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OPINION ARTICLE

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Summary of the Concluding Round Table Discussion at the 2nd International Workshop on the Klinefelter Syndrome Münster, Germany, March 10–12, 2016.

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The Klinefelter syndrome: current management and research challenges

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Following the 1st International Workshop on the Klinefelter Syndrome in 2010 (Juul *et al.*, 2011), the 2nd IWKS took place in Münster, Germany from March 10 to 12, 2016 and was organized by the Centre of Reproductive Medicine and Andrology of the University of Münster. During the program, talks were presented by leading researchers in the field followed by lively discussions among the 120 participants.

The talks comprehensively covered basic and clinical aspects of the syndrome. The basic aspects included the mechanisms of X chromosome inactivation (Joost Gribnau, Christine Disteche), sex chromosome evolution in the primate lineage (Gabriel Marais), epigenetics (Joana Viana), gene expression studies (Liborio Stuppia), and animal models (Art Arnold, Armin Raznahan, Joachim Wistuba). Among the clinical aspects, current views on early/prenatal diagnosis (Frank Tüttelmann) and transitional care (Niels E. Skakkebæk, Alan Rogol) were reviewed. A large part of the workshop was devoted to comorbidities – with focus on cardiovascular and metabolic problems (Michael Zitzmann, Anders Bojesen) as well as osteoporosis (Alberto Ferlin). Neuropsychological, behavioral, and socioeconomic aspects were discussed (Hanna Swaab, Anne Skakkebæk, Nicole Tartaglia). Divergent experiences and opinions on fertility preservation and optimal time for TESE were presented (Sabine Kliesch, Hervé Lejeune). Following new findings on testicular steroidogenesis (Manuela Simoni), testosterone replacement in infants and young children (Carole Samango-Sprouse) and age-specific recommendations for management of patients with Klinefelter Syndrome (KS) met with great interest (Anders Juul). Differences and similarities between KS and Turner syndrome provided further insights into disorders of sex chromosome aneuploidies (Claus H. Gravholt). Finally, results of the 'dsd-LIFE study' on quality of life, satisfaction with care and needs of adolescents and men with KS (Birgit Köhler) and the European COST Initiative on DSD including KS (Olaf Hiort) were presented (for details of the program see www.klinefelter2016.de).

The purpose of the concluding round table was to discuss – based on the presentations and interactions of this workshop – the shortcomings of current care of patients with KS and to indicate future directions for patient management and research.

As an introduction, tribute was paid to Harry F. Klinefelter (1912–1990), who first described this syndrome in 1942 (Klinefelter *et al.*, 1942). Of the 120 workshop participants, only Alan Rogol and Eberhard Nieschlag had met Harry Klinefelter in person, the former as one of his medical teachers at John Hopkins in Baltimore and the latter at an International Klinefelter Symposium in Murnau in the Bavarian Alps in 1983 (Bandmann *et al.*, 1984).

SCREENING FOR EARLY DIAGNOSIS

The discussion was opened by arguments for and against neonatal screening for KS as this question had come up repeatedly during the workshop. *Alan Rogol* gave the reasons for screening all male newborns for 47,XXY (and perhaps all children for sex chromosome aneuploidy): primarily in anticipation of services required in childhood, such as early treatment of deficits encountered in speech, behavior/regulation of emotion, physical findings, delayed childhood milestones. Furthermore,

for information of parents, doctors, and health care practitioners as well as school and pre-school personnel (as much as parents wish, for there is a risk of stigmatization). An overarching service/education that pediatricians have to offer is anticipatory guidance, and newborn screening would allow pediatricians to provide this guidance to families concerned with KS.

