REVIEW

The multi-omic landscape of sex chromosome abnormalities: current status and future directions

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Abstract

Sex chromosome abnormalities (SCAs) are chromosomal disorders with either a complete or partial loss or gain of sex chromosomes. The most frequent SCAs include Turner syndrome (45,X), Klinefelter syndrome (47,XXY), Trisomy X syndrome (47,XXX), and Double Y syndrome (47,XYY). The phenotype seen in SCAs is highly variable and may not merely be due to the direct genomic imbalance from altered sex chromosome gene dosage but also due to additive alterations in gene networks and regulatory pathways across the genome as well as individual genetic modifiers. This review summarizes the current insight into the genomics of SCAs. In addition, future directions of research that can contribute to decipher the genomics of SCA are discussed such as single-cell omics, spatial transcriptomics, system biology thinking, human-induced pluripotent stem cells, and animal models, and how these data may be combined to bridge the gap between genomics and the clinical phenotype.

Key Words

- sex chromosome abnormalities
- Turner syndrome
- Klinefelter syndrome
- genetics

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Introduction

Sex chromosome abnormalities (SCAs) are chromosomal disorders with either a complete or a partial loss or gain of sex chromosomes. The most frequent SCAs include Turner syndrome (TS; 45,X) and the sex chromosome trisomies, Klinefelter syndrome (KS; 47,XXY), Trisomy X syndrome (47,XXX), and Double Y syndrome (47,XYY) (Fig. 1A) (1). SCAs are associated with an increased morbidity and mortality (1). Notably, the syndromes share overlapping comorbidities and phenotypic traits, indicating that shared genetic mechanisms may exist, although syndrome-specific genetic alterations may contribute to traits isolated to the specific syndrome. Understanding the genomic basis for SCAs is an ongoing quest, and today our understanding of how sex chromosome dosage alterations shape the genome and affect molecular pathways leading to the clinical phenotype of SCAs is still very limited. However, during the past decade, it has become clear that the molecular underpinnings of SCAs are complex. A picture of a profoundly genome-wide imbalance has been drawn, which goes far beyond the simple sex chromosomal gene-dosage theory.

In this review, we provide an updated summary of current insights into the genomics of SCAs and we discuss the future direction of research that can contribute to decipher the genomics of SCAs by bridging the gap between the genotype and the clinical phenotype.

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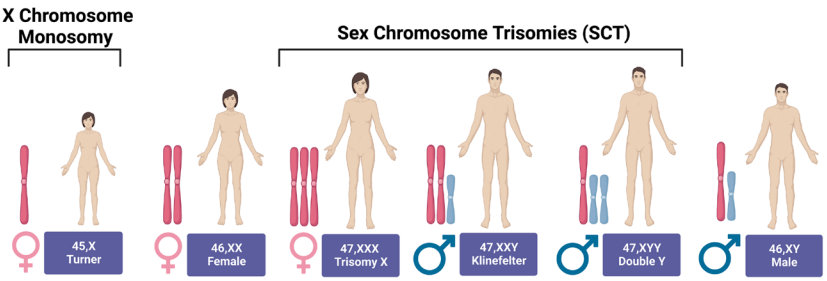

B

Karyotype	PAR1	X homolog	Y homolog	XCIE	XCI
X	1	1	0	1	1
XX	2	2	0	2	2
XXX	3	3	0	3	3
XXY	3	2	1	2	2
XYY	3	1	2	1	1
XY	2	1	1	1	1

C

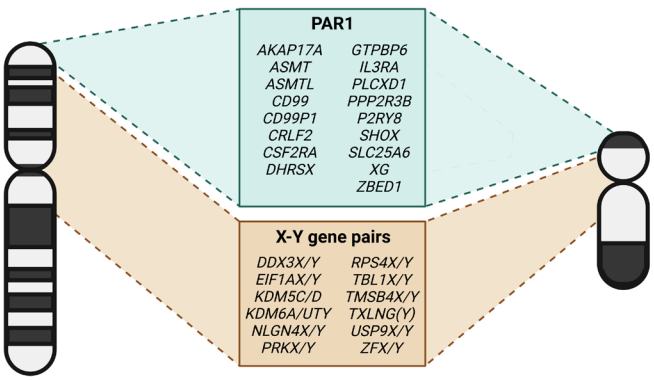

Figure 1
(A) The most frequent karyotype of Turner syndrome (TS; 45,X), Trisomy X syndrome (47,XXY), Klinefelter syndrome (KS; 47,XXY), and Double Y syndrome (47,XYY) is depicted together with karyotypically normal females (46,XX) and males (46,XY). The X chromosome and Y chromosome are shown in pink and blue color, respectively. (B) The copy number of PAR1 genes (genes located on the pseudoautosomal region 1), X and Y homologs, XCIE (genes that escape from X chromosome inactivation), and XCI (genes that are inactivated) according to karyotype. (C) The X and Y chromosome with depictions of the PAR1 genes and X and Y homologs. This figure was created with BioRender.com.

