

## Supernumerary sex chromosome abnormalities – new developments and future trajectories - A summary of the 2022 3rd International Workshop on Klinefelter syndrome, XYY and Trisomy X

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**Abstract**

The 3rd International Workshop on Klinefelter Syndrome, Trisomy X, and 47,XYY syndrome was held in Leiden, the Netherlands, on September 12-14, 2022.

Here, we review new data presented at the workshop and discuss scientific and clinical trajectories. We focus on shortcomings in knowledge and therefore point out future areas for research.

We focus on the genetics and genomics of supernumerary sex chromosome syndromes with new data being presented. Most knowledge center specifically on Klinefelter syndrome, where aspects on testosterone deficiency and the relation to bone, muscle and fat was discussed, as was infertility and the treatment thereof. Both Trisomy X and 47,XYY syndrome are frequently affected by infertility.

Transitioning of males with Klinefelter syndrome was addressed, as this seemingly simple process is in practice often difficult.

It is now realized that neurocognitive changes are pervasive in all supernumerary sex chromosome syndromes, which was extensively discussed. New intervention projects were also described and exciting new data concerning this was presented.

Advocacy organizations were present, describing the enormous burden carried by parents when having to explain their child's specific syndrome to most professionals whenever in contact with health care and education systems. It was also pointed out that most countries do not have health care systems that diagnose patients with supernumerary sex chromosome syndromes, thus pinpointing a clear deficiency in the current genetic testing and care models.

At the end of the workshop a roadmap towards the development of new international clinical care guidelines for Klinefelter syndrome was decided.

## Introduction

The 3<sup>rd</sup> International Workshop on Klinefelter Syndrome, Trisomy X, and XYY was held in Leiden, the Netherlands, on September 12-14, 2022. This event followed successful prior workshops in 2010 in Copenhagen, Denmark (1) and in 2016 in Münster, Germany (2). It was organized by professor Hanna Swaab and her local organizing team at the University of Leiden, as well as an international scientific organizing team. Over three days participants presented the latest developments within the field and clinicians and researchers from many different fields met and exchanged ideas. The workshop expanded on the previous meetings by including research on Trisomy X and XYY syndromes. Representatives from different advocacy organizations also attended the workshop. Here we present a summary of topics from keynote speakers, however must also acknowledge additional oral and poster presentations of new science on myriad topics, many from promising young investigators.

## Genetics of Supernumerary Sex Chromosome Syndromes

The session on genetics of supernumerary sex chromosome syndromes revealed great progress during the past couple of years in understanding potential molecular underpinnings for the different phenotypic traits. The pathophysiological thinking seems to be moving away from searching for single candidate genes, like the first gene, the *SHOX* gene, shown to be involved in growth many years ago (3,4), and since extensively studied (5). Currently, the picture emerging focuses more on X chromosomal escape genes, including genes from the pseudo-autosomal region (PAR) and other genes with Y chromosome homologues, thought to be likely candidate genes, both for involvement in Klinefelter syndrome, but also Turner syndrome, and the other supernumerary sex chromosomal syndromes (6-10). In addition, it is now clear that the DNA methylation landscape is altered in a genome-wide fashion (6,9-11) and that indeed also the coding RNA transcriptome as well as the non-coding transcriptome is pervasively changed in several tissues (12,13). Armin Raznahan and Anne Skakkebaek gave two keynote lectures on the genetics of primarily Klinefelter syndrome, but also on the wider picture of sex chromosome abnormalities and each presented new unpublished data that added to the complex picture. These lectures were followed by oral presentations each adding to the complex genetics/genomics of

supernumerary sex chromosome syndromes, utilizing both sampling of multiple tissues, induced pluripotent stem cells (iPSC) and comparative studies from individuals with several syndromes (14). The emerging picture also shows that more studies will be needed adding a temporal dimension, i.e. understanding how genomics change during development, and a multi-tissue approach, since it is becoming increasingly clear that each tissue carries its own coding and non-coding transcriptome and DNA methylation signature – a unique genetic fingerprint. The coming years are likely to hold major advances in understanding of sex chromosome dosage effects on the human genome given the rapid recent expansion of new techniques for genomic analysis including spatial and single cell transcriptomics, organoids, iPSC's and other techniques in a multi-tissue and temporal fashion (Figure 1)(15).

