2nd International Workshop on Klinefelter Syndrome

March 10–12, 2016
Münster, Germany

Venue

Factory Hotel

An der Germania Brauerei 5
48159 Münster, Germany

Program Organizing Commitee

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Germany

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Germany

www.klinefelter2016.de

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Welcome!

On behalf of the program and the local organizing committees, I would like to welcome you to the 2nd International Workshop on Klinefelter Syndrome.

In 2010, the first International Workshop on Klinefelter Syndrome was held in Copenhagen with great success. Over the past few years, our knowledge of Klinefelter Syndrome has significantly improved. The newly discovered clinical phenotypes suggest that the presence of a supernumerary chromosome leads to a multifactorial disease, in which hypogonadism is only one facet amongst others.

In view of the steadily emerging fields of clinical and scientific research on Klinefelter Syndrome, we have decided to organize the 2nd International Workshop in Münster. We hope to have compiled a program that will cover the different aspects of Klinefelter Syndrome ranging from X inactivation to neuropsychological effects. Renowned experts will share their most recent studies, but the 2-day event will also give young scientists and clinicians an opportunity to present their recent work during the oral communication and poster session. The workshop will be summed up in a Round table discussion, addressing emerging future clinical and scientific topics and tasks pertinent to Klinefelter Syndrome.

We hope that you will experience a stimulating workshop, which hopefully will not only broaden your knowledge on Klinefelter Syndrome and but also will lead to new collaborations.

The workshop will take place in Münster, a city of science, city of sculptures, city of the Peace of Westphalia, city of bicycles and Hanseatic city. Last but not least Münster has been awarded as one of the most livable cities in the world and is definitely worth spending some additional days enjoying cultural or social events. A first short impression will be given to you during our guided bus tour on Thursday evening.

Yours sincerely,

Jörg Gromoll, PhD

Organization of the Workshop

Venue

The workshop will be held at the Factory Hotel at the Germania (former beer brewery) - Campus. The Campus resembles beside the hotel several pubs and restaurants as well as the Cloud Lecture hall, where the workshop will take place. Everything is within very short walking distance. Free internet will be available at the lecture hall and the hotel.

For more information please visit the hotel or the lecture hall homepage.

Factory Hotel

http://www.factoryhotel-muenster.de/en/welcome

Cloud Lecture Hall

http://www.factoryhotel-muenster.de/en/meet/raeume/cloud

Registration and Information Desk

There will be a registration and information desk at the foyer of the Cloud lecture hall.
Mrs. Barbara Fischer-Rittmeyer and her colleagues will be happy to assist you concerning transportation, accommodation etc.

Media and Speakers' slide preview

In the foyer of the Cloud lecture room you will find assistance for uploading your presentation. Every presentation should be uploaded latest during the preceding session.

Poster Exhibition

The Poster session will be on Friday, March 11 from 1.30 – 2.30 pm at the Cloud lecture hall. We request that every presenting author should be available during this time at his poster.

Exhibition and Information

In the foyer there will be booths from industry and Klinefelter Organizations.

Transportation

If you need assistance in organizing transportation or changing your travel plans, please refer to the information desk.

Welcome Reception

There will be a guided tour of Münster with a Double-decker bus on Thursday. The bus will start at 7.30 pm in front of the Factory Hotel. The tour will be approx. 1, 5 hours and a food box containing typical Westphalian food and drinks will be served during the bus ride. In addition, one of the Münsterland's landmarks - the Kiepenkerl - who was an itinerant trader with a traditional basket on his back, will join us and give us some more information of Münster.

Thereafter, everyone is free to meet in the pubs or restaurants surrounding the Factory Hotel

Dinner

Dinner will be served on Friday, starting at 7 pm at the Mole Restaurant, which is situated between the Factory Hotel and the Cloud Lecture hall. A dinner buffet will be served.

Additional tickets for the dinner (30€/ticket) can be obtained at the information desk on Thursday only.

The Centre of Reproductive Medicine and Andrology (CeRA), Münster, Germany

Director and Head of the Institute of Reproductive and Regenerative Biology:
Professor Stefan Schlatt, PhD
Chief Clinician, Head of Department of Clinical Andrology:
Professor Sabine Kliesch, MD

The **CeRA** consists of the Institute of Reproductive and Regenerative Biology and the Department of Clinical Andrology of the University Hospital Münster (UKM). Scientists perform basic and translational research with strong focus on physiology, fertilization, embryo development and regeneration of cells and reproductive tissue and act back to back with the Clinical Andrology. The clinical services focus on male infertility, hypogonadism and sexual medicine. We provide modern diagnostics and all endocrine, medical and surgical treatment options to male patients of all ages. Treatment of couple infertility is jointly offered by the Clinical Andrology and the Department of Gynecology and Obstetrics, with all modern ART procedures available in our Fertility Center at UKM (http://campus.uni-muenster.de/cera/).
Since 1987, the CeRA acts as **WHO Collaboration Center (WHO CC)** for Research in Male Reproduction and since 1994 as official **Training Center for Andrology of the European Academy of Andrology (EAA)**. As WHO CC its activities were originally focused on research in **Male Contraception** and the editorial assistance in compilation of the **WHO Manual on Semen Analysis** (last 5th edition 2010), which was also translated into German by CeRA scientists. Standardization of semen analysis is still an important task and Professor Kliesch is member of the Federal Medical Board for quality control in semen analysis. The **Quality Control Program** of the German Society of Andrology (QuaDeGA) is hosted at CeRA. More than 20 courses and workshops in practical semen analysis are offered annually by CeRA staff providing training for urologists, andrologists, gynecologists, young MDs and medical students.

The CeRA has a strong focus on translational research in **male infertility and hypogonadism**, including the emerging field **of reproductive genetics**. The **Münster EXAKT** study on the Klinefelter Syndrome is an essential part of these activities. Novel areas for research were promoted in recent years, such as the **ageing male germ cell**, the effects of **embryo culture media** and innovative aspects in **sperm physiology and selection**. The CeRA has been a pioneer in **fertility preservation** both in male adults and adolescents and recently started collaborative research with several European partners to create novel stem cell based options for fertility preservation in prepubertal boys and founded the network ANDROPROTECT®. The variety of clinical services and research topics creates a scenario in which male reproductive health is considered throughout the entire life span.

Workshop Programm

Thursday, March 10		**Page**
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	Increasing prenatal diagnoses of KS: controversies in clinical counselling – Frank Tüttelmann, Germany	10
	Epigenetic signatures in the brain of KS patients - Joana Viana, UK	11
	X Chromosome regulation - Christine Disteche, USA	12
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	Neuroimaging Genomics of Sex Chromosome Aneuploidy- Armin Raznahan, USA	14
	The XXY- mouse models and their clinical counterpart - Joachim Wistuba, Germany	15
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Speaker Abstracts

Joost Gribnau

Department of Reproduction and Development, Erasmus MC, University Medical Center Rotterdam, Netherlands

Joost Gribnau received his PhD at the Erasmus University Rotterdam (1999), and did his postdoctoral training at the Whitehead Institute for Biomedical Research / MIT (Cambridge, USA). In 2004 he started his own research group at the Erasmus MC, and became professor of Epigenetics in 2012. In 2015 he was appointed chair of the department of Developmental Biology at the Erasmus MC. He is EMBO member since 2015. Research in his laboratory focusses on questions in the field of sex chromosome and stem cell biology. Currently, his research group studies the mechanisms directing initiation of X inactivation, and spreading of the long non-coding RNA Xist, in the context of embryonic development and cell differentiation. His research group also hosts the Erasmus MC iPS core facility, and is involved in the development of novel technologies to study the epigenome.

Activation of X Inactivation

In mammals, gene dosage of X chromosomal genes is equalized between the sexes by random inactivation of either one of the two X chromosomes in female cells. XIST/Xist is an X-linked non-coding gene playing a crucial role in this X chromosome inactivation (XCI) process. XIST/Xist RNA accumulates on the future inactive X chromosome and initiates silencing in cis. Silencing is robust, involving a plethora of epigenetic modifications, and only a few genes on the X chromosome escape the inactivation process. In the initial phase of XCI, a counting and initiation process determines the number of X chromosomes per nucleus, and elects the future inactive X chromosome. This process is directed by X-linked activators and autosomally encoded inhibitors of the XCI process. In my seminar I will discuss our new insights in the mechanism by which these factors direct activation of X inactivation. I will also present data that shed new light on the mechanisms involved in XCI mediated gene silencing and escape of XCI.

Frank Tüttelmann
Institute of Human Genetics, University of Münster
Münster, Germany

Frank Tüttelmann, MD, started his scientific career at the Centre of Reproductive Medicine and Andrology. He is certified Clinical Andrologist of the European Academy of Andrology, a specialist in human genetics, and senior physician and deputy director of research and teaching at the Institute of Human Genetics, Münster, Germany. His primary research topic is reproductive genetics with a strong focus on male infertility including Klinefelter Syndrome. The majority of his publications deals with genetic causes of spermatogenic failure. He founded the International Network for Young Researchers in Male Fertility (INYRMF) and received several prizes for his research.

Increasing prenatal diagnoses of KS: controversies in clinical counselling

Klinefelter Syndrome (KS) is the most common chromosome aneuploidy in males with a prevalence at birth of 1-2/1000 newborn boys. To date, the majority of KS subjects are diagnosed after puberty because of clinical symptoms, e.g. delayed puberty, undervirilisation, and infertility. During pregnancy, first trimester screening by ultrasound, which is applied routinely, is not able to detect KS. In contrast, KS may be discovered incidentally upon invasive prenatal diagnostics like amniocentesis performed for other reasons, e.g. advanced maternal age. However, because the invasive procedures have an inherent risk for miscarriage, they are not performed routinely. Recently, non-invasive prenatal testing (NIPT) has been introduced, which utilises free foetal DNA that circulates in the maternal blood. The respective tests were first only able to detect trisomies of chromosomes 21, 13 and 18 that lead to severe malformations and mental disability. NIPT was quickly developed further to include detection of the sex chromosomal constitution. This may be clinically valuable if the mother is carrier of an X chromosomal disease and invasive testing would only be performed if the fetus is male. However, the test may also be used to detect the sex of the fetus early on just for curiosity of the parents. Depending on the setting, this additional information can be “bought” as an add-on to the basic test. Any genetic test should be accompanied by respective counselling, which may, however, be brief at best in daily practice. In combination with NIPT most likely becoming routine in the very near future, this leads to more and more unsolicited or at least unsuspected findings of sex chromosomal aberrations. Thus, a large need for counselling arises. One enlightening example is the highly variable rate of induced abortions after prenatal diagnosis of KS, which may range from as low as 10% to as high as 70%. The latter seems high considering the high variability of the KS phenotype, in a large proportion currently not even diagnosed throughout life. It is well known that health professionals providing genetic counselling influence the parents’ decision against or towards pregnancy termination with the pregnancy more likely to continue if the counselling is given by a specialized geneticist. In conclusion, NIPT leads to a steep increase in prenatal diagnoses of KS which poses challenges to all disciplines involved in the diagnostic procedures and especially counselling.

Joana Viana
University of Exeter Medical School
Exeter, Devon, EX2 5DW, UK

Joana graduated in Cell and Molecular Biology at the Universidade Nova de Lisboa, Portugal, in 2011. She completed an MSc in Neuroscience at King's College London in 2012. During this period, she developed a research project under the supervision of Dr Ruth Pidsley and Prof Jonathan Mill. The primary aim of her project was to access global methylation differences in post-mortem brain samples from schizophrenia patients and controls. From September of 2012 and January 2013 Joana worked as a research technician with the Psychiatric Epigenetics Group at King's College London. She is currently finalizing her PhD under the supervision of Prof Jonathan Mill and Dr Eduarda Santos. Her project consists in modelling epigenetic responses to schizophrenia-associated environmental risks and antipsychotic medication.

Epigenetic signatures in the brain of KS patients

J. Viana, R. Pidsley, C. Troakes, H. Spiers, C. C. Wong, S. Al-Sarraj, I. Craig, L. Schalkwyk, J. Mill

Klinefelter Syndrome (KS) is the most common sex-chromosome aneuploidy in humans. Most affected individuals carry one extra X chromosome (47,XXY karyotype) and the condition presents with a heterogeneous mix of reproductive, physical and psychiatric phenotypes. Although the mechanism(s) by which the supernumerary X chromosome determines these features of KS are poorly understood, skewed X chromosome inactivation (XCI), gene-dosage dysregulation, and the parental origin of the extra X chromosome have all been implicated, suggesting an important role for epigenetic processes. We assessed genomic, methylomic and transcriptomic variation in matched prefrontal cortex and cerebellum samples identifying an individual with a 47,XXY karyotype who was comorbid for schizophrenia and had a notably reduced cerebellum mass compared with other individuals in the study (n = 49). We examined methylomic and transcriptomic differences in this individual relative to female and male samples with 46,XX or 46,XY karyotypes, respectively, and identified numerous locus-specific differences in DNA methylation and gene expression, with many differences being autosomal and tissue-specific. Furthermore, global DNA methylation, assessed via the interrogation of LINE-1 and Alu repetitive elements, was significantly altered in the 47,XXY patient in a tissue-specific manner with extreme hypomethylation detected in the prefrontal cortex and extreme hypermethylation in the cerebellum. This study provides the first detailed molecular characterization of the prefrontal cortex and cerebellum from an individual with a 47,XXY karyotype, identifying widespread tissue-specific epigenomic and transcriptomic alterations in the brain.

Christine M. Disteche
University of Washington
Seattle WA98195, USA

Christine Disteche is a Professor in Pathology and Adjunct Professor in Medicine (Division of Medical Genetics) at the University of Washington in Seattle. She has made many contributions to the study of mechanisms of regulation of the sex chromosome in mouse and human. Her laboratory has published significant papers on epigenetic and structural features associated with regulation of the X chromosome. She also works on genes that escape X inactivation, which play a role in sex differences and contribute to phenotypes in individuals with Klinefelter Syndrome.

X chromosome inactivation and escape from inactivation

Complex mechanisms of dosage compensation regulate the mammalian X chromosome due to the presence of one X copy in males and two in females. X chromosome inactivation silences one X chromosome in females in early development, leading to specific molecular and structural changes on the inactive X chromosome. We have shown that the inactive X chromosome forms a bipartite structure within the nucleus. Genes that escape X inactivation are located at the periphery of the condensed inactive X chromosome. Our allele-specific analyses using RNA-seq have shown that genes that escape from X inactivation vary between tissues. This causes sex-specific differences that manifest as differential gene expression. We have found that significant sex bias in gene expression is associated with escape from X inactivation in human tissues. In addition, individuals with an abnormal number of X chromosomes show abnormal expression of X-linked and autosomal genes.

Arthur P. Arnold
University of California
Los Angeles, USA

Arthur P. Arnold has long studied biological factors that make males and females different, and has introduced and studied several animal models of sexual differentiation of the brain and other tissues. His current focus is on the direct differentiating roles of the sex chromosomes, XX vs. XY, and effects of sex chromosome aneuploidy.

Studies of the Mouse Models of Klinefelter Syndrome: Cognitive and Physical Traits

A.-P. Arnold, S.M. Williams-Burris, S.M. Aarde, J.D. Jentsch, E. Vilain, S.A. White, H.E. Hrncir, R. Mackie, M. Ruiz-Sundstrom, J.D. Martinez, G. Beroukhim, D.C. Yu, Y. Zhang

We have studied two mouse models of Klinefelter Syndrome (KS), the C57BL/6 XY* model that compares XY and XXY males, and the MF1 strain Sex Chromosome Trisomy (SCT) model, which generates XY and XXY mice, each genotype with testes (males) or ovaries (females). Mice are studied either gonadally intact or after gonadectomy (GDX) in adulthood. We have measured non-behavioral traits in SCT mice, including body weight and adiposity before and after GDX, and bone parameters. Sex and genotype affect body weight and percent body fat over the lifespan where males > females and XXY > XY. These effects are only significant in intact animals or those gonadectomized and treated with testosterone, suggesting that gonadal hormones may be necessary for the expression of the genetic differences. Femur length was greater in GDX males than intact males, and GDX XXY males had longer femurs than GDX XY males. Gonadally intact XY males had greater bone density than all other groups, but GDX XY and XXY did not differ in bone density. We also measured cognitive and behavioral phenotypes in several tests of the XY* model: 1) ultrasonic pup vocalizations as a model of speech and language delay, 2) novel object recognition as a test of memory, 3) reward response to gauge the interest in a palatable food (sweetened condensed milk, SCM) used as a reinforcer, and 4) reversal learning as a test of executive functioning including working memory, attention, and impulse inhibition. There were no differences in novel object memory between XY* and XX^{Y}*. In both intact and GDX mice, the XY* males preferred 3% SCM more than the XX^{Y}* mice, but both genotypes preferred 10% SCM equally. Intact XX^{Y}* mice required significantly more trials to reverse learning than the intact XY* mice, suggesting an executive function deficit that may models that seen in KS men. Reversal learning did not differ in GDX XY* vs. XX^{Y}*. We are expanding these and other behavioral tests to the SCT model, with the eventual goal of identifying X genes that make XXY mice different from XY, in phenotypes that model those found in KS men.

Funding: NIH grants R01HD076125 and T32HD07228

Armin Raznahan
Child Psychiatry Branch, National Institutes of Mental Health
Bethesda, MD, USA

Armin Raznahan, MD, PhD, is a Lasker Clinical Research Scholar and Chief of the Developmental Neurogenomics Unit. His research combines neuroimaging, genomic and bioinformatic techniques to better understand the architecture of human brain development in health, and in neurogenetic disorders that increase risk for psychiatric symptoms. Clinically, Dr. Raznahan works as a Child Psychiatrist within the NIH Clinical Center Psychiatry Consultation Liaison Service. He has a degree in Medicine and a PhD in Biological Psychiatry from King¹s College University London, UK. He has completed residencies in pediatrics and psychiatry, and a specialist fellowship in child and adolescent psychiatry at the Maudsley Hospital, London, UK.