The full PubMed database was searched (without time restrictions), in October 2022, using the keywords 'sex chromosome disorders,' 'sex chromosome aberrations,' 'sex chromosome abnormalities,' 'Turner syndrome,' 'Klinefelter syndrome,' '47,XYY,' and '47,XXX' as search terms in combination with 'DNA methylation,' 'transcriptome profile,' 'epigenetics,' and 'genomics'. Relevant articles were obtained and reviewed as well as other articles selected by the authors.

The human sex chromosomes, gene dosage, and X chromosome inactivation

The evolution of the X and Y chromosome is central in understanding potential molecular underpinnings of the phenotype of SCAs and identifying candidate genes. The human sex chromosomes are thought to have evolved from a pair of homologous autosomes over the past 300 million years (2). During this evolutionary process, the Y chromosome gained a sex-determining locus (*SRY*) and lost 97% of the ancestral genes due to the repression of recombination, resulting in highly dimorphic sex chromosomes (3, 4). To compensate for this imbalance in gene dosage between sexes, an important dosage compensation mechanism evolved: X chromosome inactivation, an epigenetic silencing of one of the X chromosomes in cells with more than one X chromosome (5). However, the X chromosome inactivation process has been shown to be incomplete with approximately 12–15% of the X-linked genes consistently escaping inactivation, and an additional 8–10% showing variable escape dependent on tissue type and individual (6, 7). Escape genes are not fully expressed from the inactivated X chromosome (Xi) (6, 7) but tend to be expressed at levels between 10 and 95% of the expression levels seen from Xa (6, 7). The genes in the pseudoautosomal region 1 (PAR1) (*AKAP17A*, *ASMT*, *ASMTL*, *CD99*, *CD99P1*, *CRLF2*, *CSF2RA*, *DHRSX*, *GTPBP6*, *IL3RA*, *P2RY8*, *PLCXD1*, *PPP2R3B*, *SHOX*, *SLC25A6*, *XG*,

and *ZBED1*) (Fig. 1B and C) belong to the escape genes, as do X–Y homolog pairs outside the PAR1 region of the X chromosome (*DDX3X*, *EIF1AX*, *KDM5C*, *KDM6A*, *NLGN4X*, *PRKX*, *RPS4X*, *TBL1X*, *TMSB4X*, *TXLNG*, *USP9X*, and *ZFX)* (Fig. 1B and C) (6). This gene pool shared between the X and Y chromosomes may be dosage sensitive as indicated by the evolutionary favored copy number of two (8). Therefore, these genes have been highlighted as potential candidate genes for the phenotype seen in SCAs. Unlike the genes in the PAR1, genes located in the pseudoautosomal region 2 (PAR2) (*SPRY3*, *SYBl1*, *IL9R*, and *CXYorf1*) are subjected to X chromosome inactivation and are also being silenced on the Y chromosome (6, 9). Therefore, these genes are not considered putative genes for the phenotype of SCAs.

SHOX (short stature homeobox gene) is an example of how gene dosage of PAR1 genes can affect the phenotype of SCAs. This gene explains part of the differences in the height of SCAs since decreased and increased copy number of the *SHOX* gene is associated with reduced height in TS and increased height in 47,XXX, KS, and 47,XYY, respectively (10, 11). Although height increases with an increasing number of sex chromosomes and *SHOX* copies in the abovementioned SCAs, it cannot be applied to 49,XXXXY males, 48,XXXX females, and 49,XXXXX females, showing that the increasing sex chromosome count and the copy number of the *SHOX* gene affect the height in a nonlinear fashion (11). So far, *SHOX* is the only gene that has been linked to a specific phenotypic trait of SCAs. However, recent studies have also highlighted the gene *ZFX* as a gene playing a significant role in SCAs. *ZFX* encodes a transcription factor with transcriptional activator properties and thousands of binding sites in the genome (12). *ZFX* has been identified by two studies to have a potential key role in regulating gene expression in SCAs by organizing networks where several X chromosomal genes are highly co-expressed with several sex chromosome dosage-sensitive autosomal genes (13, 14). However, more studies are needed to establish the role of *ZFX*.

Interestingly, several of the X–Y homologous gene pairs and some of the PAR1 genes have been demonstrated to have regulatory genomic functions being involved in ubiquitination, chromatin modification, transcription, translation, and splicing (8). The altered dosage of these genes, as seen in SCAs, might therefore result in disturbed genomic functions with the potential to increase the vulnerability to diseases. Over the past 5–10 years, several studies have emerged demonstrating such imbalance of the methylome and transcriptome in SCAs (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). Most of these studies have been performed on peripheral blood samples, or cells derived from blood, from patients with TS and KS but few including 47,XXX and 47,XYY also exist. Collectively, these studies illustrate global methylation alterations as well as global gene expression alterations affecting both the X chromosome and the autosomes. Moreover, it has been illustrated that more than 75% of the identified differentially expressed genes (DEGs) are located on the autosomes in TS and KS, while it is less than 30% in 47,XXX and 47,XYY (14). In addition, the loss and gain of a sex chromosome have been shown to have an asymmetric impact on the genome. Both the number of autosomal and X-linked DEGs and differentially methylated positions are much higher in TS than in KS and Trisomy X (13, 14, 18) compared to same-sex controls. This impact on the genome has been shown to follow an inverse pattern, with preferential hypomethylation in TS and preferential hypermethylation in KS, and with DEGs displaying an inverse expression pattern in TS and KS, respectively. Furthermore, the altered transcriptome seen in SCAs does not only include coding RNAs. Also, autosomal and X chromosomal non-coding RNAs (e.g. microRNAs, circular RNAs, and lncRNAs) are reported to be differently expressed in SCAs (13, 16, 18, 21, 27, 28, 29, 30, 31, 32).

