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Autoimmunity and Klinefelter's syndrome: when men have two X chromosomes

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Abstract

Similar to other autoimmune diseases, systemic lupus erythematosus (SLE) predominately affects women. Recent reports demonstrate excess Klinefelter's among men with SLE and a possible under-representation of Turner's syndrome among women with SLE as well as a case report of a 46,XX boy with SLE. These data suggest that risk of SLE is related to a gene dose effect for the X chromosome. Such an effect could be mediated by abnormal inactivation of genes on the X chromosome as has been demonstrated for CD40L, or by genetic polymorphism as has been demonstrated for Xq28. On the other hand, a gene dose effect could also be mediated by a gene without an SLE-associated polymorphism in that a gene that avoids X inactivation will have a higher level of expression in persons with two X chromosomes.

Keywords

Systemic lupus erythematosus; Genetics; X chromosome

1. Introduction

The dual and discrete nature of human gender is a common and pervasive part of both experience and culture (for example Mother Tongue and Father Land). However, the existence of numerous and varied disorder of sex differentiation leads some to the conclusion that, in fact, gender is a continuum [1]. Sex determination is a complex process determined at least in part by the sex chromosome complement imparted to the developing organism, molecular events involving transcription factors that promote formation of germ cells, migration of these cells to the urogenetal ridge and formation of either a testis or an ovary. Sex determination of the embryo then sets in motion sex differentiation, also a complicated series of molecular events [1]. The components of sex determination and differentiation can be considered as genetic sex, phenotypic sex, and gender identification [2]. In this review, we describe the biology of X chromosome, the dose–effect of X chromosome number and the risk for developing lupus, abnormal X chromosome inactivation in female lupus patients, and finally what is known about the genetics of X chromosome in lupus.

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2. Biology of sex chromosomes

The biology of the sex chromosomes is complicated and incompletely understood [3]. The presence of X and Y determines genetic sex with testes developing in the presence of a Y chromosome, and ovaries developing in the absence of Y. The X and Y chromosomes pair during meiosis and undergo genetic crossover within the pseudoautosomal regions located on the telomeric ends of each chromosome. Each X pseudoautosomal region has complementary sequence in the corresponding region on Y, and thus meiotic pairing is possible. There are only a few genes within the pseudoautosomal regions [2,4]. On the Y chromosome, *sry* gene that encodes the testes determining factor lies perilously close to the pseudoautosomal region. So, an unequal crossover may result in the *sry* gene on an X chromosome. 46,XX individuals carrying such an X chromosome will be phenotypic, but usually sterile males. A 46,XX male with severe early childhood onset SLE has been reported [3]. In persons with more than one X chromosome, one X chromosome is inactivated by the gene product of the *Xist* gene with resultant production of the Barr body [5]. Of interest, X chromosome inactivation is not an all-or-none phenomenon. Of the 2000 or so X-linked genes on the X chromosome about 10% are not inactivated by X inactivation of the chromosome on which they lie. Thus, these genes maintain two active, transcribed and translated copies in individuals with two X chromosomes.

The common disorders of sex differentiation involving chromosomal abnormalities are Turner's syndrome (female 45,XO) and Klinefelter's syndrome (male 47,XXY). Henry H. Turner described the syndrome bearing his name in the 1930s from the Endocrinology Clinic at the University of Oklahoma Health Sciences Center [6]. Turner's syndrome mostly commonly presents with short stature or amenorrhea, and virtually all girls with Turner's syndrome come to medical attention [7]. Henry F. Klinefelter, who went on to practice rheumatology in Baltimore and was a clinical faculty at Johns Hopkins University [8], was a medical student in the 1940s when he helped describe a new syndrome [9]. Klinefelter's syndrome men are tall, have a gynecoid body habitus and are hypogonadal with small firm testes [10]. While Klinefelter's syndrome commonly presents with impotence or infertility, unlike Turner's syndrome, the great majority of persons with 47,XXY are not diagnosed [11].

3. Sex chromosome abnormalities and SLE

As part of our ongoing effort in SLE genetics, we have studied sex chromosome abnormalities in familial and sporadic SLE. Among a group of 843 SLE patients from 378 families in which at least two members had SLE, there were 76 men with SLE. Of these 76,2 (2.6%) had Klinefelter's syndrome with 47,XXY karyotype. Then, among the 138 SLE men with sporadic disease, 3 (2.2%) had Klinefelter's syndrome [12]. Of interest, only one of these five men had been diagnosed clinically with Klinefelter's, however, the greater majority of men with Klinefelter's syndrome do not come to medical attention and receive a diagnosis [11]. A 47,XXY karyotype is found in about 17 per 10,000 live male births [13]. Thus, in the total cohort of 213 SLE men, 5 (2.4%), or 1 in 42, had Klinefelter's syndrome. This represents a 14-fold increase over the general population. Based on these results, we predict that 47,XXY males have the same risk of SLE as 46,XX females from the same ethnic group (see Ref. [12] for discussion).