Neuroimaging-Genomics of Sex Chromosome Aneuploidy

Sex chromosome aneuploidies (SCAs) increase risk for a range of neurodevelopmental morbidities. Better understanding this elevated risk requires clarifying (i) which brain systems are sensitive to changes in X- and Y-chromosome dosage, and (ii) which gene-sets are most likely to mediate the effects of SCA on brain development. We specify brain systems that are anatomically altered by changes in X and Y-chromosome dosage through high-resolution structural neuroimaging in humans (n= 300, karyotypes: XO, XX, XXX, XY, XXY, XYY, XXYY) and mice (n= 90, karyotypes: XO, XX, XY, XXY) with assorted SCAs. We study candidate transcriptomic drivers of SCA phenotypes by (i) modeling gene dosage effects in beadarray measures of gene expression in human SCA lymphoblastoid cell-lines (LCLs), and (ii) merging maps of altered brain anatomy in murine SCA with publically-available atlases of brain gene-expression. Both humans and mice show replicable evidence for regionally-specific X chromosome aneuploidy effects on brain anatomy, involving systems that are critical for adaptive social functioning in each species. In humans, we identify a strong overlap between X- and Y- chromosome supernumeracy effects within the cortex and subcortex – suggesting a role for dosage-sensitive X-Y chromosome homologous gene pairs. Our transcriptomic analyses in human SCA LCLs prioritize sex chromosome genes by dosage sensitivity and reveal order-of-magnitude differences amongst SCAs in the degree of autosomal gene dysregulation. In mice, distributed brain regions with differing profiles of anatomical change across XO, XX, XY and XXY groups show distinct patterns of gene-expression which point towards a striking interplay between sex-chromosome and sex-steroid effects on brain development. Our multimodal cross-species approach provides a set of highly articulated and falsifiable hypotheses regarding specific pairings of gene-sets and brain-regions which may mediate the neurodevelopmental impairments that can accompany altered X and Y chromosome dosage. Testing these hypotheses in tractable model systems will further advance the translational science of SCA.

Joachim Wistuba
Centre of Reproductive Medicine and Andrology, University Hospital of Münster
Münster, Germany

Joachim Wistuba started his career at the department of Zoology at the University Münster before he specialized in reproductive biology and endocrinology. He has worked in the field with various animal species for more than 15 years, dealing with testicular development and physiology, questions of sperm competition and its testicular reflections and translational approaches using animal models for male reproduction. His current research focus is on an experimental mouse model for Klinefelter Syndrome as well as on in vitro models for germ cell differentiation. His work on Klinefelter Syndrome was twice awarded by the German Society of Endocrinology with the Dietrich-Knorr Price.

The XX^{Y*}- mouse models and their clinical counterpart

Klinefelter Syndrome (KS, 47,XXY) provokes features as infertility, hypogonadism, gynecomastia, disturbed bone metabolism, diabetes, vascular and cardiac problems and cognitive deficits. Although morbidity and mortality are increased, to date, the disorder is strongly underdiagnosed, perhaps because the phenotype is heterogeneous and the combination and severity of the symptoms highly variable. Generally, a loss of germ cells and hypergonadotropic hypogonadism are observed. Genetic and epigenetic changes were related to the clinical phenotype of patients exhibiting an affected DNA methylation profile and differentially expressed X chromosomal and autosomal genes. However, clinical studies are limited. Thus, it is of advantage that mouse models for KS are available resembling the human disorder. Molecular mechanisms and the actual influence of the genetic impact of the second X chromosome - not addressable in patients - can be examined in these mice. For example, in mice only few genes escape from silencing of the second X chromosome making the analysis feasible compared to the more complex situation in patients. However, these few most relevant escapee genes which mice and men share, i.e. Utx, Kdm5c, Eif2s3x and Ddx3x are also inducing a phenotype very similar to human KS. Analyzing the expression of these genes in 41,XX^{Y*} male mice, we found tissue- and gene- but also development-specific expression profiles rendering genotype-phenotype relations more complex than thought so far. Physiologically, data from mouse models elucidated that the disturbed bone metabolism and the cognitive deficits have a genetic background and are not only metabolic consequences of the disorder. In addition, experimental exploration using the animal models revealed the germ cell loss to start already during the intrauterine life period and the steroidogenic Leydig cells to function normally although being hyperactivated, i.e. not to be causative for hypogonadism, a finding which was later confirmed in patients. The latter finding pointed to a possible problem of testicular blood supply which we could recently demonstrate by stating that the testicular vascularization in 41, XX^{Y*} mice is disturbed. Taken together, the evidence obtained in the mouse models for KS suggests that the further use of these animals for the examination of the effects of the presence of a supernumerary X chromosome in a male environment might be of importance also for future therapeutic developments.

Gabriel Marais
Université Lyon
Villeurbanne, France

Gabriel Marais did a PhD on bioinformatics for whole genome analysis, one of the first in France. This last 10 years, he and his research group at LBBE in Lyon have worked on the evolution of sex chromosomes in animals and plants. This research effort has included several studies on human and non-human primate sex chromosomes and have addressed questions about how recombination suppression between X and Y, the degeneration of the Y, the gene conversion within the Y and the X dosage compensation have evolved.

The evolution of dosage compensation and the implications for human X aneuploidy syndromes

How and why female somatic X chromosome inactivation (XCI) evolved in mammals remains poorly understood. It has been proposed that XCI is a dosage-compensation mechanism that evolved to equalize expression levels of X-linked genes in females (2X) and males (1X), with a prior twofold increase in expression of X-linked genes in both sexes ("Ohno's hypothesis"). Whereas the parity of X chromosome expression between the sexes has been clearly demonstrated, tests for the doubling of expression levels globally along the X chromosome have returned contradictory results. However, changes in gene dosage during sex-chromosome evolution are not expected to impact on all genes equally, and should have greater consequences for dosage-sensitive genes. We have worked on RNA-seq data from different human tissues/organs and on data on protein complex genes from the HPRD database. We show that, for genes encoding components of large protein complexes (≥ 7 members) - a class of genes that is expected to be dosage-sensitive - expression of X-linked genes is similar to that of autosomal genes within the complex. We also explore the contribution of dosage-sensitive genes to X aneuploidy phenotypes in humans, such as Turner (X0) and Klinefelter (XXY) Syndromes. X aneuploidy in humans is common and is known to have mild effects because most of the supernumerary X genes are inactivated and not affected by aneuploidy. Only genes escaping XCI experience dosage changes in X-aneuploidy patients. We combined data on dosage sensitivity and XCI to compute a list of candidate genes for X-aneuploidy syndromes. These data support Ohno's hypothesis that XCI acts as a dosage-compensation mechanism, and allow us to refine Ohno's model of XCI evolution. We also provide a list of candidate genes for X-aneuploidy syndromes, which could serve for future studies.

Claus Højbjerg Gravholt
Aarhus University Hospital
Aarhus C, Denmark

Currently working as a consultant and professor at Aarhus University Hospital, Denmark, Department of endocrinology and Internal Medicine, and the Department of Molecular Medicine. I have worked clinically and scientifically with sex chromosome abnormalities for the last 20 years. I have performed clinical, genetic, epidemiological and experimental studies. I have published more than 10 original publications and review papers. I am active participant in the international Turner and Klinefelter syndrome research community, as well as national and international research societies.
My main research activities are in the endocrinology of Turner and Klinefelter Syndrome, focusing on GH, IGFs, androgens and estrogens, but also aspects of diabetes and metabolism, epidemiology, cardiology, and genetics.

Turner syndrome – the other side of the coin?

While Klinefelter Syndrome (KS) is the most frequent male sex chromosomal disorder (150 per 100,000 males), Turner syndrome (TS) is the second most frequent female sex chromosome disorder (50 per 100,000 females). KS have an extra X chromosome (47,XXY) and persons with TS typically lack a X chromosome (45,X), and as such perhaps these two conditions could be seen as opposites? The typical male suffering from KS has traditionally been described as tall, with narrow shoulders, broad hips, sparse body hair, gynecomastia, small testicles, with androgen deficiency, azoospermia and decreased verbal intelligence. However, the phenotypic spectrum is very broad. The typical female with TS is described as short, with a broad chest and altered body composition, estrogen deficiency, gonadal dysgenesis and thus infertility, with normal intelligence and a range of external stigmata which is often present. However, just as for KS, the phenotypic spectrum is wide. While the divergence in height and the presence of some phenotypic characteristics like micrognathia and sensorineural deafness in TS, are readily explained by the number of active SHOX genes (1 active in TS, 3 active in KS, and of course 2 active in both normal males and females), there are a number of traits that are actually comparable between KS and TS. For example, the occurrence of hypergonadotropic hypogonadism and resulting sex hormone deficiency, germ cell deficiency, type 2 diabetes which is seen with 4-fold increased frequency in both TS and KS, osteoporosis, neurocognitive difficulties (although the neurocognitive profile of KS and TS is clearly not similar), and also a reduction in lifespan, although again, there are probably different causes for this in KS and TS. Although the SHOX gene has been firmly established as causing some of the phenotypic traits in both KS and TS, there has since the description of the SHOX gene, been a paucity of new genes or genetic mechanisms described in both syndromes. We dearly lack the description of such genes or genetic mechanisms in order to further our understanding of both the similarities and discrepancies between these two important syndromes. Comparative analyses of clinical cohorts of KS and TS with inclusion of genomic variables would likely lead to new discoveries. I will focus on some of the things that have been learnt from TS research and discuss how we could possibly learn from this in the KS context. In summary, TS is not only the other side of the coin of KS. A number of traits are clearly opposites, but other traits are clearly similar or at least cannot be seen as opposites.

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Olaf Hiort; M.D.; Ph.D. was trained in Paediatrics, and subspecialities Neonatology, Paediatric Endocrinology and Diabetes, and Laboratory Medicine. He is Professor of Paediatrics at the University of Lübeck and leads the Division of Experimental Paediatric Endocrinology and Diabetes in the Department of Paediatrics and Adolescent Medicine. His research interests are in sex development and calcium- and phosphorus metabolism, where he established also specialised care programmes for patients with rare conditions. He is currently chairing the European Network "DSDnet" and is elected to the European Commission Expert Group on Rare Diseases.

The European COST Initiative on DSD

The European Program on Cooperation of Science and Technology (COST) funds the formation of networking activities with Horizon 2020. In November 2013 the COST Action DSDnet (www.dsdnet.eu) was started and currently 23 European countries as well as additional international partner countries participate. DSDnet encompasses five Working Groups (WGs) which compile consented information on (a) clinical approaches, (b) genetics and biology, (c) laboratory aspects, (d) perception of research, and (e) dissemination of information. We have laid the grounds for consented manuscripts on sharing of genetic information and laboratory assessment. A survey on the current status of Centres of Reference for DSD in the different countries was launched. And a proposal for clinical assessment is in preparation. The incorporation of young investigators was propelled forward by a Training School on "Holistic Care in DSD" and the instalment of Short Term Scientific Missions (STSM). DSDnet is a tool to provide access to training, education and research for DSD. It aims to form a clinical ERN for urogenital rare conditions. Furthermore, it provides the basis for further structured international research grant applications.

Michael Zitzmann
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Professor Michael Zitzmann, born in 1964, is a high school teacher specialized in the fields of endocrinology, diabetology, sexual medicine and andrology at the University Clinics of Münster, Germany. Couples with fertility problems form a major part of the patient clientele as well as men with chronic inherited hormone disorders requiring life-time attention, especially the Klinefelter Syndrome. In addition, he is also focusing on endocrinological andrology: boys with pubertal disorders up to older men with a variety of sexual and metabolic problems seek his advice, which is interrelated to diabetes mellitus, thyroid disorders and problems of the pituitary gland.
Michael Zitzmann has developed treatment programs for the induction of fertility in hypogonadal men. He is largely involved in the research of the interplay of genetic features, epigenetic regulation, sex hormones, psychological characteristics and obesity treatment.

Cardiovascular risks in Klinefelter Patients

The mortality among men with KS is increased due to endocrine and metabolic diseases (primarily diabetes mellitus) as well as diseases in the circulatory, respiratory, digestive, and nervous system. Focusing on cardiovascular abnormalities, males with KS have an increased risk of left ventricular diastolic dysfunction, sinus node dysfunction and impaired cardiopulmonary performance, chronotropic incompetence, and possibly increased intima-media thickness. These aspects have, to a larger degree, been reported on a case-report-basis, but not all of them.

Chronotropic incompetence was described in a cohort of KS men vs controls to be impaired. KS patients exhibited lesser capacity to increase their heart rate upon physical exercise into an adequate range to cope with exercise strain. It has been speculated that this leads to a phenotype of left ventricular diastolic dysfunction and impaired cardiopulmonary performance.

In addition, our group was able to describe changes within the rhythmogenic setting of KS patients (n=132 vs controls) as shortening of the so-called QTc interval. KS patients often exhibit a shortening this interval into the pathophysiological range below 360 ms, which increases the likelihood for them to further deteriorate their cardiac rhythm into ventricular tachycardia. We have demonstrated in our description that the phenotype of short QTc intervals is especially prominent in KS men with paternal origin of the supernumerary X chromosome. In addition, expression rates of certain genes, so-called PAR-1 genes present on both the X and the Y chromosomes and thus in KS putatively active in three copies were strongly and positively related to shorter QTc times: CD99, P2RY8 and SLC25A6. These genes are related to inflammation and cardiac development.

Noteworthy, an increased QTc interval has been observed in 20-30% of girls and women with Turner syndrome (carrying only one sex chromosome, 45,X). In contrast, in men with KS (carrying three sex chromosomes), we found the reduced QTc interval in 11%. This further supports an association between sex chromosomal constitution and QTc interval. The results were later confirmed by a similar approach from a Danish group.

Cardiovascular research in KS patients is about to enter clinical application and it is quite imminent to do so, as patients seem to be at risk. Within the Danish cohort, the patient with the shortest QTc interval died suddenly, shortly after participating in the study, from ventricular fibrillation.

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International Collaborations:
IMPACT (Identification of Men with a genetic predisposition to ProstAte Cancer: Targeted screening in men at a higher genetic risk and controls) – Coordinator in Denmark
CIMBA (Consortium of Investigators of Modifiers of BRCA1/2)

Metabolic risks in Klinefelter Syndrome

In 1969, an increased prevalence of diabetes in Klinefelter Syndrome (KS) was described, but remained somewhat unnoticed for 30 years, until we and others described a high prevalence of metabolic syndrome (MetS), abdominal obesity and insulin resistance. Epidemiologic studies revealed an excess in both hospital admissions and mortality from diabetes, underpinning the important clinical consequences for patients with KS. In 2006 we described a striking incidence of abdominal obesity, insulin resistance and metabolic syndrome in KS patients. 44% had MetS and had on average 10% more abdominal fat for the same BMI value compared to an age-matched (non-slim) control group. Lipids were slightly skewed - significantly higher triglycerides, total cholesterol, LDL-cholesterol and significantly lower HDL-cholesterol. Others have subsequently corroborated these data both in adult KS patients and in pre-pubertal KS patients as well. The causes of these metabolic disturbances are not fully elucidated, but genes, sex-hormones and physical activity are definitely some the major determinants. As most KS are (or become) hypogonadal and are offered testosterone replacement therapy, the influence of hypogonadism and the effect of testosterone therapy on metabolism in KS, will be the main focus of this talk. There is an inverse relation between testosterone and insulin resistance – although most of the effect is caused by abdominal obesity. Likewise (but opposite direction), a relation between insulin resistance and hypogonadism has been repeatedly shown. The relation is circular and reflects a vicious circle of hypogonadism - abdominal obesity- insulin resistance.
In other patient groups and in experimental settings, testosterone treatment have shown effect on abdominal fat, muscle strength and insulin sensitivity and conversely testosterone depletion (for controlling prostate cancer) have shown increase in abdominal fat mass and increased insulin resistance. High quality data on testosterone treatment in KS are sparse and there is a lack of randomized controlled trials (RCT) to give evidence for a positive effect on the metabolic disturbances. Non-randomized trials have shown minor effect on insulin resistance and lipids (triglyceride). I will show data from a small RCT, where 2 x 6 months cross over study with testosterone and placebo, resulted in a significant improvement in body-composition during the testosterone treatment.

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Alberto Ferlin, MD, has a specialization in Endocrinology and Metabolism and a PhD in Endocrinological and Haematological Sciences. He is Associate Professor of Clinical Pathology at the University of Padova, Head of the Specialized Regional Centre for Klinefelter Syndrome, coordinates the Molecular Biology Lab and the PGD Program of the University Hospital of Padova. He is President-elected of the Italian Society of Andrology and Sexual Medicine. Principal research fields include molecular biology and genetics of male infertility, cryptorchidism and testicular cancer; role of FSH in the regulation of spermatogenesis and pharmacogenetics of male infertility; molecular and clinical aspects of Klinefelter Syndrome; testis-bone cross-talk. Author of more than 170 publications on international journal, impact factor (IF) is >800, citations are >5000, H-index is 39.

Osteoporosis risks

Klinefelter Syndrome (KS) is associated with decreased pubertal peak bone mineral density (BMD) and accelerated bone loss during adulthood. Decreased bone mass is detected in up to 40% of patients and it has been traditionally related to low testosterone levels. However, testosterone replacement therapy does not necessarily increase bone mass in these patients and low BMD can be observed also in patients with normal testosterone levels. Indeed, different mechanisms in KS contribute to reduced bone mass and osteoporosis risk, irrespectively of testosterone levels. Androgen receptor (AR) gene CAG polymorphism, X chromosome inactivation, INSL3 and vitamin D levels are hypothesized to cooperate with and modulate the effect of testosterone on the bone. AR gene CAG polymorphism seems to modulate the sensitivity to testosterone and previous studies have related it to some clinical aspects of KS, including BMD as demonstrated by our group. INSL3 has an anabolic role on bone metabolism by acting on osteoblasts and INSL3 levels are low in KS. Therefore, low INSL3 concentrations might represent a possible new pathogenic mechanism for reduced bone mass in KS. We also recently focused on vitamin D and found that its levels are significantly lower in KS patients with respect to controls. The percentage of osteopenia/osteoporosis in subjects with 25-hydroxyvitamin D deficiency was higher with respect to subjects with normal 25-hydroxyvitamin D and was not related to the presence/absence of low testosterone levels. Importantly, subjects treated with calcifediol or testosterone + calcifediol had a significant increase in lumbar BMD after treatment, whereas no difference was found in testosterone -treated group. Therefore, these data highlight that low 25-hydroxyvitamin D levels seem to have a more critical role than low testosterone levels in inducing low BMD in KS subjects. Furthermore, vitamin D supplementation seems to be more effective than testosterone replacement therapy alone in increasing BMD. Finally, we recently studied by proximal femur QCT analysis the structural basis of low bone mass and its consequences on bone strength in KS. We found that KS and women had similar bone strength, both significantly lower than elderly men. This similarity emerged however from different structural traits: KS had thinner femoral neck cortex, partially compensated by a denser trabecular compartment and larger bone dimensions (i.e. higher moments of area and bone mass).