When comparing the alterations seen in the methylome with that seen in the transcriptome of SCAs, it is evident that these alterations are not overlapping but rather complementary for most genes (13, 15, 16). Hence, it has been speculated that the altered methylome may be a compensation for the altered sex chromosome dosage, perhaps viewing the methylation changes as an organism's attempt to dampen the effect of altered sex chromosome dosage. Such a mechanism has been seen in families with inherited 7q31.1 microdeletion affecting *IMMP2L*, where healthy carriers have reduced DNA methylation levels of *IMMP2L* compared to affected offspring (33).

Interestingly, recent findings also indicate that inactive X chromosomal genes may be seen as candidate genes contributing to the phenotype of SCAs. Expression analyses of X-linked genes annotated as inactive show that the expression of these genes decreased with increasing number of X chromosomes. This suggests a compensatory mechanism in SCAs by which increasing the X chromosome number may lead to partial

transcription repression of these genes from the single active X chromosome in patients with two or more X chromosomes (14).

Potential mechanistic modifiers underlying phenotypic variability in SCAs – mosaicism and global modifiers

TS, KS, 47,XXX, and 47,XYY present with a broad phenotypic variability, with patients presenting a wide range of clinical traits across a spectrum (variable expressivity) (Fig. 2) and with some patients showing a specific phenotypic trait, whereas others do not show this trait (incomplete penetrance). Currently, there is a huge gap in translating to what extent and how potential individual genomic variation affects the individual phenotypic presentations of SCAs. However, it is likely that many of the same mechanisms explaining variation in penetrance and expressivity in other diseases are at play.

Mosaicism

A phenotypic effect of mosaicism is known from Mendelian diseases where mosaicism can result in variable expressivity or reduced penetrance (34, 35) (Fig. 2). Among SCAs, mosaic karyotypes are well-known (36, 37, 38, 39, 40), with TS and 47,XXX having the highest frequencies (>30%)(36,37), and KS and 47,XYY having the lowest frequencies of mosaicism (6–7 and 11%, respectively) (38,39). Although studies investigating the phenotypic differences between mosaic and non-mosaic SCAs are sparse, the general picture is that SCA patients with a mosaic karyotype seem to have a more favorable phenotype compared to non-mosaic patients (36, 37, 39, 40, 41, 42, 43). This is also reflected in the later age of diagnosis seen in mosaic patients (1). More specifically, TS females with a 45,X/46,XX karyotype have been reported to have a significantly lower mortality and a more favorable phenotype regarding height, reproductive, and cardiovascular phenotype than females with 45,X karyotype (36, 41). In 46,XX/47,XXX patients, the overall morbidity is lower compared to 47,XXX patients, with 46,XX/47,XXX having only significantly more registrations than controls in 4 out of 19 ICD-10 chapters (congenital malformation, perinatal, urogenital, and skin) in an epidemiological evaluation of the syndrome (40). Among KS patients, a karyotype of 46,XY/47,XXY is associated with a more favorable phenotype compared to 47,XXY regarding testicular volume, mean total sperm count, azoospermia, and luteinizing hormone and estradiol levels (43). In addition, patients with a 46,XY/47,XYY karyotype are less severely affected than patients with a 47,XYY karyotype, illustrated through epidemiological studies of hospital diagnoses, where 47,XYY, but not 46,XY/47,XYY, have an increased overall risk of hospital diagnosis and risk of having any medication prescribed (39).

The karyotypes of SCAs are in most cases based on a conventional cytogenetic analysis of peripheral blood samples. However, it is important to be aware that the degree and distribution of mosaicism may differ between different tissues with the potential to modify the phenotype (34, 35). The true prevalence of mosaicism in SCAs may therefore be underestimated. In line with this, it has been hypothesized that TS patients with a 45,X karyotype may have some degree of undiscovered