In the same study, among 768 women with SLE, not a single patient with Turner's syndrome was found. Subsequently, we have identified a family in which two sisters have SLE, and one of them also has Turner's syndrome. However, this patient and family were identified by canvassing physicians caring for SLE patients for a patient with both SLE and Turner's syndrome, not by our usual recruitment methods. There are only 2 previous case reports of Turner's syndrome and SLE occurring together [14,15]. Thus, 45,XO is certainly not over-

represented among women with SLE, although the present data do not allow determination of the relative risk of SLE in 45,XO women compared to 46,XX women, analogous to that made for Klinefelter's syndrome.

Based on these data, we have found that there is a gene dose effect for the X chromosome such that 46,XX women and 47,XXY men share a similar risk of SLE. Furthermore, we hypothesize that the risk of SLE is similar between 45,XO women and 46,XY men. Thus, the risk of SLE may be more related to the number of X chromosomes instead of sex.

As mentioned above a single 46,XX male child has been reported with severe SLE from an early age [3]. To our knowledge, this is the only other report of a sex chromosome abnormality other than Klinefelter's or Turner's syndrome in SLE. Similar to the Turner's syndrome patients, however, such patients could ultimately prove to be highly informative if a critical portion of the X chromosome can be identified as associated with disease based on deletion and triplication [3].

There are no reported data in other female predominate auto-immune diseases concerning the presence of Klinefelter's syndrome among men with such diseases. Clearly, the relationship of 47,XXY to other disease such as multiple sclerosis, Sjögren's syndrome, rheumatoid arthritis, scleroderma, autoimmune thyroid disease, among others, will need to be investigated. Such studies could be among large cohorts of men with these diseases, or population-based.

Another abnormality of the sex chromosome appears to be acquired and is associated with several autoimmune diseases. X chromosome monosomy of peripheral blood lymphocytes is increased in women with primary biliary cirrhosis [16] as well as scleroderma or autoimmune thyroid disease [17]. Furthermore, the X chromosome loss in primary biliary cirrhosis is not random [18]. These data suggest that haplotype deficiency in lymphocytes may play a role in the female predilection for these diseases. However, a recent study found no increase in X monosomy in women with SLE compared to matched controls [19]. The difference in X monosomy, as well as data that Turner's syndrome women with particular X chromosome loss have increased rates of autoimmune thyroid disease [20], suggests the mechanisms by which X chromosome biology influences SLE are distinct from those influencing these other autoimmune diseases.

4. X chromosome inactivation

To adjust for the genetic imbalance on the X chromosome between males (XY) and females (XX), one of the X chromosomes is silenced and inactivated in mammalian female diploid cells. This is accomplished by paternal X chromosome imprinting in some lower mammals, which ensures inactivation of the X chromosome from the father side at all times. X chromosome inactivation in higher mammals is accomplished by the process of random X inactivation rather than imprinting. In this process, X inactivation is random, so that either the maternal or the paternal X chromosome is inactivated in a given diploid cell. In contrast to paternal imprinting, random X inactivation has the advantage of minimizing the effects of mutations, if present, on either the paternal or maternal X chromosomes, since diploid cells are mosaic with either paternal or maternal X inactivation [21]. Therefore, this is more compatible with the natural selection process and is thought to be a logical evolutional alternative to inactivation by paternal imprinting [22].

The process of X chromosome inactivation is complex, and remains incompletely understood. The process of X chromosome inactivation starts by counting the number of X chromosomes and allowing X inactivation only if more than one X chromosome is present per diploid cell [23]. This is followed by selecting which X chromosome will be inactivated. The counting and selection process are regulated by a locus called the X-inactivation center (*Xic*), and appears

to occur during transient pairing of the two X-inactivation centers in both X chromosomes [24]. The *Xist* genetic locus, located within the *Xic* is critical for the X inactivation process. The X chromosome that is selected for inactivation expresses *Xist* RNA that accumulates over the chromosome from which it is transcribed [5].