However, based on his experience in Denmark, *Claus H. Gravholt* felt that it was premature to screen large populations for KS, as we do not yet have evidence that the different treatment options that we can provide are efficient and reduce morbidity, mortality, and improve outcome. There is no proof yet that early diagnosis and treatment is of advantage. Therefore, before a general program for all neonates is initiated, the merit of such screening should be explored in well-conducted investigations of defined populations. In general, one can consider population-based genetic screening if a condition is an important health problem with a latent early symptomatic stage, has a well-understood natural history, and there exist accepted treatments with associated facilities for providing diagnosis and treatment (Grosse *et al.*, 2009). These requirements are fulfilled by KS to some extent, but a formal proof of improved long-term adult outcomes is lacking. It may prove challenging to accumulate such evidence because of the rarity of the syndrome. Therefore, *Claus H. Gravholt* advocated large collaborative RCTs across Europe in order to answer some of these questions.

Birgit Köhler would also opt for a pilot study of early screening in boys. Measures should be taken to make the diagnosis of KS earlier as early special support for education can be given and testosterone therapy can be started in puberty in patients with testosterone deficiency. Fertility issues can be tackled before the age of 25 with possibly better results.

The current assumptions concerning the incidence of the KS in the general population are based on older extrapolations from genetic and statistical data, as *Eberhard Nieschlag* indicated. Although the sensitivity and specificity of KS diagnosis have improved, it remains unclear whether still only 25% of all patients are properly diagnosed and 75% remain undetected despite high morbidity and mortality, and thus frequent contact with doctors (Nieschlag, 2013). Screening of all male newborns could resolve this conundrum.

COUNSELING

Frank Tüttelmann, who had presented a talk on 'Increasing prenatal diagnosis of KS: controversies in clinical counseling' was provocatively questioned when he anticipated the birth of the last KS patient. He emphasized in his answer that non-invasive prenatal diagnoses concerning the sex chromosomes were currently hampered by high false-positive rates. However, these were technical issues that will most likely be resolved in the near future. Nevertheless, even if prenatal screening was to be routinely applied, KS would remain a diagnosis in which, in a large percentage of expectant mothers/parents, will decide on having the boy, in contrast to terminating the pregnancy. The rate of pregnancy termination heavily relies on post-test counseling (Meschede *et al.*, 1998).

In response to an opinion poll taken by the chairman Eberhard Nieschlag to determine whether individuals in the audience would or would not recommend terminating a pregnancy of an unborn baby with KS *Nicole Tartaglia* offered an important third choice that was not presented, namely providing counseling with updated, accurate information so that a woman and her partner could make their own decision about what was right for them (Tartaglia *et al.*, 2015).

FERTILITY

Hervé Lejeune concluded from the current literature (e.g. Aksglaede *et al.*, 2013) and the presentations and discussions during this workshop that: (i) TESE-ICSI provide similar results in KS as in men with non-obstructive azoospermia with normal karyotype, concerning sperm retrieval rate, pregnancy rate, miscarriage rate, and children's health. (ii) The experience of the surgeon and the biologist is important for the success of TESE. Micro-TESE performed by trained surgeons results on average in higher sperm retrieval rate than open biopsy; and (iii) The age range giving rise to higher chances of sperm retrieval is 15–30 years (Plotton *et al.*, 2015; Rohayem *et al.*, 2015). Conversely, some important issues remain to be investigated properly:

1 Whether previous testosterone treatment, even withdrawn for at least 6 months at the time of TESE is or is not deleterious to the sperm retrieval rate. This could be investigated, first retrospectively, by investigating the modality of the previous testosterone treatment (type, dose, and duration), and prospectively by randomizing young patients to different treatment modalities (usual treatment, low-dose treatment leaving the gonadotropin levels within the normal range, no treatment). This study will resolve the question whether it is necessary to perform TESE before initiating testosterone therapy or is it safe to wait until paternity is wished.
2 Whether a treatment designed to increase intra-testicular testosterone secretion (hCG, clomiphene, aromatase inhibitors) is efficient or not in increasing sperm retrieval rates. This could be investigated prospectively by randomized double-blind clinical trials vs. placebo. Multicenter studies would be useful to obtain enough statistical power; however, an effort of standardizing the practice of TESE-ICSI among the different centers will be necessary.
3 Identification of predictive markers of successful TESE would be helpful and should be developed.

Sabine Kliesch emphasized that such multicenter trials would require standardization of the techniques used in the participating centers and a strong collaboration between the involved clinicians and biologists to reconcile currently controversial conclusions from different studies (Plotton *et al.*, 2015; Rohayem *et al.*, 2015).