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1965, Graduated Medical School, University of Copenhagen
1974, Doctor of Medical Sciences, University of Copenhagen.
1982 Professor of Paediatrics , University of Copenhagen
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Testicular changes during childhood and puberty in Klinefelter Syndrome (KS)

N. E. Skakkebaek, S. B. Winge, E. Rajpert-De Meyts, K. Almstrup, L. Aksglaede and A. Juul

The processes that lead to azoospermia in men with 47,XXY KS start early in life. The fetal KS testis contains numerous germ cells and the transition from gonocytes to pre-spermatogonia is not disturbed. However, already in early childhood a progressive loss of spermatogonia begins. The published data on testicular histology is limited. Usually it originates from specimens from KS patients undergoing surgery for urogenital diseases, which themselves may influence germ cell development. Therefore results must be taken with a grain of salt. However, taken together our data suggests that by the onset of puberty in KS boys only few seminiferous tubules contain germ cells. On the other hand, at that point in development of a KS boy, the architecture of the testis is relatively homogeneous. In the beginning of puberty some temporary increases in size of the testicles can be seen, reflecting some increase in tubular diameter, although testicular size rarely exceeds 6-8 ml, before it decreases again. Around mid-puberty dramatic testicular changes occur, resulting in total hyalinization of the majority of the seminiferous tubules. Usually, few tubules only escape hyalinization. Most of these contain Sertoli cells only, either fully differentiated Sertoli cells (type A tubules) or undifferentiated Sertoli cells (type B tubules) although, a few tubules containing spermatogenic cells are often also preserved in adult men. Interestingly, only the undifferentiated Sertoli cells contain Barr bodies (X heterochromatin), not the fully differentiated Sertoli cells. The testicular changes are matched by conspicuous changes in inhibin B levels, which most often are normal until the almost complete hyalinization of seminiferous tubules during puberty leaves circulating inhibin B levels unmeasurable and FSH significantly increased. In addition, Leydig cell insufficiency and formation of Leydig cell clumps are reflected by relatively low normal or subnormal testosterone levels and severely increased LH. The testicular phenotype in KS poses several interesting research questions: What are the biological mechanisms behind the programmed cell death of most of the Sertoli cells during puberty? Do the Barr body-negative Sertoli cells in type A tubules escape apoptosis because they have lost the extra X chromosome in spite of the fact that there is no evidence of mosaicism in the patients' karyotype? Could the germ cell loss during childhood and puberty be prevented by endocrine intervention? We believe that answers to these and other questions with regard to pathogenesis of testicular failure in KS may also provide important new insight into testicular function in normal and infertile men.

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Dr. Carole Samango-Sprouse is an associate clinical professor of pediatrics at The George Washington University, is on staff at Children's National in Washington, D.C. and is an Adjunct Associate Professor in the Department of Human and Molecular Genetics at Florida International University. She specializes in rare disorders and writes on XY disorders and the positive effects of early treatment. She has published more than 90 peer-reviewed articles and writes about the link between brain, neurogenetic disorder, performance and behavior. She is on the Editorial Boards of American Journal of Medical Genetics, the Journal of Integrative Psychology and Therapeutics, BioMed Research International, and Advances in Endocrinology.

An Algorithm for Effective Care and Management of 47, XXY (Klinefelter Syndrome) from Infancy through Adolescence

47, XXY (Klinefelter Syndrome) is associated with a wide spectrum of the phenotypic profile within the central nervous systems including differences in neurodevelopmental, endocrine, and brain morphology. With proactive care, some of the more severe phenotypic characteristics described in 47, XXY can be mitigated. From nearly a decade of research, an algorithm of effective care and management for 47, XXY patients will be described in infancy, preschool, and adolescence. To describe a neurodevelopmental theorem of what produces the most positive outcomes within the boys with XXY. Contrastingly, to describe what factors we have observed that contribute to lesser achievement and more compromised outcome in neurodevelopmental domains. Previously published work has detailed the effects of biological treatment, testosterone supplementation, and Familial Learning Disabilities (FLD). These factors, in addition to timing of ascertainment, and access to care will be analyzed to delineate between a “high risk” and “lower risk” algorithm of impairment associated with 47, XXY. By reporting comprehensive neurodevelopmental data including cognition (verbal and non-verbal), behavior, and neuromotor (fine and gross) we characterize a prudent route to successful long-term outcomes. Our data suggests that postnatally diagnosed males with 47, XXY who do not receive testosterone supplementation, nor services targeting known areas of deficit (i.e. speech and language, fine motor control, etc.) with a profile further compounded by FLD are at the highest risk to demonstrate moderate-to-severe differences associated with 47, XXY. On the contrary, prenatal diagnosis, intervention services where necessary, regular follow-up appointments, testosterone supplementation and a negative history of FLD can lead to the most optimistic outcomes for a newborn with 47, XXY. Individual variations to treatment and paradigms for optimal outcome for boys with XXY will be discussed linking brain, behavior and neurodevelopmental outcomes to biological deficiencies.

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Alan D. Rogol, MD, PhD received his university degree in chemistry from MIT. He received an MD and PhD (Physiology, endocrinology) from Duke University and completed training in pediatrics and endocrinology at Johns Hopkins Hospital and the National Institutes of Health. His academic career began at the University of Virginia in 1975, rising to the ranks of Professor of Pediatrics, Chief Division of Endocrinology and Professor of Pharmacology. He is presently Professor, Emeritus with continuing research interests on growth and adolescent maturation as well as the endocrinology of physical activity and sport training. In 2015 he received two distinguished career awards from the Human Growth Foundation and from the Pediatric Endocrine Society (US).

Transition to adult care for adolescents/emerging adults with Klinefelter Syndrome: Much more than just Testosterone

A. D. Rogol, S. Ryan

The transition from pediatric to adult-focused health care is a process that should occur for all adolescents/emerging young adults regardless of their health and medical needs. This process([L. transition (-ovis), a passing over, from transitus, pp of transire, to pass over]) should begin well before the singular event of transfer ([L. transferre; trans, across and ferre, to bear]) and may be particularly problematic for those individuals with long-term special health care needs due to on-going chronic conditions and illness. There are a number of medical and psychosocial issues that are specific for those emerging adults with KS, including the specific endocrine issues of fertility, osteoporosis, and requirements for testosterone replacement, concerns for cardiovascular disease and the metabolic syndrome, and venous stasis, increased risk for breast cancer and anterior mediastinal tumors, autoimmune diseases, as well as psychosocial needs around educational attainment, cognitive deficits and emotional/behavioral problems. The educational plans that are developed during transition have direct implications for choice of vocation because certain educational or learning deficits are likely to affect future employment, indicative of the unavoidable intertwining of these two issues. In addition, mental health and behavioral issues are prevalent and will likely affect both continuing education and vocation. These are related not only to the specific karyotype, but also must take into account the normal developmental trajectory from childhood to adolescence to emerging adulthood. Continuity of care is a main goal for all adolescents. The process often requires more planning and many more details, the records of multiple physicians and other health care professionals. Indeed, it may be that the adolescent will still require multiple specialty physicians as an emerging adult and thus the process may become diffuse as it often is in the pediatric age group. The age of the transition is not fixed, but its planning should start at a time when the early adolescent is seemingly competent to begin the process of self-management and should continue on a skills-based “curriculum” appropriate for the patient’s developmental level. For a number of chronic conditions this is usually in the 12 to 13 years age-range; however, for those with Klinefelter Syndrome this early age may be problematic because of cognitive and behavioral deficits.

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Anne Skakkebæk, MD, PhD started her scientific career at the Department of Endocrinology, Aarhus University Hospital. She is under specialist training in Clinical Genetics at the Department of Clinical Genetics, Aarhus University Hospital. Her primary research topic is Klinefelter Syndrome and other sex chromosome disorders. In 2013 she defended her PhD entitled "Klinefelter Syndrome – Brain, Behavior and Genetic Aspects. The majority of her publications deal with different aspects of Klinefelter Syndrome with a special focus on neuropsychological, neuroanatomical, genetic and epigenetic aspects of the syndrome. She is currently a post doc at the Department of Molecular Medicine, continuing with genomic studies of Klinefelter Syndrome.

Neuropsychology and socioeconomic aspects

The neuropsychological phenotype (NP) in Klinefelter Syndrome (KS) have been intensively investigated, leading to a more nuanced description of KS. Although, the NP shows variability within KS, the majority of KS suffer from learning disability, dysfunctional inhibitory control, working memory deficiencies and below average intelligence. KS is also associated with a characteristic personality profile with higher level of neuroticism and lover levels of extraversion, openness and conscientiousness. The determinants of cognitive deficits among individuals with KS are not well understood. In a recently submitted work, we assess the impact of intelligence, personality and social engagement on cognitive performance among KS. These data suggest that memory deficits in KS are largely a function of general intelligence and that KS and lower executive function combine to reduce social skills or that the lower level of social engagement may exert a negative influence on their executive function. There has been a growing interest for understanding the neurobiology underlying the NP in KS. Brain imaging studies indicate that grey and white matter volumes is smaller in KS and that these differences is located primarily to subcortical areas, insula and medial temporal cortices, however without correlation to neurocognitive measures, suggesting that the NP seen in KS may be related to the microarchitecture of the brain, rather than the mesoscopic anatomical brain level. Recently, a few functional neuroimaging studies have been performed to further investigate the neurological underpinnings of the NP in KS. Our recently submitted data, suggest that auditory and motor systems in KS are affected, however further studies is needed to characterize the exact functional nature of the KS brain. Our knowledge about the genotype–NP correlation in KS is still very sparse. Preliminary genome wide DNA methylation data suggest that the extra X chromosome has an impact on DNA methylation, and thereby may have an effect on gene expression and on the NP seen in KS. Much less is known of the social and economic implications of living with KS. Register study of the Danish cohort of KS men found that KS men have fewer partnerships, lower educational level, lower income and were retired at an earlier age. Furthermore, KS have increased mortality. The NP seen in KS may influence the socioeconomic status, which further can be linked to poorer health related outcome and increased mortality.

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Prof. Dr. Hanna Swaab is clinical neuropsychologist, clinical psychologist and psychotherapist, head of the outpatient department and dean of the faculty of social and behavioral sciences of Leiden University. Her research program is focused on neurodevelopmental disorders.

Sophie van Rijn
Sophie van Rijn has a PhD in Psychology, and for the last 13 years her line of research has focused on the cognitive and behavioral phenotype of individuals with 47,XXY and 47,XXX. She is currently appointed as an associate professor at the department of Clinical Child and Adolescent Studies at Leiden University, where she has set up a research lab for the study of developmental risks in children with these conditions. Using techniques from the field of neuroscience, such as neurocognition, MRI, eyetracking, and neurophysiological parameters, she has contributed to the understanding of neurodevelopmental mechanisms driving risk for social problems and psychopathology in children and adults with an extra X chromosome.

Social problems and underlying cognitive mechanisms

Children and adolescents with an extra X chromosome like in Klinefelter Syndrome appear to be at risk for problems in social and language development and for problems in regulation of emotion and behavior. In Klinefelter there is high risk for meeting the criteria for language disorder, Attention Deficit Disorder, Autism Spectrum Disorder and for psychotic symptoms. The focus of our research program is on the neurocognitive mechanisms that are related to developmental risk for social dysfunction and psychopathology. Finding associations between neurocognitive mechanisms and behavioral symptoms might help to understand the effect of an extra X chromosome on development and to design interventions that target vulnerable developmental mechanisms. We evaluated cognitive performance within the domain of language, executive function and social cognition to further understand problems in self-regulation and risk for social problems and psychopathology. Addressing social language we found that discriminating emotions in tone of voice was hampered and that language lateralization was not typical and associated with disorganization in thought and language. Deficits in verbal abilities seem to be associated with increased risk for autism traits. Focusing on the executive functions that might underpin the regulation of thought and behavior, we found that disorganization was associated with problems in mental flexibility and inhibition. Mental flexibility was also found to be associated with the risk for autism traits. In children with an extra X chromosome (boys and girls) we found deficits in inhibition, mental flexibility, sustained attention and visual working memory. Especially inhibition difficulties were associated with parental report of higher levels of thought problems, aggression and rule breaking behavior in daily life. To further understand the problems in social behavior we examined the spontaneous orientation towards facial expressions (social attention) with an eye tracking paradigm presenting emotional social situations. Individuals with Klinefelter Syndrome fixated less on the eye region of faces when compared to controls, and did not show the typical tendency, to first fixate on the eyes when presented with a face. High levels of arousal were found in response to emotional situations, suggesting problems in successful down regulation of emotional distress. Learning about the mechanisms that are related to problems in self-regulation and social behavior, it might be possible to improve methods of neurocognitive assessment for Klinefelter in order to identify individual developmental risk and to improve treatment to tailor made interventions that support social development and quality of life.

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Nicole Tartaglia, MD is a pediatrician who specializes in developmental-behavioral and medical features of and treatments for neurogenetic disorders, including sex chromosome aneuploidy, fragile X syndrome, and 22q deletion syndrome. She is the founder and director of the eXtraordinarY Kids Clinic at Children's Hospital Colorado, an interdisciplinary clinic for children and adolescents with sex chromosome variations. Current research by Dr. Tartaglia and her team include studies of social-emotional development and autism spectrum disorders in sex chromosome disorders, and an NIH-funded placebo-controlled trial evaluating psychological and motor effects of testosterone therapy in adolescents with Klinefelter Syndrome in early puberty. She is also very active in research on clinical trials of new medications for Fragile X syndrome. She has received several awards for research and clinical care, including the Patricia Jacobs Lifetime Achievement Award from AXYS and a Champion of Hope award for Medical Care of Rare Diseases from the Global Genes Project.

Behavioral and Social Phenotypes in Boys with 47,XXY and 47,XYY

Children with 47,XXY/Klinefelter Syndrome and 47,XYY can have a complex neurobehavioral phenotype, including increased risks for difficulties in many areas. Behavioral profiles on standardized assessments show elevations in both groups in areas of attention, emotional liability, social problems, anxiety, and somatic complaints compared to controls; however the reported behavioral problems in XYY are broader and cause more significant impairments. When comparing ADHD symptoms using parental-report of DSM-IV and DSM-5 symptoms, 36% of XXY and 76% of XYY samples met ADHD diagnostic criteria. Behavioral symptoms with more significant attentional problems and distractibility were common in XXY, compared to XYY where hyperactive and impulsive behaviors were also an important part of the ADHD profile. In other studies we have compared domains of social responsiveness, autism symptoms, and autism diagnostic criteria using standardized assessments including the Social Responsiveness Scale, Social Communication Questionnaire, Autism Diagnostic Interview – Revised, and Autism Diagnostic Observation Scales. While both XXY and XYY show an increased risk for social deficits and a shared profile on the SRS with preservation of social motivation, diagnostic rates of ASD are 3-fold higher in XYY compared to XXY. Expression of the Neuroligin-4 gene from the Y chromosome (NLGN4Y) in boys with XYY correlates with overall social responsiveness and autism symptoms. Clinical experience at the eXtraordinarY Kids Clinic in Colorado, USA comparing clinical visits and diagnostic impressions summarized from the first 1000 patient visits including XXY and XYY will be presented. Similarities and differences in the behavioral phenotypes of XXY and XYY may provide clues into the etiology of phenotypic features, and help to differentiate those aspects that may be due to sex chromosome trisomy, androgen deficiency (seen only in XXY), or excess Y chromosome gene product. Implications of these findings on counseling families and direct clinical care will also be discussed.

Liborio Stuppia
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Liborio Stuppia is full professor of Medical Genetics at the "Gabriele d'Annunzio" University of Chieti, Italy. He is the Director of the Department of Psychological, Health and Territorial Sciences, head of the Laboratory of Molecular Genetics and member of the Italian National Committee for Biosecurity, Biotechniques and Life Sciences. His research activity has been mainly devoted to the investigation of the genetic basis of male infertility, in particular to the study of Yq microdeletions and to the analysis of gene expression profiling in testes of infertile males. More recently, Liborio Stuppia is focusing his activity on the study of Amniotic Fluid Stem Cells as a model for the study of human Primordial Germ Cells.

Transcriptome of KS testis

Klinefelter Syndrome (KS) is the most common abnormality of sex chromosomes (47,XXY karyotype) and represents the first genetic cause of male infertility. Despite the high prevalence of this condition and the number of studies carried out in order to identify the pathogenesis of testicular damage, so far the mechanisms leading to KS testis degeneration are still not completely defined. A great help in the study of the molecular basis of KS testis degeneration can be provided by studies aimed to the comparison of gene expression profiles of testis biopsies obtained from KS patients as compared to those of normal subjects. To this aim, our group carried out a microarray-based analysis of global testis transcriptome in KS patient and in normal controls. This analysis evidenced that, compared to controls, KS patients showed the differential up- and down-expression of 656 and 247 transcripts, the large majority of which expressed by Sertoli cells (SCs) and Leydig cells (LCs). Functional analysis of the deregulated transcripts indicated changes of genes involved in cell death, inflammatory response, lipid metabolism, steroidogenesis, blood-testis-barrier formation and maintenance, as well as spermatogenesis failure. Taken together, these data highlight the modulation of hundreds of genes in the somatic components of KS patient testis. The increased LCs steroidogenic function together with the impairment of inflammatory pathways and blood testis barrier structure, result in increased apoptosis. These findings may represent a critical roadmap for therapeutic intervention and prevention of KS-related testis failure.