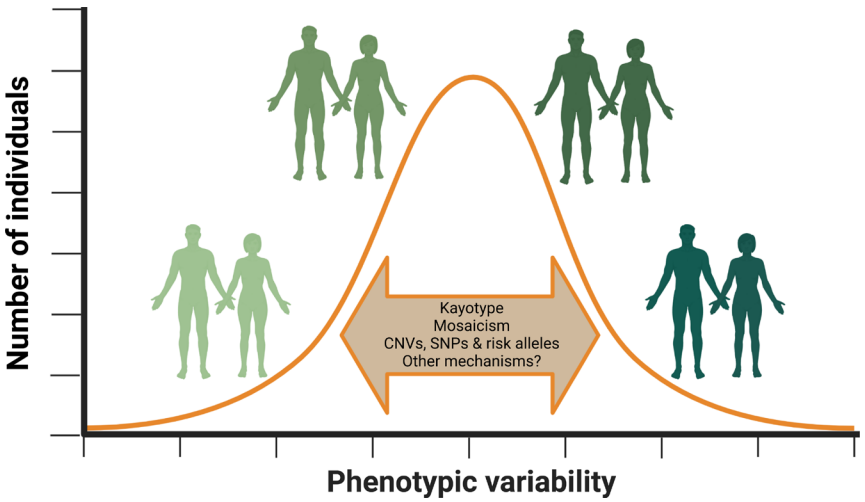

Figure 2
SCAs are associated with several clinical phenotypic traits that show a huge range of inter-individual variation, even in subjects with the same karyotype. Several genetic modifiers (e.g. mosaicism, copy number variations (CNVs), and single-nucleotide polymorphisms (SNPs)) have been suggested to be implicated in the inter-individual variation seen. Thus, many of the phenotypic traits seen in SCAs may be seen as a spectrum following a normal distribution curve, like that of the general population, only shifted to the left (e.g. intelligence in KS) or right (e.g. height in KS), rather than a binary classification. This figure was created with BioRender.com.

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mosaicism, since up to 99% of 45,X fetuses are believed to be spontaneously aborted very early in pregnancy (44, 45, 46, 47). This is supported by evidence that the 45,X cell line is caused by mitotic errors leading to the loss of an X chromosome in diploid conceptions rather than by meiotic errors (46). However, although substantial evidence supports this mosaic hypothesis, a recent study has questioned it. In this study, it was described that the majority of TS patients with a 45,X karyotype, did indeed have this 45,X by FISH analysis of cells with different embryonic origin (mesoderm (lymphocytes), ectoderm (buccal cells), and endoderm (rear-tongue epithelial cells))(48). However, these findings do not exclude mosaicism in cells/tissues not available for study. Furthermore, technical factors may affect the results of the study such as the longer time needed to culture fibroblast than lymphocytes, *in vitro* competition between different cell lines, etc.

To unravel the potential role of mosaicism in the phenotype of SCAs, additional studies mapping the degree of mosaicism in different tissues of SCA patients in relation to phenotypic traits are needed.

Individual genomic variations as modifiers of the phenotype of SCAs

Besides mosaicism, individual genomic variations such as single-nucleotide polymorphisms may contribute to the phenotypic variability seen in SCAs (Fig. 2). The polygenic model is known from the inheritance of complex traits such as mood disorders, obesity, diabetes, and osteoporosis, all with an increased prevalence in SCAs (49, 50, 51, 52). However, little effort has been directed toward investigating the impact of such genetic variations on the phenotypic variability seen in SCAs. Deciphering if such a polygenic model could explain some of the phenotypic variability seen in SCAs concerning mood disorders, obesity, diabetes, etc. will be important, as it may be useful in predicting the risk of a specific trait in the individual SCA patient, thereby enabling risk stratification.

Furthermore, evidence of a 'two-hit' model for phenotypic traits of TS has been found. A single copy of the X-linked escape gene, *TIMP1*, combined with *TIMP3* risk alleles, located on chromosome 22, synergistically increases the risk for bicuspid aortic valve (53, 54). This may be part of the explanation for why the bicuspid aortic valve is only found in a proportion of TS females (approximately 28%) with the highest prevalence of bicuspid aortic valve seen in the 45,X karyotype (42). The combination of *TIMP1* haploinsufficiency and *TIMP3* risk alleles has also been suggested to weaken the aortic wall and thereby leading to aortopathy in TS (53, 54). However, future functional studies are needed to validate the combinatorial effect of *TIMP1* haploinsufficiency and *TIMP3* risk alleles.

Future directions – from single cells and deep phenotyping to animal models

Based on the earlier discussion, it is evident that a considerable amount of knowledge has already been obtained about the genomics of SCAs. The variable multi-tissue phenotypes seen in SCAs may result from complex genetic heterogeneity affecting multiple organ systems. This means that a plethora of different cell types may be affected by SCAs. Hence, genomic and transcriptomic data at single-cell resolution and in a spatial context may be a prerequisite to gain a further understanding of the genomics of SCAs. Below we will focus on new molecular techniques and approaches that can help to advance and refine our knowledge of the genomics of SCAs and the genotype–phenotype associations. Furthermore, we discuss the use of human-induced pluripotent stem cells (hiPSCs) isolated from SCAs and animal models as possible methods to gain further insight into the genomics of SCAs.

