter's are more prone to developing autoimmunity than typical XY males: studies show that men carrying one or more extra X chromosome had an equivalent risk to women of developing lupus or Sjogren's syndrome, two diseases that disproportionately affect women[9]. Furthermore, XCI works by methylating gene promoters and deacetylating histones to form a Barr body, which is the tightly packed, inactive chromosome.

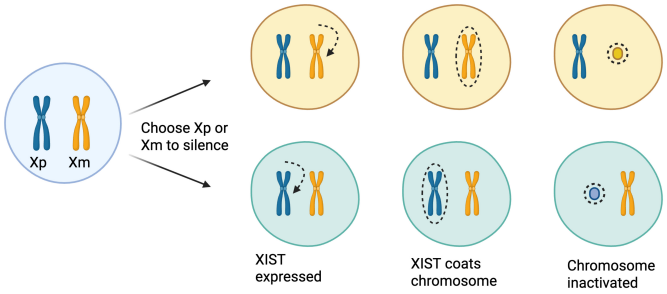

Fig. 1 The figure depicts the random silencing of one X chromosome. *XIST* is expressed in either the maternal or paternal chromosome (labeled X_m and X_p respectively), which then coats the chromosome, silencing its expression. Adapted from B.K Sun, H. Tsao. X-Chromosome Inactivation and Skin Disease. Journal of Investigative Dermatology. 128, 2753-2759 (2008).

Regardless, XCI does not fully resolve female genetic imbalance because the process is imperfect and some genes escape inactivation. In fact, in certain cells, about 15-23% of genes from the inactive X chromosome escape XCI, leading to biallelic expression of X-linked genes[9]. This results in the overexpression of specific X-linked genes in certain female tissues. XCI escape varies between genes and individuals, manifesting in detectable sex differences in gene expression and phenotypic traits. As observed in mammalian cells, some escape genes are common to multiple tissues while others are tissue specific. In 2015, researchers used a mouse model to observe genes that escaped XCI using a RT-PCR approach. The study used the Patski cell line, derived from embryonic kidney mouse cells, as well as mouse brain, spleen, and ovary tissue to examine gene expression[10]. The gene *Rlim* escaped both XCI in Patski cells and XCI in brain cells, while genes such as *Shroom4* and *Car5b* escaped XCI only in the Patski cell line, thus indicating a variation of gene expression depending on the cell/tissue type[10]. Indeed, the findings reflect the unique expression pattern of the genes. For example, some genes that escaped XCI in certain tissues would have functions related to that tissue. All 6 genes observed to escape XCI in the brain had brain-related functions such as myelination[10]. But, of the 33 genes to escape ovary XCI, only around half (15) were identified as ovary-specific, suggesting still the variation of expression in escapement[10]. The degree to which these discoveries are generalizable to human XCI remains uncertain given marked differences in XCI initiation and the

extent of escape across species.

The tissue-specific context of genes is especially important in XCI analysis. Data collected in 2017 from a systematic survey of human XCI using three complementary RNA sequencing based approaches suggested strong evidence for tissue-specific escape of the *KAL1* gene, its function causing X-linked Kallmann syndrome[11]. *KAL1* demonstrated exclusive expression in the lungs, which is in line with the strong female bias detected in lung expression[11]. In the same study, CLIC2 showed considerable inactive X expression only in skin tissue[11]. This data implies that XCI can vary from tissue to tissue, but further analyses are required to fully assess this notion. Again in the same study, an analysis of sex-biased expression found 60 genes to be biased towards females, implying there e*XIST*s a potential, genetic contributing factor to sex-specific differences in disease[11]. In a study done on fetal tissues (muscle, kidney, neural tissue), 12% of genes showed tissue-specific expression, but specifically, neural tissue exhibited a noticeably greater degree of tissue-specific escape[12]. Interestingly, this may suggest that the brain may have a more permissive XCI landscape than other tissues[12]. It should be noted though that the majority of genes in all studies exhibited rather stable XCI patterns across tissue types, but the specific genes that escaped varied. So, it can be generalized that XCI is quite consistent across human tissues, but certain genes may exhibit tissue-specific expression.

More importantly, the X chromosome is known to contain the most immune-related genes in the entire human genome, strongly suggesting the correlation between XCI and autoimmunity[9]. For example, Toll-like receptor genes, *TLR7* and *TLR8*, are closely located on the X chromosome and are crucial in the innate immune system responses[9,13]. They are responsible for pathogen recognition and are directly involved in the inflammatory responses of the immune system[13]. Studies have found that there is a significantly larger amount of *TLR7* protein in female blood compared to males, which may account for the increased sensitivity in female immune systems[9]. Subsequently, this suggests that if TLRs, or any other immune-related genes, were part of the genes on the inactive X chromosome that escapes XCI, a more severe immune response in women could definitely occur, explaining the high rate of autoimmune disease in females. This hypothesis has been supported using a single-cell RT-PCR approach, demonstrating *TLR7* escapes from XCI in plasmacytoid dendritic cells (pDC) causing biallelic expression of *TLR7*[14]. Biallelic expression of *TLR7* frequencies range from 7 to 45% in certain female cells, influencing an increase in interferon-α (IFNα) reception[14]. This strongly suggests a correlation between XCI and autoimmunity predisposition in women.

Other noteworthy genes located on the X chromosome are *CD40L* and *CXCR3*. *CD40L* initiates a signaling pathway by binding to the CD40 receptor, activating macrophages, promoting T cell responses for the adaptive immune system, and

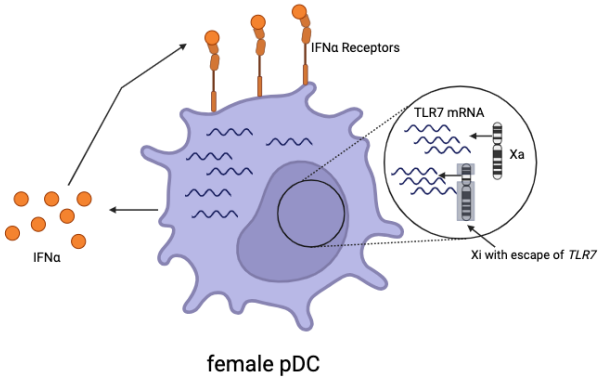

Fig. 2 Figure depicts the proposed *TLR7* escape pathway on the X chromosome. Pictured is biallelic expression of *TLR7*, increasing IFNα reception. Adapted from S. H. Hagen, F. Henseling, J. Hennesen, H. Savel, S. Delahaye, L. Richert, S. M. Ziegler, M. Altfeld. Heterogeneous escape from X chromosome inactivation results in sex differences in type I IFN responses at the single human pDC level. Cell reports. 33, (2020).

stimulating B cell activation, proliferation, and differentiation into antibody-producing plasma cells[15]. This gene is known to increase antigen presentation and pro-inflammatory responses in dendritic cells, B cells, and macrophages. CXCR3 is a G protein coupled receptor active on T cells, and regulates the migration and function of the cells. The G protein is activated by chemokines which direct the amplification of immune cells to inflammation sites, increasing protein production and pro-inflammatory cytokine secretion[15]. Interestingly, both genes have been shown to escape XCI, increasing their gene dosage in females, therefore increasing antibody production[9]. In fact, an overexpression of *CD40L* has been observed in both healthy females and individuals with SLE, suggesting an increased predisposition to autoimmunity in women[15].