### **Klinefelter Syndrome, Hypogonadism, Bone, Muscle, and Fat Metabolism**

The risk of osteoporosis is significantly elevated in men with Klinefelter syndrome (16,17) and easily attributed to the ensuing hypogonadism that most experience. However, this relation is not always clear cut. Many studies have found diminished bone mineral density (BMD) and increased risk of vertebral fractures in subjects with Klinefelter syndrome (18-20). Testosterone replacement therapy (TRT) increases BMD in men with Klinefelter syndrome (21,22), but there are no studies showing that the fracture rate actually is diminished. However, this is inferred from other conditions of male hypogonadism and from observational studies (21,23-25). Studies using high resolution pQCT, a CT based method that allows the study of the microarchitecture of bone, have shown that the microarchitecture is changed with reduced trabecular density at the tibia (26) and lower cortical BMD at the radius, but with an increase during TRT (27). In addition another study demonstrated that in young adults with Klinefelter syndrome treated from adolescence measures from high resolution pQCT improved in most, but not all (28). Alberto Ferlin summarized recent data and concluded that the relation between testosterone, BMD and fracture is not clear in men with Klinefelter syndrome, and that more research will be needed to better determine this relation, as well as pointing towards the best biomarkers to evaluate the effects of TRT (29). In addition, other compartments such as skeletal muscle and fat are also influenced by TRT and several studies have determined that muscle mass is universally reduced and fat mass increased in men with Klinefelter syndrome starting during childhood (30-32) and into adulthood (29,33,34). A

study in children showed a positive effect of oxandrolone treatment on fat mass (35) and in adults a small randomized controlled trial (RCT) also showed decreases in fat mass during active treatment (36). Finally, a recent meta-analysis concluded that TRT exerts positive effects on bone, muscle and fat mass (22). Of particular note, it is even more evident that the relation between testis function and bone/skeletal muscle metabolism in subjects with Klinefelter syndrome cannot be limited to testosterone levels, as other characteristics might be involved, such as the expression and function of the androgen receptor, insulin-like factor 3 (INSL3) and 25-hydroxy vitamin D levels (37).

### **Klinefelter Syndrome in Transition**

Transition of young adolescents with Klinefelter syndrome to the adult clinic remains a problem. Anders Juul presented new data concerning anthropometry, endocrine and metabolic changes during the pubertal transition and discussed when and how to start TRT. Concerns regard the appropriate time to initiate TRT remain controversial without good data to guide clinicians (38). There is a clear need for additional studies, preferentially RCT's, focusing on advantages and drawbacks to early versus late start of TRT, including focus on issues like fertility and neurocognition.

### **Supernumerary Sex Chromosome syndromes, Fertility and Testicular Function**

Klinefelter syndrome has been associated with infertility since the original description been associated and is considered one of the hallmarks of the syndrome (39). Great progress has been seen in recent years and today many men with Klinefelter syndrome can be offered TESE (testicular sperm extraction) micro-TESE, with a recovery rate of about 40%, pregnancy rate of 43% per intracytoplasmic sperm injection (ICSI) cycle and a cumulative pregnancy rate of 16% by ICSI (40-42). Research is ongoing in understanding the causes for the poor functioning of the testes (41,43). Jörg Gromoll presented a keynote lecture with a novel view of testicular function in men with Klinefelter syndrome, suggesting that disturbed vascularization contributes to the observed testicular hormone resistance (44). The cause of the hyalinization of the testes with subsequent