Xist RNA expression is followed by recruitment of polycomb group proteins to the *Xist* RNA coated X chromosome [22,25]. Polycomb group proteins are necessary to maintain X chromosome inactivation throughout cell division. Indeed, the presence of polycomb group proteins such as polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2) triggers a number of epigenetic modification in the X chromosome that result in a heterochromatin that is inaccessible for transcription [22]. Most notably, the inactive X chromosome is marked by histone H3 methylation at lysine 9 and lysine 27, and hypoacetylation of histones H3 and H4 [26]. In addition, promoter sequences in the X chromosome are heavily methylated resulting in transcriptional silencing [27]. Both histone modifications and promoter DNA methylation that characterize the inactive X chromosome are heritable changes that are carried over and maintained throughout cell division.

This DNA methylation suppresses gene expression by a number of mechanisms. These include stearic hindrance of transcription factor binding, and recruitment of methylcytosine binding proteins resulting in an increase in chromatin density [28]. Methyl CpG binding protein 2 (MECP2) binds methylated DNA and recruits histone deacetylases, which by deacetylating histone tails, increase the charge attraction between methylated DNA and histones, therefore increasing chromatin density resulting in chromatin that is transcriptionally inaccessible.

Therefore, DNA methylation is thought to play a central role in maintaining and stabilizing X chromosome inactivation. Indeed, treating proliferating cells with the DNA methylation inhibitor 5-azacytidine, results in reversal of X inactivation and gene expression from the previously inactivated X chromosome [29]. These data imply that if DNA methylation is defective, then a potential exists for reactivating the inactive X chromosome, resulting in duplication of gene expression levels of X chromosome genes in females but not in males who only have one X chromosome.

Can a gene dose effect on the X chromosome, therefore, explain the predominance of lupus in females? Indeed, we believe the answer is yes. In fact, DNA methylation is defective in CD4 + T cells from lupus patients [30]. Do T cells from female lupus patients over-express X chromosome genes compared to male lupus patients and compared to normal females? Before we discuss this, we would like to summarize the literature that supports a central role for defective T cell DNA methylation in the pathogenesis of lupus.

DNA methylation is an enzymatic reaction that refers to the addition of a methyl group to the 5th carbon in the cytosine ring within CG dinucleotide pairs. This process is mediated by a group of enzymes, known as DNA methyltransferases. DNA methyltransferases 3a and 3b are responsible for establishing the pattern of DNA methylation *in utero* and are known as *de novo* DNA methyltransferases [31]. On the other hand, the DNA methyltransferase 1 maintains DNA methylation patterns during mitotic cell divisions [32–34]. In lupus CD4+ T cells, DNA is globally hypomethylated, resulting from reduced expression of DNA methyltransferase 1 [35,36]. Indeed, T cells from active lupus patients over-express a number of methylation sensitive genes such as *ITGAL* (CD11a), *PRF1* (perforin), and *TNFSF7* (CD70) [37]. Promoter sequences of the aforementioned genes are hypomethylated in lupus T cells, and become hypomethylated to the same extent in normal T cells treated with DNA methylation inhibitors such as 5-azacytidine [30]. Indeed, normal human T cells treated with 5-azacytidine over-express the same genes (CD11a, CD70, and perforin), similar to T cells from active lupus patients [30], and become autoreactive *in vitro* [38]. T cells treated with 5-azacytidine are

capable of killing autologous or syngeneic macrophages [38,39], and induce immunoglobulin production in co-culture assays with autologous B cells [40]. Further, T cells treated with 5-azacytidine results in autoimmunity when adoptively transferred into syngeneic mice [41]. These mice develop a lupus-like disease characterized by glomerulonephritis, alveolitis, CNS disease, and anti-histone and anti-dsDNA autoantibody production [41]. Studies from the MRL/lpr lupus-prone mouse also demonstrate defective DNA methylation and reduced Dnmt1 expression in CD4+ T cells, and over-expression and hypomethylation of CD70 gene [42]. These data powerfully support an important role for defective T cell DNA methylation in the pathogenesis of lupus.