Another exception to XCI is known as skewed XCI, where the random 50/50 selection of the paternal or maternal X chromosome is disturbed, causing a predominance of one parent's chromosome and genes in each cell. There are two main categories of skewed XCI; primary nonrandom XCI and secondary nonrandom XCI[4]. In primary nonrandom XCI, the bias for a particular chromosome is due to genetic modifications or polymorphisms which disturb the process of randomness itself[4]. But, the process of selective pressures that influence female cells to prefer one parent's chromosome over the other is known as secondary nonrandom XCI. One X chromosome may contain a particular gene, or genes, that prove to give an advantage, or disadvantage, to the cell during embryogenesis, and after many rounds of cell division, the ratio between the paternal and maternal X chromosome may favor the expression of one

or the other chromosome[4]. Skewed XCI has been observed to influence the appearance and severity of X-linked traits or diseases, such as Duchenne muscular dystrophy and hemophilia A, in females if a higher proportion of cells choose to inactivate the wild-type chromosome, favoring a mutant[4]. It would not be surprising if this skewing could also interfere with immune related genes linked to the X chromosome, supposing the favored, mutant chromosome contains an abnormally high amount of pro-inflammatory immune genes. It is crucial for the fetus to survive through embryogenesis, making stronger immune responses favorable during the process. In fact, this is yet another selective pressure that influences the development of autoimmunity in women. Evolution is the ongoing race to survive and pass on offspring, the latter being a feat only capable by women. To bear a child while also maintaining the life of the mother requires a more sensitive immune system, supporting the continual survival and transmittance of genes in a species. Although an overexpression of proinflammatory cytokines could increase the chances of survival for a mother and fetus, it would encourage a harsher immune response, promoting autoimmunity. For the purposes of this paper, the first definition of skewing, primary nonrandom XCI, will be used because it is unknown whether selective pressures influenced a preference for a parent's chromosome in the studies and literature used.

Common autoimmune diseases in women in relation to XCI

This study gathers quantitative data on thyroid autoimmune diseases, but it is important to mention other common autoimmune diseases that disproportionately affect women. Systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc) are three of the most common autoimmune diseases in women from adolescence to middle age, and each disease primarily targets women more than men. SLE is an autoimmune disease with multiorgan system manifestations and around 85% of patients are women[16]. The sex bias ratio of RA is 3:1 when comparing women to men[17]. In RA, two main autoantibodies initiate the disease: the rheumatoid factor and the anti-citrullinated protein antibodies[17]. These autoantibodies precede clinical manifestation of RA by many years, explaining the later onset of disease in most patients[17]. The sex bias ratio in SSc can be as high as 11:1[18]. The disease is characterised by extensive fibrosis of the skin and internal organs which often proves fatal.

Patients with RA and SSc have been found to have a significantly greater amount of XCI skewing than patients without the disease[17]. Interestingly, SLE starkly differs, exhibiting a reduction in XCI skewing as observed in patients[16]. However, the genetic mechanism behind female bias in SLE is compensated by the fact that it has been revealed that XCI gene escape in B cells of SLE patients is remarkably high[16]. Immune related genes that revealed cell type-specific biallelic expression were *BTK, NONO, AP1S2*, and more, although the significance is unknown because these genes have not been commonly reported in autoimmune diseases[16]. All four B cell types tested in a study done in 2021 showed a reduction in both *XIST* RNA and H2AK119Ub enrichment on the inactive X chromosome, providing evidence of abnormal XCI maintenance in SLE patients[16]. H2AK119Ub is essentially a repressive marker laid by the inactive X chromosome. Overall, the strong female bias exhibited by these diseases is reflective of the X chromosome's impact, especially since immune-related genes are enriched on it.

XCI's role in other potential causes of female autoimmunity

Pregnancy and Autoimmune Diseases

There are a handful of other factors that influence the development of autoimmune disorders in females that could potentially be linked to XCI, such as hormones and other forms of epigenetics. XCI may well be a factor of the expression of female-related hormones, most notably estrogens and progesterone (P4). These hormones which are more predominant in women interact directly with immune cells, significantly increasing proinflammatory cytokine production[19]. This spike of immune induced inflammation contributes to the greater instances of autoimmunity in females. Pregnancy, a unique female experience, causes hormonal shifts that influence the immune system. Hormonal and immunological changes are crucial during pregnancy to ensure a healthy birth, but these changes can prove to be detrimental to the mother. During the reproductive cycle, estrogens and P4 reach their highest levels during pregnancy. These hormones stimulate the synthesis of progesterone-induced binding factor (PIBF) by lymphocytes, which are crucial in the innate and adaptive immune response[20,21]. As PIBF increases throughout the pregnancy, they promote the differentiation of CD4+ T cells into T cell type 2, cells which secrete large amounts of anti-inflammatory cytokines[21]. This suggests pregnancy lowers the effects of inflammatory diseases, many of which are associated with autoimmunity, which sounds positive, but can cause striking effects after pregnancy. The birth of the child essentially acts as a stop signal for PIBF, which then drops dramatically postpartum, significantly lowering the rate of production of anti-inflammatory cytokines in the mother[20]. This drastic change in the immune system within such little time increases the likelihood and severity of inflammatory responses in the body after pregnancy. Moreover, genes that regulate estrogens and P4 can be found on the X chromosome[22]. It can be assumed that these genes could also be a part of the 15-23% which escape XCI, exacerbating a more intense immune response in females.

