REVIEW

New developments and future trajectories in supernumerary sex chromosome abnormalities: a summary of the 2022 3rd International Workshop on Klinefelter Syndrome, Trisomy X, and XYY

Claus H Gravholt[1,2,3], **Alberto Ferlin**[4], **Joerg Gromoll**[5], **Anders Juul**[6,7], **Armin Raznahan**[8], **Sophie van Rijn**[9], **Alan D Rogol**[10], **Anne Skakkebæk**[2,3,11], **Nicole Tartaglia**[12] and **Hanna Swaab**[9]

[1]Department of Endocrinology, Aarhus University Hospital, Aarhus, Denmark
[2]Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark
[3]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[4]Department of Medicine, Unit of Andrology and Reproductive Medicine, University of Padova, Padova, Italy
[5]Centre of Reproductive Medicine and Andrology, Münster, Germany
[6]Department of Growth and Reproduction Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark
[7]Department of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
[8]Section on Developmental Neurogenomics, National Institute of Mental Health Intramural Research Program, National Institutes of Health, Bethesda, Maryland, USA
[9]Clinical Neurodevelopmental Sciences, Leiden University, Leiden, The Netherlands and TRIXY Center of Expertise, Leiden University Treatment and Expertise Centre (LUBEC), Leiden, The Netherlands
[10]Department of Pediatrics, University of Virginia, Charlottesville, Virginia, USA
[11]Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark
[12]Department of Pediatrics, Developmental Pediatrics, University of Colorado School of Medicine, Aurora, Colorado, USA

Correspondence should be addressed to C H Gravholt: claus.gravholt@clin.au.dk

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 centre specifically on Klinefelter syndrome, where aspects on testosterone deficiency and the relation to bone, muscle and fat were 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 in practise is often difficult.
It is now realized that neurocognitive changes are pervasive in all supernumerary sex chromosome syndromes, which were extensively discussed. New intervention projects were also described, and exciting new data concerning these were 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

Key Words

- Klinefelter syndrome
- testosterone
- anti-Mullerian hormone
- trisomy X syndrome
- 47,XYY syndrome

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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.

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Introduction

The 3rd 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 Prof Hanna Swaab and her local organizing team at the University of Leiden, as well as an international scientific organizing team. Over 3 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 but must also acknowledge additional oral and poster presentations of new science on myriad topics, many from promising young investigators.

Genetics of supernumary 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, that was 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 and also Turner syndrome, and the other supernumerary sex chromosomal syndromes (6, 7, 8, 9, 10). In addition, it is now clear that the DNA methylation landscape is altered in a genome-wide fashion (6, 9, 10, 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 Skakkebæk gave two keynote lectures on the genetics of primarily Klinefelter syndrome and 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 to add 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 the 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, iPSCs and other techniques in a multi-tissue and temporal fashion (Fig. 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 was most experienced. However, this relationship 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, 19, 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 as a consequence of TRT. However, this is inferred from other conditions of male hypogonadism and from observational studies (21, 23, 24, 25). Studies using

