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Review

Chromosome Imbalance as a Driver of Sex Disparity in Disease

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Abstract

It has long been recognized that men and women exhibit different risks for diverse disorders ranging from metabolic to autoimmune diseases. However, the underlying causes of these disparities remain obscure. Analysis of patients with chromosomal abnormalities, including Turner syndrome (45X) and Klinefelter syndrome (47XXY), has highlighted the importance of X-linked gene dosage as a contributing factor for disease susceptibility. Escape from X-inactivation and X-linked imprinting can result in transcriptional differences between normal men and women as well as in patients with sex chromosome abnormalities. Animal models support a role for X-linked gene dosage in disease with *O-linked N-acetylglucosamine transferase* (*OGT*) emerging as a prime candidate for a pleiotropic effector. *OGT* encodes a highly regulated nutrient-sensing epigenetic modifier with established links to immunity, metabolism and development.

Key words: dosage compensation, Turner syndrome, Klinefelter syndrome, imprinting, O-GlcNAcylation

Introduction

Men and women have different susceptibilities for a number of diseases including cognitive disorders, metabolic, cardiovascular and autoimmune disease. Although sex hormones account for some of the differences in predisposition to disease, it is unlikely to be the only causative factor. Intriguingly, observations of patients with sex chromosome abnormalities have implicated gene dosage of X-linked genes as a potential factor contributing to sex disparities in disease development. For example, a subset of Turner syndrome females have increased prevalence of cognitive disorders, visceral adiposity and atherogenic lipid profiles similar to men, whereas Klinefelter males have a predisposition to systemic lupus erythematosus (SLE) similar to women.

Turner syndrome (TS) is a chromosomal disorder caused by complete or partial loss of an X chromosome (45X karyotype), occurring in ~1/2500 live born females [1]. TS females exhibit many characteristic features, including short stature, gonadal dysgenesis, and organ abnormalities [2]. Interestingly, manifestations of many TS associated features are quite variable. For example, congenital cardiovascular and renal defects, as well as deficits in neuropsychological function are present only in smaller subsets of patients [3, 4]. The wide range of phenotypes exhibited by TS females is largely due to the fact that there are a number of genes on the X chromosome that escape X-inactivation and are normally biallelically expressed in 46XX females. In humans, ~15% of X-linked genes escape X-inactivation [5] and presumably, haploinsufficiency for one or more of these genes could cause the variable phenotypes associated with TS.

About 60-80% of TS females inherit their X chromosome from their mothers (X^M) with the re-

maining having an X chromosome of paternal origin (X^P) [6-9]. Intriguingly, characteristics such as cardiovascular disease, lipid metabolism, visceral adiposity and cognitive function have been associated with the parental origin of the single X chromosome in TS patients [6, 9-19]. In other words, a TS patient has an increased likelihood of these features depending on whether her X chromosome was inherited from her mother or father. These observations suggest that there are unique genes expressed exclusively from either the X^M or X^P, and led to the hypothesis of X-linked imprinted genes [11].

Klinefelter syndrome (KS) is the most common sex chromosome abnormality in men, occurring in about 1 in 660 newborn boys. These males have an extra X chromosome with the karyotype 47XXY. KS is greatly under diagnosed. Only ~25% of men with KS are diagnosed, with the mean age of diagnosis in the mid-30s. KS men are tall, have a gynecoid body habitus and are hypogonadal [20]. One intriguing feature of KS men is that, similar to women, they are 14 times more likely to develop SLE than normal XY males [21, 22].

In this review we present observations from TS and KS patients that highlight the importance of X-linked gene dosage in the susceptibility of disease. Here, we focus on the parent-of-origin effects in TS and consider how the mouse has been used to model these TS-like effects. Additionally, we discuss how activation of genes on the silenced X chromosome in females could lead to sex disparities in the predisposition to lupus. Finally, we summarize the emerging evidence that differential expression of the versatile epigenetic regulator *O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT)* from the X chromosome may contribute to the gender disparities in disease susceptibility.

Sex chromosome dosage compensation

In mammals sex is determined by the presence or absence of a Y chromosome. Whereas females normally have two X chromosomes, males have an X and Y chromosome. Because of the imbalance in the number of chromosomes between males and females, mechanisms of dosage compensation have evolved. Dosage compensation is achieved in a two-fold manner in mammals; (1) by inactivation of one of the two X chromosomes in females [23] and (2) upregulation of X-linked genes to balance the expression levels between X-linked and autosomal genes [24-28].

Female cells undergo a process of random X-inactivation so that approximately half of the cells have an inactive X^M and half an inactive X^P. The process of X chromosome inactivation is largely mediated by the expression of the long non-coding RNA *Xist* [29, 30], which acts to recruit repressive complexes silencing one X chromosome [31]. DNA methylation is then necessary to maintain the silenced X chromosome [32, 33].