However, in 2021, it was also observed that autoimmunity was frequently found in childhood or postmenopausal women, life stages when estrogen levels are lower[9]. Estrogen's significance to autoimmunity is therefore uncertain and requires greater research. In addition, the birth control pill is a popular alternative to pregnancy, with 25% of women aged 15 to 44 claiming the pill as their contraceptive of choice[23]. But, this widely used contraceptive has its own setbacks regarding hormones, as they mainly act to prevent ovulation. The pill essentially mimics the body's naturally producing progesterone and estrogens, and these ingested hormones are able to suppress the feedback mechanisms of P4 and estrogens[23]. As stated above, the reduction in hormones is bidirectionally linked to the immune system. P4 is especially targeted as the hormone that prevents pregnancy, and so as it is reduced, the anti-inflammatory cytokines whose regulation is dependent on progesterone may also see a reduction. This could lead to an increase in inflammation from the immune system, though no studies have confirmed this hypothesis yet.

It can be generally concluded that pregnancy has varying effects on individuals, as data has reflected both increases and decreases of autoimmune conditions due to pregnancy. For example, a study using the Danish Civil Registration System identified women born between 1960 and 1992 and performed a data linkage analysis to identify whether certain pregnancy delivery types influenced subsequent maternal autoimmune disease[24]. The cohort of 1,235,639 women aged 14 years or older were split into the following groups: women who had their first child by vaginal delivery, those who performed a caesarean section (CS), women who had abortions, and a control group of women who had no previous pregnancy. Researchers found the risk of autoimmunity in women one year after vaginal delivery and CS to be significantly higher compared to the year following abortion[24]. Further, women who were pregnant had a higher incidence of autoimmune disease than those who had no pregnancy records, implicating pregnancy could very well contribute to the risk of autoimmunity[24]. However, the risk of autoimmunity decreased between the 3rd and 10th year following vaginal delivery, complicating the matter. It could be that fetal microchimerism, low levels of fetal cells persisting in the mother, can increase the pathogenesis of autoimmune disease because technically the fetal cells are foreign even though they developed within the mother, thus affecting the immune system[24].

That said, the effects of pregnancy on RA actually induce improvement or even remission of disease activity in 65% of patients[25]. As stated earlier, the differentiation of CD4+ T cells into T cell type 2 during pregnancy increases the release of anti-inflammatory cytokines which, in the case of RA, can improve symptoms. Yet, the median number of affected joints significantly increased from 8, during pregnancy, to 10 joints at 6 months postpartum, demonstrating the fleeting nature of remission[25]. It is inconclusive whether these positive effects can also be demonstrated in other autoimmune diseases.

MicroRNAs

As stated earlier, epigenetic factors are key to XCI, but they are coincidentally linked to autoimmunity too. Recently, studies have highlighted microRNAs (miRNA) as important regulators of the immune response. miRNAs are noncoding RNAs involved in post-transcriptional gene regulation and function to repress, or outright degrade, the mRNA before translation. Regulatory T cells (Treg) function to maintain immune homeostasis through negative feedback by acting as brake pedals in order to prevent overreactions, or in other words, autoimmunity. However, miRNAs are highly expressed in these cells, specifically miRNA 15/16 clusters[26]. There is a staggering number of miRNAs on the X chromosome itself, around 118, compared to only 4 on the Y chromosome[27]. The difference is astonishing. Again, it is important to note how large the X chromosome is compared to the Y chromosome and other autosomal chromosomes. The difference is further exaggerated because of the fact women have two X chromosomes. XCI may have a strong influence on miRNA expression, since it is probable that genes which code for miRNAs that act on the immune system are able to escape XCI. In the case they do escape, this would lead to even more miRNA interfering with the expression of Treg cells, which would lead to the inhibition of "brake pedals" which control inflammatory responses. This may heavily contribute to autoimmune responses in females due to the lack of repressors on inflammation.

Numerous studies have been conducted on specific X-linked miRNAs and their correlation with autoimmune diseases. *XIST* itself is positively regulated by the miRNA cluster miR-374a[27]. The most intensely studied X-linked miRNA in autoimmune diseases is miR-223, but its function is rather multifarious; miR-223 is context-dependent, meaning it has been observed to both enhance and decrease inflammation, but more studies have shown it represses inflammation. A study done on RA and miRNA expression found miR-223 to be noticeably overexpressed in CD4+ naive T-lymphocytes of all patients with RA[28]. Compared to healthy donors, miR-223 did not consistently express even after TCR stimulation, suggesting the overexpression is associated with the pathology, not a consequence, of T-lymphocyte activation in RA patients[28]. Little is known about the miRNA's function in T-lymphocytes though there is a clear connection between it and autoimmunity. In another case, using a mouse experimental autoimmune encephalomyelitis as an animal model of MS (multiple sclerosis) provided evidence for the contribution of miR-223 in autoimmune inflammation of the central nervous system in mice[29]. In addition to significant increases of miR-223 levels in CD4+ and CD11 cells, miR223 was identified in spinal cords, spleen, lymph nodes, and bone marrow of mice, suggesting a complex role[29]. miR-223 was also observed to

suppress Th1 and Th17 cell differentiation[29]. These findings may not be directly generalizable to humans, but it is still significant due to the fact these are mammal cells. It should be noted that although these studies on miR-223 are rather inconclusive, these studies nonetheless show miRNAs are able to escape the X chromosome, and this leaves the door open for other possible escapees to study. Moreover, certain miRNAs could be potentially useful as markers for disease to enhance disease diagnosis and treatment efficacy.

Methods

This article reviews literature on what is currently known about XCI, its link to autoimmunity in women, the implications of these studies, and the current advancements research is working towards concerning this issue. Literature review was obtained from sources all across the globe through Google Scholar. The diverse sources were collected from studies published in the United Kingdom, Japan, and Argentina, to name a few. The variety of sources selected all encompass XCI escapement and skewing in relation to the female immune system. The next section uses sources that focus on XCI and thyroid autoimmune diseases, as there are a greater number of studies done on their relation. This inspired the research on the thyroid organ itself and its unique relationship with the immune system, and its potential correlation to XCI.

More recent studies were preferably used, but because there is limited data on XCI's relation to autoimmune disease, some sources were used from over two decades ago. Older studies were authenticated with recent studies by comparing data from sources, observing if they aligned with each other. Overall, a total of 44 papers were reviewed and are included in the cited literature. Papers were assessed based on quality—experimental, primary studies—with a preference for recency. Narrative literature synthesis is the backbone of this article's hypothesis, that XCI increases the risk of autoimmunity in women, therefore contributing to female autoimmune bias. Literature with data from experiments were typically preferred, however, general information regarding XCI and autoimmune conditions were used from 10 secondary, review articles that provided comprehensive overviews. Studies that relied on press releases were excluded to maintain integrity of the paper. In the following section, studies were qualified for the inclusion criteria if they provided experimental evidence supporting the correlation of XCI to Graves' Disease and Hashimoto's Thyroiditis. Studies for the thyroid-autoimmune relationship had to include a genetic analysis of patients to support their claims. In addition, studies reviewed required statistical analyses which fit the sample size to further validate their claims. Studies were also excluded if they did not pertain to thyroid autoimmune diseases, lacked evidence to support XCI skewing in autoimmunity, or the focus of the study was irrelevant to autoimmunity prevalence among women. Connecting data from different sources through narrative synthesis is especially emphasized in XCI discussion. In both data sets used for quantitative data, human participants were the main subjects of the study, which raised questions of ethical implications. It was crucial to evaluate the quality and ethical standard each study was put to. In both experiments, subjects provided informed written consent to the researchers, and the experiments themselves were approved by their respective local ethics committees in Osaka and the United Kingdom.