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Sabine Kliesch, MD, started her scientific career at the former Institute of Reproductive Medicine 1991 and the Department of Urology 1995, University Münster. She is specialized in urology, andrology and medical tumor therapy. Since 2008, she is the Head of the Department of Clinical Andrology of the Center of Reproductive Medicine and Andrology (CeRA) and responsible Director of the WHO Collaboration Centre and the EAA Training Centre within the CeRA. The clinical unit offers all modern diagnostic and treatment modalities in andrology, including endocrine, medical and surgical options in andrology, offering a life-course approach from childhood to the ageing male. Her research topics comprise the impairment of human spermatogenesis, early diagnosis and late effects of testis cancer, the optimized diagnosis and treatment of male infertility and hypogonadism as well as the preservation and restoration of male fertility. The research activities focus on clinical and translational research aspects. She published more than 240 peer reviewed original articles, reviews and book chapters in the field of andrology and received several prizes for her research.

Fertility preservation in adolescent and adult Klinefelter patients

Klinefelter patients experience in more than 95% hypergonadotropic azoospermia. Only in rare cases semen analysis is positive for spermatozoa and thus treatment of infertility remains extremely difficult. While by means of microsurgical testicular sperm extraction (mTESE) overall sperm retrieval rates could be improved in recent years from 35 to 60%, predictive parameters to foresee successful sperm retrieval so far did not exist.
Very recent studies focused on identification of predictive parameters in adolescent and adult patients with Klinefelter´s syndrome (KS): we analysed the clinical data of 135 patients with non-mosaic Klinefelter's syndrome aged 13–61 years who underwent micro TESE procedure for fertility preservation at our institution since 2008. Among them 50 late pubertal adolescents ≤19 years were analysed. Specifically a history of cryptorchidism, age at mTESE, bi-testicular volumes, serum levels of LH, FSH and testosterone (T). Inhibin B, AMH and INSL3 were analysed in the adolescent subgroup. All patients were asked to provide a semen sample prior to surgery. The results showed that younger age and near-normal serum T levels were positive predictors of successful sperm retrieval in mTESE procedures. When an age cut off of ≤25 years was applied, the success rate was 41% in the younger vs. 25% in the older patient group (p=0.029). Normal to moderately elevated LH (≤17.5 U/l) with T-levels ≥7,5 nmol/l predicted TESE success in 53%, whereas a combination of high LH (>17.5 U/l) with low T (<7.5 nmol/l) was successful only in 9%. Neither testicular volumes, FSH nor Sertoli cell markers were predictive of mTESE success (Rohayem *et al*., Andrology 2015). More aggressive treatment attempts such as subcapsular orchiectomy did not result in higher success rates (37%) (Fedder *et al*., Urology 2015), while an age around 24 years could be confirmed recently (Plotton *et al*., JCEM 2015). Overall pregnancy rates after TESE-ICSI procedures are reported in 23% of couples, with 10-15% of TESE patients becoming fathers, irrespective of the underlying cause of azoospermia (DIR data registry Germany, JRE 2015). So far in KS patients about 200 child births after TESE-ICSI procedures are reported throughout the literature. In summary, young age above 15 and below 25 years with robust Leydig cell function results in the highest sperm retrieval rates in mTESE procedures for fertility preservation in adolescent and adult men with Klinefelter´s syndrome.

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Klinefelter Syndrome and TESE-ICSI

H. Lejeune, S. Giscard d'Estaing, B. Cuzin, A. Brosse, M. Benchaib, J. Lornage, R. Ecochard, F. Dijoud, I. Plotton.

Most patients with Klinefelter's syndrome (KS) are azoospermic and were previously considered definitively sterile. Since 1996, the possibility of a biological paternity has been demonstrated by applying TESE-ICSI to 47,XXY homogenous karyotype KS. Reviewing the published series of TESE, a mean sperm retrieval rate (SRR) of 50% was found, similarly to that in non-obstructive azoospermia with normal karyotype. In case of successful TESE, the pregnancy rate was as high as 50% and the rate of miscarriage was similar to the other TESE-ICSI. Among the born children, the sex ratio was normal and the karyotype of the born children was normal, except for one case. These data were in agreement with the physiopathology of focal spermatogenesis arising from spermatogonia that lost one X chromosome, giving rise to a clone of 46,XY spermatogonia able to progress through the spermatogenic process. The only prognostic factor regularly found was patient age, with a decrease in positive TESE with aging. A statistical threshold was found at approximately 35 years in most studies. In the light of this inverse relationship between age and SRR, several authors suggested testicular biopsy in adolescent patients with KS. However, we recently demonstrated in a prospective study that the SRR is similar in young (15-24 y; SRR=52%) and adult patients (25-40 y ; SRR=62,5; P=0.73). FSH, Inhibin B and testicular volume were not predictive of positive TESE. Testosterone was marginally significant in only few studies. In our series, a previous testosterone treatment, withdrawn at least 6 month before TESE had no deleterious effect on SRR. The usefulness of treatments designed to increase the intra-testicular testosterone production has not been adequately investigated to date. Micro-TESE by trained surgeons could give rise to higher SRRs (57%) than standard open biopsy (42%), however the ranges of published SRRs are large ([47–69%] and [28–57%] respectively). In conclusion, the SRR and pregnancy rate are similar in non-mosaic 47,XXY KS and in non-obstructive azoospermia with normal karyotype. The SRR is higher between 15 and 35 years of age. Trained surgeons reported higher SRR with micro-TESE. An eventual deleterious effect of previous testosterone treatment and the efficacy of a medical treatment increasing intra-testicular testosterone production should be further investigated.

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Anders Juul MD, PhD, DMSc graduated from university of Copenhagen in 1991. He is trained as paediatric endocrinologist in Copenhagen, Denmark. He subsequently trained and was EAA-certified as clincal andrologist. His research interests include growth and pubertal disorders as well as genetic, epigenetic and environmental influences on gonadal function throughout life. He has published more than 300 scientific papers and supervised more than 20 PhD students. In 2015 he received the prestigious ESPE research award. He is clinical professor at the University of Copenhagen, head of the department of Growth and Reproduction, and leader of the newly established research centre EDMaRC.

47,XXY Klinefelter Syndrome: Clinical characteristics and age-specific recommendations for medical management.

A. Juul, L. Aksglæde, E. Rajpert-De Meyts, N. Jørgensen and N. E. Skakkebaek

Klinefelter Syndrome was originally described in 1942 as a syndrome characterized by "Gynaecomastia, Aspermatogenesis without A-Leydigism, and Increased Excretion of Follicle-Stimulating Hormone". The phenotypic characteristics were based on nine males aged 17 to 38 years of age (Klinefelter *et al*., JCEM 1942). Since then several descriptions of phenotypic characteristics have been published, covering not only the adult phenotype, but clinical features that can be observed from birth and onwards. These may include increased prevalence of cryptorchidism, micropenis, increased linear growth, cognitive, social, behavioural and learning disabilities in childhood. In puberty testicular growth occurs with the onset of puberty, but the testes usually shrink again to the typical adult volume of 3-6 ml. In adulthood, gynaecomastia, osteopenia/osteoporosis, eunuchoid body proportions, sparse body hair, lowered libido, increased body fat and risk of metabolic syndrome are observed. The most consistent findings in adult patients are the small testes and azoospermia. However the phenotype is highly variable ranging from an almost normal appearance to a significantly affected individual. Decreased awareness of this syndrome among health professionals and a general perception that the majority of patients with 47,XXY share the classic textbook phenotype results in a highly under-diagnosed condition with up to 75% of the patients left undetected. This is important to have in mind, as most phenotypic descriptions are based on those who are diagnosed, whereas the phenotype of patients who never have a diagnosis remains unknown. Appropriate medical management of KS patients is closely dependent on the age of the patient. In childhood, management of cryptorchidism, speech, social and learning disabilities are predominant features, whereas gynecomastia, metabolic syndrome, testosterone insufficiency, and fertility issues are predominant features in adolescent and adult life. It is also important to keep in mind that KS patients have an increased risk of mediastinal germ cell tumors (especially in childhood) and breast cancer (in adulthood).

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Manuela Simoni, MD, PhD, trained as clinical endocrinologist at the Unit of Endocrinology of the University of Modena, Italy between 1982 and 1990 and, thereafter, as molecular endocrinologist at the Institute of Reproductive Medicine of the University of Münster, Germany, where she was Professor for Endocrinology and Molecular Biology of Reproduction from 1998 to 2008. Current positions: full professor and chair for Endocrinology at the University of Modena and Reggio Emilia; Director of the Clinical Unit of Endocrinology at the NOCSAE Hospital, Director of the School of Specialization in Endocrinology, Deputy Director of the of the Department of Biomedical, Metabolic and Neural Sciences and Director of the Centre for Genomic Research of the University of Modena and Reggio Emilia. Her research interests are gonadotropin and androgen action, testicular function, genetics of male infertility, endocrinology and pathophysiology of reproduction. She is member of several societies, including the European Academy of Andrology (EAA) and the European Society of Endocrinology (ESE, serving as the Secretary) and is active in the editorial boards of several journals in the fields of endocrinology and reproduction.

Testicular steroidogenesis in response to human chorionic gonadotropin stimulation in Klinefelter Syndrome by liquid chromatography – tandem mass spectrometry

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Men with Klinefelter Syndrome (KS) show hypergonadotropic hypogonadism, but the pathogenesis of hypotestosteronemia remains unclear. Testicular steroidogenesis in KS men was evaluated over three decades ago after human chorionic gonadotropin (hCG) stimulation, but inconclusive results were obtained. Intriguingly, some recent studies show increased intratesticular testosterone concentrations in KS men. To analyze serum steroid profile, as a proxy of testicular steroidogenesis, after hCG stimulation in KS compared to eugonadal men. Prospective, longitudinal, case-control, clinical trial. 13 KS patients (36±9 years) not receiving testosterone (T) replacement therapy and 12 eugonadic controls (32±8 years) were enrolled. Serum steroids were measured by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) at baseline and for 5 consecutive days after intramuscular injection of 5000 IU hCG. Progesterone (P), 17OH-progesterone (17OHP), T and estradiol (E2) showed a significant increase ($p<0.001$) after hCG stimulation in both cohorts. On the contrary, androstenedione (A) and dehydroepiandrosterone did not increase after hCG stimulation. The 17OHP/P ratio increased in both groups ($p<0.001$), the T/A ratio (17ßHSD3 activity) did not increase after hCG in any group and the E2/T ratio (aromatase activity) increased significantly in both groups ($p=0.009$ in KS and $p<0.001$ in control). Luteinizing-hormone decreased after hCG in both groups ($p=0.014$ in KS and $p<0.001$ in control) while follicle-stimulating hormone decreased only in eugonadal men ($p<0.001$). This study demonstrates for the first time using LC-MS/MS that Leydig cells of KS men are able to respond to hCG stimulation and that the firsts steps of steroidogenesis are fully functional. However, the T production in KS men is impaired, possibly related to reduced dehydrogenase activity due to an unfavorable intratesticular metabolic state.

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Birgit Köhler, PhD, is a Senior Paediatric Endocrinologist at the Department of Paediatric Endocrinology, Charité, Berlin, Germany. Her scientific interest is genetics and improvement of holistic interdisciplinary care of persons with variants of sex development. At present she is the head of the clinics for variants of sex development at Charité. She is the coordinator of the European study dsd-LIFE, which evaluates outcomes of treatment and care QoL and patients' views of persons with different biological variants of sex development (http://www.dsd-life.eu/). She received the Henning Anderson price of the European Society of Paediatrice Endocrinology and holds the certificate International Fellowship of of Pediatric and Adolescent Gynaecology (IFEPAG).

QoL and satisfaction with care of adolescents and men with Klinefelter Syndrome - First results from the European study dsd-LIFE

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dsd-LIFE is a comprehensive clinical outcome study investigating medical, surgical, psychosocial and ethical issues to improve treatment and care of patients with the different diagnoses included in the umbrella term disorders/differences of sex development (dsd). The multidisciplinary dsd-LIFE consortium consists of 15 experienced European scientists in the areas endocrinology, psychology, surgery, gynaecology, urology and ethics. The study is conducted in Germany, United Kingdom, France, Sweden, the Netherlands and Poland.
Patients >16 years with the following diagnoses were invited to participate: Turner syndrome, Klinefelter Syndrome, congenital adrenal hyperplasia (CAH), impaired testosterone synthesis (e.g. 5-alpha-reductase-2 deficiency, 17-beta-HSD-3 deficiency, LH-receptor defects), impaired androgen action (complete androgen insensitivity, CAIS; partial androgen insensitivity, PAIS), dysgenesis of the testes or ovaries, mixed gonadal dysgenesis, karyotype 46,XY/46,XX, 46,XX males, ovotestes, hypospadias. 220 persons with Klinefelter Syndrome have participated in the study. Here we present the first results on QoL and satisfaction with care of adolescents and adults with Klinefelter Syndrome from 6 European countries (http://www.dsd-life.eu)

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Prof. Nieschlag has been president of the German Society of Endocrinology, the German Society of Andrology, the German Society of Reproductive Medicine, the International Society of Andrology and the European Academy of Andrology. From 2007 to 2011 he was president of the European Society of Endocrinology.

Round Table: Klinefelter Syndrome: current management and challenges

E. Nieschlag (chair), A. Ferlin, C. Gravholt, J. Gromoll, B. Köhler, A. Rogol, H. Lejeune, J. Wistuba and Workshop participants

Based on the shortcomings of current KS patient care - as evidenced by this workshop - the panel will discuss future directions for early diagnosis, for treatment options to enhance quality of life and reduce sequelae of comorbidities, for improved chances of paternity as well as for better integration, socialization and vocational success. A further issue will be strategies to educate non-endocrinologists and non-andrologists to increase chances for diagnosis of undetected KS-patients.

Short Oral Presentations Abstracts

Fertility preservation in Klinefelter boys: an update after 6 years' experience

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In the UZ Brussel we started to offer cryopreservation of testicular tissue to Klinefelter boys in 2009. In an initial retrospective study performed in seven non-mosaic 47,XXY adolescents, aged 13–16 years no sperm cells were observed after masturbation or after penile vibrostimulation or electro ejaculation. Subsequently, testicular sperm extraction (TESE) was performed with the aim of finding sperm cells and if not preserving the spermatogonia by testicular tissue cryopreservation. The presence of spermatogonia was reported in five patients, although only one patient presented spermatogonia in seminiferous tubules showing a normal architecture. We continued offering this option to Klinefelter boys and are presenting an update of the success of cryopreserving sperm cells and/or spermatogonia in this abstract. A retrospective study was conducted in 20 non-mosaic 47,XXY adolescents, aged 12–19 years, who were invited for an experimental testicular tissue banking program during their follow-up at the Paediatric Endocrinology Department of the UZ Brussel between 2009 and 2015. Paraffin-embedded testicular tissue was sectioned and stained with periodic acid Schiff (PAS)/ haematoxylin staining, and immunostaining was performed for Mage-A4. The presence of spermatogenesis and/or spermatogonia was evaluated. Motile sperm cells were isolated after an enzyme treatment on testicular tissue in one boy and were cryopreserved for future fertility options. In all the other boys, no sperm cells were found. When no spermatogonia were found in the first paraffin-embedded tissue piece, an additional vial was thawed to perform immunostaining for MAGE-A4. Spermatogonia were observed in nine boys with an occurrence of 0.2 up to 34.5% tubules having positive cells. Remarkably, spermatogonia were not observed in the testicular biopsy from the boy in which motile sperm cells were found. Fibrotic areas were observed in all but one patient. The cryopreservation of sperm cells was achieved in 5.0% of the patients, while spermatogonia were found in 45.0% of patients. The fibrotic process was also already initiated in the testicular tissue from these adolescent boys. To improve the changes of preserving spermatogonia, testicular tissue should be cryopreserved at a younger age in Klinefelter boys.

Hypothetical higher setting of hypothalamus pituitary testis axis in infants with non-mosaic Klinefelter Syndrome

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Klinefelter Syndrome (KS) is a genetic disorder due to the presence of an extra X chromosome in male subjects. It is characterized by a variety of subtle, age-related clinical signs, which evolve into hypergonadotropic hypogonadism. The hypothalamic–pituitary–gonadal (HPG) axis is transiently activated in infancy; it is still unknown when testicular damage occurs. To evaluate the HPG axis and the hormonal and clinical pattern of KS boys. 142 KS boys and 78 controls aged 0 to 11,9 years were recruited. Serum FSH, LH, Testosterone (T), Estradiol (E2), Inhibin B (INHB), Sex Hormone Binding Globulin (SHBG) and Anti-Müllerian Hormone (AMH) were determined. Height, weight, testicular volume and penile length were assessed. The entire population was analyzed after being splitted in 3 different groups: 28 infants aged 0-12 months (group 1); 153 children aged 1 to 8 years (group 2) and 39 children aged 8 to 11,9 years. Our data showed LH and FSH physiologic pattern both in KS and in control population. Moreover, their serum levels appeared significantly higher in KS than in euploid boys in group 1 ($p<0,05$ for both hormones). Testosterone raise during minipuberty was significantly higher in KS than in controls ($p<0,05$), whereas it did not show any difference in group 2 and 3. Before pubertal period INHB was significantly higher in KS than in controls in group 1 and 2 (respectively $p<0,0001$ and $p<0,05$). AMH appeared higher in KS than in controls in all groups but the difference did not reach statistical significance. No significant differences in E2, testicular volume and penile length were found. No tubular or interstitial damage was found in KS infants. The presence of gonadotropins and testosterone higher levels during minipuberty may be interpreted as an altered setting of hypothalamic-pituitary-gonadal axis in KS infants. It seems that the presence of a relative central resistance could be present during their first months of life.
This study was funded by the Italian Ministry of Health and the Italian Medicines Agency (AIFA): research project MRAR08Q009 on rare diseases.