Despite being on the inactive X, many genes remain active. In humans, ~15% of genes on the X chromosome escape X-inactivation, whereas in mice only ~3-6% escape [5, 34, 35]. Some of these escape genes have a Y chromosome paralog, resulting in equal expression from both sexes, and homologous pairing during meiosis. These regions of homology between the sex chromosomes are known as pseudoautosomal regions (PAR) [36]. Other escape genes are expressed exclusively from the X chromosome, exhibiting higher expression in females [5, 34, 35]. Therefore, in TS patients, who only have one X chromosome, transcription of a number of genes is lower as compared to normal females. Conversely, in KS patients, who have two X and one Y chromosomes, transcription of genes in the PAR is greater than in normal males. Some of these genes have been directly correlated to specific disease characteristics. For example, haploinsufficiency of *SHOX* (short stature homeobox-containing gene) contributes to the short stature of TS females [37], however, overexpression of *SHOX* in KS males is associated with taller height [38].

In addition to X-inactivation, a second form of dosage compensation maintains a balance between X-linked and autosomal gene expression by doubling transcription from the active X chromosome [24-28]. Whereas little is known about the mechanisms coordinating upregulation of the X chromosome in mammals, this concept has been well documented in *Drosophila*. Similar to humans, *Drosophila* males and females are distinguished by their XY or XX karyotypes, respectively. In contrast to mammals, *Drosophila* males upregulate expression of their single X chromosome, and females maintain two active X chromosomes. In male somatic cells of *Drosophila* the male-specific lethal (MSL) complex, which is comprised of proteins and non-coding RNAs, is targeted to the X chromosome and is necessary for transcriptional upregulation. Importantly, the component males absent on the first (MOF) specifically acetylates histone H4 lysine 16 leading to opening of chromatin and increased expression [39]. Although further research is essential to elucidate the mechanisms of X-upregulation in mammals, insights from *Drosophila* can help in understanding the mammalian system. In fact, many of the MSL components have orthologues in humans, and MOF containing complexes are largely evolutionarily conserved [40].

X-linked imprinting

Genomic imprinting is an epigenetic phenomenon in which expression of a small subset of genes is dependent upon the parental origin of the chromosome [41]. Therefore, some genes will be expressed from the paternal allele, whereas others will be expressed from the maternal allele. One key feature of imprinted genes is the presence of differential DNA methylation that marks the parental origin of the allele [42]. Imprinting of autosomal genes has been well established and loss of imprinting of many genes causes human congenital diseases such as Beckwith-Wiedemann, Silver-Russell, Prader-Willie and Angelman syndromes [31, 43].

Due to the imbalance of X chromosomes between males and females, expression of X-linked imprinted genes would differ between the two sexes. While females have both a maternal and paternal X chromosome, males inherit only a maternal X. Therefore, if a gene were expressed exclusively from the paternal allele, it can only be expressed in females (figure 1A). However, if a gene were expressed from the maternal allele, expression levels would be higher in males than in females (figure 1B). The presence of imprinted X-linked genes could be one potential factor for differences in disease susceptibility between males and females.

One way to assess X-linked imprinting and its potential contributions to disease susceptibility is through analysis of TS patients. Phenotypic comparisons of TS females that are monosomic for X^M or X^P have given us insights into the potential role of X-linked imprinting in human disease and cognitive function. Additionally, the observed parent-of-origin effects in TS patients has led to the discovery of X-linked imprinted genes in mice. It should be noted, however, that due to small sample sizes and potential cryptic Y-chromosome mosaicisim of patients, there has been controversy as to whether phenotypes could be attributed specifically to parental origin of the X chromosome [44].