Data Extraction and Results

Thyroid autoimmune diseases have been observed to have a strong correlation with XCI. Graves' disease is an autoimmune disorder that is essentially a precursor to hyperthyroidism. The immune system denotes the thyroid gland as foreign, and mistakenly produces too much thyroid hormone in an attempt to attack the gland. On the opposite end of the spectrum, Hashimoto's disease is an autoimmune disease which reduces the hormone production of the thyroid gland, leading to hypothyroidism. The instances of these thyroid autoimmune conditions in females can be as high as 10:1 compared to males[1].

In 2015, a study done by doctors from the Osaka School of Medicine measured the degree of skewed XCI in female patients with Graves' Disease (GD) and Hashimoto's Thyroiditis (HT). The study was done by gathering 120 women with GD and HT with differing cases of severity as the experimental group, and 49 healthy women with no clinical history of autoimmunity and thyroid autoantibodies as the control[30]. To complete an accurate analysis of XCI skewing, researchers collected blood samples in EDTA tubes, isolating genomic DNA from peripheral blood mononuclear cells, and followed it up with a polymerase chain reaction (PCR)[30]. This DNA was digested with the restriction enzyme HpaII, a methylation sensitive enzyme, to obtain PCR templates, targeting the CAG repeat region of the androgen receptor gene. Researchers included a male as an enzyme digestion control because male X chromosomes are unmethylated, unlike female X chromosomes. The number of CAG repeats are essentially a marker of a maternal or paternal active X chromosome. Generally, when the X chromosome is inherited from the father, the number of CAG repeats increases in subsequent generations, while a maternally inherited X chromosome observes that CAG repeats remain stable, or even decrease through generations[31]. Following 30 cycles of amplification, the products went through gel electrophoresis in 8% denaturing polyacrylamide gels for 120 minutes at 250 V[30]. The gels were stained with ethidium bromide and pictured under ultraviolet light to differentiate between maternal and paternal skewing through the CAG repeat number. To quantify the degree of XCI skewing as a percentage, researchers briefly measured the density of each band from the two alleles, dividing the density of allele A by the sum of allele A and allele B, multiplying the quotient by 100[30].

A calculation of 50% skewing would be interpreted as random or normal XCI, whereas 100% is completely skewed[30].

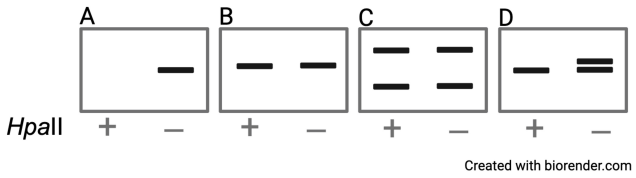

Fig. 3 (A) Male control (B) Unable to distinguish the two bands because CAG repeats were the same (C) Not skewed (D) Skewed. HpaII lane indicates the presence or absence of HpaII. The figure presents the electrophoresis results of the CAG repeat experiment on female individuals, including a male control, with GD and HT. Adapted from N. Ishido, N. Inoue, M. Watanabe, Y. Hidaka, Y. Iwatani. The Relationship Between Skewed X Chromosome Inactivation and the Prognosis of Graves' and Hashimoto's Diseases. THYROID. 25, (2015).

In both patients with GD and HT, the severity of the disease increased as skewing increased[30]. In this study, severity of disease was defined by the prevalence of antibodies and the use of/resistance to medication, though the exact definition differs between diseases. GD patients were categorized by their clinical history of thyrotoxicosis and elevated levels of antithyrotropin receptor antibody (TRAb) as follows: 45 patients with intractable GD who were treated with methimazole for at least five years and remained positive for TRAb were considered severe, and 32 patients with GD in remission who maintained a euthyroid state and were negative for TRAb for more than two years without medication were not considered severe[30]. HT patients were positive for antithyroid microsomal antibody and/or antithyroglobulin antibody were sorted into the following groups: 20 patients who developed moderate to severe hypothyroidism before 50 years of age and were treated daily with thyroxine were considered severe, and 23 patients who were untreated and euthyroid over 50 years of age were considered mild[30]. These definitions of severity for GD and HT are consistent across the study. When the cutoff for skewed XCI was set to 65%, patients who suffered intractable GD had a significantly higher degree of skewed XCI (66.7%) than those with GD in remission (25%) using a chi square analysis test with p-values of less than 0.05, specifically $p = 0.0033$[30]. Similarly, skewing was higher in patients with severe HT (76.5%) compared to patients with mild HT (41.2%) with a relaxed cutoff of 60% and a p-value of 0.0342. This means there is evidence for a relationship between skewed XCI in cells and the severity of the autoimmune disease. However, as researchers increased the cutoff beyond 60% skewing, the difference of skewing was calculated as insignificant[30]. To make up for these discrepancies, other factors which influence autoimmunity should once again be noted, such as XCI escapement, hormones, miRNAs, etc. Nonetheless, the study is a great leap towards figuring out the relationship between XCI and autoimmunity, but it may be limiting due to the acknowledgment that all of the subjects tested were females of Japanese descent, and the sample size was small. In spite of that, another study on skewed XCI and thyroid autoimmune diseases was conducted in the United Kingdom with a much larger female data set of 309 GD patients, 490 HT patients, 325 caucasian controls, 273 caucasian GD families, and a meta-analysis of 454 GD, 673 HT, and 643 caucasian controls[32]. Using CAG repeats as indicators of skewing, similar to researchers in Osaka, the UK study analyzed significantly increased XCI skewing in females with GD and HT compared to controls, and the cutoff for skewing was defined as 80% or greater[32]. Severity of disease is not explicitly defined in this study, but is instead indicated through the rate of auto-antibody positive status[32]. In fact, HT subjects with extremely skewed XCI showed higher rates of thyroid autoantibody-positive status, a direct indicator of increased autoimmune instances in relation to XCI[32]. This study established the notion of increased XCI skewing in females with Graves' disease and Hashimoto's thyroiditis with 95% confidence intervals. This furthers the idea that XCI is able to interfere with immune related genes on the X chromosome and contribute to autoimmunity prognosis, because the same relationships between XCI and autoimmune disease severity were observed in two very culturally different female sample groups.