A Multidisciplinary Model of Early Fertility Preservation in Klinefelter Patients: Description of a Program

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Klinefelter Syndrome is the most common genetic disorder compromising male fertility, and is characterized by masculine phenotype, extra sex chromosomes, affecting 1/500 to 1/1000 newborn males. Previous studies of its physiopathology have shown a dramatic loss of germ cells including spermatogonial stem cells (SSC) following the onset of puberty. To establish a multidisciplinary referral program to offer clinical and experimental fertility preservation options to Klinefelter patients of all ages. Newly diagnosed Klinefelter patients at any age including prenatal, infancy, prepubertal, adolescence and adult are referred by either pediatric endocrinologists or medical genetics consultants to a male reproductive medicine and surgery clinic. After initial consultation, each patient is enrolled in a long term follow up program to monitor his endocrine profile (Testosterone, FSH, LH, E2, Inhibin B and AMH), pubertal development (Tanner stage) and testicular structure to detect early fibrosis with Elastography and Ultrasound. At Tanner stage III or higher, one step fertility preservation is offered, including semen collection (by penile vibration stimulation or electro ejaculation), microsurgical testicular sperm extraction (micro TESE) and SSC cryopreservation. The extracted sperm is stored in a clinical setting for future IVF/ICSI and his testicular tissue containing SSCs is stored in our experimental autologous testicular tissue bank for possible future use for in vitro or in vivo spermatogenesis trials. From December 2014 to January 2016, 10 patients have been enrolled in this program. Two patients (11 & 13 years old; none-mosaic XXYY and XXY respectively) went through electro ejaculation and semen was collected successfully. However; no sperm was found in their semen. Micro TESE was performed immediately in both testes of each patient and no testicular sperm were found in either specimen by an embryologist presented in the operating room to evaluate the ejaculate and testicular biopsy samples. A biopsy from each testis was stored to preserve SSCs. Diagnostic pathology performed by a dedicated testicular pathologist confirmed the absence of testicular sperms at all and presence of spermatogonia in less than 10% of tubules in both patients. We have established an effective, comprehensive and safe multidisciplinary team approach for potential early fertility preservation in Klinefelter boys.

Expansion of the language phenotype in Klinefelter Syndrome and children with XXYY

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Klinefelter Syndrome (47,XXY) and its variant (48, XXYY) occur in approximately 1:650 males. In addition to a distinct physical phenotype, children with these disorders also exhibit language-based learning disabilities. Previous research has identified weaknesses in basic receptive and expressive language skills in these children. No systematic examination has been conducted yet of higher-level language skills, which includes the ability to process figurative language, and the ability to make inferences and predictions. The latter are important academically as they are needed to process narratives and learn more complex content. The primary goal of this study was to examine more critically the various linguistic abilities of children with 47, XXY and 48 XXYY, to include higher-level language abilities. 49 boys with XXY and 27 boys with XXYY were recruited through the eXtraordinarY Kids Clinic, a multi-disciplinary clinic for children with sex chromosome aneuploidy. Receptive and expressive semantic, syntactic, and higher-level language skills were assessed using standardized measures. Semantic, syntactic and higher-level language composites were created and hierarchical regression was used to predict higher-level language skills. Children with XXY had normal overall semantic and syntactic skills, but deficient higher-level language abilities. Children with XXYY scored significantly lower than children with XXY across all three domains. In children with XXY, semantic and syntactical skill both explain significant amounts of unique variance in higher-level language skills ($p<.01$). In children with XXYY, syntax explained unique variance in higher-level language skill, but semantics did not. Children with XXY were deficient in higher-level language abilities even though their semantic and syntactic skills were normal. Children with XXYY had deficits across all three domains. Different underlying language skills seem to predict higher-level skills in each group. Both semantics and syntax are predictive in XXY, while it is mostly syntactic processing that predicts higher- level language skill in XXYY. In the XXY group, additional non-linguistic factors such as attention, non-verbal reasoning skills, and executive function may add additional explanatory variance. Implications for educational and social interventions are discussed.

Disturbed testicular vascularization in 41, XX^{Y}* mice: a functional analysis using contrast enhanced ultrasound

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Earlier findings in Klinefelter Syndrome (KS) patients suspected Leydig cell disturbance to provoke serum testosterone deficiency. Addressing this issue in a KS mouse model (41,XX^{Y}*), we found LCs in contrast to be hyperplastic and hyper-reactive. Interestingly, also intratesticular testosterone (ITT) concentrations were comparable to controls. In addition, we could confirm this in a cohort of patients, therefore excluding insufficient ITT levels as the cause of hypogonadism. Recently, it was reported that arteries in KS patients are altered and impairing circulation. We hypothesized changes in testicular vascularization might be involved in the endocrine phenotype and utilized our 41,XX^{Y}* mouse model to evaluate the testicular blood supply functionally. We therefore performed a study in which an enhanced ultrasound based analysis of the testicular blood flow rate in 41,XX^{Y}* mice was conducted. Adult male 41,XX^{Y}* (n=5) and littermate mice (n=6) underwent ultrasound analyses with the Non-Targeted Contrast Agent Vevo MicroMarker. The agent containing gas filled micro-bubbles was administered intravenously for lower body perfusion. After initial perfusion, micro-bubbles were destroyed by high ultrasound pressure and the reperfusion period was documented and analysed. In parallel, electrocardiograms (ECGs) were taken. Afterwards mice were sacrificed and testes removed for histological analysis of the vascularization. Whilst ECGs did not reveal differences in heart function between the groups, the reperfusion time for testes was significantly increased in 41,XX^{Y}* mice (XX^{Y}* 28.8± 1.69s; XY* 19.9± 2.8s) Testes of 41,XX^{Y}* mice (XX^{Y}* 4.6 ± 0.10mm²; XY* 11.1 ± 0.34mm²) and the area covered by blood vessels (XX^{Y}* 0.025 ± 0.003mm²; XY* 0.040 ± 0.002mm²) were significantly smaller as revealed by histology. These functional data strengthen the assumption that the observation made previously and pointing to an affected vascular system in the disturbed testicular tissue of males with supernumerary X contributes to the endocrine phenotype seen in KS. Furthermore, a close relation between blood vessel formation and spermatogonial stem cell niches was reported and thus, the altered vascularization could also be involved in germ cell loss observed in KS. However, further studies have to be undertaken that confirm our observation also clinically.

Transcriptome analysis of testis tissue from pre-pubertal Klinefelter boys

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Klinefelter Syndrome (KS) is caused by the presence of an additional X chromosome in men. Adult KS men are azoospermic or cryptozoospermic due to degeneration of the seminiferous tubules. This degeneration accelerates during puberty, but already in childhood, fewer germ cells are present. However, very little is known about the process that leads to testicular degeneration, in part because testicular tissue is extremely hard to obtain as biopsies very rarely are performed in pre-pubertal KS boys. Here, we report RNAseq transcriptome analysis of paraffin-embedded testicular tissue samples from 5 pre-pubertal KS boys aged 9 to 14 years and 4 control boys aged 7 to 10 years (3 autopsies and one biopsy from a boy with leukemia performed due to suspicion of testicular relapse). The pre-pubertal KS samples contained either few (3 samples) or no germ cells (2 samples), whereas the control samples all had normal histology and germ cells in the tubules. A small amount of RNA was extracted from all biopsies, which was technically challenging as biopsies were fixed in either Stieve or Cleland fixatives. Subsequently, sequencing libraries were prepared from the RNA and sequenced on an Illumina HiSeq. Sequencing reads were trimmed and aligned to the reference genome. The mean library size was 478654 reads. Using an unadjusted p-value of 0.01, we identified 206 and 18 genes respectively down- and up-regulated in the pre-pubertal KS testis compared to control pre-pubertal testis. Among the up-regulated genes were genes well-known to be expressed in KS tissues, like XIST, genes well-described in relation to testicular function, like INSL3, but also less described genes, such as JAK3 and IQSEC1. Using immunohistochemistry, JAK3 was found to be expressed in normal adult Leydig cells and Sertoli cells, whereas IQSEC1 was expressed in peritubular cells and blood vessels. Pathway analysis of the differentially expressed genes indicated that RAS/MAPK signaling was affected and that genes escaping X chromosome inactivation were over-represented in the KS testis. Our results for the first time describe the transcriptome of the pre-pubertal KS testis and point towards an involvement of testicular somatic cells in the degeneration of KS tubules.

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Klinefelter Syndrome co-morbidities induced by increased gene dosage and altered interactome activity

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Men with Klinefelter Syndrome (KS) have increased risk of numerous other disorders, co-morbidities. Known co-morbidities are breast cancer, type II diabetes, osteoporosis and psychiatric disorders. These are caused by altered hormonal dosage and gene expression fluctuations induced by the aneuploidy. X chromosome inactivation (XCI) to some extent compensates the extra gene dosage. Yet, XCI varies between individuals, which presumable cause the varying clinical presentation of KS. Thus, diagnosis and clinical supervision keeps being challenging both due to varying presentation and still insufficient characterization of the syndrome. Here we present a study combining data-driven epidemiology and system biology to improve the understanding of KS co-morbidities and the molecular interplay causing their co-morbidities.

KS co-morbidities were extracted from the Danish National Patient Registry including 6.2 million patients and 65 million hospital encounters. A KS co-morbidity network was built by extracting disease-associated proteins and linking them by observed protein-protein interactions. Gene expression data was generated from KS patients and controls, and was integrated on the network to identify complexes changed in activity in KS.

A total of 78 co-morbidities were extracted from Danish electronic patient records. These covered both well-known (e.g. infertility, osteoporosis, and gynecomastia) and clinically still non-established KS co-morbidities (e.g. pituitary gland hypofunction, dental caries, and other endocrine disorders). The molecular interplay of the KS co-morbidities was investigated by building a protein network consisting of significantly interconnected sub-networks each covering KS co-morbidities. Certain nodes in the network was of immediate interest as they were either associated to several co-morbidities themselves or through their interaction partners connected multiple co-morbidities. A total of 182 transcripts were deregulated in KS compared to controls of which thirteen were X-encoded. Integration of this data on the protein network identified functional active complexes in KS and/or controls.

Data-driven epidemiological analyses of patient records revealed yet not clinically established KS co-morbidities. Integrative systems biology shed light on the molecular interplay between KS co-morbidities and revealed central proteins for their interconnectivity, which improves the understanding of KS.

Effects of long-term treatment with testosterone undecanoate injections (TU) in patients diagnosed with Klinefelter's syndrome (KS) following detection of osteoporosis

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KS is underdiagnosed with less than 10% of cases identified by puberty and less than 20% ever diagnosed during life. We describe a cohort of men with KS, diagnosed at advanced age. The majority of the patients had been referred by an orthopedist to an urologist to be checked for hypogonadism after a diagnosis of osteoporosis. Open-label, single-center, cumulative registry study of 40 men with testosterone levels between 7.3 and 12.1 nmol/L (mean: 10.1±1.5). Mean age: 47.4±6.8 years (min.: 33; max.: 60). All men received TU/12 weeks after an initial interval of 6 weeks. Mean follow-up: 64±22 months, median 73.5 months. Anthropometric and metabolic parameters were measured every 3 months. Bone mineral density (BMD) was assessed every 3 months; variation was expressed as a T-score of measurements of the spine (L2–4) and femoral neck. Testosterone (ng/mL) increased from 10.1 to trough levels between 16 and 18 nmol/L ($p<0.0001$). Weight decreased from 98.5±12.7 to 85.4±6.3 kg by 18.6±5.2%, waist circumference from 101.5±6.2 to 95.8±4.7 cm, BMI from 29.01±3.75 to 24.98±1.58 kg/m^2 ($p<0.0001$ for all). 32 men had osteoporosis. The mean T-score increased progressively from -3.39±0.51 to -1.23 ($p<0.0001$ vs baseline). Glucose levels and HbA_{1c} remained unchanged with mean levels below 5.4 mmol/L and 5.5%, respectively, at all time-points. Only 2 patients with only 18 months treatment duration still fulfilled criteria for osteoporosis at the last observation, all other men fell into the category of osteopenia. Lipids (mmol/L): Total cholesterol (TC) decreased from 6.14±0.6 to 4.75±0.25, LDL from 3.51±1.13 to 2.03±0-76 and triglycerides from 2.65±0.29 to 2.11±0.09. HDL increased from 1.15±0.36 to 1.54±0.44 ($p<0.0001$ for all). Both systolic and diastolic blood pressure decreased slightly but significantly from 131.2±6.4 to 125.2±3.6 and 77.6±5 to 73.5±5.4 mmHg, respectively ($p=0.0001$ for both). C-reactive protein (CRP) declined from 2.01±3.6 to 0.1±0 ($p<0.01$). Prostate volume increased slightly but significantly by 3.9±0.37 ml but prostate size remained small. Testicular volume decreased slightly from 8±4.14 to 6.89±1.45 (left, $p<0.05$) and from 8.43±4.06 to 7.56±1.59 (right, $p<0.01$). Normalizing serum testosterone in patients with a late diagnosis of KS improved body composition including BMD and metabolic parameters. To identify KS, knowledge and awareness among orthopedists is highly important.

Poster presentation Abstracts

Posters

GENETICS

P01

Klinefelter Syndrome is associated with high recurrence of copy number variations on the X chromosome with a potential role in the clinical phenotype

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Klinefelter Syndrome (KS) is the most frequent sex chromosomal disorder in males, characterized by at least one supernumerary X chromosome (most frequent karyotype 47,XXY). This syndrome presents with a broad range of phenotypes. The common characteristics include small testes and infertility, but KS subjects are at increased risk of hypogonadism, cognitive dysfunction, obesity, diabetes, metabolic syndrome, osteoporosis, and autoimmune disorders, which are present in variable proportion. Although part of the clinical variability might be linked to a different degree of testicular function observed in KS patients, genetic mechanisms of the supernumerary X chromosome might contribute. Gene-dosage effects and parental origin of the supernumerary X chromosome have been suggested to this regard. No study has been performed analyzing the genetic constitution of the X chromosome in terms of copy number variations (CNVs) and their possible involvement in phenotype of KS. To analyze CNVs of the X chromosome in KS subjects, we performed a SNP arrays analysis on 94 KS and 85 controls, including 42 46,XX females ad 43 46,XY males. KS subjects have more frequently than controls X-linked CNVs (39/94, [41.5%] with respect to 12/42, [28.6%] of females, and 8/43, [18.6%] of males, $p < 0.01$). The number of X-linked CNVs in KS patients was 4.58 ± 1.92 CNVs/subject, significantly higher with respect to that found in control females (1.50 ± 1.29 CNVs/subject) and males (1.14 ± 0.37 CNVs/subject). Importantly, 94.4% X-linked CNVs in KS subjects were duplications, higher with respect to control males (50.0%, $p < 0.001$) and females (83.3%, $p = 0.1$). Half of the X-linked CNVs fell within regions encompassing genes and most of them (90%) included genes escaping X-inactivation in the regions of X–Y homology, particularly in the pseudoautosomal region 1 (PAR1) and Xq21.31. This study described for the first time the genetic properties of the X chromosome in KS and suggests that X-linked CNVs (especially duplications) might contribute to the clinical phenotype

P02

AXYON: An online patient registry including Patient Reported Outcome measures for individuals with Sex Chromosome Aneuploidies.

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Patient-centered outcomes (PCOs) are defined as clinical outcomes most important to patients and families. PCOs are increasingly emphasized in clinical research in the USA since the 2001 Affordable Care Act established the Patient-Centered Outcomes Research Institute (PCORI) to support patient-centered comparative effectiveness research. This emphasis led to developing standardized patient reported outcome (PRO) measures for assessment of physical symptoms, psychosocial functioning, and quality of life (QoL). Examples include National Institutes of Health Toolbox and Patient-Reported Outcomes Measurement Information System (PROMIS). To identify PCOs in SCAs, the US-based advocacy group, AXYS, received PCORI funding through Genetic Alliance to launch a patient-reported registry, AXYS AXYON Registry (AAR). AAR aims to globally collect patient-reported data for all SCAs about diagnosis, symptoms, treatments and QoL, including standardized PRO measures. Respondents may opt to disclose contact information. AAR formally launched December 2015. This project describes data collected the first month. AAR utilizes Genetic Alliance's online registry system, Platform for Engaging Everyone Responsibly (PEER). Respondents provide consent and answer up to 650 questions including demographics, diagnoses, medical and family history, developmental/psychological history, and social factors (education, work, etc.) Standardized PRO questions address peer relations, anxiety, depression, fatigue, life satisfaction, daily living skills, and QoL. Goal is 2000 respondents. From 29/Dec/15 to 20/Jan/16, there are 116 respondents including 51 XXY, 28 XXX, 8 XYY, 15 XXYY, 1 XXXY, 6 mosaics. Age range 2-72, median age 17. Origins are 76 USA, 7 AUS, 6 CAN, 2 NLD, 2 GBR, 2 DEU, 1 ZAF and 1 NZL. 98 respondents gave an email address to re-contact and 18 declined re-contact. AAR captures a wide range of self-reported data about diagnosis, symptoms, comorbidities, treatments and QoL for persons with SCA. Strengths of AAR include an opportunity for researchers globally to query a large dataset with longitudinal data, validate PRO measures in SCA populations, compare PRO measures in SCA to other populations, and target research recruitment. Weaknesses include that the survey is lengthy and responses may not be clinically reliable due to self-report. Furthermore, AAR is grant-funded and it is unclear how maintenance and data access will continue at grant completion.

P03

Do men with Klinefelter Syndrome show a different epigenetic aging signature than healthy males and females?