Transcriptomics at single-cell resolution and spatial transcriptomics

Single-cell RNA sequencing (scRNA-seq) provides transcriptomic information at single-cell resolution, allowing for the detection of small genomic changes and cellular heterogeneity (Fig. 3). This method further allows for trajectory (pseudotime) analyses of cell differentiation and cell–cell communication based on intercellular interaction patterns. In addition, epigenetics can also be investigated at the single-cell level with single-cell assay for transposase-accessible chromatin), allowing for analysis of gene regulatory mechanisms dependent on cell type and stage. However, tissue dissociation protocols and high cellular quality are a prerequisite for these methods. To overcome these limitations, an alternative method is single nuclei sequencing (snRNAseq), profiling gene expression from isolated nuclei rather than entire cells. This alternative

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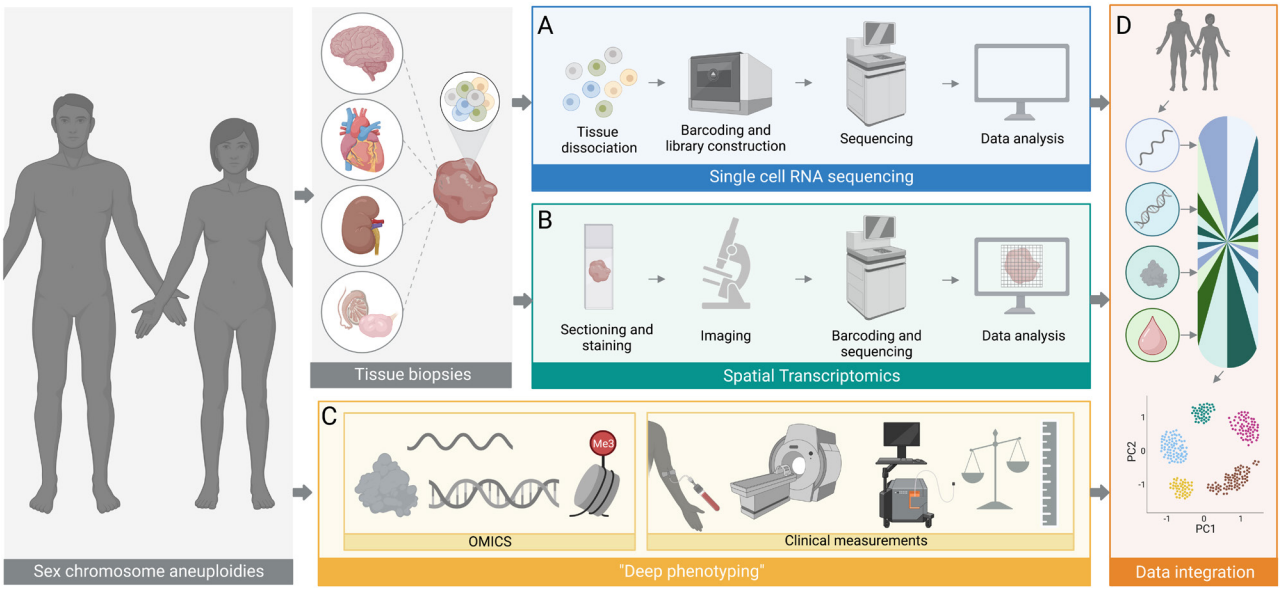

Figure 3
Multi-tissue and multi-omics approach at the single cell level to better deep phenotype SCAs. From SCA individuals, representative tissue biopsies should be obtained from relevant tissues. Biopsies can then either be dissociated to a single-cell suspension, barcoded and sequenced to obtain single cell omics (A) or sectioned, stained, imaged and barcoded prior to sequencing for spatial RNA expression (B). If possible, clinical measurements should be obtained at the same time for the same individuals (C). Multi-omics and clinical measurements can be combined into an integrated dataset, maintaining both the single cell resolution and the spatial information, thus, making it possible to identify specific molecular signatures for each SCA in a tissue and cell-specific phenotypic context (D). This figure was created with BioRender.com.

excludes cytoplasmic transcripts, resulting in a ~20–40% reduction in total captured genes dependent on sample type. However, it can still allow for the distinction of cell populations that could otherwise risk neglection (https://kb.10xgenomics.com/hc/en-us/articles/360054702612-How-does-the-Gene-Expression-data-compare-between-whole-cells-and-nuclei-).

In multiple organs, tissue architecture and localization of cellular subtypes are of interest to understand the effects of transcriptional variation. Here, the application of spatial transcriptomics allows for visualization of spatial gene expression and tissue heterogeneity (Fig. 3). However, the spatial context comes at the expense of single-cell resolution, as current technologies only offer subcellular resolution. By integrating spatial data with scRNAseq of the same cell types, this can, to some degree, be overcome with cell type deconvolution algorithms (Fig. 3) (55).