Discussion

Researchers in the UK study came to hypothesize that because XCI happens at such an early stage of development, when mature T-cells encounter the skewed X chromosome after the female has grown, they would not be able to recognize the cells and deem it foreign, leading to an autoimmune reaction in the thyroid[32]. Nonetheless, both studies confirmed increased XCI skewing in female subjects with GD and HT, although hypotheses on the matter differ. It is amazing how these two groups of women from nations so far from each other experienced the same genetic issue of XCI furthering progression and severity of autoimmunity, showing just how universal the issue of autoimmunity and women reaches out to.

There is growing evidence supporting the compelling, bidirectional relationship between the thyroid gland and the immune system, although the thyroid is traditionally considered part of the endocrine system. The thyroid functions as an important body and hormone regulator, releasing thyroid hormones like T4 (L-thyroxine) and T3 (3,5,3′-triiodo-L-thyronine), the deionized and active form of T4, which contribute to metabolism, growth, and development in practically all tissues[33]. Because these hormones circulate through the entire body, they could potentially play a role in immunity. T3 and T4 have been reported in monocytes, granulocytes, natural killer cells, mast cells, and lymphocytes, although their functions and mechanisms in these

Table 1 The table summarizes and compares key aspects of the Osaka and United Kingdom studies.

Study	Osaka	United Kingdom
Sample size	169 Japanese females, unrelated. 77 with GD, 43 with HT, and 49 healthy controls	1392 Caucasian females, 273 families also screened. 417 with GD, 590 with HT, and 385 healthy controls
Definition of skewing	XCI skewing is defined as greater than or equal to 65%, though the study also relaxed the cutoff to 60% for severity in HT patients. Random skewing is represented by 50%, while completely skewed is represented by 100%.	XCI skewing was defined as 80% or greater of a specific paternal X chromosome being inactivated, while extreme skewing was defined as 90% or greater.
Statistical tests used	• Chi square analysis • Fisher's exact test • Mann-Whitney U-test • Probability values of less than 0.05 were considered to be significant	• Cochran's Q • Odds ratio calculated with I^2 metric • Modified Egger test • 95% confidence interval • Random effects models used due to presence of heterogeneity (I^2 of 75% or greater)
Autoimmunity type	Graves' Disease and Hashimoto's Thyroiditis	Graves' Disease, Hashimoto's Thyroiditis, and Autoimmune Thyroid Disease
Main findings	• No significant difference in the mean degree of skewing in AITD patients with control, or of GD and HT patients and controls • Intractable GD patients have significantly higher XCI skewing degree than those in remission • With relaxed cutoff, severe HD patients have a higher degree of skewing than mild HD patients	• Confirmation of increased XCI skewing and extreme skewing in female subjects with GD and HT in the largest sample collection and meta-analysis of currently available data from UK

immune system cells have not yet been solidly confirmed[33,34]. At the genomic level, T3 regulates gene expression of growth hormone (GH), hairless (Hr), sonic hedgehog (Shh), and other genes, by binding to thyroid receptor proteins $TR\alpha$and $TR\beta$, which act as ligand-regulated transcription factors, showing the multifaceted functions of T3[34]. In avian natural killer cells, T3 enhances the responses to IL-2 (interleukin 2) and the IL-2 receptor, a cytokine known to increase natural killer cell proliferation and activation[33]. Although this may not translate to mammalian cells, this suggests T3 is able to uniquely function for the immune system. In the cytoplasm, T4 has been shown to enhance interferon-γ (IFN-γ) activity in HeLa cell lines, and although these cells are not directly linked to the immune system, they do not contain the classical nuclear receptors, so any type of signal transduction must have been through an alternate receptor type[33]. This observation has profound implications for the immune system because IFN-γ activates natural killer cells, thereby enhancing the innate immune system which then affects the activation of the adaptive immune system[34]. For females with an increased dosage of certain proinflammatory

genes, these thyroid hormones may indirectly set off a severe immune reaction via natural killer cells because the overactivated adaptive immune system would trigger increased inflammation. This reveals a correlation between the thyroid and the prognosis of autoimmunity. Furthermore, the ability of T4 to bind to alternative receptors suggests a possibility for the hormone to bind to receptors in monocytes, granulocytes, natural killer cells, mast cells, and lymphocytes, which can contribute to a severe immune reaction. Both hypothyroidism and hyperthyroidism can alter the functions of chemotaxis, phagocytosis, generation of reactive oxygen species, and cytokine synthesis and release in immune system cells because of altered thyroid conditions[33]. In addition, both are often associated with ovarian cycle irregularities, which in turn affect other hormones such as estrogen, the hormone's influences on the immune system mentioned earlier.

Receptor expression patterns and tissue-specific effects are able to affect TH signaling in different tissues. The best known effects of TH on cellular proliferation and differentiation require the presence of nuclear thyroid hormone receptors (TR), which are encoded by the genes $TR\alpha$and $TR\beta$[35]. These genes are able to generate protein isoforms TRα1, $TR\beta$1, and $TR\beta$2 which are the main hormone-binding isoforms found throughout the body. TRα1 is predominantly found in bone, gastrointestinal tract, cardiac and skeletal muscle, and the central nervous system; $TR\beta$1 is found more in the liver and kidney; $TR\beta$2 is most abundant in the hypothalamus, pituitary gland, cochlea, and retina[35]. These receptors can all be found in the same tissue, but at different concentrations which can affect how TH is sent and received from the thyroid gland. For example, intestinal epithelial cells express both $TR\alpha$and $TR\beta$, but rely on the TRα1 receptor for cell proliferation[36]. In addition, mice with a deletion of $TR\alpha$have shown a markedly decreased heart rate, whereas mice with a deletion of $TR\beta$ have a normal heart rate and contractile function[37]. In addition, TH transporter proteins on the cell membrane regulate the entry of thyroid hormones into cells, which varies by tissue and controls the local availability of TH. These ATP-dependent proteins include monocarboxylate transporters MCT8 and organic anion transporter proteins[38]. At the cellular level, gluconeogenesis, lipogenesis, insulin signaling, adenylate cyclase signaling, cell proliferation, apoptosis, and cellular immunity have been found to be regulated by TH[39].

As of now, research on the relationship between the immune and endocrine system is often difficult to interpret, given the dynamic and nuanced interplay between these systems built to adapt to physiological changes. Continuing to evaluate this relationship could have important implications for the future of autoimmunity and inflammation.

