Intragenomic Conflict and Immune Tolerance: Do Selfish X-Linked Alleles Drive Skewed X Chromosome Inactivation?

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Abstract

In mammalian females, diploid somatic cells contain two X chromosomes, one of which is transcriptionally silenced, in a process termed X chromosome inactivation (XCI). Whereas XCI is largely random in placental females, many women exhibit skewed XCI (SXCI), in which the vast majority cells have the same X chromosome inactivated. SXCI has serious health consequences, associated with conditions ranging from Alzheimer’s to various autoimmune disorders. SXCI is also associated with outcomes of pregnancies, with higher rates of recurrent spontaneous abortion in women with SXCI. Here, I suggest that SXCI could be driven by selfish X-linked alleles. Consistent with the association of SXCI with autoimmunity, I first note the possibility that recurrent spontaneous abortion could reflect immune rejection of fetuses inheriting alleles from the largely silenced maternal X chromosome. Preferential abortion of fetuses carrying silenced X-linked alleles implies a transmission advantage for X-linked alleles on the largely expressed chromosome, which could drive the emergence of X-linked alleles that make the chromosome resistant to XCI. I discuss the evolutionary dynamics, fitness tradeoffs and implications of this hypothesis, and suggest future directions.

Key words: intragenomic conflict, dosage compensation, epigenetics.

Introduction

X chromosome inactivation (XCI) in XX female mammals involves terminal transcriptional silencing of one of the two X chromosomes in each cell during early development (Lyon 1961). Patterns of XCI differ across mammalian lineages (Dupont and Grimbau 2013). In marsupials, the paternal X chromosome is preferentially silenced in the soma. In humans, XCI is stochastic, with no clear parent-of-origin effect. In mice, XCI varies across tissues, with stochastic XCI in embryonic tissues but paternal XCI in extraembryonic tissues including the placenta (see Haig 2006 for a treatment of intragenomic conflict [IGC] and the evolutionary origins of XCI systems). Stochastic XCI is often described as "random"; however, in humans and mouse some females can show skewed XCI (SXCI) with the same chromosome being silenced in most cells. In a few percent of women, skew can reach 95% (Brown and Robinson 2000; Sharp et al. 2000) (while various authors prefer different cutoffs for SXCI, the dynamics described here do not depend on particular cutoffs in obvious ways).

SXCI can arise in several ways. SXCI can arise either at the time of initial XCI (called “primary” SXCI) or due to differential survival of cells expressing the two different X chromosomes (“secondary” SXCI; Plenge et al. 2002). Primary SXCI may be due to random chance, due to the inherent stochasticity involved in random inactivation at an early developmental stage with few cells (stochastic primary SXCI), a likelihood that may be increased by loss of aneuploid cells (Lau et al. 1997).

Primary SXCI may also arise due to genetic differences between the X chromosomes leading to different propensities to inactivation (genetic primary SXCI). Secondary SXCI is generally thought to arise due to differences in survival of cells expressing the two X chromosomes. For instance, if one of the two X chromosomes contains a strongly deleterious X-linked allele, cells expressing this X chromosome may have a lower survivability, leading to a preponderance of surviving cells expressing the other chromosome. Notably, this can sometimes ameliorate disease symptoms by leading to a preponderance of cells expressing the nonmutant chromosome, for example as in Rett Syndrome (Plenge et al. 2002).

The current argument focuses on genetic primary SXCI. The best characterized case of genetic primary SXCI occurs in Mus musculus and related species, in which mild to moderate SXCI is largely determined by a single X-linked locus called Xce (Cattanach and Papworth 1981; Calaway et al. 2013).
Heterozygotes with different Xce allele pairs show predictable SXCI, with straightforward hierarchies of dominance with respect to propensity to chromosome inactivation (Cattanach and Papworth 1981; Calaway et al. 2013). The extent of the genetic contribution to SXCI skew in humans is more poorly understood, although some findings suggest the possibility of heritability (Pegoraro et al. 1997; Renault et al. 2007; Wong et al. 2011). For instance, it was found that females with autism who show SXCI are more likely to have mothers with SXCI (Talebizadeh et al. 2005).