hypogonadism and infertility is unknown. There is a loss of spermatogonia from infancy (45), while hyalinization of the seminiferous tubules does not occur probably until mid-puberty (46). At the beginning of puberty, testes grow to approximately 4-8 mL and thereafter shrink to the pathological adult size of <4 mL (46). Testes may be malfunctioning already during intra-uterine life, since micropenis seen in some newborn males with Klinefelter syndrome may be a result of decreased testosterone production *in utero* (47). The genetics behind the demise of the testes in Klinefelter syndrome is incompletely understood. Previous studies of testis have investigated genetic, epigenetic and transcriptomic changes in Klinefelter syndrome on bulk testis tissue (48-51), or at single cell level using single cell RNAseq (scRNAseq) (52-54). None of these studies have convincingly pinpointed disease mechanisms or candidate genes, probably due to small sample sizes and lack of clinical and pathological information necessary to understand the spectrum of non-obstructive azoospermia.

Alan Rogol presented an overview of fertility and hormonal function in XYY and Trisomy X syndromes, with recognition of the limited research on this topic. Clinical data show that fertility is affected to a certain degree in these syndromes and also that many fewer become fathers and mothers possibly also due to socio-economic factors (55-58). Clinical studies among 47,XYY males have shown that the pubertal maturation, testicular histology, and spermatogenesis is most often normal, although epidemiological data suggest that some testicular dysfunction is frequent (56). Small testes, although most have normal-sized or even large testis, decreased spermatogenesis, spermatogenic arrest, subfertility, and sterility have been reported (59-61). It appears that XY pairing and recombination usually occur normally in 47,XYY, with the extra Y chromosome being lost during spermatogenesis (62,63), so that many 47,XYY men have fathered chromosomally normal children. In Trisomy X syndrome, premature ovarian failure (primary ovarian insufficiency) is more prevalent than in controls (55,64), and there are many case reports of both primary and secondary amenorrhea. Anti-mullerian hormone (AMH) levels as a marker of ovarian reserve is low in adolescents (65), however the significance of these levels to risk of POI or future fertility remain unclear. Further, cases of precocious activation of the hypothalamic-pituitary-ovarian axis (with and without signs of early puberty), lower ovarian volumes, and early-onset menarche has been reported in small sample sizes compared to controls (66,67). It was concluded that there is a great need for additional research on puberty and fertility in both XYY and Trisomy X syndromes.

## **Supernumerary Sex chromosome Syndromes - Neurocognitive and Behavioural Development**

The keynote lectures by Sophie van Rijn and coworkers and Nicole Tartaglia focused on the recent developments over the last couple of years of the TRIXY study and the eXtraordinary Babies Study, and presented exciting new data in the largest cohorts of very young children with Klinefelter Syndrome, Trisomy X, and 47,XYY studied to date (68-71). While it is known that all supernumerary sex chromosome syndromes present with an increased risk for an altered neurocognitive phenotype, autistic traits, ADHD symptoms and socio-emotional and behavioural issues, results of these studies show emergence of early signs of these neurocognitive and behavioural diagnoses within the first years of life. Interestingly, both studies found that when comparing developmental profiles between SCT conditions (i.e. KS vs 47,XYY vs Trisomy X), there were more similarities than differences, pointing to the need to emphasize the effects of aneuploidy itself on neurodevelopment compared to previous large emphasis on hormonal effects. Increases in non-invasive prenatal genetic testing (NIPT) practices in the USA has allowed for prospective study of a new cohort of over 275 infants identified in the prenatal period, with new detailed descriptions of developmental trajectories, milestone acquisition, and early medical problems such as feeding disorders and atopic conditions that can guide pediatric care. Coupled with a bank of biological samples, translational and longitudinal studies of this cohort have great potential to understand predictors of phenotypic variability in SCT conditions. Studies from Leiden detailing the first interventions to improve social-emotional difficulties in both young children and adult men were presented, which is most exciting, because prevention and treatment will hopefully lead to a better quality of life for these patients (72,73).