Previous genomic studies of SCAs have measured DNA methylation and RNA expression in bulk tissue, comprising thousands or millions of heterogeneous cells, thereby partly neglecting cell type compositions, specialized cell populations and spatial context. Currently, only a few scRNAseq studies have been performed on SCAs to overcome these challenges. These include a study on blood from a single 47,XXY male, finding distinct cell subpopulations with gene expression associated with various phenotypical traits (56). However, more scRNAseq studies have been performed on testicular tissue ($n=3$), identifying dysregulated testicular pathways in KS patients (57, 58, 59). These studies have established that Sertoli cells are the most affected testicular cell type in KS (58, 60), with a subset lacking *XIST* expression, resulting in loss of X chromosome inactivation (57).

Applying scRNA-seq and spatial transcriptomics in future studies of SCAs would be highly relevant as it may widen our understanding of the genomics of SCAs and perhaps lead to the discovery of specialized cell populations so far undiscovered. In addition, an atlas for various karyotypes and tissues would be highly advantageous in the understanding of SCAs, spanning from embryo to adulthood.

Deep phenotyping by a multi-omics approach

A large share of our knowledge about the genomics of SCAs has come from the implementation of exploratory omics-based methods used for the analysis of DNA (genomics), DNA methylation (methylomics), gene expression (transcriptomics), and protein abundance (proteomics) (13, 14, 15, 16, 17, 18, 21, 61). A common element of these omics-based techniques is the enormous

output generated, resulting in high-dimensional datasets where each individual, SCA patient or control, is described by a very large number of measured variables. Historically, when implementing omics-based acquisition in studies of SCAs, a substantial focus has been on associating changes in single genes to phenotypic traits. This approach can be characterized as a reductionist biology approach where a hypothesis test is done on one variable at the time, independently of the other variables. The significant variables resulting from this analysis can then be linked to phenotypic traits by correlation or regression, or in a more unbiased approach, by using all significant variables as input for functional enrichment analysis, for example, gene set enrichment analysis (62). Unfortunately, these classic approaches do not seem to fully appreciate or describe the complexity of the molecular pathways that are perturbed in SCAs, and as such, makes it difficult to adequately describe SCA genotype–phenotype links.

To better elucidate important biological pathways in SCAs, a simultaneous multi-omics approach, followed by an integrated multivariate analysis, could prove essential in understanding how underlying changes – perhaps subtle – in networks of gene-regulatory processes such as DNA methylation, gene regulation by non-coding RNAs and post-translational modifications influence the SCA phenotype, and the heterogeneous manifestation of the phenotype within the different SCAs. To date, a majority of the published SCA studies that have applied one or several omics-techniques, have used blood, or cells derived from blood, as the sample material. However, the changes in molecular pathways in blood cells cannot be expected to explain clinical traits such as differences in neuropsychology, body composition, endocrinology, and immune system (15, 16). Based on the substantially distinctive expression profiles of different tissues, also within SCAs (27), it seems plausible that the different SCA groups will have a tissue-specific impact that will depend on tissue and cell-type susceptibility to the specific SCA. Thus, understanding tissue and cell-type specific impact will not only require a multi-omics approach, but also a multi-tissue approach – possibly at a single cell level – to describe SCA biological pathways and to characterize the phenotype as a manifestation hereof.

Several methods exist to carry out a data-driven analysis in a systems biology context. One of the most well-known methods is the weighted gene correlation network analysis (WGCNA) (63). With this method, it is acknowledged that the expression and translation of single genes are not independent. Instead, co-expressed genes or proteins, based on correlation, are grouped into larger networks (modules). This approach has been used successfully to identify gene networks that were associated with TS, KS, and the number of X chromosomes (13, 21, 64). Thus, WGCNA makes it possible to associate clinical traits, continuous and categorical, with the identified networks. However, WGCNA is not well-suited for a combined and integrated analysis of multi-omics datasets. Here, specialized methods using dimensional reduction techniques to identify pathway signatures of correlated variables across different omics, while also discriminating the different SCAs, could prove useful. Some of these methods have been described in detail and implemented in various software packages including the mixOmics package for R (65).

For the earlier approach to be successful, it will require larger sample sizes, several tissue biopsies and well-measured clinical traits for each subject. Unfortunately, SCA cohorts often consist of a small number of subjects and different clinical measurements are obtained in different cohorts in different studies. As such, analyses become prone to clinical heterogeneity, noise and overfitting. Thus, a multicenter approach, with standardized biopsy and clinical measurements, across different countries, maybe a way forward to increase the sample size number and in this way find significant and robust genotype–phenotype links. As this might not be plausible based on national rules or the availability of certain tissues, novel SCA model systems, as described below, could instead act as valuable alternatives to drive SCA research forward.