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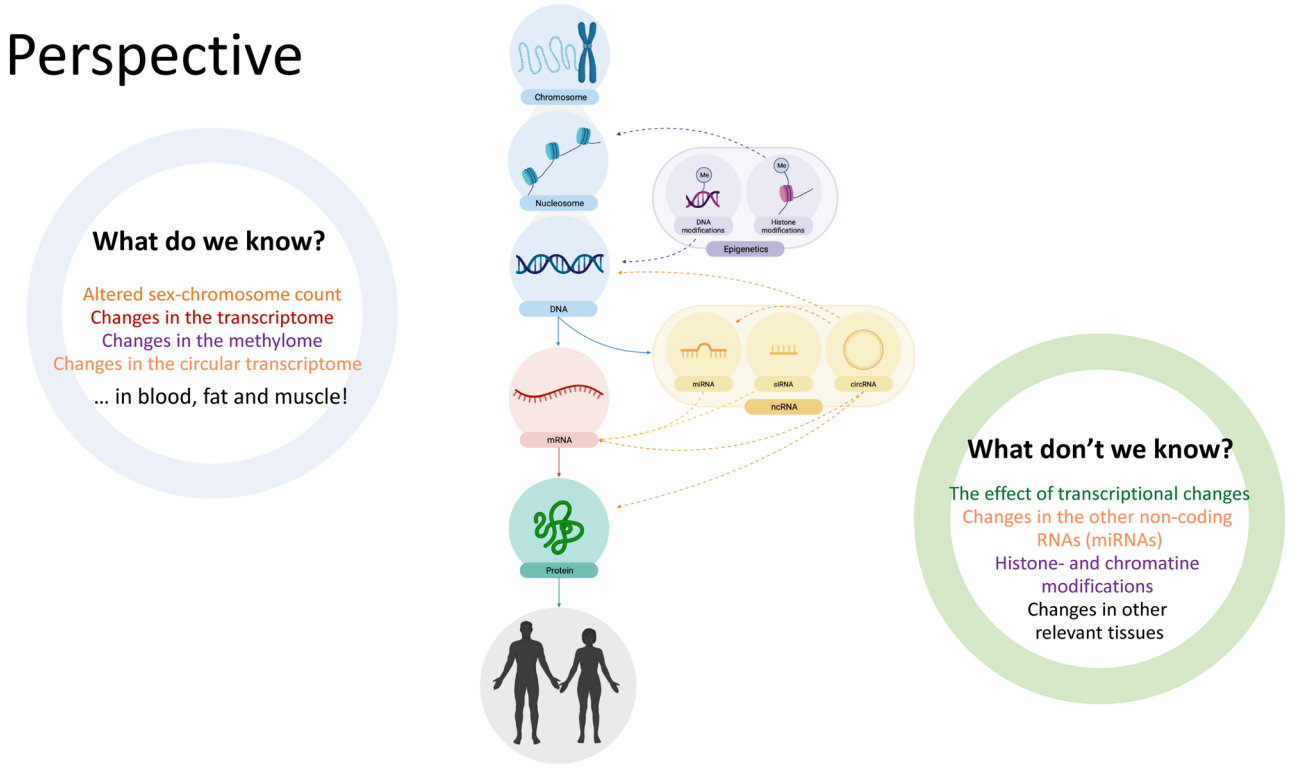

Figure 1
The current status of knowledge concerning the genomics of Klinefelter syndrome, 47,XYY and trisomy X. It has been realized that in addition to the altered chromosome count, there are changes in transcriptome, the non-coding transcriptome and the methylome across different tissues. It remains to be understood how these changes affect the phenotype and 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 needs to be achieved.

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, most but not all parameters determined with high-resolution pQCT improved (28). Alberto Ferlin summarized recent data and concluded that the relationship between testosterone, BMD and fracture is not clear in men with Klinefelter syndrome and that more research will be needed to better determine this relationship, as well as point 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, 31, 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 relationship 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 regarding the appropriate time to initiate TRT remain controversial without good data to guide

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clinicians (38). There is a clear need for additional studies, preferentially RCTs, focusing on advantages and drawbacks to early vs 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 testicular sperm extraction (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, 41, 42). Research is ongoing in understanding the causes of 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, 49, 50, 51), or at single-cell level using single-cell RNAseq (scRNAseq) (52, 53, 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, 56, 58, 58). Clinical studies among 47,XYY males have shown that the pubertal maturation, testicular histology and spermatogenesis are 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, 60, 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 are low in adolescents (65); however, the significance of these levels to risk of POI or future fertility remains 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 have 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, 69, 70, 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. Klinefelter syndrome vs 47,XYY vs trisomy X), there

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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 have 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 paediatric 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. The life course is characterized by late diagnosis, many un-diagnosed cases, non-specific increases in morbidity covering all ICD-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, 75, 76, 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 five-fold 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 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 out 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, 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, inspire more research in areas that

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specifically are underserved and 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 on 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.

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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