### **Impact of X and Y on life course**

The full natural course for those with supernumerary sex chromosome syndromes is not clear. Claus H. Gravholt presented comparative data on males with Klinefelter syndrome and 47,XYY syndrome and pointed out that many aspects of the two syndromes are indeed quite similar. Late diagnosis, many un-diagnosed cases, non-specific increases in morbidity covering all ICD-10

(International Statistical Classification of Diseases and Related Health Problems version 10) chapters, increased medicinal use again covering all types of medication, a poor socio-economic trajectory with early retirement in many instances, and increased risk of criminality and increased mortality (56,57,74-77). Different studies have also shown similarities in the neurocognitive profile Klinefelter syndrome with deficits in cognitive functioning including language, and executive functioning (78,79), and slightly decreased IQ (80). Also an increased referral to psychiatric treatment has been found (81). A survey for sex-chromosome alterations among patients with schizophrenia found a four- to fivefold excess of patients with Klinefelter syndrome (82). Similarly, neurocognitive changes are described among males with 47,XYY syndrome (83). But while boys with Klinefelter syndrome have significantly smaller whole-brain volumes on MRI, males with 47,XYY seem to have normal brain volume (84,85).

### **Advocacy and Supernumerary Sex Chromosome Syndromes**

A number of people representing different advocacy organizations attended the workshop and participated very constructively and with great enthusiasm. They raised questions related to schooling, anxiety, quality of life, the lack of international consensus, and the general lack of medical professionals with knowledge of supernumerary sex chromosome syndromes. For example, they described the enormous burden they carry having to explain their child's specific syndrome to most medical professionals and educators whenever in contact with health care and education systems. They also pointed to the fact that most countries do not have health care systems that "catch" (diagnose) patients with supernumerary sex chromosome syndromes, thus pinpointing a clear deficiency in the current genetic testing and care models.

### **New International Guidelines**

At the closing of the meeting a session on the need for new multidisciplinary care guidelines for Klinefelter syndrome was held. Alberto Ferlin, who, together with Michael Zitzmann, had chaired the European Academy of Andrology's recent guidelines (40), presented these guidelines which are a major leap forward in generating a uniform platform for improved care for all males with



Klinefelter syndrome. Shanlee Davis and Lise Aksglaede presented areas of care that also needed to be acknowledged and improved concerning care of patients with Klinefelter syndrome and Claus H. Gravholt presented a roadmap towards a new international set of guidelines, with focus on inclusion of all invested parties and societies around the world through a transparent process. The goal will be to bring together the Klinefelter syndrome medical and research community leaders to develop international, up-to-date and evidence-based recommendations for medical care for boys and males with Klinefelter syndrome throughout the lifespan. Focus should be on all areas of Klinefelter syndrome, including fertility, neurocognition and psychological features, co-morbidity, socio-economic aspects and others, including the increased number of subjects with a diagnosis of Klinefelter syndrome following prenatal screening. We discussed that advocacy groups should also be involved in the process. The guidelines should serve as a benchmarking tool, should inspire more research in areas that specifically are underserved and should be endorsed by as many professional societies as possible.

During the session it also became clear that there is a grave need for international guidelines in other areas, such as 47,XXX and 47,XYY syndromes, but also the rarer supernumerary sex chromosome syndromes. Presently, it was deemed premature to develop such guidelines due to insufficient data, however the processes from the Klinefelter syndrome project will serve as a model for future work for these conditions.

At the closing of the meeting, it was decided that the fourth international workshop on Klinefelter, 47,XXX and 47,XYY syndrome is to be held in Padua, Italy in 2025. Alberto Ferlin is going to arrange this next workshop.

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Figure 1:**

The current status of knowledge concerning especially the genomics of Klinefelter syndrome, but also 47,XYY and Trisomy X. It has been realized that in addition to the altered chromosome count, there are changes in transcriptome, the non-coding transcriptome and the methylome across different tissues. It remains to be understood how these changes affect the phenotype, specific candidate genes need to be identified, and further genomic changes, like histone and chromatin modifications, also need to be addressed. In addition, an understanding of the temporal changes from fetal life to old age need to be achieved.