In humans, SXCI is associated with a myriad medical conditions, including ovarian and esophageal cancers, autism, Rett syndrome, Alzheimer’s, Klinefelter Syndrome, mental disabilities, neurodevelopmental disorders, diseases of metabolism, and others conditions (Plenge et al. 2002; Brix et al. 2005; Iitsuka et al. 2001; Ozbalkan et al. 2005; Ozcelik et al. 2006). Two associations are of particular importance for the current argument. First, autoimmune disorders are more frequent in women with SXCI (Stewart 1998). Briefly, Stewart argues that because generation of self-tolerance to proteins encoded by an allele requires expression in the thymus, reduced expression of alleles on a largely silenced X chromosome could lead to reduced self-tolerance of these alleles, leading to autoimmune responses to these proteins (fig. 1). (See Ngo et al. 2014 for a recent review of these and other issues contributing to sex biases in autoimmune disorders.) Second, some data indicate that SXCI in a mother affects the outcome of pregnancies (Lanasa et al. 1999; Sangha et al. 1999). For instance, two meta-analyses have concluded that SXCI is associated with a higher degree of recurrent spontaneous abortions (Su et al. 2011, 2015), and other work suggests an association between maternal SXCI and homosexuality in biological sons (Bocklandt et al. 2006), although further work on both questions is certainly needed.

These considerations suggest that fetuses inheriting more highly expressed X-linked alleles could experience lower levels of spontaneous abortion and therefore enjoy a transmission advantage (fig. 1). This advantage could lead to emergence of X-linked alleles that render X chromosomes resistant to XCI.
leading to SXCI-favoring alleles. Here, I develop this argument and consider various potential consequences including reduced overall maternal fitness and consequent emergence of suppressors of SXCI, effects on sexual orientation, and alleles promoting secondary reinforcement of SXCI. I conclude by discussing predictions of the model, placing the model in the context of general models of IGC, and suggesting future directions.

**Immune Tolerance and the Skewed Reproductive Outcome of SXCI**

Transplant rejection is a major barrier to various medical procedures, and may occur even when the tissue donor is a close relative. Particularly in light of the fact that 50% of a fetus' genes are foreign to the mother, a successful pregnancy may thus be seen as a rarity—a successful allograft. However, many pregnancies do not succeed, and a substantial fraction of spontaneous abortions, particularly in cases of recurrent spontaneous abortion, may involve immune rejection (Guerin et al. 2009).

The associations of SXCI with both autoimmune disorders and recurrent spontaneous abortion raise the question of whether these two associations might be related. The most compelling hypothesis for the observed association between SXCI and autoimmune disorders is that proteins expressed from the primarily inactivated X-chromosome are incompletely tolerated, leading to an autoimmune response (Stewart 1998; Kast 1977; Chitnis et al. 2000). This has potentially important implications for pregnancy, given that each targeted X-linked allele has a 50% chance of being inherited by a given fetus. If fetal expression of these targeted alleles triggers the maternal immune response, SXCI could lead to a higher rate of spontaneous abortion (and other complications) specifically targeting fetuses carrying large numbers of alleles from the silenced X chromosome. Note that this explanation differs from previous suggestions that deleterious X-linked alleles would drive both SXCI and lower survivability of male fetuses inheriting the deleterious allele(s) (Lanasa et al. 1999) or to problems in oogenesis including increased production of aneuploid offspring (Robinson et al. 2001).

**Transmission Advantage and the Origins of SXCI**

These considerations suggest that an X-linked mutation that leads the chromosome to be resistant to XCI, thereby leading to SXCI by preferential silencing of the other (nonmutant) chromosome, could gain a transmission advantage. In particular, if X chromosomes carrying such an SXCI-favoring allele were preferentially expressed, their alleles would be preferentially tolerated both in the female and in fetuses, leading to greater success of pregnancies. Notably, females homozygous for the SXCI-favoring allele would be expected to return to rough balance of XCI (as is seen among Xce alleles in mice, Cattanach and Papworth 1981), so these individuals would not suffer reduced pregnancy success. Thus, as with many similar demonstrated mechanisms of transmission advantage by “spite,” SXCI-favoring alleles would not act spitefully towards other copies of the same allele.

At the same time, it is worth noting that this mechanism for transmission advantage is not completely airtight. Because of meiotic recombination between X chromosomes, fetuses carrying the SXCI-favoring allele would be expected to also carry alleles from the opposite X chromosome at genomically distant loci. However, given the overall small number of observed chiasmata per chromosome per generation (e.g., Broman et al. 1998), fetuses that maternally inherit the SXCI-favoring allele would also inherit mostly highly-expressed alleles across the X chromosome, and thus would be expected to experience less immune rejection on average than fetuses that inherit the wildtype (nonSXCI-favoring) allele (see Box 1 for an explicit treatment of these issues).