Using human-induced pluripotent stem cells to elucidate the molecular underpinnings of SCAs

As mentioned above, getting tissue biopsies from multiple phenotypic target organs from patients with SCAs can be a major challenge. Inaccessibility of the relevant tissue (brain, heart, aorta, etc.) can potentially make it difficult, or in the worst case, impossible to map out the full picture of the genome of SCAs and their relation to the phenotype. The possibility of deriving hiPSCs from peripheral blood mononuclear cells or skin fibroblasts, and further differentiate these into organotypic cells (66) such as cardiomyocytes or neurons, provides a unique opportunity to elucidate the molecular pathways involved in the neurophenotype and cardiac phenotype of SCAs. A recent study has utilized this technique to differentiate 47,XXY-hiPSCs into germ cells

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(67). Another study using KS and other supernumerary sex chromosome aneuploidy induced pluripotent stem cells (iPSCs) provided the first evidence of an X-dosage-sensitive autosomal transcription factor (*NRF1*), and that this transcription factor is a key regulator of the X-linked zinc finger protein *ZFX*. Thus, iPSCs can be used as an *in vitro* modeling of X chromosome abnormalities in an attempt to gain further knowledge about how altered sex-chromosome dosage impact the transcriptome and methylome in different cell types (64).

The potentials of animal models in SCAs

Animal models have been useful as a tool to investigate the genetics behind diverse diseases and clinical manifestations in humans. In SCAs, various mouse models have been established including mouse models of TS and KS (reviewed in (68, 69)). Some of the phenotypic traits of SCAs are seen in these models such as germ cell loss, impaired cognition, and Leydig cell hyperplasia in the mouse model of KS. However, other phenotypic traits are not seen in these models. For example, monosomy X female (39,XO) mice have a less severe phenotype than TS females since the XO mice are fertile and embryonically viable. The phenotype discrepancy between humans and mice may be due to their genetic differences. The number of both PAR1 genes and X–Y gene pairs is lower in mice than in humans (8, 70). Moreover, 12–25% of the X-linked genes escape from inactivation in humans (6, 7), while it is only approximately 3–7% in mice (71). Therefore, one may speculate whether the genes implicated in the phenotypic trait of SCAs are PAR1 genes, X–Y gene pairs, and/or escape genes found only in humans and not in mice.

Another possible model organism for SCAs is the zebrafish. In 2013, a high-quality sequence assembly of the genome of zebrafish (*Danio rerio*) showed that more than 70% of the human genes have at least one zebrafish ortholog, while at least 80% of the human disease-associated genes have one (72). Accordingly, the zebrafish is a valuable vertebrate model organism for the study of genes involved in human disorders and clinical manifestations. Although zebrafish do not have sex chromosomes, the majority of the human PAR1 genes and X–Y gene pairs have at least one zebrafish ortholog (Fig. 4A), making it a qualified model organism

A

	Candidate gene in SCAs	Zebrafish ortholog	Chromosome location in the zebrafish genome
PAR1 genes	*AKAP17A*	*akap17a*	9
	ASMT	*asmt* *asmt2*	9 17
	ASMTL	*asmtl*	9
	CD99	*cd99*	1
	CD99P1	-	-
	CRLF2	-	-
	CSF2RA	-	-
	DHRSX	*dhrsx*	1
	GTPBP6	*gtpbp6*	6
	IL3RA	-	-
	PLCXD1	*plcxd1* *si:dkey-66a8.7* *zgc:112023*	6 6 6
	PPP2R3B	*ppp2r3b*	9
	P2RY8	*p2ry8*	9
	SHOX	*shox*	9
	SLC25A6	*slc25a6*	9
	XG	-	-
	ZBED1	-	-
X-Y gene pairs	*DDX3X/Y*	*ddx3xa*[1] *ddx3xb*[2]	9 6
	EIF1AX/Y	*eif1axb*[1] *eif1axa*[2]	24 5
	KDM5C/D	*kdm5c*[2]	8
	KDM6A/UTY	*kdm6a*[2] *kdm6al*[2]	9 6
	NLGN4X/Y	*nlgn4xb*[1] *nlgn4xa*[2]	9 1
	PRKX/Y	*prkx*[2]	1
	RPS4X/Y	*rps4x*[2]	7
	TBL1X/Y	*tbl1x*[2]	9
	TMSB4X/Y	*tmsb4x*[2]	9
	TXLNG(Y)	*txlng*[1]	11
	USP9X/Y	*usp9*[2]	9
	ZFX/Y	*zfx*[2]	24