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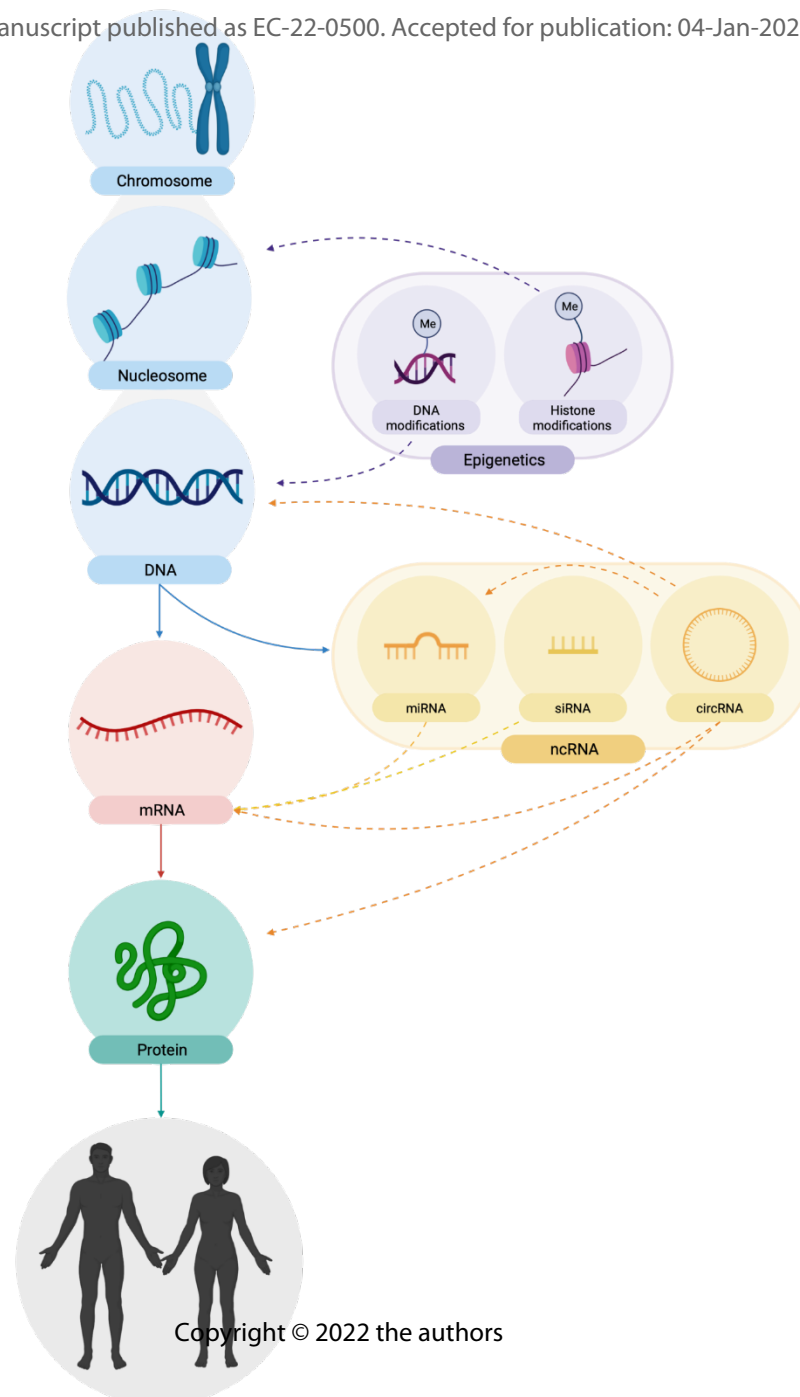




# Perspective

**What do we know?**

Altered sex-chromosome count  
 Changes in the transcriptome  
 Changes in the methylome  
 Changes in the circular transcriptome  
 ... in blood, fat and muscle!



**What don't we know?**

The effect of transcriptional changes  
 Changes in the other non-coding RNAs (miRNAs)  
 Histone- and chromatine modifications  
 Changes in other relevant tissues