Currently, advancements are being made in relation to female predisposition to autoimmunity. XCI was discovered relatively recently in 1991 and was groundbreaking for the field of epigenetics[40]. Now, XCI is under intense study, as much of the molecular basis of the process still remains unknown yet its effects are prevalent in all females. In February of 2025, the immunogenic complex of *XIST* RNP (riboneucleoprotein) was investigated, and researchers found its RNA sequence overloaded with UU dinucleotides, a type of single-stranded RNA (ssRNA) composed of two consecutive uracil bases[41]. This is significant because these ssRNAs act as ligands for *TLR7* activation, which as stated before, are responsible for inflammatory reaction, and there is more *TLR7* in female blood than males[9,41]. This raises the question of whether *XIST* RNA can have functions other than silencing the X chromosome, such as acting as ligands to activate immune responses. Earlier in 2024, 30 proteins of *XIST* RNP were found to serve as autoantigens in human autoimmune diseases. They were found to be targeted by autoantibodies[3]. When a cell goes through apoptosis, these *XIST* RNPs will be exposed to the body, influencing an immune reaction. There may be other proteins or molecules that interact with *XIST* that researchers have not discovered yet which may also influence female predisposition to autoimmunity. Further research is needed to advance XCI mechanism understanding and its relation to autoimmunity in females.

Non-genetic Factors

Though this paper focuses on the genetic factors of autoimmunity in females, non-genetic factors are also important when discussing autoimmunity as they pertain to a broader range of patients.

Environmental impacts on autoimmunity are extremely diverse and can have varying effects on different individuals. The role of pollutants and pesticides in autoimmunity is an emerging issue within epigenetics and immunology as a whole. The industrial solvent trichloroethylene (TCE) is a drinking water pollutant that has been implicated in CD4+ T cell-mediated autoimmunity[42]. To determine whether continued TCE exposure during development had an effect on autoimmunity, researchers Byrum et al. conducted a whole genome reduced representation bisulfite sequencing of their mouse model to evaluate methylation of CpG sites in female mice exposed to a control, TCE from gestation until 37 weeks, or TCE until 22 weeks. They found that continuous developmental exposure to TCE altered 274 CpG sites in promoters and CpG islands. Continued exposure to differentially methylated binding sites of PcG proteins in effector/memory CD4+ cells, indicating exposure may alter CD4+ T cell function in adulthood[42]. In a different study, a sample of 668 male farmers were examined to determine the association between a lifetime of pesticide use and serum antinuclear autoantibodies (ANA) on their Hep-2 cells[43]. Their findings provide evidence that moderate to higher levels of ANA are associated with past use of specific organochlorine pesticides, increasing the risk of systemic autoimmune diseases in these farmers[43].

In addition, lifestyle habits can contribute to the onset

of autoimmune disease. For example, the "Western Diet"—associated with processed foods, snacks, soft drinks, and "fast food"—has been studied to better understand its effect on inflammation. Western diets are rich in refined sugars, salt, white flour, and additives, yet lack necessary fibers, vitamins, minerals, and antioxidants that keep the body healthy[44]. These diets are particularly energy-dense with high glycemic indexes which spike blood glucose, promoting weight gain. A 2004 study observed the dietary patterns of 732 women, finding a positive relation between Western dietary patterns and concentrations of C-reactive protein (CRP), interleukin-6 (IL-6), E-selectin, soluble intercellular adhesion molecule 1, and soluble vascular cell adhesion molecule 1 proteins[44]. These correlations are significant because CRP and IL-6 are markers of systemic inflammation and predictors of cardiovascular diseases in women[44]. This study supports the claim that certain dietary patterns may affect the immune system, particularly to induce inflammation.

Closing Thoughts

Historically, sex differences in health and disease concerning women have been largely ignored, proving to be a universally harmful issue for women that is difficult to resolve. Females are the primary victims of autoimmune disease, and the only target of X-inactivation. XCI is the process of randomly silencing one X chromosome, be it paternal or maternal, in cells of individuals with more than one X chromosome. XCI is imperfect, leading to 15-23% of genes escaping inactivation, causing an imbalance of gene expression. Skewing is a phenomenon in which one parent's chromosome is favorably expressed over the other. Research done on thyroid autoimmune diseases (Graves' Disease and Hashimoto's Thyroiditis), and the known influences hormones and epigenetics have on the immune system suggests that XCI may be an important contributor to the disproportionate ratio of autoimmunity in women compared to men. It is also important to note that although XCI can be associated with the female autoimmune bias, it is not the sole cause of the phenomenon. Other factors of autoimmunity include production of hormones, miRNA interference, and the thyroid organ itself. In the wake of this information of autoimmunity and XCI, one final question arises; what does this mean for the future health of women? While XCI occurs in every female cell and, according to current research, cannot be controlled or mitigated, this paper is in support of treatment catered specifically towards women with autoimmune diseases should be of major concern, and development of such treatments should be continually investigated and funded for. Such treatments could include genetic therapy that works to target genes on the X chromosome, such as *CD40L* and *CXCR3*, or development of immunity regulators directed towards the X chromosome. Suppressants aimed to mitigate the *TLR7* pathway can be tested in females with severe autoimmunity to observe if inflammation is able to decrease using such medications. An experiment using mice as an animal model can potentially lead to the emergence of such suppressants. For instance, female mice with autoimmune disease symptoms would be the experimental group and healthy female mice would be the control. The experimental group could be divided into two subgroups: those treated with the *TLR7* suppressant, and the other without. It would be expected that the former group would show a significant reduction in autoantibody levels, mirroring that of healthy mice, whereas the latter group would continue to develop autoimmune disease symptoms. A study like this would effectively test the proposed *TLR7*-autoimmunity relationship in females. It could also be useful to evaluate promising molecular biomarkers of autoimmunity that could be tied to the X chromosome in order to track the prognosis of autoimmunity in women. With this, researchers would be able to predict major genetic risks and plan preventative therapies for the patients. These severe discrepancies between female and male immune systems cannot be overlooked to ensure every person, regardless of their sex, will find treatment that works for them. Therefore, further research on XCI should be continually supported to better understand and spread awareness of the implications this phenomenon may have on children, friends, and mothers.

Acknowledgments

I wrote this paper out of my own experience dealing with autoimmunity—severe atopic dermatitis and alopecia areata—all my life. Through researching for this paper, my personal struggle seemed to bridge across to a wider cohort, the cohort of women all around the world. This paper created a solace of scientific understanding where, for the first time, I could find peace with my circumstances.