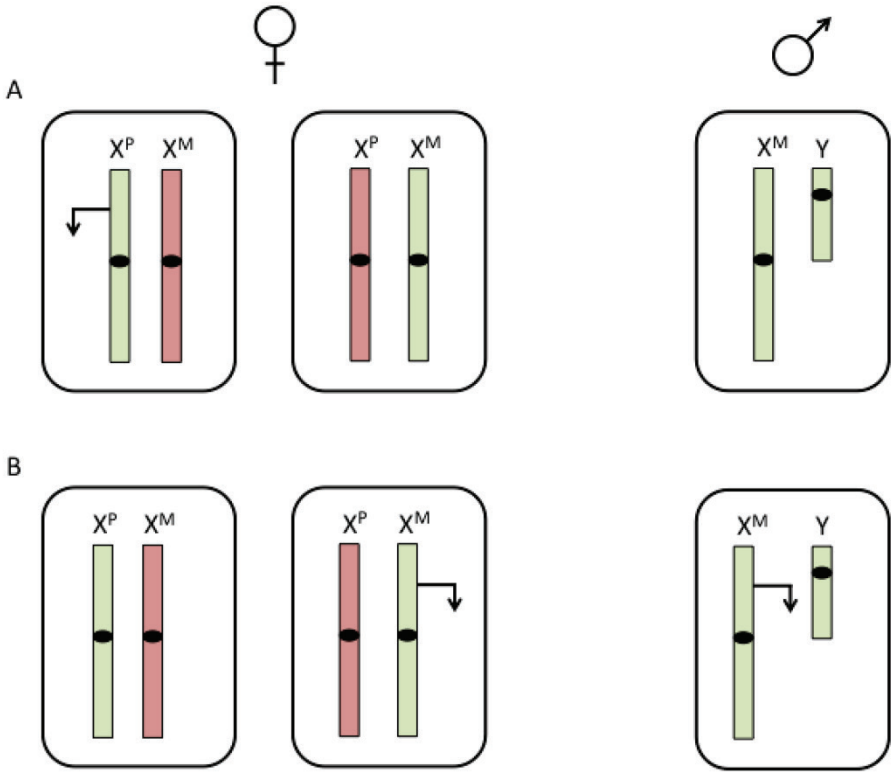

Figure 1. Expression of an X-linked imprinted gene. X-linked imprinting would result in differential expression between males and females. (A) A paternally expressed X-linked imprinted gene would only be expressed in about half of female cells and not expressed in male cells. (B) A maternally expressed X-linked imprinted gene would be expressed in all cells of a male and only half the cells of a female. The inactive X chromosome is represented in red, the active X chromosome is represented in green and transcription is represented by an arrow.

Parent-of-origin effects in Turner syndrome

Neuropsychological differences between X^M and X^P TS females

Impaired social cognition of TS females has been linked to the parental origin of the X chromosome. Using a questionnaire based approach to measure social cognition, it was reported that X^M individuals scored significantly higher on measures of social cognitive dysfunction as compared to X^P females. Similarly, normal males scored significantly higher than normal females, indicating poorer social cognition for both males and X^M TS females [11]. Accordingly, a diagnosis of autism appears to be found more predominantly in X^M versus X^P TS patients [11, 12]. X^M individuals also had a significantly lower verbal IQ and impaired behavioral inhibition [11]. However, a more recent study was unable to replicate the findings that X^M TS females had poorer social cognition in a larger but younger cohort of patients [45]. Intriguingly, an additional study assessing verbal skills found that X^P subjects rather than X^M individuals had a significant decrease in verbal skills [13]. Hence, it still remains inconclusive whether or not there are X-linked imprinted genes involved in social cognition and verbal abilities.

Parent-of-origin effects have also been implicated in long-term memory. Verbal forgetting rate had been measured by assessing the ability of subjects to recall a story after delay. X^M individuals were more likely to forget verbal material than X^P females [15]. Interestingly, using the Rey figure copying task, in which a subject is asked to draw a figure from memory, X^P individuals scored significantly lower than X^M individuals [15]. Therefore, whereas X^M females have deficits in verbal memory, X^P individuals have deficits in visuospatial memory [15].

Studies of brain morphology comparing X^M and X^P TS females have suggested that imprinted X-linked genes are involved in brain development and anatomy [14, 16, 17]. These studies indicated that X-linked imprinted genes act to influence development in a region-specific manner. For example, X^M females were reported to have a significant decrease in gray matter volume bilaterally in the caudate nuclei and thalamus, as well as decreased white matter volume in the temporal lobes [17]. Differences in volume have also been reported in the superior temporal gyrus in TS patients based on the parental origin of their X. Specifically, in X^M females the right and left superior temporal gyrus were significantly larger than that of X^P females [14, 16]. Similarly, a recent study has reported an increase in gray matter volume in the superior frontal and pericalcarine regions along with decreased white volume matter in the latter in X^M TS females [14]. This same study also reported a bilateral increase in cortical thickness in the temporal and parietal lobes of X^P TS individuals [14]. Although parent-of-origin effects are present in the brain morphology of TS patients, future research is necessary to determine if these morphological differences result in cognitive defects.

Eye disorders and hearing impairment in X^M and X^P females

Parent-of-origin effects have been described for eye disorders and sensorineural hearing loss, as both have significant increased prevalence in X^P as compared to X^M patients [9, 19]. The greater frequency of these disorders could potentially be due to differences in brain morphology between the two groups (as previously discussed) or due to monoallelic maternal expression of X-linked genes necessary for normal eye and hearing function.

Physical and structural anomalies in X^M and X^P females

Cardiac anomalies as well as neck webbing have also been associated with the parental origin of the X chromosome. X^M females were reported to have a significant increase in cardiac abnormalities and neck webbing as compared to X^P females [18]. The authors hypothesized that these features could be caused by abnormal development of the lymphatic system [18]. However, a more recent and larger study was unable to detect any significant differences in the presence of cardiac anomalies between X^M and X^P individuals [9]. Interestingly, Sagi et al. reported a significant increase in renal abnormalities in X^M females as compared to X^P females [9]. Similarly, Chu et al. observed more renal anomalies in X^M than X^P patients, though the differences were not statistically significant [18]. It therefore remains controversial as to whether some of the physical and structural anomalies associated with TS have a parent-of-origin component.