While attempts are being made to obtain and preserve testicular spermatozoa from adolescent KS patients, *Joachim Wistuba* also addressed approaches to cryopreserve testicular tissues from KS boys and adolescents who are not presenting with testicular gametes (Davis *et al.*, 2015; Gies *et al.*, 2016). Thus, medicine is making a promise that those tissues could offer an option for in vitro differentiation in 20 years, when such boys might have the wish to become fathers. However, to date, no reliable method for human in vitro spermatogenic differentiation is available. Thus, there is a medical as well as an ethical obligation for research. Here, animal models could be a worthy tool in development and efforts to keep this promise.

Concerning the question whether KS fathers would carry a great risk for producing aneuploidy offspring, *Stefan Schlatt* was of the opinion that this risk should not be greater than in the

general population as only euploid spermatogonia could produce viable spermatozoa. *Alberto Ferlin*, however, suggested that genetic counseling should be offered to the couple, as a higher risk of producing unbalanced spermatozoa has been reported in some studies, albeit not in others. More research on aneuploidy rate of spermatozoa is desirable.

Michael Zitzmann suggested a study or registry for children born after TESE-ICSI to KS fathers as the knowledge about these children and their genetic setting – except that their karyotypes are mostly normal – is rather limited and the medical profession has a responsibility for them. The karyotype of these children is assumed to be normal, but actually, information about this topic is far from complete. Also, the altered epigenetic setting on KS X chromosomes, an issue that was highlighted during the workshop, might be passed on to their daughters. In addition, altered epigenetic settings in KS can also be assumed for PAR regions on the Y chromosome and autosomes. These could also be transmitted to offspring.

TESTOSTERONE AND OTHER TREATMENTS

Although testosterone is routinely administered to patients with KS, the effects and outcomes of this treatment have not been evaluated in terms of evidence-based medicine. Why does the body composition of KS males remain distorted, with a higher fat mass and a lower muscle mass, despite very long-term treatment with appropriate testosterone supplementation? Is this because we are not able to supplement testosterone with sufficient precision, or is it because the body composition of KS is inherently changed, perhaps because of the chromosomal imbalance? Why do so many men with KS end up with limited education and in early retirement? Is it because of late diagnosis, with ensuing lack of focus on KS-specific problems during school years, or to limited intellectual capacity or is it because of poor testosterone supplementation and other supporting treatment? To overcome these fundamental therapeutic questions, *Claus H. Gravholt* suggested randomized controlled trials (RCT) to determine the efficacy of testosterone on different aspects of health (bone, heart, metabolism, etc.), on psychological parameters, on puberty induction, and in relation to fertility. Also, the available testosterone preparations and dose regimen should be compared in RCTs for their suitability for KS patients. In this context, it should also be important to study side effects, as different testosterone preparations (e.g. injectable vs. transdermal) may have different safety profiles (Layton *et al.*, 2015).

Alberto Ferlin also emphasized that the endpoints of testosterone therapy including levels of testosterone (and LH) obtained under treatment are not well supported by RCTs. Which parameter is the best marker of androgenicity? He also returned to his talk on 'Optimized treatment for osteoporosis' and pointed out that KS men have a high risk for osteoporosis when testosterone levels are either low or normal (or near normal), probably because low T is not the only cause of low BMD in these subjects (low vitamin D and low INSL3 may contribute) (Ferlin *et al.*, 2015). T replacement therapy is not fully efficient in increasing BMD or maintaining it. Studies on combining testosterone treatment with vitamin D (and calcium supplementation) are lacking, and more importantly, studies on the use of other agents for osteoporosis, such as bisphosphonates, in conjunction with testosterone treatment have never been performed. A multicenter clinical trial could be considered. Aside from BMD, other microarchitecture features of bone and bone strength, as well as fracture risk of KS subjects (including other risk factors for osteoporosis) are not well investigated and RCTs are advocated.