I would like to thank my mentor Kate Gilbert, a PhD candidate from Brown University, for guiding me through the research process, and providing me with indispensable knowledge and resources. Her support pushed me to research deeper and to keep going with this paper. Biorender was used for 3 figures in this review, proving to be an especially helpful tool during the process of writing this article. I would also like to commend and thank my mother who, whilst I was writing this paper, was diagnosed with hyperthyroidism. While battling with her thyroid issues and even going through surgery, she still made time to access literature for me while consistently reminding me to keep going. I am beyond grateful for her support.

References

1 S. Koppala and V. Guruprasad, *Overview of autoimmunity: classification, disease mechanisms, and etiology*.

2 F. Miller, *The increasing prevalence of autoimmunity and autoimmune disease: an urgent call to action for improved understanding, di-*

agnosis, treatment, and prevention, https://doi.org/10.1016/j.coi.2022.102266.

3 D. Dou, Y. Zhao, J. Belk, Y. Zhao, K. Casey, D. Chen, R. Li, B. Yu, S. Srinivasan, B. Abe, K. Kraft, C. Hellstrm, R. Sjberg, S. Chang, A. Feng, D. Goldman, A. Shah, M. Petri, L. Chung, D. E. Lundberg, A. Wutz, P. Utz and H. Chang, *Xist ribonucleoproteins promote female sex-biased autoimmunity*, https://doi.org/10.1016/j.cell.2023.12.037.

4 B. Sun and H. Tsao, *X-chromosome inactivation and skin disease*, https://doi.org/10.1038/jid.2008.145.

5 R. Adrianse, K. Smith, T. Gatbonton-Schwager, S. Sripathy, U. Lao, E. Foss, R. Boers, J. Boers, J. Gribnau and A. Bedalov, *Perturbed maintenance of transcriptional repression on the inactive X-chromosome in the mouse brain after Xist deletion*, https://doi.org/10.1186/s13072-018-0219-8., pg. 50, 2018,.

6 K. Plath, S. Mlynarcztk-Evans, D. Nusinow and B. Panning, *Xist RNA and the mechanism of X Chromosome inactivation*, https://doi.org/10.1146/annurev.genet.36.042902.092433., , 2002,.

7 Y. Markaki, J. Chong, Y. Wang, E. Jacobson, C. Luong, S. Tan, J. Jachowicz, M. Strehle, D. Maestrini, A. Banerjee, B. Mistry, I. Dror, F. Dossin, J. Schoneberg, E. Heard, M. Guttman, T. Chou and K. Plath, *Xist nucleates local protein gradients to propagate silencing across the X chromosome*, https://doi.org/10.1016/j.cell.2021.10.022.

8 A. Loda and E. Heard, *Xist RNA in action: past, present, and future*, https://doi.org/10.1371/journal.pgen.1008333.

9 A. Youness, C. Miquel and J. Gury, *Escape from X Chromosome Inactivation and the Female Predominance in Autoimmune Diseases*, https://doi.org/10.3390/ijms22031114.

10 J. Berletch, W. Ma, J. Shendure, W. Noble, C. Disteche and X. Deng, *Escape from X inactivation varies in mouse tissues*, https://doi.org/10.1371/journal.pgen.1005079.

11 T. Tukiainen, A. Villani, A. Yen, M. Rivas, J. Marshall, R. Satija, M. Aguirre, L. Gauthier, M. Fleharty, A. Kirby, B. Cummings, S. Castel, K. Karczewski, F. Aguet, A. Byrnes, G. Consortium, T. Lappalainen, A. Regev, K. Ardlie, N. Hacohen and D. MacArthur, *Landscape of X chromosome inactivation across human tissues*, https://doi.org/10.1038/nature24265.

12 A. Cotton, L. Lam, J. Affleck, I. Wilson, M. Penaherrera, D. McFadden, M. Kobor, W. Lam, W. Robinson and C. Brown, *Chromosome-wide DNA methylation analysis predicts human tissue-specific X inactivation*, https://doi.org/10.1007/s00439-011-1007-8.

13 C. Huret, L. Ferray, A. David, M. Mohamed, N. Valentin, F. Charlotte, M. Savignac, M. Goodhardt, J. Gury, C. Rougeulle and C. Morey, *Altered X-chromosome inactivation predisposes to autoimmunity*, https://doi.org/10.1126/sciadv.adn6537.

14 S. Hagen, F. Henseling, J. Hennesen, H. Savel, S. Delahaye, L. Richert, S. Ziegler and M. Altfeld, *Heterogeneous escape from X chromosome inactivation results in sex differences in type I IFN responses at the single human pDC level*, https://doi.org/10.1016/j.celrep.2020.108485.

15 Z. Feng, M. Liao and L. Zhang, *Sex differences in disease: sex chromosome and immunity*, https://doi.org/10.1186/s12967-024-05990-2., pg. 1150, 2024,.

16 S. Pyfrom, B. Paneru, J. Knox, M. Cancro, S. Posso, J. Buckner and M. Anguera, *The dynamic epigenetic regulation of the inactive X chromosome in healthy human B cells is dysregulated in lupus patients*, https://doi.org/10.1073/pnas.2024624118.

17 S. Kanaan, O. Onat, N. Balandraud, G. Martin, J. Nelson, D. Azzouz, I. Auger, F. Arnoux, M. Martin, J. Roudier, T. Ozcelik and N. Lambert, *Evaluation of the X chromosome inactivation with respect to HLA genetic susceptibility in rheumatoid arthritis and systemic sclerosis*, https://doi.org/10.1371/journal.pone.0158550.

18 J. Broen, I. Wolvers-Tettero, L. Bon, M. Vonk, M. Coenen, R. Lafayatis, T. Radstake and A. Langerak, *Skewed X chromosome inactivation impacts T regulatory cell function in systemic sclerosis*, https://doi.org/10.1136/ard.2010.129999.

19 J. Aisemberg, C. Vercelli, M. Bariani, S. Billi, M. Wolfson and A. Franchi, *Progesterone is essential for protecting against LPS-induced pregnancy loss. LIF as a potential mediator of the anti-inflammatory effect of progesterone*, https://doi.org/10.1371/journal.pone.0056161.

20 M. Lim, C. Ku, T. Tan, Y. Lee, J. Allen and N. Tan, *Characterisation of serum progesterone and progesterone-induced blocking factor (PIBF) levels across trimesters in healthy pregnant women*, https://doi.org/10.1038/s41598-020-59452-y.

21 J. Szekeres-Bartho, Z. Faust, P. Varga, L. Szereday and K. Kelemen, *The immunological pregnancy protective effect of progesterone is manifested via controlling cytokine production*, https://doi.org/10.1111/j.1600-0897.1996.tb00492.x.

22 D. Fairweather and N. Rose, *Women and Autoimmune Diseases. Emerging Infectious Diseases*, https://doi.org/10.3201/eid1011.040367.