Adiposity and lipid metabolism in X^M and X^P individuals

In general, men have more visceral fat and atherogenic plasma lipids than women [46], making metabolic phenotypes a good candidate for X-imprinting effects. Young X^M patients have been observed to be significantly overweight as compared to X^P pediatric patients [9]. However, the X^P pediatric patients in this study were reported to have higher total and low-density lipoprotein cholesterol levels

[9]. A study of adult TS patients did not observe a difference in total body mass composed of fat, though the X^M group had higher levels of abdominal fat and visceral fat as compared to the X^P group [10]. Additionally, X^M adults had a more atherogenic lipid profile, with higher triglyceride and low-density lipoprotein cholesterol levels [6, 10]. The conflicting observations of these studies could be due to the difference in ages of the subjects. Nevertheless, both studies support a role for imprinting of X-linked genes in metabolic regulation.

Mouse models of sex chromosome imbalance

The 39XO mouse

Unlike their human counterparts, mice monosomic for the X chromosome (referred to as 39XO) appear grossly normal and fertile. These differences in phenotypes between 39XO mice and TS females is most likely due to the fact that only ~3-6% of genes escape X-inactivation in the mouse, whereas about ~15% escape in humans. Therefore, many more genes exhibit decreased expression in TS patients than 39XO mice. Nevertheless, these mice possess some more subtle phenotypes similar to TS characteristics.

The 39XO mice were developmentally delayed early in gestation and had a reduction in body weight as well as a decreased germ cell population [47-50]. Additionally, 39XO mice had a higher frequency of hearing loss and decreased thyroid activity and body temperature [4, 51]. Studying these mice has some advantages that could help clarify many of the controversies in the human literature; firstly, mice possess a single X chromosome without the potential of Y chromosome mosaiscism. Secondly, the use of mice has the benefit of studying large, age-matched populations on the same genetic background.

Parent-of-origin effects in 39XO mice

39XO mice can be derived such that they specifically inherit a maternal or paternal X chromosome ($39X^MO$ and $39X^PO$, respectively). $39X^PO$ mice are produced by mating a wild type male with a female containing a large inversion in her X chromosome (In(X)1h mutant) [4] (figure 2A). If crossing over within the large inversion occurs, the mother produces gametes without X chromosomes [52]. To generate $39X^MO$ mice, wild type females are mated with *Patchy fur* (*Paf*) mutant males (figure 2B). *Paf* males have a mutation at the PAR, which results in nondisjunction during spermatogenesis, producing sperm without sex chromosomes [4, 53].

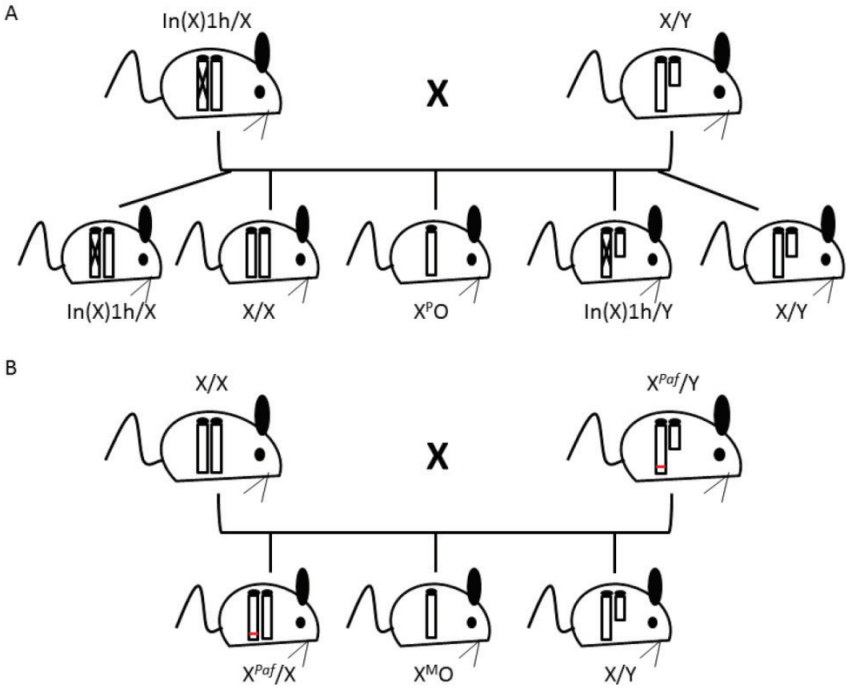

Figure 2. Mating schemes to derive monosomic X mice. (A) $39X^PO$ mice are derived by mating an In(X)1h mutant female (In(X)1h/X) with a wild type male. The In(X)1h sex chromosome contains a large inversion (represented by an X in the chromosome). If crossing over during meiosis occurs within the inversion, gametes without an X chromosome will be produced. (B) $39X^MO$ mice are derived by mating a wild type female (X/X) with a *Paf* mutant male (X^{Paf}/Y). The *Paf* mutation (represented by a red line) is located at the pseudoautosomal region and causes nondisjunction during spermatogenesis, producing sperm without a sex chromosome. Genotypes of mice are indicated below.