Frank Tüttelmann suggested analyzing the CAG repeat in the androgen receptor gene to predict testosterone treatment effects in KS (Zitzmann *et al.*, 2004) and wondered which measure should be used in heterozygous KS men (X-weighted mean?).

Nicole Tartaglia who had given a talk on 'Behavioral and social phenotypes in 47,XYY or 47,XXY boys' addressed a concern raised by an audience member: it seemed researchers were trying to make boys with KS superhuman by giving them testosterone, rather than fostering acceptance that they may not be leaders or the best in their class. 'The goal of testosterone treatment in adolescents with KS is not to make them superhuman, but to replace testosterone to a normal level for their age and development (Rogol & Tartaglia, 2010). Testosterone is not a "cure" for the neurodevelopmental effects of KS, and even with appropriate testosterone therapy, there will still be a higher rate of differences in learning and behavior because of the effects of the extra X chromosome on brain development. When adolescents are treated with testosterone, they describe many of the same effects of hypogonadal men when they are treated – such as improvement in energy level/stamina, attention span, mood, and general well-being. These are all very important areas for teenagers to be successful in school and socially. In my opinion, the goal of treatment is to help them do the best they can to reach their potential without testosterone deficits, but testosterone is not going to cure all learning or other psychosocial issues associated with KS'.

Concerning the discussion on testosterone therapy during puberty, *Birgit Köhler* had the impression that there was some fear of giving testosterone at this age and suggested randomized controlled trials to investigate a possible benefit of early testosterone therapy in patients with testosterone deficiency. She would prefer treatment with testosterone gel as it can be given in more physiological doses.

However, *Carole Samango-Sprouse* felt confident that early hormonal treatment (EHT) is helpful to these boys as each and every boy was seen by his pediatric endocrinologist prior to beginning treatment. She believes that EHT is not a cure but does help significantly to minimize the boys' developmental challenges and behavioral issues based on her and other publications (Rogol *et al.*, 2014; Samango-Sprouse *et al.*, 2015).

GENETIC AND BASIC RESEARCH

Joachim Wistuba addressed research issues with special regard to animal models. When trying to understand the effects of a supernumerary X in the male physiological environment, the KS patient is extremely difficult to investigate because of the enormous complexity and heterogeneous phenotypic appearance. In the mouse models, fewer than 10 escapee genes are sufficient to induce a phenotype resembling the human KS as well as an animal model can do (Wistuba *et al.*, 2010; Tüttelmann *et al.*, 2014). The experimental work in animal models is therefore indispensable to enable genotype–phenotype correlates, as well as to understand basic physiological and metabolic changes associated with an extra X chromosome in a male environment. Only if these effects are explored in a less complex mammalian system, conclusions might be transferred to the human disorder.

However, this does not disengage the scientific community from analyzing the human/clinical aspects of KS further, but should rather result in an intensive translational approach between disciplines.

Christine M. Disteche made a plea for further basic research on the roles of specific genes and of epigenetics in phenotypes of Klinefelter patients. She indicated that research focused on cell types relevant to Klinefelter is important and suggested that one should consider novel methods to generate specific human tissues which have made tremendous progress in the last few years in terms of combining biological approaches with engineering approaches.

Frank Tüttelmann suggested investigations on the causes underlying the phenotypic heterogeneity in KS. Does the influence of – potentially undetected – mosaicism, for example, testicular mosaicism explain foci of spermatogenesis? The role of the parental origin of the supernumerary X needs further elucidation: so far relevant studies are contradictory and underpowered and do not permit convincing conclusions.

Jörg Gromoll raised the issue of the increasing gap between the recent exciting gain of knowledge on the X chromosome with respect to X inactivation (Disteche & Berletch, 2015; Maduro *et al.*, 2016), escapee genes, and their organ-specific expression pattern compared to the somewhat outdated genetic diagnosis of KS by karyotype analysis only. To close this gap and make use of the emerging new technologies and knowledge, it would be necessary to obtain more information on the origin and haplotype of the X chromosome. He suggested that buccal smears from at least one parental side should be obtained, which would enable the precise origin of the supernumerary X chromosome (paternal/maternal and identical or different). This would allow the influence of the X chromosomal origin on the heterogenic phenotypical appearance of KS patients to be studied in midterm.