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Men with Klinefelter Syndrome (KS) have a unique genetic constellation, which leads to complex clinical phenotype. Epidemiologic studies revealed that KS patients have a reduced lifespan as well. Dominant causes of death (endocrine disorders, diseases of the circulatory and respiratory system etc.) are strongly associated with the syndrome (1, 2). The elevated morbidity and mortality rates are likely influenced by an interaction between genetic, hormonal and socioeconomic factors (1). Recently it was shown that aging can be monitored with a surprisingly high accuracy by determination of only a few methylation marks in blood DNA. Accordingly a biological age marker, beside of telomere length, is available, enabling large scale studies in humans (3). Based on the previous description of a significantly reduced lifespan in KS patients we studied possible differences between DNA-methylation levels of three age-associated genes in patients with KS to those in healthy controls. This should address to which extent men with Klinefelter Syndrome are prematurely aged due to their unique genetic constellation. Whole blood samples were obtained from 132 Klinefelter patients, male (n=50) and female (n=50) age matched healthy controls from the EXACT study (4). Methylation levels at three genes (ASPA, ITGA2B and PDE4C) were determined by bisulfite pyrosequencing of genomic DNA. The biological age was calculated by applying a formula established by Weidner *et al.* (3). Chronological age and calculated age show a significant strong correlation with each other in all three study groups (Pearson r KS: 0,807; male controls: 0,865; female controls: 0,871). Mean values for difference between chronological and calculated age (age deviation) are +7,95 years ±7,14y for KS, for males +6,51y ±7,14y and for female controls +8,99y ±5,67y. Between groups no significant difference can be found. Using a novel methylation analysis of three genes in blood samples, biological age can be calculated with high accuracy.
The lack of significant difference between the three groups (KS, male/female controls) indicates that Klinefelter men do not undergo an accelerated aging process at an epigenetic level.

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P04

Prenatal diagnosis of Klinefelter Syndrome – using the Danish national screening program for Down syndrome

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In 2004-2006 a national prenatal screening for Down syndrome was instituted in Denmark. The screening consists of nuchal translucency (NT), measured by ultrasound, maternal serum-free beta human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and maternal age. Using these parameters a risk is calculated to assess the need for invasive procedures. If the risk of having Down syndrome is above 1:300, amniocentesis or chorionic villus sampling will be offered to identify the fetus karyotype. Using this method not only Down syndrome fetuses are identified, but also other chromosome abnormalities are discovered. Recent research has shown that the incidence of sex chromosome abnormalities is higher in countries with screening for Down syndrome. To examine the significance of NT, β-hCG and PAPP-A in predicting pregnancies with Klinefelter Syndrome (KS) and to describe the natural history of these pregnancies. Further we will investigate the outcome and estimate the detected prenatal prevalence. All pre- and postnatal KS karyotypes diagnosed in Denmark from 1/1-2008 to 31/12-2012 were retrieved from the Danish Central Cytogenetic Register and cross-linked with the Danish Fetal Medicine Database to obtain information regarding maternal age, ultrasonic findings and biomarkers. Matched controls were identified from the same database. 34 with KS was identified, 27 pre- and 7 postnatally diagnosed. The detected prenatal prevalence was 19 per 100.000 male fetuses (13% of the expected). The median fetal NT, measured between week 11+3 and 13+6, was significantly increased compared to controls (2.3mm vs. 1.7mm, p=0.001); The median PAPP-A, measured between week 8+0 and 13+6, was significantly lower 0.5 (0.2-2.4), p=0.001). Free β-hCG was significantly lower 0.6 MoM (0.2-1.5), p=0.001. Maternal age at the NT scan was significantly higher compared to controls (34 vs. 30 years, p=0.001). 13 out of 27 (48%) prenatally detected fetuses resulted in induced abortion. The percentage detected is much lower than anticipated, with only 13% of the expected 47,XXY being diagnosed prenatally. Thus using the Down syndrome algorithm testing does not seem to be a sufficient method in detecting KS pregnancies. Low PAPP-A, increased NT and reduced free β-hCG were seen in KS pregnancies compared to normal pregnancies, suggesting that the prenatal development of KS differ from normal development.

P05

Gene Expression Patterns in Relation to the Clinical Phenotype in Klinefelter Syndrome

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Klinefelter Syndrome (KS) is the most common chromosome disorder in men (47,XXY), exhibiting a phenotype with marked variation and increased morbidity. The supernumerary X chromosome may contribute to the pathology. To elucidate whether differential gene expression patterns can be detected in KS patients and whether these are related to pathologies. EXAKT (Epigenetics, X chromosomal features and Clinical Applications in Klinefelter Syndrome Trial) is a Münster-based prospective non-interventional project involving 132 Klinefelter men and their parents assessing a range of cardiovascular, inflammatory and metabolic factors in comparison to age-matched male (n=50) and female controls (n=50) involving genetic features. Predefined hypotheses: differential gene expression patterns exist in KS patients vs male controls and are related to the clinical phenotype. Differential expression of 36 X chromosomal and autosomal genes put KS patients into a unique genetic setting vs male controls and / or female controls. The KS cohort exhibited increased insulin resistance and an enhanced inflammatory status, a procoagulatory status, higher waist circumference, dyslipidemia and an altered cardiac rhythmogenic setting (shorter QT-interval partly located within pathological range) vs male controls (all $p<0.001$). Clinical dyshomeostasis was associated with expression patterns of dysregulated genes (all $p<0.01$). Paternal origin of the supernumerary X chromosome was a confounder regarding insulin resistance and cardiac phenotype ($p<0.05$). In testosterone-treated KS patients, the pathophysiological pattern persisted, albeit depending on inflammatory-regulating gene expression ($p<0.001$). The supernumerary X chromosome may contribute to a number of pathologies in KS. The pattern of gene expression is altered in KS and the degree of differential gene expression is related to the clinical phenotype. This is observable independently from testosterone substitution which may have attenuated responses in KS.

P06

Congenital underandrogenisation in Klinefelter Syndrome

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Klinefelter Syndrome (KS) is characterized by 47,XXY karyotype and usually an unequivocal male genital appearance at birth. Several case reports, however, report genital ambiguity in KS. It remains unclear if genital ambiguity ranging from female genital appearance to severe hypospadias is a coincidence or related to KS. We have screened patients with KS and genital ambiguity for additional genetic abnormalities with array comparative hybridization, MLPA, and a next generation sequencing approach involving 83 DSD-related genes. Results: In one case of KS a deletion within the AR-gene was detected on one X chromosome, leading in conjunction with X-inactivation to partial androgen insensitivity syndrome, while otherwise no genetic abnormality was detected. Conclusion: This demonstrates that genital ambiguity in KS may have an additional genetically determined reason and this should be considered in the diagnostic approach.

ANIMAL – MODEL's

P07

The 41,XX^{Y}* male mice, a reliable model for human Klinefelter Syndrome

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Klinefelter Syndrome (KS) is the most frequent male chromosomal (1-2:1000) disorder in men (karyotype 47,XXY). The supernumerary X chromosome originates from aberrant gametes, which derive from germ cells resulting from meiotic non-disjunction. Males with a supernumerary X chromosome exist in many mammalian species and generally present a similar phenotype. The hope to generate animal models for KS from such male mammals in order to address its pathophysiology experimentally was jeopardized by the fact that the condition provokes infertility and thus is only enabling anecdotal descriptive studies for a long time but not large scaled systematic settings. The opportunity to establish a KS mouse model occurred when male mice with a mutated Y chromosome (B6Ei.Lt-Y* strain) were discovered. The mutants carry a Y* chromosome in which the centromere is delocalized to a distal position. 40,XY* males are phenotypically normal and fertile but mating with 40,XX females provoked meiotic sex chromosomal non-disjunction and allowed for the production of mice with chromosomal aberrations (such as XXY, XXY*, XXY*Y, XY*Y, XYY*X, XYY*, XY*X) in a staggered four –generational breeding scheme. Of these, 41,XXY and 41,XX^{Y}* males closely resemble the features of KS. Use of these models for experimental research on the genetic condition was intensified when methods became available which enabled karyotyping living animals. Fluorescence in situ hybridization and Xist expression are tools for the detection of the presence of a supernumerary X chromosome from d1 post-partum to adulthood, therefore also enabling developmental studies. In our studies, we could demonstrate that male 41,XX^{Y}* mice are an adequate model for the disorder. However, as those males are already an outcome of the first breeding step and occur in 25-30% of the male offspring, the use of 41,XX^{Y}* males allows for animal numbers that enable experiments of a statistically reasonable size. Currently, we are running two breeding colonies of approx. 600 animals in total which provide approx. 40-50 41,XX^{Y}* males for experiments per year. As male and female littermates are also used experimentally, this animal model is extremely efficient also in terms of the 3R principle asked for in animal ethics. Studies employing our model have brought novel insights on cognition, escapee genes, timing of germ cell loss, Leydig cell function and testicular vascularization which might serve as a template for further clinical analyses.

P08

Altered testicular vascularization during development in 41,XX^{Y}* mice

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Testosterone (T) deficiency is a major feature of Klinefelter Syndrome (KS) and as intratesticular T levels are comparable to controls and Leydig cell function was proven to be normal at least in in vitro assays, testicular vascularization changes came into focus as a potential factor contributing to hypergonadotropic hypogonadism. In addition, germ cell loss could be provoked by disturbed testicular vessel formation. We therefore performed a study in which the distribution and size categories of testicular blood vessels in juvenile (1, 3, 5, 7, 10, 21dpp) and adult (15wpp) 41,XX^{Y}* mice and healthy littermates with 40,XY* (n≥5 per group and developmental stage) were analysed. Blood vessels were detected and assigned to size categories (I<80µm², II=80-272µm², III=273-411µm², IV=412-574µm², V=575-661µm², VI=662-2052µm², VII=2053-5062µm², VIII=>5062µm²) each representing maximum values of sizes found in the respective developmental stage in 41,XX^{Y}* animals. In addition, for each size category the blood vessel / testis surface ratio was determined to correct for the smaller testes of 41,XX^{Y}* mice. Differences in size of the largest noticeable vessels were observed in all stages. The largest blood vessels found in 41,XX^{Y}* were always smaller than those of 40,XY* mice. Blood vessel area was significantly smaller in KS mice of 10dpp ($p<0.05$) and 15wpp ($p<0.01$). Furthermore, a significant lower number of smaller and middle sized blood vessels (<1000µm²; $p<0.001$) in adult KS mice was detected. Calculated blood vessel / testes ratio as an indicator for the degree of vascularization did not show any significant differences in the juveniles. Nonetheless, degree of vascularization was elevated in adult KS males ($p<0.01$). KS mice exhibited a higher degree of vascularization regarding middle and larger sized vessels (>80µm²; $p<0.05$). Our data indicate an impaired vascularization in testes of males with a supernumerary X chromosome already during juvenile development; a feature which might contribute to the pathological endocrine phenotype of KS.

ENDOCRINOLOGY and METABOLISM

P09

Metabolic and lipoproteic alterations in patients with Klinefelter Syndrome

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Patients with Klinefelter Syndrome (KS) are at higher risk of cardiovascular disease (CVD) and metabolic abnormalities. However, there is still controversial evidence on which metabolic or endocrine risk factor contribute significantly to CVD risk. To investigate the contribution of quantitative and qualitative lipoprotein abnormalities, including LDL density and oxidation, to the CVD risk in KS patients naïve of any prior hormone replacement therapy. In this preliminary study, 30 patients with KS, 30 age-matched male and 40 female controls were studied. Anthropometric data and fasting blood samples were collected for each individual. Full lipid profile and qualitative lipoprotein analysis by density gradient ultracentrifugation was carried out, and plasma oxidized LDL levels measured. In patients with KS, fasting glycaemia, glycated hemoglobin, HOMA index, LH, FSH, total testosterone, SHBG and TSH were also evaluated. Qualitative lipoprotein analysis showed a unique lipid profile in KS: an increase in HDL and VLDL cholesterol, and large LDL particles, associated with a reduction of small, dense LDL when compared with male controls, and an increase of VLDL and LDL particles when compared with female controls. KS patients in the two tertiles with greater waist circumference (waist circumference >91.3 cm) had a highly atherogenic lipid profile characterized by higher triglyceride, lower HDL cholesterol, increased prevalence of small and dense LDL. Furthermore, oxidized LDL's levels correlate with small and dense LDL fractions studied by ultracentrifugation. Finally, multiple logistic regression analysis showed that low levels of testosterone are significantly associated with increased waist circumference. With increasing waist circumference patients with KS have increased plasma triglycerides, reduced HDL cholesterol, small, dense and oxidized LDL, a highly atherogenic lipid profile. The increased waist circumference is, at least partly, modulated by the low plasma testosterone levels. There may be clinical implications of the testosterone replacement therapy in these patients: it decreases waist circumference and therefore positively influences lipid profile, but it might be associated with an increase of small, dense LDL and a decrease of HDL. Other studies will be required to analyze the effects of the testosterone therapy on the lipid profile and on the cardiovascular events.

P10

Proximal femur strength, cortical thickness and bone structure in Klinefelter Syndrome

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Klinefelter Syndrome patients (KS) frequently show low bone mass, which could have multiple etiologies. The structural basis of low bone mass and its consequences on bone strength are almost not known, but analogies in bone microstructure and strength between KS and aging women have proposed by studying distal tibia by HRpQCT. The aim of this study was to compare proximal femur strength and bone structure of KS with elderly women and men. Proximal femur QCT analysis was performed on 18 KS (mean age 44±8 years) and compared with 89 elderly women (76±6 years) and 39 elderly men (79±5 years). QCT-based estimates of proximal femur strength were obtained with a personalized Finite Element procedure previously validated in-vitro and in-vivo under loading conditions corresponding to 10 fall directions to span accidental conditions. Bone structure analysis included trabecular and cortical volumetric bone mineral density (Tb.vBMD, Ct.vBMD), and cortical thickness (Ct.Th.), mapped to 18 sectors covering the whole femoral neck. Femoral neck length and cross-sectional area were calculated. KS and women had similar bone strength (KS: 2981±514 N, W: 2822±627 N, Mann-Whitney P=0.14), both significantly lower (P<0.001) than elderly men (4176±985 N). Bone cortex was significantly thinner in KS patients with respect to women (P<0.05 in 13 out of 18 sectors). Ct.vBMD was equivalent in KS and women, whereas Tb.vBMD was instead higher in KS (P=0.003). Femoral neck was significantly larger in KS patients (CSA 25% higher, P<0.001). We showed for the first time that, at proximal femur, KS and elderly women are similar in terms of bone strength. This similarity emerged however from different structural traits: KS had thinner femoral neck cortex, partially compensated by a denser trabecular compartment and larger bone dimensions (i.e. higher moments of area and bone mass).

P11

Will steroid measurements affect the outcomes of clinical trials? Comparison between immunoassay and mass spectrometry in men with Klinefelter Syndrome undergoing human chorionic gonadotropin stimulation test

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Liquid-chromatography tandem mass-spectrometry (LC-MS/MS) was developed in parallel to Immunoassays (IA) and represents the gold standard for steroid assays. Recently, our group has demonstrated that in men with Klinefelter Syndrome (KS) Leydig cells respond to human chorionic gonadotropin (hCG) stimulation, even if the testosterone (T) production is impaired, using only LC-MS/MS. To compare IA and LC-MS/MS performance, evaluating steroidogenesis after hCG stimulation in men with KS compared to control volunteers. Longitudinal, prospective, case-control clinical trial, in which 14 KS patients (36±9 years) not receiving T replacement therapy and 13 eugonadic control men (32±8 years) were enrolled. Serum steroids were evaluated at baseline and for 5 days after intramuscular injection of 5000 IU hCG using both routinely IA (chemiluminescent microparticle immunoassay, radioimmunoassay and competitive immunoenzimatic assay) and LC-MS/MS. Progesterone (P), 17-Hydroxy-Progesterone (17OHP), androstenedione (A), T and estradiol (E2) were significantly higher using IA compared to LC-MS/MS ($p<0.001$, $p=0.043$, $p<0.001$, $p<0.001$ and $p<0.001$, respectively). IA and LC-MS/MS showed a direct correlation for 17OHP and T measurement ($r=0.850$, and $r=0.979$, respectively), and a moderate concordance ($\rho=0.844$ and $\rho=0.894$). A linear relationship was not excluded for T measurement ($p=0.590$) in spite of significant proportional and systematic errors. On the contrary, a poor correlation ($r= 0.826$, $p<0.001$) with a poor strength ($\rho c=0.347$) of the agreement between the 2 methodologies was evident for A measurement. On the other hand, the two methodologies found the same significant 17OHP and T increasing-profile, although smoothed with IA. A linear regression between IA and LC-MS/MS performances is pointed out, although IA seems to be less specific than LC-MS/MS, with an overestimation trend of sex steroids levels. Moreover, IA sensitivity seems to be poor when a slight hormonal change has to be detected, such as after hCG stimulation.

P12

Is serum estradiol (E2) really increased in patients with Klinefelter Syndrome (KS)? Results from a meta-analysis study

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KS has been classically described as characterized by hyperestrogenism and elevated serum E2 together with increased gonadotropins and low-to-normal serum testosterone (T). In literature, data on increased serum E2 are not solid. The aim of this study is to meta-analyse data from studies evaluating serum E2 in both KS and healthy subjects (HS) in order to verify if E2 is increased in KS. An extensive MEDLINE was performed using 'PubMed' with the following key words: 'KS' and 'E2' or 'T' or 'sex steroids' from 1946 to January 2015 (Current Contents-ISI was used for searching oldest studies). All studies (case-control, case-series, case-reports) reporting E2 measurement were considered. Controlled-studies were used for meta-analysis, the others only for reviews. Only serum E2 at baseline (no ongoing treatments) was included. Meta-analysis was conducted according to the PRISMA statement using RevMan. Out of 956 articles, 26 case-control studies, 15 case-series and 21 case-reports had data on serum E2. A total of 878 KS and 1000 HS were included in the meta-analysis. Serum E2 was significantly higher in HS than in KS, with a mean difference of 7,93 pg/mL (CI:2,24,13,61; p=0,006), with a chi-squared=688,32 (I-square=97%). Serum T was significantly lower in KS than in HS, with a mean difference of -2,79 ng/mL (CI:-3,46,-2,11;p<0,001), with a chi-squared=198,29 (I-square=89%). Data from case-series and case-reports confirmed that E2 is not above the normal range in KS. Serum E2 is not increased in KS and is significantly lower than in HS in this meta-analysis. The limits of this study are the heterogeneity of methods for steroids measurement and the lack of studies having the comparison of serum E2 between KS and HS as primary endpoint. The traditional belief that KS is associated to elevated E2 should be reconsidered together with some pathophysiological and clinical issues.