CD40L is a T cell co-stimulatory molecule that plays an important role in T cell-B cell interaction. Indeed, the interaction between CD40L on T cells and CD40 on B cells is important for immunoglobulin class switching [43]. Further, CD40L-CD40 ligation is involved in B cell proliferation, germinal center formation, and increasing the sensitivity of B cells to cytokine stimulation. CD40L is over-expressed on both T cell surface and in the serum in lupus patients [44–46]. Serum CD40L levels correlate with both anti-dsDNA production and disease severity in lupus patients [46]. The gene encoding for CD40L is located on the X chromosome. Gene dose balance of CD40L expression in normal males (46,XY) and females (46,XX) is maintained by random X chromosome inactivation in female cells. This is maintained by epigenetic changes including heavy DNA methylation of the inactive X chromosome as discussed above. In fact, the CD40L promoter sequence is demethylated in normal male CD4 + T cells, while females have one methylated and one demethylated alleles, reflecting random X chromosome inactivation (as reflected by heavy methylation) in one of the two X chromosomes [45]. When normal CD4+ T cells are treated with the DNA methylation inhibitor 5-azacytidine, the normally methylated CD40L allele demethylated and the expression of CD40L is doubled in T cells from females compared to males [45].

The normal physiology described for CD40L is disrupted in patients with SLE, however. In CD4+ T cells from active lupus patients, both CD40L alleles are demethylated and CD40L is expressed as twice as much as compared to male lupus patients, or normal female CD4+ T cells [45]. This suggests an explanation for the gene dose effect observed on the X chromosome in lupus patients; and, therefore, may be part of the explanation for the higher prevalence of lupus in patients with Klinefelter's syndrome that is discussed earlier. Since DNA methylation, and therefore X inactivation is defective in lupus patients, male patients with Klinefelter's syndrome (47,XXY) have two CD40L alleles that are available for transcription, similar to female patients (46,XX). By this mechanism, males with Klinefelter's syndrome (47, XXY) may be at a risk of SLE similar to females, and considerably higher than in karotypically normal males (46,XY).

Another example for an X chromosome gene copy number excess resulting in autoimmunity is the duplication of TLR7 in mice. In BxSB mice, the presence of the *Yaa* genetic locus on the Y chromosome is implicated in accelerated autoimmunity in male mice [47]. Recently, it was discovered that the *Yaa* locus is a 4-megabase genetic region at the telomeric end of X chromosome that is translocated onto the Y chromosome in *Yaa* bearing mice [48,49]. Therefore, BxSB mice have two copies available for transcription of this genetic element – one on the X chromosome and the other on the Y chromosome; whereas, females have one copy since one X chromosome is randomly inactivated. The gene in this locus that is responsible for the autoimmune phenotype acceleration in male BxSB mice was confirmed to be TLR7. Indeed, the expression of TLR7 was increased by twofolds in CD19 B cells from *Yaa* strains compared with B6 controls [49]. To confirm that TLR7 duplication is responsible for the *Yaa* effect, transgenic mice that over-express TLR7 were generated and showed that increased TLR7 dosage was sufficient to induce autoimmunity [50].

In human lupus, however, TLR7 duplication has so far not been evident in a study of 99 unrelated SLE patients [51]. We have studied 22 SLE men from 11 families in which at least 2 men have SLE. So, these men all share a Y chromosome with one other man with SLE. We did not find a translocation of a segment of the X chromosome containing the TLR7 gene on to the Y chromosome in these men with familial male SLE. Thus, a *yaa* gene equivalent among men with is either not present at all, or is very uncommon.

5. X chromosome genetics

Our knowledge about DNA methylation and X chromosome in lupus, would suggests that a genetic polymorphism on the X chromosome might help explain the female predominance in this disease. One candidate gene for such an effect is methyl CpG binding protein 2 (MECP2) which is located on chromosome Xq28. This gene product plays a critical role in mediating the repressive effect of DNA methylation on transcription. MECP2 binds methylated CG dinucleotides and recruits histone deacetylases, which induce a chromatin configuration that is inaccessible for transcription. Further, MECP2 recruits DNA methyltransferase 1 and therefore allows for DNA methylation outside of the replication fork during DNA synthesis [52].

We have studied single nucleotide polymorphisms (SNPs) within the *MECP2* gene that were genotyped in two large cohorts of lupus patients and controls. These studies established and confirmed that the genetic association between *MECP2* and lupus is two ethnically divergent lupus cohorts [53]. These data, might help explain the observed over-expression of methylation sensitive genes in lupus T cells, and potentially explain the predominance of lupus in females. A genetic association with one non-synonymous SNP in interleukin-1 receptor-associated kinase 1 gene (*IRAKI*) has been recently reported in childhood-onset lupus [54]. *IRAK1* is located on Xq28 and is closely located to *MECP2*. *IRAK1* is also an interesting candidate gene for lupus, as it has important functions in the innate immune response.

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