**Implications for Evolution of Linked and Unlinked Loci**

**Suppressors of SXCI**

IGC occurring at one locus in a genome can have important implications for the evolution of the rest of the genome. For instance meiotic drive of sex chromosomes can lead to emergence of autosomal or sex-linked suppressors, and the emergence of sexually antagonistic alleles in regions flanking sex-determining loci can lead to local recombination suppression (Charlesworth 1991). How is an SXCI-favoring allele expected to influence evolution of the genome?

The first possibility is straightforward. Since a higher rate of immune rejection of fetuses will very likely reduce overall maternal fitness, a cost shared by the entire genome, suppressors of SXCI could emerge across the genome (This case is similar to the case of genome-wide selection for meiotic drive suppression, Tao et al. 2007.) The second possibility is more subtle. The advantage of the SXCI-favoring allele is dependent on immunogenic differences between proteins expressed from alleles from the two different X chromosomes: In each female heterozygote for the SXCI-favoring allele, the SXCI-favoring allele gains a transmission advantage insofar as the immunological self tolerance to the alleles on the commonly-expressed X chromosome (on which it falls) does not cover alleles from the other (rarely-expressed) X chromosome. The alleles at other loci that are in cis to the SXCI allele also increase their transmission advantage insofar as they differ from the allele on the other X. However, this is a double-edged sword, since the same allele will impose an additional cost when it is in trans to the SXCI allele. As such, a novel X-linked allele will only be clearly favored when it arises in cis and tightly linked to the SXCI allele.
Tradeoffs between reduced maternal fitness, leaky immune targeting and transmission advantage

I here derive the conditions under which an SXCI-causing allele that induces an increased rate of spontaneous abortion in heterozygotes will be favored, considering the overall increase in the abortion rate (a), the difference in the rate of spontaneous abortions of fetuses carrying and not carrying the SXCI-causing allele (2k), the overall reduction in maternal fitness (s), and the degree of recombination across the entire X chromosome (R).

If SXCI causes increased fetal immune rejection in mothers heterozygous for a SXCI-causing allele, resulting in an increase a in the rate in spontaneous abortions across all fetuses, with increases of a(1 – k) and a(1 + k), respectively, for fetuses carrying and not carrying the SXCI-causing allele, then the fraction of offspring born that carry the SXCI-causing allele is (1 – a(1 – k))/(2 – 2a) = 1/2(1 + (ak)/(1 – a)), or a fraction ak/(1 – a) greater than under equal transmission. If SXCI causes the mother an overall reduction in fitness of s, then the SXCI-causing allele enjoys an overall fitness advantage if and only if (1 – s)(1 + (ak)/(1 – a)) > 1, which can be rewritten as a > s/(s + k – sk) (eq. 1).

In the model, the increase in spontaneous abortions is due to fetal inheritance of alleles on the lowly-expressed X chromosome, which are poorly tolerated. Fetuses carrying and not carrying the SXCI-causing allele have different susceptibilities to abortion because on average they inherit different proportions of the lowly-expressed X chromosome. This imbalance is due to loci near the SXCI locus being cotransmitted, thus the extent of the skew is a function of the fraction of the X chromosome that is nonrecombinant (pnr). To calculate the expected pnr, consider that the probability that a locus a distance x away from the SXCI-determining locus is within the nonrecombinant region is e⁻rx, where r is the recombination rate per unit length. The average length of the nonrecombinant region in each direction from the SXCI-determining locus is then ∫₀⁻∞ e⁻rdr = (1 – e⁻rx)/r and ∫₀⁻∞ e⁻rdr = (1 – e⁻r(l⁻x)/r for a locus a fraction l of the way along the chromosome from the nearest chromosome end. If the total recombination across the chromosome is Nr = R, the fraction of the chromosome within the nonrecombinant region is equal to pnr = (2 – e⁻Rx – e⁻R(l⁻x))/R. These values are plotted for a SXCI-determining locus at the middle and end of the X chromosome in figure 2A.

Notably, assuming that spontaneous abortion is elevated roughly in proportion to the number of immune-targeted X-linked alleles carried by a fetus, the relative rates of spontaneous abortion of fetuses carrying and not carrying the SXCI-causing allele are 1 – pnr and 1 + pnr, which implies that pnr = k. Combining with equation (1) thus yields critical values for a relative to the overall reduction in maternal fitness s and the degree of recombination across the X chromosome R (fig. 2B).

Reinforcers of SXCI

Another possibility for the origin of SXCI is that differential immune tolerance of the two X chromosomes could be produced by an X-linked allele whose expression led to greater cell survival in the thymus, producing secondary SXCI specifically in the thymus. However, by leading to immune targeting of an X chromosome that is expressed in half of cells outside the thymus, such an allele would be expected to lead to even greater autoimmune complications. Still, on the genetic background of a primary SXCI driver, additional mutations leading to increased SXCI in the thymus could enhance the transmission advantage.