P13

Effects of long-term treatment with testosterone undecanoate injections (TU) in patients diagnosed with Klinefelter's syndrome (KS) following detection of osteoporosis

A. Haider, K.S. Haider, G. Doros, F. Saad, L. Gooren

see Oral presentation Abstracts on page 46

P14

Is intratesticular lactate and creatine content a reliable biomarker of testicular dysfunction in men with Klinefelter Syndrome?

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Introduction: Klinefelter Syndrome (KS) is a genetic condition caused by additional copies of the X chromosome. It has a prevalence of 1 in 660 men turning KS one of the most frequent sex chromosome disorder. It also has an 11% incidence in azoospermic men. KS is also associated with a higher risk of developing metabolic diseases and alterations in body fat composition. Notably, the mechanisms responsible for these phenotypes remain largely unknown. We aimed to study the metabolic profile of testicular biopsies from KS men seeking fertility treatment. Testicular biopsies from men with conserved spermatogenesis previously subjected to vasectomy (control) (46, XY) (n=6) and KS (47, XXY) (n=6) men were collected and analysed by proton high- resolution magic-angle spinning magnetic resonance. Quantitative PCR and Western blot were used to study gene and protein expression of relevant enzymes and transporters associated to glycolysis. Testicular tissue from KS individuals presented decreased mRNA levels of glucose transporter 1, lactate dehydrogenase A and phosphofructokinase 1. Moreover, glucose transporter 3 mRNA levels were increased and alanine aminotransferase protein levels were decreased in testicular tissue from KS men. Notably, intratesticular lactate and creatine content were severely decreased in KS men. Our data shows that the testicular metabolic profile of KS men is severely altered. Among the several differences detected, we highlight the possible role of intratesticular lactate and creatine concentration as a reliable biomarker of testicular dysfunction in men with Klinefelter Syndrome. Our results provides evidence that Sertoli cell population may have a role in the infertility detected in KS men and identifies new therapeutic targets for the treatment of young, pre-pubertal KS patients.

P15A

Testosterone in infants with 47, XXY has positive short-term effects on body composition

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Prenatal diagnosis of sex chromosome aneuploidies, including Klinefelter Syndrome (KS), is rapidly increasing since the commercialization of non□invasive prenatal screening, while evidence□based management in infancy is lacking. Previous studies have found a reduced testosterone surge in infants with KS and observations of poor penile growth and hypotonia may be secondary to androgen insufficiency. Boys and men with KS are also known to have a high prevalence of abnormal body composition and insulin resistance, possibly mediated by androgen deficiency. The objective of this ongoing study (NCT02408445) is to evaluate the short□term effects of testosterone on body composition and motor development in infants with KS. Infants 6□15 weeks of age with karyotype 47,XXY were randomized 1:1 to testosterone (T) 25 mg intramuscularly every four weeks for three doses or no treatment. Air displacement plethysmography (PeaPod) was performed at baseline and 3 months later by an investigator blinded to randomization status. Percent body fat (%BF) z□scores were calculated for age and the change between baseline and the final study visit was compared between treatment groups. Six infants have completed the study. The average age of enrollment was 66±28 days; all subjects were born average for gestational age at term and 5/6 were solely breastfed. The average %BF at baseline was within the normal range (z-score -0.36). The mean change in %BF z-scores in infants who did not receive T (n=3) is +1.23, while the mean change in %BF z-scores in infants who did receive T (n=3) is □0.26 (p=0.06). Preliminary data on body composition and motor development will be presented for all nine subjects who will have completed the study by the time of presentation. In untreated infants with 47,XXY, %BF increased by over 1 standard deviation in a three month period, while infants who received a short course of testosterone followed a more normal curve with minimal change in z-scores. Although preliminary, these data suggest body composition follows an abnormal trajectory of excessive %BF gain in early infancy in boys with KS and supplemental testosterone prevents this. These data are too premature to evaluate if the impact on body composition is a transient or sustained effect of testosterone therapy, or if this surrogate marker translates to clinically important outcomes, such as development and metabolic programing.

P15B

Low Inhibin B correlates with features of Metabolic Syndrome in prepubertal boys with Klinefelter Syndrome

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Klinefelter Syndrome (KS) is the most common sex chromosome aneuploidy, occurring in ~1/650male births. The phenotypic spectrum includes nearly universal testicula insufficiency in adulthood, high prevalence of metabolic syndrome (MetS), and increased mortality from cardiovascular diseases. Testicular insufficiency, predominantly testosterone deficiency may contribute to MetS and increased cardiometabolic risk. The objective of this study was to evaluate whether gonadal function was related to the MetS phenotype in prepubertal boys with KS. In this double-blind, placebo-controlled clinical trial (NCT00348945), 93 boys with KS, 4-12 years were randomized to oxandrolone or placebo for a 2 year period. This reports includes analysis of baseline physical examination and fasting laboratory data for boys who were <9.5 years of age, tanner 1 pubertal development (n=59). MetS was defined as meeting at least 3 of the following criteria: waist circumference >75%ile for age, fasting triglycerides >97 mg/dl, HDL <50 mg/dl, blood glucose >110 mg/dl, and systolic or diastolic blood pressure >90%ile for age and height. At least 1 feature of MetS was present in 81% of subjects, while full MetS criteria were met in 17% of subjects. Total testosterone by mass spectroscopy was less than the reference range in 47% of subjects. 18% of subjects had an INHB <5%ile for age. In a logistic regression model, low INHB was significantly associated with the probability of meeting at least 3 MetS criteria (p=0.047). An INHB <50 ng/dl yields a sensitivity of 83.3% and specificity of 79.2% for meeting full criteria for MetS. Cardiometabolic risk markers and gonadal insufficiency are prevalent in prepubertal boys with KS. This study is the first to suggest an association between impaired Sertoli cell function (low INHB) and cardiometabol phenotype. Whether testicular dysfunction is the cause of MetS or both the testicular dysfunction and the higher risk cardiometabolic profile are secondary to an underlying mechanism, such as variable genotype expression, cannot be determined from this cross-sectional data. Future analysis will examine two year longitudinal data to further investigate this relationship.

P16

Increased plasma concentration of PAI-1 in Klinefelter Syndrome is not alleviated by testosterone supplementation therapy

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A marked increase in thrombotic events in 47, XXY Klinefelter Syndrome (KS) is reported. In KS central obesity, overall increased morbidity and a skewed sex hormonal balance might partially explain such an association. However, studies on the haemostatic balance and the effect hereon by testosterone supplementation therapy (TT) in KS are lacking. We hypothesize that KS is associated with an impaired fibrinolytic capacity leading to greater thrombosis proneness. An ELISA calibrated against the NIBSC 07/114 standard was used for analysis of plasminogen activator inhibitor-1 (PAI-1) antigen in blood samples from 56 KS males not receiving TT (U-KS), 69 KS males receiving TT (T-KS) and 143 control males. Groups were compared by one-way ANOVA and linear regression was applied to adjust for any effect of testosterone on PAI-1. PAI-1 (ng/mL) median (25-75 percentiles) was 67.8 (50.2-106.6) for U-KS, 70.2 (47.8-123.0) for T-KS and 50.6 (34.5-74.9) in controls. Higher mean PAI-1 was found for U-KS compared with controls ($p=0.002$) and T-KS compared with controls ($p<0.001$). No difference was observed between U-KS and T-KS ($p=1.0$). PAI-1 was inversely correlated with total testosterone in U-KS and controls but not in T-KS. PAI-1 was inversely correlated with free testosterone in controls but not in KS. Higher values of PAI-1 were seen for both U-KS and T-KS compared with controls adjusting for testosterone level. PAI-1 is elevated in KS compared with controls possibly reflecting impairment of the fibrinolytic capacity. This effect is independent of testosterone and maintained albeit normalising testosterone levels in KS by TT.

P17

Effects of 6 month testosterone treatment on body composition and glucose metabolism in Klinefelter Syndrome. A randomized, double-blind, placebo-controlled, cross-over study

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Patients with Klinefelter Syndrome (KS) are hypogonadal and have a high incidence of metabolic disease, and epidemiological studies report an increased mortality due to diabetes and cardiovascular disease. Testosterone treatment of other hypogonadal patients with type 2 diabetes primarily improves insulin sensitivity in obese patients, which indicates that improvements in insulin sensitivity may largely depend on the amount of "modifiable fat." Whether such observations extend to KS patients is presently unknown, since no formal studies are at hand. In a randomized, double-blind, placebo-controlled, cross-over study, 13 KS patients (age: 34.8 (22-56) yrs.); BMI: 26.7±8.8 (kg/m2)) received Andriol® 160 mg per day or placebo treatment for 6 month. Thirteen age-(age: 34.8(21-53) yrs.) and BMI (27.0±6.9 (kg/m2)) matched healthy controls were recruited. DEXA scan, abdominal CT scan and a 3-h hyperinsulinemic euglycemic clamp were performed after each period of treatment and once in controls. Testosterone naïve KS were comparable to controls with respect to total lean (61.0±12.1 vs. 64.0±12.9 (kg), P=0.37) and body fat mass (26.8±16.8 vs. 21.3±15.2 (kg), P=0.14)), whereas visceral fat mass was increased (3.5± 2.4 vs.2.3±1.9 (kg), P=0.05), as was both total abdominal and intra-abdominal fat by CT scan (both p<0.01). Testosterone treatment decreased total body fat (26.8±16.8 vs. 24.6±15.3 (kg), P=0.01) and abdominal fat by CT (533±408 vs. 495±366 (cm3), P=0.04). There was no change in lean body mass or VO2max during 6 month treatment. Total glucose disposal was similar between T naïve KS and controls (8.9±1.1 vs. 10.3±0.6 (µmol/kg/min), P=0.28), and there was no effect of T on total glucose disposal in KS (8.9±1.1 vs. 8.6±1.0 (µmol/kg/min), P=0.82). The first randomized study of testosterone supplementation in Klinefelter syndrome shows that testosterone treatment for 6 month leads to favorable changes in body composition with reductions in fat mass, including abdominal fat mass, but not to changes in glucose homeostasis. We speculate that longer term treatment would lead to greater changes in body composition and eventually to an increase in insulin sensitivity.

P18

The variant FSHB -211G>T attenuates serum FSH levels in the supraphysiological gonadotropin setting of Klinefelter Syndrome – an update

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Hypergonadotropic hypogonadism represents the endocrine hallmark of Klinefelter Syndrome (KS). Single-nucleotide polymorphisms (SNPs) located within the FSHB/FSHR genes have been shown to impact serum follicle-stimulating hormone (FSH) levels and reproductive parameters in men. To investigate the effect of FSHB c.-211G>T (rs10835638), FSHR c.2039G>A (rs6166) and FSHR c.-29G>A (rs1394205) on endocrine and reproductive parameters in untreated and testosterone-treated KS patients. A total of 309 non-mosaic KS individuals between 18-65 years were retrospectively selected and genotyped. Associations of genotypes and endocrine/reproductive parameters in untreated and testosterone-treated KS patients were assessed. If available, associations with testicular biopsy and (microsurgical-) testicular sperm extraction [(m-)TESE] was assessed. In the untreated group (n=248) the FSHB c. 211G>T T-allele was significantly associated with reduced serum FSH levels (-6.5 U/l per T-allele, p=1.3x10-3). TT-homozygotes displayed serum FSH levels about 60% lower compared to GG-homozygotes. Testosterone (T) treatment (n=150) abolished the observed association. When analyzing patients before and under T treatment (n=89) gonadotropin levels were similarly suppressed. In histological evaluation of testicular biopsies (n=146), frequency of complete spermatogenesis (presence of elongated spermatids) did not differ between FSHB -211 G>T genotypes. (GG vs. GT/TT, 24% vs. 25%, respectively, p=n.s.). Testicular sperm extraction techniques (n=138) resulted in comparable success rates [TESE (n=31, performed until 2009) vs. mTESE (n=96, performed from 2009) vs. combination (n=11), 39% vs. 34% vs. 27%, respectively]. Sperm retrieval rate did not differ between FSHB -211 G>T genotypes [GG (n=104) vs GT/TT (n=34), 35% vs. 35%, p=n.s.]. FSHR SNPs did not exhibit any significant influence in any group. In a hypergonadotropic setting such as KS the effect of FSHB c. 211G>T serum FSH levels is markedly pronounced (compared to normal or infertile men). Gonadotropin suppression under testosterone treatment seems to be independent of the genotype. At the testicular level, no significant impact on testicular biopsy/sperm retrieval rate was observed for FSHB/FSHR SNPs. However, the FSHB c.-211G>T genotype appears to be a key determinant in the regulation of gonadotropins in different reproductive-endocrine pathophysiologies.

P19

Expression of estrogen receptors in testicular tissue of individuals with Klinefelter Syndrome

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Infertility affects about 10–20% of couples and genetic abnormalities are thought to account for 15%–30% of male factor infertility. Due to a genetic defect a wide range of physiological processes including hormonal homeostasis, spermatogenesis, and sperm quality may be affected resulting in a total or partial impairment of male fertility. Estrogens play important roles in the regulation of testes development and spermatogenesis, through the interaction with their specific receptors. Individuals with Klinefelter Syndrome (KS) exhibit particularly high serum estradiol (E2) levels at the beginning of puberty and throughout the adult life. We aimed to identify and evaluate estrogens receptors expression in testicular tissue of men with KS comparing with 46XY karyotype. Human testicular biopsies were obtained from twelve men: six with KS and 47XXY karyotype (KS group) and six with conserved spermatogenesis and 46XY karyotype (Control group). The mRNA expression of three estrogen receptors (G protein-coupled estrogen receptor 30 (GPR30), estrogen receptor α (ERα) and estrogen receptor β (ER β)) was evaluated in each biopsy. ERβ transcripts are the most abundant in testicular tissue of 46XY men. Notably, testicular GPR30 transcription in KS men was approximately twelve times higher. Since GPR30 is essential to mediate estrogen' effects over steroidogenesis, our data illustrates that GPR30 may underpin the testicular alterations observed in KS men.

P20

Cardiovascular risk factors in Klinefelter patients and healthy controls: a prospective clinical trial

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Klinefelter Syndrome (47,XXY; KS) is a very common chromosome disorder, affecting 1:600 men. Klinefelter men have been described to exhibit clinically relevant metabolic patterns related to a pro-inflammatory status, resulting in a high prevalence of insulin resistance and cardiovascular impairment. Testosterone deficiency in form of primary hypogonadism is a common feature in these men. A prospective clinical trial involving Klinefelter patients (n=132), assessing a wide area of cardiovascular, inflammatory and metabolic factors as well as sex steroids and questionnaires in comparison to age-matched healthy male and female controls (2 x n=50). A significant range of genetic and epigenetic investigations completes the approach. Here, we present novel clinical data comparing Klinefelter patients to healthy male controls in regard to cardiovascular and metabolic parameters. Klinefelter patients had a higher waist circumference and Body Mass Index in comparison to controls. Further on, decreased insulin sensitivity, higher levels of triglycerides and lipoprotein type a as well as lower concentrations of HDL-cholesterol were found in patients. Levels of high-resolution c-reactive protein were elevated in Klinefelter patients. Consequently, the prevalence of the Metabolic Syndrome according the Harmonized Criteria was markedly higher in Klinefelter men than in controls (52/130 vs 5/50). Corroboratingly, carotid artery intimia-media thickness was increased and flow mediated dilatation of the brachial artery was decreased in patients vs controls. These differences were statistically significant. Metabolic disadvantages of patients were further enhanced by low testosterone concentrations and already present in the sub-cohort younger than 40 years. In addition, we report a novel description of pathologically shortened 12-lead ECG QTc time in Klinefelter Syndrome (see other abstract). Men with the Klinefelter Syndrome exhibit an unfavorable pattern of cardiovascular risk factors in comparison to healthy male controls. This picture is already present in younger patients and enforced by testosterone deficiency.

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FERTILITY and SPERMATOGENESIS

P21

Characterization of Sertoli cells in the 41, XX^{Y*} mouse model for Klinefelter Syndrome during development

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A major feature of Klinefelter Syndrome (KS) is germ cell loss and disturbed testicular architecture and function. The substantial loss of germ cells at birth points to disturbed interaction between somatic and germ cells. Amongst other key mechanisms, the gender related fate of somatic cell differentiation might be affected. Fate decision is determined during fetal development when bipotential somatic cell precursors mature either into a female granulosa or a male Sertoli cell (SC). Recently, a reversibility of SC determination and differentiation throughout life was suggested, meaning that sex related differentiation has to be maintained actively in both sexes. These processes involve the transcriptional regulator Dmrt1 as a key factor of testicular male sex maintenance and on the other hand the ovarian transcription factor Foxl2, being essential for sex maintenance in the ovary. Dmrt1 additionally controls the switch between mitosis and meiosis in male germ cells at the transcriptional level. SCs fulfill numerous other functions, i.e. signal transduction, germ cell niche and blood-testis barrier formation. All these functions differentiate during development and might be affected under a given genetic condition. In the current study we aimed at the determination and differentiation of SCs in our $41,XX^{Y*}$ mouse model. We analyzed the Dmrt1/Foxl2 regulatory system as well as the transcriptional expression of SC markers like FSH receptor, Amh, Gdnf, Claudin5, Rhox5 and Cxcl12 postnatally (d1 post-partum - adult stage, n>3). Dmrt1 protein expression pattern in SCs was found to be similar to controls. However, whilst Dmrt1 was observed in spermatogonia of the control tissues, none of the remaining germ cells of $41,XX^{Y*}$ mice was positive. Only in a few tubules with focal spermatogenesis, also immunopositive spermatogonia in $41,XX^{Y*}$ males, indicating correct expression in these foci, were detected. No Foxl2 expression in SCs was observed. The protein abundances were consistent with mRNA expression during postnatal development. Other SC markers showed altered transcription profiles in $41,XX^{Y*}$ mice pointing to compensatory (due to germ cell loss Gdnf, Cxcl12) or delayed expression (due to maturation defects Amh, Claudin5, Rhox5). Conclusively, the differentiation of SC in $41,XX^{Y*}$ appears to be sex specific but altered compared to controls and future studies should aim at a full transcriptome to provide deeper insights on the SC function in KS.