Parent-of-origin effects have been described in the 39XO mouse model. 39X^{P}O embryos had delayed postimplantation development, were smaller than 39X^{M}O females and displayed poor development of the ectoplacental cone [54, 55]. These observations suggest that preferential expression of genes from the X^{M} is necessary for normal embryogenesis. Interestingly, mice normally inactivate the paternal X chromosome in extraembryonic tissues [56]. Taken together, these data indicate that maternally expressed genes from the X chromosome could be required in extraembryonic tissues for normal development.

The 39XO model has also replicated some of the parental origin effects on cognitive function. Using a Y-maze based serial reversal learning paradigm, which tests the attentional and inhibitory processes required to switch from a pre-potent correct response to a previously incorrect response, 39X^{M}O mice displayed significant deficits in reversal learning as compared to 39X^{P}O mice [57]. These findings indicate that having only a maternal X chromosome led to difficulties in inhibiting response to a previously correct but now incorrect cue, and forming new associations with the previously incorrect but now correct cue [57].

Identification of X-linked imprinted genes in the mouse

Analysis of gene expression in 39X^{M}O and 39X^{P}O neonatal and embryonic mouse brains, has led to the identification of an X-linked imprinted gene cluster [57, 58]. Using Affymetrix microarrays *Xlr3b*, *Xlr4b* and *Xlr4c* were identified as being maternally expressed [57, 58]. The homologous region in the human, mapping to Xq28, contains a pseudogene with sequence homology to the *Xlr3* gene family and has a high concentration of loci involved in neurodevelopmental pathologies [57, 58]. This pseudogene is most closely related to another human X-linked gene, *FAM9B* [57]. Although expression status of *FAM9B* in the brain has not been determined, deletions within this gene have been described in cases of autism and schizophrenia [59, 60].

Another X-linked imprinted gene has been identified in the mouse by comparing gene expression profiles of male and female blastocysts [61]. The X-linked gene, *Rhox5,* was determined to be expressed only in female blastocysts. Interestingly, imprinting of *Rhox5* switches during development, with paternal expression observed from the 8-cell until the blastocyst stage and maternal expression observed at E7.5 [61]. *Rhox5* does not have an obvious human orthologue.

The 'four core genotypes' mouse model of X-linked gene dosage and disease susceptibility

Another mouse model that uncouples sex hormones from sex chromosomes has correlated X-linked gene dosage with metabolic disorders associated with sex chromosome abnormalities. In mammals, sex determination is largely controlled by dosage-dependent nuclear accumulation of the Y-linked gene *Sry*, an architectural transcription factor that serves as a 'trigger for maleness' [62, 63]. This property of vertebrate sex determination has been exploited in the development of a model referred to as the four core genotypes mouse model. With this model, the *Sry* gene has been deleted from the Y chromosome and a functional *Sry* transgene was inserted onto an autosome, thus uncoupling testes determination from the Y chromosome [64, 65]. This allows generation of mice that can be XX or XY with female gonads and mice that are XX or XY with male gonads. The normal patterns of dosage compensation and inheritance of X^{M}Y are expected to occur independent of gonadal phenotype. The four core genotypes mouse model has been used to tease apart the influence of sex chromosomes from sex hormones for a variety of traits. Interestingly, mice with two X chromosomes had a greater body weight and more body fat than mice with an X and Y chromosome, regardless of gonadal sex. Moreover, when placed on a high fat diet these XX mice were faster to gain weight, and developed fatty liver and insulin resistance [66]. These results suggest that X-linked gene dosage plays a role in metabolic disease risk, irrespective of sex hormone levels. Accordingly, a high prevalence of type 2 diabetes has been reported in TS women, as well as KS men [67, 68].

OGT as a candidate X-linked gene associated with TS phenotypes

Aside from confirmed X-linked imprinted genes, transcriptional analysis of 39XO mice, as well as normal male and female mice, has uncovered a number of genes that exhibit both sex disparities in gene expression and dysregulation with X monosomy [69, 70]. Of interest, the gene encoding OGT, located in humans at Xq13.1 and in mice at XqD, is one such gene. Being located on the X chromosome, *OGT* is under the control of dosage compensation mechanisms [71, 72] (figure 3). However, *Ogt* has been identified as having decreased expression in 39XO mouse liver as well as sex dependent differences in expression in liver, adipocytes and placenta [69, 70, 73], suggesting that *Ogt* is either imprinted or escapes X-inactivation in these tissues.