Tradeoff between Transmission Advantage and Reduced Maternal Fitness

Notably, while an SXCI-favoring allele may gain a transmission advantage, it is also expected to suffer somewhat from the reduction in overall individual maternal fitness. First, overall maternal health and thus fitness may be reduced: SXCI may promote autoimmune conditions, and in addition overall fitness may be reduced insofar as SXCI uncovers deleterious mutations by rendering them effectively haploid. Second, fetuses carrying the SXCI-favoring allele may also carry alleles that provoke a maternal immune response either due to chance inheritance from the father, or because of meiotic recombination on the X chromosome (see above, and Box 1).

SXCI, Birth Weight, and the Biology of Sexual Orientation

The notion that the state of the maternal immune system has implications for the outcome of pregnancies is by now widespread (Sangha et al. 1999; Blanchard 2001). In males, it has been found that weight at birth is negatively correlated with the number of previous male offspring of the mother (Blanchard and Ellis 2001), which could reflect increasing maternal immune response to male-specific proteins (e.g., male-specific histocompatibility antigen, HY, Blanchard and Ellis 2001). Similarly, homosexual males also tend to have a larger number of older biological brothers, and a contribution of immune-based response has also been proposed (Blanchard and Boggaert 1996). Other work has found that mothers of homosexual males exhibit more SXCI (Bocklandt et al. 2006).

How can these findings be synthesized in the current context? If SXCI leads to increasing strength of immune response with subsequent pregnancies, this could contribute to decreasing birth weight and phenotypic differences in later
births. The failure of the number of older sisters to predict male birthweight is not necessarily predicted by the current model, although it might be expected that older brothers would have a larger effect than older sisters since in sons all cells express maternal X alleles (as opposed to half of cells in daughters).

**Testable Predictions of the Model**

The current model makes several testable predictions. First, if offspring carrying lowly-expressed alleles are more likely to be spontaneously aborted, we would expect to see an overrepresentation of maternally-inherited highly-expressed alleles among surviving offspring, a test that could be performed in humans as well as mice, but which has not to my knowledge. We might also expect to see lower birth weights to mothers with SXCI, and particularly among offspring carrying lowly-expressed maternal alleles. Finally, if intolerance of lowly-expressed alleles increases with the number of previous pregnancies with fetuses carrying these alleles, we would expect that the genotypes of older siblings would influence rates of spontaneous abortions and birth weights.

**SXCI and Other Forms of IGC**

The current model clearly draws inspiration from a wide variety of work on IGC (e.g., Tao et al. 2007; Moore and Haig 1991; Haig 1996; Burt and Trivers 2009); however, the site of the conflict here differs from some previous models. Whereas success and resource allocation in pregnancy often focuses on conflicts between the maternal and paternal alleles in the fetus (Haig 1993), this model posits conflict between the two maternal alleles. This model does echo models that envision alleles expressed in the mother that specifically favor fetuses inheriting the same allele. For instance, Haig argued for positive green-beard interactions across the placenta for an allele shared between mother and fetus (Haig 1996). Similarly, in the “zygotic drive” model of Rice et al. (2008), competition between sex chromosomes in the heterogametic sex could lead to sex-linked alleles that harm offspring carrying the opposite sex chromosome.

The current model also stands at a rare position vis-a-vis mechanisms of IGC. Although allele-specific expression and transmission advantage are recurrent themes in IGC, the two are often treated separately. IGC models invoking allele-specific expression generally focus on somatic phenotypes that increase individual or inclusive fitness without conferring a direct transmission advantage (Moore and Haig 1991; Haig 1996), while IGC models of transmission advantage generally focus on germline processes that generally do not increase (and sometimes decrease) overall fitness (Tao et al. 2007; Burt and Trivers 2009). In the current model, these two themes are intertwined, as the model envisions that chromosome-wide biases in allelic expression could lead to indirect transmission advantage.

**Conclusions and Future Directions**

Given the importance of SXCI for a variety of diseases and complications of pregnancy, it is crucial to understand the potentially varied causes and mechanisms by which SXCI arises and the mechanisms by which it affects phenotype. The current model makes a number of predictions that are testable by relatively straightforward assays. In particular, studying immunological and reproductive differences in female mice with genetically-driven SXCI could answer important questions as to the broader phenotypic consequences of SXCI. Population genomic studies of regions implicated in...
SXCI may help us to understand the evolutionary forces driving SXCI-favoring alleles.

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