PATIENT CARE AND CENTERS OF COMPETENCE

KS is associated with an increased rate of multiple morbidities and increased mortality. Nevertheless, as *Joachim Wistuba* pointed out, the specific disorders are only diagnosed and treated by the respective specialists for the single disease and a holistic approach to the patient as an entity is lacking. To overcome this shortcoming, *Alberto Ferlin* requested multidisciplinary centers of competence for KS patients.

Alan Rogol reminded the panel to specifically focus on the process of transition and thus to focus on preparation for transfer to adult care, the actual transfer, and then how these emerging adults cope with the new (and very different) adult-oriented health care system as well as patients' success in the educational and vocational spheres.

The multidisciplinary clinics in the USA have been shown to be a success, as *Emily Wadsworth* pointed is correct. These provide a central location for Klinefelter patients to have all required services in one integrated clinic where their medical information can be shared more efficiently as compared to the current approach in some countries. Implementing multidisciplinary clinics in any country will primarily benefit the current younger generation of Klinefelter patients because of having support services upfront and centrally located. This entails a significant expense, but the long-term economic savings will be visible. To provide an example, as infants, children, and adolescents become adults, the economic benefit within the health and mental health system will become evident, as they naturally mature to adulthood confident and even self-aware of their syndrome. The need for support services will decrease in some areas as services are better integrated. The multidisciplinary clinics in the USA are a fantastic example of what a clinic should look like!

Nicole Tartaglia, as the director of an interdisciplinary clinic for children with XXY and other sex chromosome disorders in Colorado, USA called the eXtraordinarY Kids Clinic, definitely felt that it is advantageous for families to receive interdisciplinary care by experts who are up-to-date on research and who have experience with many previous patients (Tartaglia *et al.*, 2015). 'When new families come to our clinic, they often express that previous providers were inexperienced with KS and often could not answer whether the neurodevelopment or medical findings of their children were related to KS, whereas specialized centers provide better care and patient satisfaction, integrating recommendations for medical and psychological care. An important next step, however, is to evaluate what this clinic model actually improves for patients. Coordinating and running an interdisciplinary clinic is often an expense to hospitals, and so we need further data to support that these clinics are cost-effective, improving patient satisfaction, driving important research, and improving overall patient outcomes'.

Furthermore, *Carole Samango-Sprouse* believes that the benefits of early intervention services have been well proven in the USA in many different populations of children with special needs including Down syndrome, ASD, and speech and language delay among others. With these services, boys with XXY are likely to have fewer behavioral issues, be more successful and independent based on experience in the last 20 years. Clinical trials on early intervention services does not seem like a useful idea.

In terms of the need for specialized centers that increase awareness of KS among non-specialized medical doctors and other health care providers, it appears mandatory to increase proper diagnosis as early as possible. As *Eberhard Nieschlag* pointed out, the specialized centers depend on the transfer of pre-diagnosed patients from the periphery. Not only endocrinologists and andrologists need to be educated, but a special effort should be made to teach also non-endocrinologists/non-andrologists who deal with the various symptoms and comorbidities of KS patients and have no idea about the underlying chromosomal disorder. An important step in this direction would be if all physicians would examine the testes of their patients routinely as small testicular volume is the most consistent symptom of KS, pointing the physician in the right direction. Therefore, physical examination of the testes should be part of graduate and postgraduate training (Nieschlag, 2013).

ROLE AND BENEFIT OF PATIENT SUPPORT GROUPS

'The conference was fantastic' *Emily Wadsworth* summarized her participation in the workshop as a representative of a support group. 'The work of each and every clinician, scientist, and researcher is admirable and the parents appreciate the work each and every person does to give all Klinefelter children and adult Klinefelter patients a better quality of life. Having a representative from several support groups present at such meetings is extremely beneficial as it allows the support group

representative to elicit technical information from the workshop, and translate this information back to their Klinefelter support communities in a more easily digestible manner – a middle man approach.'

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