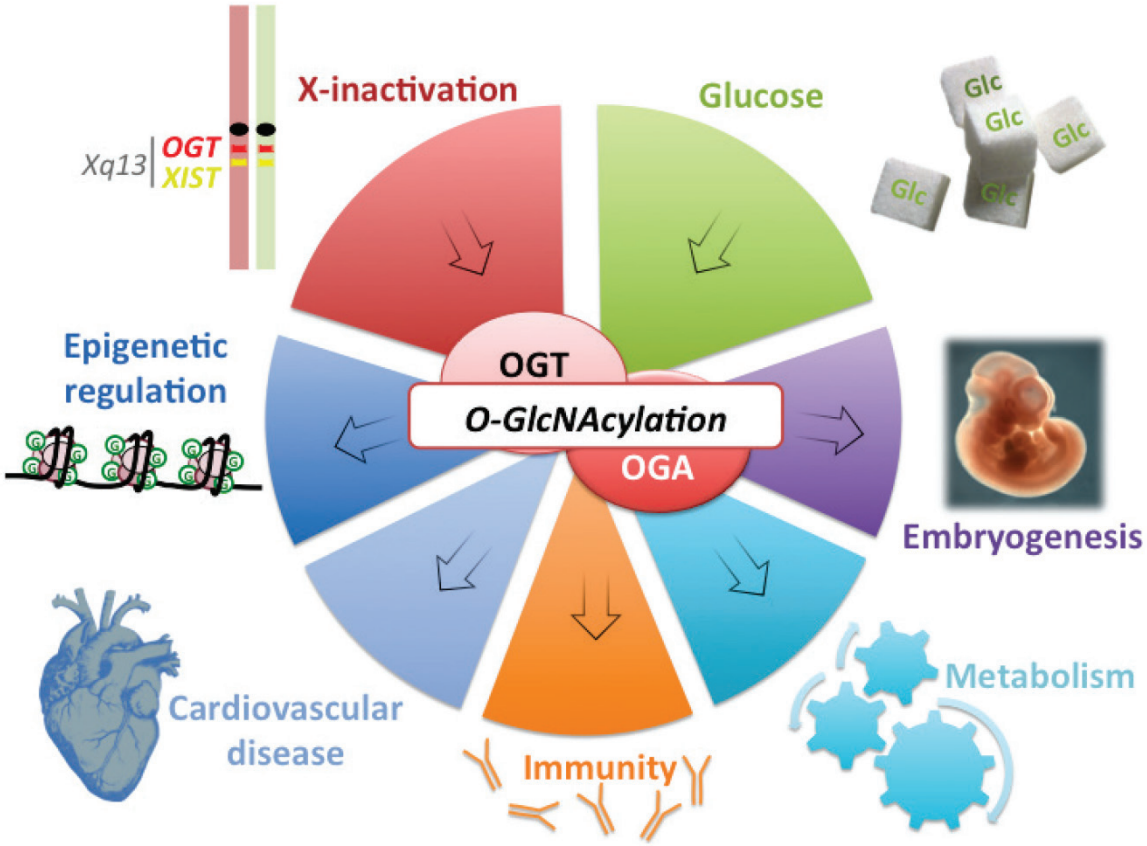

Figure 3. OGT is a good candidate connecting X-linked gene dosage to epigenetic regulation of metabolism and disease susceptibility. *OGT* is located on the X-chromosome, close to *XIST* and is subject to X-inactivation in mammalian females (silenced chromosome is depicted in red). Variation of *OGT* expression and the abundance of its substrate UDP-GlcNAc by glucose intake (Glc), modulate *O*-GlcNAc dynamism. This post-translational modification (represented by a green G) affects various processes from epigenetics to embryogenesis and immunity. *O*-GlcNAcylation is also involved in metabolism and cardiovascular defects, two of the major features of X-linked abnormalities.

OGT is a nutrient sensor that adds a single *O*-GlcNAc modification onto a variety of intracellular proteins. This modification is a key regulator of diverse cellular processes including, signal transduction, transcription and protein stability [74-76]. OGT utilizes the product of the hexosamine biosynthetic pathway, UDP-GlcNAc, as a sugar donor to add a GlcNAc moiety to serine and threonine residues of subsrates. This modification is removed by *O*-GlcNAcase (OGA or MGEA5). *O*-GlcNAcylation has been implicated in many human diseases such as type 2 diabetes, cancers, aging, cardiovascular and neurodegenerative diseases (figure 3) [74, 76]. Importantly, *O*-GlcNAcylation, and by extension *OGT* expression, plays a role in processes that contribute to parent-of-origin phentoypes in TS patients such as, gluconeogenesis, lipogenesis and cardiac metabolism [76-79].

OGT has recently emerged as a major epigenetic regulator. OGT has been found to complex with TET1,2,3 (regulators of DNA demethylation) [80-82], SIN3A and histone deacetylases (transcriptional repressors) [83], HCF-1 (transcriptional activator) [84, 85], MLL5 (histone methyltransferase) [86] and polycomb repressive complex 2 (PRC2) [87], in addition to being able to modify histones themselves [87-90]. Beyond interacting with epigenetic regulators, OGT modifies the C-terminal domain (CTD) of RNA polymerase II [91, 92]. Therefore, OGT is a general transcriptional regulator that could modulate diverse expression networks. Thus, potential dysregulation of *OGT* in TS patients could contribute to the variable phenotypes observed (figure 3).

Although OGT clearly acts as a key transcriptional regulator, how *OGT* itself is regulated to ensure proper gene dosage remains largely unknown. Because it is located close to *Xist* (figure 3), *Ogt* appears to be under tight transcriptional control in mice. Prior to widespread X-inactivation and upregulation of *Xist*, most X-linked genes are biallelically expressed in mouse embryonic stem (ES) cells. However, allelic analysis in mouse ES cells indicated that *Ogt* expres-

sion was skewed towards the paternal allele, suggesting that due to its close proximity to *Xist*, *Ogt* can be repressed prior to *Xist* upregulation [72].