23 D. Cooper and P. Patel, *Oral Contraceptive Pills*.

24 A. Khashan, L. Kenny, T. Laursen, U. Mahmood, P. Mortensen, T. Henriksen and K. ODonoghue, *Pregnancy and the Risk of Autoimmune Disease*, https://doi.org/10.1371/journal.pone.0019658.

25 Y. Man, R. Dolhain, F. Geijn, S. Willemsen and J. Hazes, *Disease activity of rheumatoid arthritis during pregnancy: results from a nationwide prospective study*, https://doi.org/10.1002/art.24003.

26 J. Dong, W. Huth, N. Marcel, Z. Zhang, L. Lin and F., *Lu. miR-15/16 clusters restrict effector Treg cell differentiation and function*, https://doi.org/10.1084/jem.20230321.

27 A. Palo, C. Siniscalchi, M. Salerno, A. Russo, C. Gravholt and N. Potenza, *What microRNAs could tell us about the human X chromosome. Cellular and molecular life sciences: CMLS*, https://doi.org/10.1007/s00018-020-03526-7.

28 V. Fulci, G. Scappucci, G. Sebastiani, C. Giannitti, D. Franceschini, F. Meloni, T. Colombo, F. Citarella, V. Barnaba, G. Minisola, M. Galeazzi and G. Macino, *miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis*, https://doi.org/10.1016/j.humimm.2009.11.008.

29 T. Satoorian, B. Li, X. Tang, J. Xiao, W. Xing, W. Shi, K. Lau, D. Baylink and X. Qin, *MicroRNA223 promotes pathogenic T-cell development and autoimmune inflammation in central nervous system in mice*, https://doi.org/10.1111/imm.12611.

30 N. Ishido, N. Inoue, M. Watanabe, Y. Hidaka and Y. Iwatani, *The Relationship Between Skewed X Chromosome Inactivation and the Prognosis of Graves and Hashimotos Diseases*, https://doi.org/10.1089/thy.2014.0318.

31 A. Gupta and S. Kushwaha, *Maternally Inherited Huntington Disease and Paternally Inherited Neurofibromatosis Type-1 in an Individual- A Rare Co-Occurrence*, https://doi.org/10.29245/2572.942X/2019/2.1244.

32 M. Simmonds, F. Kavvoura, O. Brand, P. Newby, L. Jackson, C. Hargreaves, J. Franklyn and S. Gough, *Skewed X Chromosome Inactivation and Female Preponderance in Autoimmune Thyroid Disease: An Association Study and Meta-Analysis*, https://doi.org/10.1210/jc.2013-2667.

33 P. Vito, S. Incerpi, J. Pedersen, P. Luly, F. Davis and P. Davis, *Thyroid Hormones as Modulators of Immune Activities at the Cellular Level*, https://doi.org/10.1089/thy.2010.0429.

34 J. Rubingh, A. Spek, E. Fliers and A. Boelen, *The Role of Thyroid Hormone in the Innate and Adaptive Immune Response during Infection*, https://doi.org/10.1002/cphy.c200003.

35 E. Bochukova, N. Schoenmakers, M. Agostini, E. Schoenmakers, O. Rajanayagam, J. Keogh, E. Henning, J. Reinemund, E. Gevers, M. Sarri, K. Downes, A. Offiah, A. Albanese, D. Halsall, J. Schwabe, M. Bain, K. Lindley, F. Muntoni, F. Vargha-Khadem, M. Dattani, S. Farooqi, M. Gurnell and K. Chatterjee, *A mutation in the thyroid hormone receptor alpha gene*, https://doi.org/10.1056/NEJMoa1110296.

36 M. Plateroti, K. Gauthier, C. Domon-Dell, J. Freund, J. Samarut and O. Chassande, *Functional interference between thyroid hormone receptor (TR) and natural truncated TR isoforms in the control of intestine development*, https://doi.org/10.1128/mcb.21.14.4761-4772.2001.

37 B. Gloss, S. Trost, W. Bluhm, E. Swanson, R. Clark, R. Winkfein, K. Janzen, W. Giles, O. Chassande, J. Samarut and W. Dillmann, *Cardiac ion channel expression and contractile function in mice with deletion of thyroid hormone receptor or* , https://doi.org/10.1210/endo.142.2.7935.

38 P. Strmme, S. Groeneweg, E. Souza, C. Zevenbergen, A. Torgersbrten, A. Holmgren, E. Gurcan, M. Meima, R. Peeters, W. Visser, L. Johansson, A. Babovic, H. Zetterberg, H. Heuer, E. Frengen, D. Misceo and T. Visser, *Mutated thyroid hormone transporter OATP1C1 associates with severe brain hypometabolism and juvenile neurodegeneration*, https://doi.org/10.1089/thy.2018.0595.

39 P. Yen, S. Ando, X. Feng, Y. Liu, P. Maruvada and X. Xia, *Thyroid hormone action at the cellular, genomic, and target gene levels*, https://doi.org/10.1016/j.mce.2005.11.030.

40 P. Gil-Ibez, J. Pernal and B. Morte, *Thyroid hormone regulation of gene expression in primary cerebrocortical cells: role of thyroid hormone receptor subtypes and interactions with retinoic acid and glucocorticoids*, https://doi.org/10.1371/journal.pone.0091692.

41 J. Crawford, H. Wang, D. Trejo-Zambrano, R. Cimbro, C. Talbot, Jr, M. Thomas, A. Curran, A. Girgis, J. Schroeder, A. Fava, D. Goldman, M. Petri, A. Rosen, B. Antiochos and E. Darrah, *The XIST lncRNA is a sex-specific reservoir of TLR7 ligands in SLE*, https://doi.org/10.1172/jci.insight.169344.

42 S. Byrum, C. Washam, J. Patterson, K. Vyas, K. Gilbert and S. Blossom, *Continuous developmental and early life trichloroethylene exposure promoted DNA methylation alterations in polycomb protein binding sites in effector/memory CD4+ T cells*, https://doi.org/10.3389/fimmu.2019.02016.

43 C. Parks, A. Souza Espindola Santos, C. Lerro, C. DellaValle, M. Ward, M. Alavanja, S. Berndt, L. Freeman, D. Sandler and J. Hofmann, *Lifetime pesticide use and antinuclear antibodies in male farmers from the agricultural health study*, https://doi.org/10.3389/fimmu.2019.01476.

44 E. Lopez-Garcia, M. Schulze, T. Fung, J. Meigs, N. Rifai, J. Manson and F. Hu, *Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction123*, https://doi.org/10.1093/ajcn/80.4.1029.