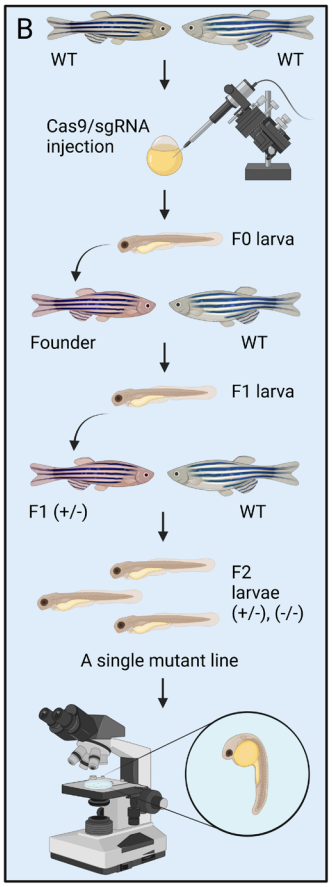

Figure 4
(A) The table shows the zebrafish orthologs to the human PAR1 genes and X–Y gene pairs and their chromosome location in the zebrafish genome. The data in this table are retrieved from the Alliance of Genome Resources (https://www.alliancegenome.org). In the table, – indicates that no ortholog is found, while 1 and 2 indicate that the gene is an ortholog to the X homolog and X–Y gene pair, respectively. (B) An example of how to genetically manipulate zebrafish and establish knockout lines using CRISPR/Cas9. Wildtype embryos are obtained by crossing adult wildtype zebrafish. Subsequently, single guide RNA (sgRNA) and Cas9 mRNA are co-injected into the one-cell stage wildtype embryos to disrupt gene function of the target gene by, for example, introducing a premature termination codon (PTC). When the injected embryos become adult fish, they are screened to identify a founder fish (F0) that can pass on the PTC to the next generations. The F1 and F2 generations are obtained by crossing F0 founders with wildtype zebrafish and crossing F1 zebrafish with wildtype zebrafish, respectively. The F2 zebrafish can be used for phenotype analysis. This figure was created with BioRender.com.

https://ec.bioscientifica.com
https://doi.org/10.1530/EC-23-0011

to study the genetics of SCAs. Data retrieved from the Alliance of Genome Resources (http://www.alliancegenome.org) (73) show that most of these orthologs are found on chromosomes 6 and 9, while the rest of these genes are more widely spread in the z ebrafish genome. Thus, targeting single candidate genes will be a potential way to examine their role in a zebrafish model of SCAs. Zebrafish have several advantages as an animal model (reviewed in (74)). For example, zebrafish have high fecundity, external fertilization, short generation time, and several organs and organ systems that are also found in humans. Additionally, their embryos are transparent and develop very rapidly outside the mother, enabling observation of the embryonic development *in vivo* and making it easy to collect and genetically manipulate them (Fig. 4B). Therefore, the consequences of haploinsufficiency and gene overdose of candidate genes can be investigated in these models. These qualities demonstrate that zebrafish as an animal model has the potential to contribute to elucidating the genotype–phenotype relation in SCAs.

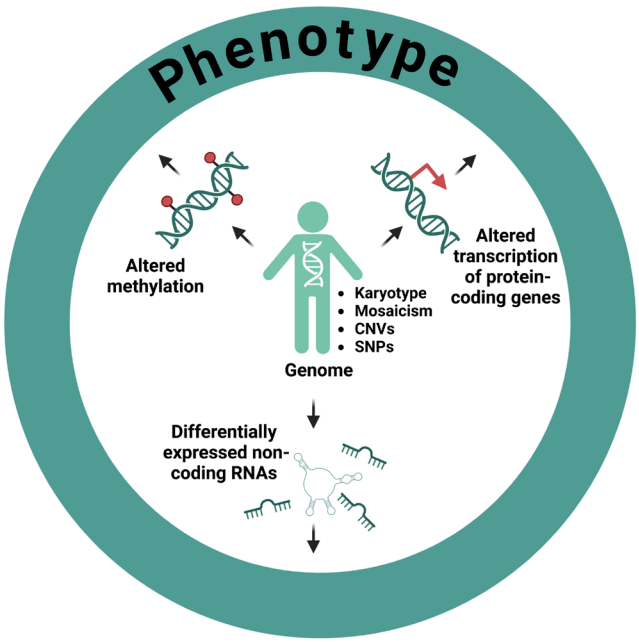

Figure 5
The molecular underpinnings of the phenotype of SCAs are complex and may include mosaicism, genomic variations (e.g. CNVs and SNPs), altered methylome and transcriptome. It has been speculated whether the altered methylation can alleviate the effect of sex chromosome loss or gain and thereby the phenotype. The altered transcription of the protein-coding genes may result in an altered proteome that may also contribute to the phenotype. Moreover, the non-coding RNAs (e.g. microRNAs, circular RNAs, and lncRNAs) have been suggested to contribute to the phenotype. This figure was created with BioRender.com.

Conclusions

Even though new technologies enabling high-throughput analysis of the methylome and transcriptome have broadened our insight into how altered sex chromosome dosages impact the human genome, much is still left unanswered. It is clear that an altered sex chromosome dosage has huge genome-wide consequences, and that the phenotype of SCAs may not merely be due to the direct genomic imbalance from altered sex chromosome gene dosage but may also be due to additive alterations in gene networks and regulatory pathways across the genome as well as individual genetic modifiers (Fig. 5). More research is required before we can translate these advances into the observed phenotype. We believe that there is a need to study phenotypically relevant tissues such as gonads, muscle, fat, and heart at single-cell resolution by a multi-omics and system biology approach to understand the spatial and temporal gene networks leading to the phenotype of SCAs across a lifespan. To reach the ultimate goal of being able to describe the genotype–phenotype association of SCAs, we advocate for national and international research collaborations.

Declaration of interest
The authors have no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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