Studies using *Drosophila* have also given us insights into potential regulatory mechanisms for human OGT. Evidence in *Drosophila* suggests that long introns can affect patterns of transcription and that *Ogt* expression is limited by splicing [93]. Similarly, the genes that encode OGT in mice and humans also contain unusually large introns. Moreover, many of the *Drosophila* dosage compensation components have orthologues in humans, and MOF containing complexes are evolutionarily conserved. One distinction between *Drosophila* MOF and human MOF complexes are the presence HCF-1 and OGT [40]. Thus, if human MOF has a role in upregulation of X-linked gene expression, OGT could be acting in a self-regulatory feedback loop.

Klinefelter syndrome and systemic lupus erythematosus

Women have an increased likelihood of developing autoimmune diseases, including SLE. In fact, about 90% of SLE patients are female [94]. One likely contributing factor is the hormonal differences between men and women. Estrogen exacerbates SLE in mouse models [95] and estrogen supplementation is associated with lupus flares in patients [96]. Nevertheless, sex hormones cannot be the only contributing factor for the sex differences in SLE as increased prevalence has also been observed in pre-pubertal girls and post-menopausal women [97, 98].

These observations have led to the hypothesis that a second X chromosome could predispose woman to SLE [22]. If this hypothesis were true, men with Klinefelter syndrome would be expected to develop SLE at a comparable rate to women. In fact, there is a 14-fold increase in the prevalence of SLE among men with Klinefelter syndrome as compared to men in the general population [21]. Therefore, males with an XXY karyotype are at a similar risk for developing SLE as females. Accumulating evidence suggests that activation of genes on the inactive X chromosome could contribute to the onset of SLE [22, 99, 100]. Thus, overexpression of X-linked genes could contribute to disease development particularly in women and KS men.

Aberrant DNA methylation patterns have been reported in CD4+ T cells from SLE patients [101-104], including hypomethylation of many X-linked genes [101]. It is therefore possible that demethylation on the silenced X chromosome could cause increased expression of X-linked genes contributing to autoimmunity in females and KS males. Accordingly, immune genes located on the X chromosome, including *CD40LG,* a T cell costimulatory molecule that plays an important role in T cell B cell interaction [105] and *CXCR3,* which encodes a chemokine receptor expressed in T cells [106], have been shown to be hypomethylated and overexpressed in women but not men with lupus [99, 100]. Of particular interest, *OGT,* which is required for T and B cell activation [107], was also identified as one of these hypomethylated/overexpressed genes [99, 100]. Tight regulation of dosage of these genes could be necessary for normal immune function. Thus, demethylation of these immune related genes on the inactive X chromosome could lead to increased expression, predisposing both women and KS men to SLE.

Conclusions and future directions

Observations from TS and KS patients highlight the importance of tight regulation of X-linked gene dosage and have given us insights into how dysregulation could contribute to sex disparities in disease development (figure 4). Parent-of-origin effects of the X chromosome in TS suggest the presence of genes whose expression depends on the parental origin of the allele. However, the confounding factors of cryptic mosaicism of the Y chromosome, and small populations of patients have made many of these studies difficult to replicate. Future studies with larger, aged-matched cohorts will be necessary to fully define parent-of-origin effects in TS. Nevertheless, these observations have led to the identification of X-linked imprinted genes in the mouse.

Further research focused on the identification of X-linked imprinted genes would not only help us tease apart the etiology of TS, but will also contribute to our knowledge of sex differences in the susceptibility to disease. Because males inherit only a maternal X, whereas females inherit both a maternal and paternal X, differences in gene expression from the X chromosome could contribute to sex specific disease risk. Interestingly, some of the features observed in X^M patients, including cognitive dysfunction, increased visceral adiposity and a more atherogenic lipid profile are also more common among males.

Although 39XO mice are grossly normal and fertile, parent-of-origin effects on cognition and the identification of an X-linked imprinted cluster in the brain make the mouse a good model for exploring X-linked imprinting. Previous studies using the 39XO mouse have focused on the brain for identification of X-linked imprinted genes [57, 58], however, many imprinted genes are tissue-specifically imprinted [108] and therefore further transcriptional analysis in a variety of tissues is warranted. Furthermore, it will

be important to ascertain the epigenetic mechanisms controlling X-linked imprinting. Whereas, allele-specific DNA methylation is a key feature for imprinting of autosomal genes [42], differential DNA methylation has not been identified at the *Xlr* imprinted cluster [109], suggesting a novel mechanism of regulation. Intriguingly, histone H1 depletion in the mouse resulted in upregulation of a number of imprinted genes, including *Xlr3b* and *Rhox5* [110]. It is therefore possible that histone H1 is involved in the epigenetic mechanism regulating imprinting on the X chromosome.

As a master regulator of diverse cellular processes *OGT* has emerged as an X-linked gene requiring a specific dosage for normal function. Although recent evidence suggests *OGT* overexpression contributes to SLE, it remains unknown if *OGT* is dysregulated in TS individuals. Because of the overlap between TS characteristics and known roles of OGT, particularly in cardiovascular function, gluconeogenesis, lipogenesis and brain development, further examination of *OGT* expression in these individuals would be of interest. Moreover, elucidating mechanisms involved in regulation of *OGT* is necessary to understand how dosage is properly maintained and how changes could contribute to disease progression. Of particular interest, the gene encoding OGT has recently been shown to have differential expression between male and female placental tissue in both mice and humans [73]. As a nutrient sensor, this sex dependent disparity in expression poises OGT to differentially interpret environmental cues early in development with potential consequences manifesting later in life.

Historically, it has been difficult to obtain the quantities and numbers of appropriate patient samples required to statistically power the identification of candidate X-linked genes contributing to disease phenotypes. However, continuously evolving technologies put us on the precipice of understanding the potential role of X-linked gene dosage in human disease. The use of primary patient fibroblasts to generate induced pluripotent stem cells followed by high-throughput transcriptional analysis will provide a platform for identifying X-linked genes that contribute to disease risk. The molecular features of products encoded by these genes will help us better understand the role of X-linked gene dosage in sex-dependent disease risk and may provide novel therapeutic targets.

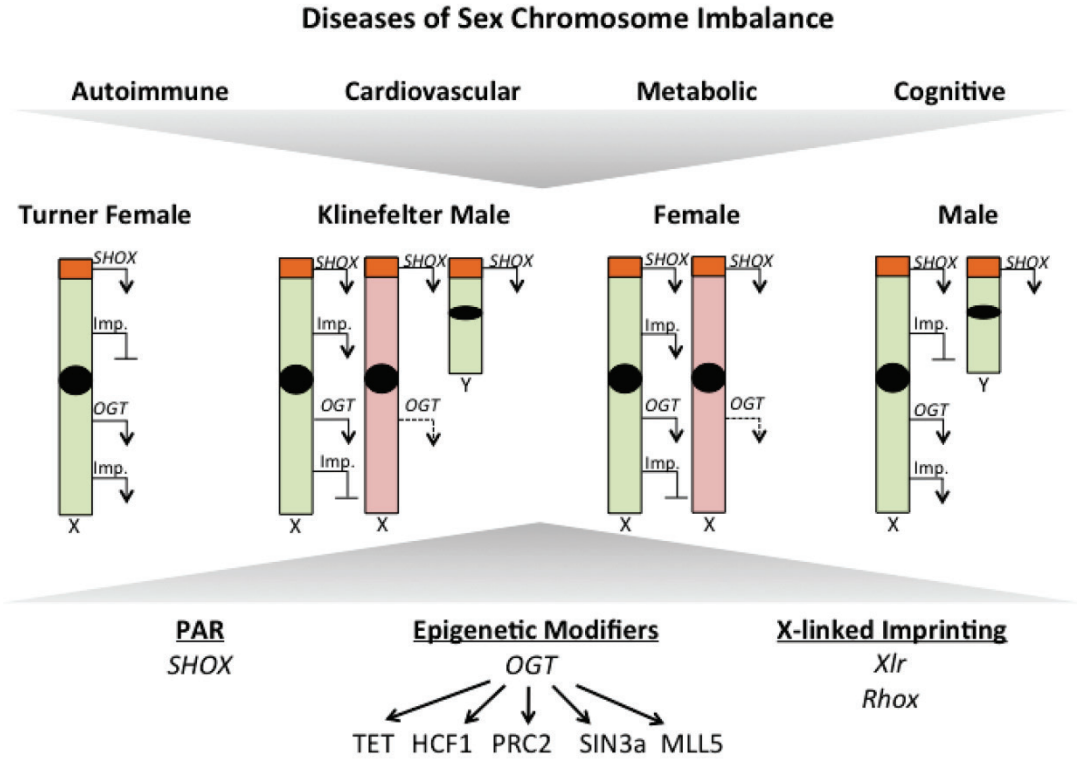

Figure 4. X-linked gene dosage contributes to sex disparities in disease. Observations from Turner and Klinefelter syndrome patients have uncovered a role for X-linked gene dosage in contributing to sex disparities in disease risk. Differences in expression of genes that escape X-inactivition, like those within the pseudoautosomal region, epigenetic modifiers, like *OGT*, and X-linked imprinted genes (Imp.) could all contribute to disease susceptibility. Active chromosomes are represented in green, inactive chromosomes are represented in red, transcription is represented with an arrow and loss of repression represented by a dotted arrow.

Abbreviations

TS: Turner syndrome; KS: Klinefelter syndrome; SLE: systemic lupus erythematosus; OGT: O-linked N-acetylglucosamine transferase; O-GlcNAc: O-linked N-acetylglucosamine; X^M: maternal X chromosome; X^P: paternal X chromosome; MOF: males absent on the first; MSL: male-specific lethal; SHOX: short stature homeobox-containing gene.

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Competing Interests

The authors have declared that no competing interest exists.

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