P22

Novel Aspects of Testicular Architecture in Adolescent Klinefelter Patients

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Data on testicular architecture of boys with Klinefelter Syndrome (KS) are scarce and it is unclear how hormonal markers and age are related to fertility. 82 KS patients (mean age 16.2±2, range 12-21 y) underwent testicular biopsies including potential sperm retrieval. Testicular histology was assessed by PAS-stainings and using the point counting method in spermatogonia-specific MAGEA4-stainings. Serum hormones were assessed prior to this. 8 young men of normal karyotype and various malignant diseases undergoing experimental germ cell preservation served as controls (mean age 14.8±2, range 11-20 y). Testicular volume in KS was lower vs controls (3±2 vs 12±11mL) and inversely related to age ($p=0.04$). The testicular interstitial tissue was prominent in KS vs controls (64% vs 28%,$p<0.001$) but did not advance with age, while the number of Sertoli-Cell-Only tubuli (SCO) regressed ($p<0.001$) and complete degraded tubuli (tubular shadows, TS) increased ($p=0.007$). FSH and LH related inversely to SCO tubuli ($p=0.001$) and positively to TS (LH:$p<0.001$, FSH:$p=0.04$), while InhibinB levels related positively to SCO ($p<0.001$) and inversely to TS ($p=0.007$). InhibinB concentrations were also associated with tubuli containing only spermatogonia ($p<0.001$) or to the level of spermatocytes ($p=0.04$). MAGEA4 staining reflected the presence of intact tubuli vs SCO-tubuli or TS (all $p<0.001$) and was related to LH levels ($p=0.003$). PAS-staining related to Inhibin B levels ($p=0.01$) and various types of tubular stages (all $p<0.003$). Testicular architecture in KS is altered already in (pre-)pubertal age of KS, exhibiting a pronounced proportion of interstitial tissue. InhibinB levels and both PAS- and MAGEA4 stainings reflect this strongest.

P23

Age and markers of Leydig cell function, but not of Sertoli cell function predict the success of sperm retrieval in adolescents and adults with Klinefelter's syndrome

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Microsurgical testicular sperm extraction (mTESE), combined with intracytoplasmic sperm injection (ICSI) represents a chance for azoospermic men with Klinefelter's syndrome (KS) to father children. The objective of this study was to identify predictive factors for the success of mTESE from adolescents and adults with KS. The clinical data of 50 late pubertal adolescents (13-19 years) and 85 adult patients (20-61 years) with non-mosaic KS, who underwent mTESE, were analysed with respect to factors, potentially predictive of active spermatogenesis; specifically a history of cryptorchidism, age, testicular volumes, serum levels of LH, FSH, testosterone (T) and estradiol at the time of surgery. Inhibin B, AMH and INSL3 were additionally analysed in the adolescents. A younger age and a near-compensated Leydig cell function were associated with higher success of sperm retrieval via mTESE: In adolescents ≥15-19 years, spermatozoa were retrieved in 45%, compared to 31% in adults; in adolescents aged 13-14 years, spermatozoa were collected in only 10%. Adolescents with an LH ≤17.5 U/L, along with a T level ≥7.5 nmol/L had the best success rate (54%), which fell to 44% with higher LH, whereas those with low T (<7.5 nmol/L), irrespective of LH had no sperm retrieval. In adults with T levels above and LH below these thresholds, the success rate was 51%, falling to 19%, if LH was higher. When T was lower than threshold, the rate was 17%. No association between testicular volumes, serum levels of FSH, Inhibin B, AMH, estradiol and mTESE success was found. A history of cryptorchidism was associated with lower retrieval rates. A window of opportunity for an approximate 50% chance to retrieve spermatozoa via mTESE exists for young, late pubertal KS patients between age 15 and young adulthood, when Leydig cell function is at its best. In these cases, referral to a centre of expertise should be considered.

P24

Sperm retrieval in subjects with Klinefelter Syndrome: preliminary results from a meta-analysis study

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Specific factors underlying successful sperm retrieval after testicular sperm extraction (TESE) in adult patients with Klinefelter Syndrome (KS) are not completely clarified. The aim of present study is to meta-analyze currently available data regarding surgical sperm retrieval in subject with. All trials reporting sperm retrial rate after TESE or micro-TESE and its specific determinants without any arbitrary restriction were included. The identification of relevant studies was performed independently by three of the authors (F.L, A.P. and A.G), and the fourth investigator (G.C.) resolved the conflicts. Overall, 19 trials were included in the study enrolling 562 patients with a mean age of 29.9±5.9 years. TESE was performed in 12 trials and micro-TESE in 5. In addition, one study was performed using fine-needle aspiration and in one a mixed TESE/micro-TESE approach was applied. Among retrieved trials, 16 studies included only non-mosaic 47, XXY subjects, whereas in 3 studies, also mosaic patients were included. Overall, 46[41-52]% of sperm retrieval rate was detected. The data were confirmed even when those studies enrolling mosaic subjects were excluded from the analysis 49[43-53]%. In addition, no difference was observed when TESE data were compared to those derived from studies applying micro-TESE technique (45[36-54]% vs. 50[46-56]%; Q=1.02; p=0.31). Meta-regression analysis showed that none of the parameters tested, including age, testis volume as well as FSH, LH and testosterone levels at enrollment, affects final sperm retrieval (not shown). No sufficient data were available to test the effect of previous or concomitant testosterone replacement therapy (TRT). Present preliminary data suggest that performing TESE/micro-TESE in subjects with KS provide a retrieval sperm rate of about 50% independent of any clinical or biochemical parameter tested. The evaluation of previous or concomitant TRT on sperm retrieval remains to be determined.

P25

Evidence for a dual role of cells characterized by the expression of the neuronal stem cell marker nestin in the testis of patients suffering from Klinefelter Syndrome

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Previous studies suggested that vascular wall cells (a subpopulation of smooth muscle cells [SMCs] and pericytes) characterized by the expression of the neuronal stem cell marker nestin, represent progenitor cells of testicular Leydig cells (LCs). Highest Nestin levels were found in case of low testosterone (Davidoff *et al*. 2004). In addition, Nestin-expressing cells are also involved in the remodeling of blood vessels (Saboor *et al*. 2015). We studied the role of nestin in the testis of Klinefelter Syndrome (KS) patients, by examining 16 patients in the age of 14 to 42 years. Morphological investigations revealed different degrees of disturbed spermatogenesis and LC hyperplasia. In all biopsies of KS patients nestin-expressing vascular wall cells were detectable. While nestin+ vascular smooth muscle cells (VSMCs) were found in each section, nestin was never detectable in extra-vascular contractile cells of the regular and fibrotic lamina propria. Small vessels (mainly capillaries) within LC clusters and especially in case of LC hyperplasia, were nestin+ more regularly. Using a newly developed approach for three-dimensional analyses of testis biopsies, we could also demonstrate a high number of bigger vessels in KS biopsies different to other testis biopsies. Changes of vasculature were previously suggested in the XX^{Y*} mouse by Tüttelmann *et al.* (2014). Interestingly, also a lot of bigger arteries, that were not in proximity to LCs, showed nestin+ VSMCs. Currently we are investigating vascular nestin expression during postnatal development in the XX^{Y*} mouse testis. Data suggest a dual role of nestin-expressing cells in KS testes. In capillaries of LC clusters they might represent progenitor cells of LCs. In bigger vessels far away from LCs they might play an important role in vascular remodeling. Thus, nestin-expressing cells might be involved in the development of two peculiarities of KS testes, LC hyperplasia and changes of vasculature.

P26

Reduced spermatogonial number and impaired differentiation of Sertoli cells in Klinefelter patients

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Klinefelter Syndrome (KS) (47, XXY) is the most frequent sex chromosome disorder. During puberty, a severe loss of spermatogonia, including the spermatogonial stem cells (SSCs), occurs leading to infertility. Therefore, cryopreservation of immature testicular tissues is offered to these patients at risk for germ cell loss. While SSC based approaches including germ cell transplantation assays and the in vitro derivation of sperm were optimized experimentally there are no clinically established protocols available for the refertilization of these patients. Also, it remains unclear whether patient-specific protocols are required due to variability in the number of germ cells and the differentiation status of individual patients. The aim of the study was to assess the absolute number of spermatogonia and the differentiation status of testicular somatic cells in patients at risk for germ cell loss. Testicular tissues from KS patients (n=19; 12-20 years) and control patients (n=6; at risk of germ cell loss due to cancer treatment; 6-14 years) were evaluated. Immunohistochemical analyses for germ cell (LIN28, UTF1, MAGEA4, and DDX-4) and somatic markers (AMH, αSMA) were performed to determine the differentiation status of the testis. Two cross-sections of each patient were evaluated to determine the absolute number of spermatogonia employing morphometric analyses. In control patients, high expression levels of AMH were detected until the age of 13 years and low levels in patients above 14 years of age. Interestingly, high expression levels of AMH even persisted in KS patients between 14 to 20 years. In control tissues, the calculated mean number of spermatogonia was 93.239 (±18.857) per 1 mm^3. In KS patients, spermatogonia were only detected in 7 (4 patients 12-14 years and 3 patients 19-20 years) out of 19 KS patients and the mean value of spermatogonia was 5.348,7 (±5.6) per mm^3.Spermatogonia were only detected in 1/3 of KS patients and the absolute number of spermatogonia was reduced about 15 fold per mm^3 compared to controls. Sertoli cells remained immature based on AMH levels even in post-pubertal patients. Based on these results, it appears likely, that protocols for the propagation and in vitro differentiation of SSCs may have to be specifically adapted for Klinefelter patients.

NEUROPSYCHOLOGY and COGNITION

P27

Klinefelter's Syndrome in Intellectually Disabled Offenders Admitted on a Forensic Psychiatry Unit

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Klinefelter's syndrome is the commonest male sex chromosome variation and is associated with varying degrees of intellectual disability. There is evidence that an association exists between this condition and offending behaviour. The response to psychological treatment among intellectually disabled offenders with Klinefelter's syndrome is less well reported in published literature. This report describes the cases of two adult males admitted on Low Secure Forensic Units within the same Hospital for people with Intellectual Disabilities. We describe their offending behaviour against the background of available information regarding the relationship between Klinefelter's syndrome and offending as well as highlight their response to treatment in the context of their forensic history. Both patients are men with mild intellectual disability and both have received treatment for affective disorder. The first case is a 38 year old man with a history of marital difficulties who had been diagnosed with primary infertility which led to the diagnosis of Klinefelter's syndrome. He was found to have threatened a family member with an air rifle following an argument. The second case is of a 59 year old man who also has a history of psychotic illness and was convicted of sexual assaults and violence. Both men were enrolled onto psychological treatment programs aimed at reducing their risk of offending and have made varying degrees of progress. Little is known about the relationship between Klinefelter's syndrome and offending behaviour, let alone response to treatment. This report highlights the complex relationship between Klinefelter's syndrome and offending behaviour as well as the benefits of psychological treatment as part of a holistic treatment approach for these patients at least in the short term.

P28

Self-Reported Education, Work and Health Status Among 53 Norwegian MEN With SCA

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Sex chromosome aneuploidies (SCA) in men are associated with multiple challenges, but little is known about self-reported functioning among men with SCA. This is the first survey of Norwegian men with SCA. The study aims to contribute to the increasing knowledge about health and functioning among men with SCA. A cross-sectional survey of self-reported education, work, and health status in 53 men with sex chromosome aneuploidies (SCA) aged 19 to 67 years, recruited from a user organization and a resource center for rare disorders. In terms of mental and physical health, men with SCA reported poorer health on all Health Survey – Short Form (SF-36) scales compared to Norwegian male norms (average effect size d = -0.92). The survey showed that only 13% had post high school-education and that 57% experienced learning difficulties. Learning difficulties were associated with significantly lower education. Only 38% reported to be working and 34% received social welfare financial benefits. The mean age of retirement due to health reasons was 42.1 years (SD = 2.7). More than half the sample (57%) reported reading and/or writing difficulties. In terms of self-reported learning style, 68% confirmed learning slower than others. The vast majority (89%) preferred learning by doing to reading and 70% confirmed feeling their memory was poorer than others. Nearly half (44%) of the participants rated everyday routines, structure, and predictability as important or very important. Our results are in line with international studies documenting low education, poor socio-economic status, and early retirement for men with SCA. Our survey also provides new insights into self-perceived physical and mental health among this group of men. Practical implications for professionals include ensuring thorough cognitive and psychological assessment, offering practical education in high school and offering flexible working hours.

P29

Neuropsychological Evaluation Of The Child With Klinefelter Syndrome

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The neurocognitive and psychological profiles of patients with Klinefelter Syndrome (KS) show considerable variability. About half of patients are affected by the most frequent problems, including language and cognitive development. The purpose of our study is to compare the cognitive and neuropsychological development (with special attention to IQ) between children with XXY karyotype and a control group, in order to identify their strengths and weaknesses and to direct the type of therapeutic intervention to the most effective way. 15 KS boys and a control group of 15 children aged 6 to 10 years, attending primary school, have been selected. The cognitive profile of the children has been assessed using the WISC (Wechsler Intelligence Scale for Children). We compared the averages of each parameter stating total IQ (TIQ), verbal IQ (VIQ), performance IQ (PIQ) between KS and controls, using Student's test for independent samples, with a confidence interval of 95% (significance level of 0.5%). TIQ was 98.7±16.2 in KS patients and 105.1±9.7 in controls (p not significant). VIQ was 93.4 ± 17.3 in KS patients and 104.3 ± 10.7 in controls (p=0.0487). PIQ was 105.8 ± 14.1 in KS and 106 ± 8.7 in controls (p not significant). 53% of KS boys showed a difference bigger than 10 points between VIQ and PIQ; meanwhile the same difference was described in the 6% of controls. The results of our study confirm already published data. Typical cognitive profile of patients with KS is characterized by a normal range of IQ. However, there is a significant difference between performance and verbal tests. In our opinion, children with KS undergo a cascade of events that should be well investigated. If an emerging language disorder is not treated in time, it can lead to develop learning disability at school age. Prenatal diagnosis allows to prevent possible future problems and to take care promptly to reduce the discomfort of affected children, considering that the environmental factor can affect the prognosis.

GUIDELINES and NETWORKS

P30

Assistance Of Children And Adults With Klinefelter Syndrome: Proposal Of Guidelines

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Physicians should take account of all the problems by which pediatric and adult subjects with Klinefelter Syndrome (KS) could be affected. Pediatric geneticists and endocrinologists should be involved in Klinefelter patients' care, respectively in childhood and adulthood. Even though no conclusive treatment is possible, a large number of management options can be provided. Being each period of life different, peculiar clinical and biochemical management strategies are indicated. Physicians should take note of auxologic data, testicular volume, penile length and pubertal stage during each clinical evaluation. Postnatal period: pediatric geneticist assessment, blood tests for general and hormonal evaluations, endocrinologic consultation and first ultrasound testis analysis. First childhood (1-6 years): ultrasound testis analysis repeated only in the presence of cryptorchidism or testicular and hormonal diseases. Surgical assessment in the presence of cryptorchidism. Speech therapist evaluation of 3-year-KS boys, bone age and neuropsychiatric evaluation. Second childhood (6-10 years): same approach, except for surgical and speech therapist evaluation. Pre-pubertal and pubertal period (10-14 years): thyroid and mammary echo tomography and color-Doppler (the latter in the presence of suspected gynecomastia). DXA scan every 2-3 years and dental examination. At G3-G4 pubertal stage semen collection should be considered. At the age of fourteen endocrinologists are in charge of Klinefelter adults' clinical management. 14-25 years of age: blood and ultrasound tests annually, DXA every two years. 25-50 years of age complete cardiologic assessment (ECG, echocardiography and epiaortic ultrasound) and prostate function evaluation. After 40 years of age, annually prostate ultrasound in men under androgen substitution therapy. Sex specialist and/or a psychiatrist evaluation for all adult KS. Mammary echo tomography every two years; DXA every 18 months. After 50 years of age: in addition to previous evaluations, abdominal ultrasound. At whichever age KS is diagnosed, a psychological support is strongly recommended, in order to improve the processing of the received information. The psychological support is suggested both from the patient and family members. The communication of the diagnosis should be customized according to the personality of the patient and to the family background and it must be told before eighteen years of age.

P31

A KING for Klinefelter Syndrome: The SIAMS task force

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Klinefelter Syndrome (KS) is a fascinating condition for clinicians and researchers due to the variety of open questions still waiting for an answer. KS is one of the most frequent chromosomal disorders, occurring in 1:500 to 1:1000 live male births. Although significant research has been conducted, KS remains frustratingly underdiagnosed with a remarkable portion of cases being unidentified, among which only 10% are in the prepubertal age while 25-50% in the adulthood. As a consequence, medical research results often become clouded due to the relatively small number of patients reported in scientific papers. To overcome this difficulty, the Italian Society of Andrology and Sexual Medicine (SIAMS) relayed to the expertise of Italian researchers and clinicians in this field to constitute an outstanding working group on KS. Thus, a network named KING (Klinefelter ItaliaN Group), aiming at sharing the know-how and collecting KS patients, to improve the knowledge of this syndrome, was created. KING is composed by fifteen high-specialized Endocrinology and Andrology units, either academic or institutes for treatment and research (IRCCS), located throughout Italy. Each unit has a principal investigator and a KING coordinator has been identified. It has been created a common data register for an initial retrospective and registration study. Each KING unit has collected retrospective demographical data from KS patients among those regularly attending the units, after written informed consent has been obtained. Results: Up to now, four hundred and two KS from 12 out of 15 units have been registered. Their mean age was 41.6±13.0 years (range:8–76years). Only seventeen KS were diagnosed before the age of 18 years. Finally, the estimated total number of KS will be nearly 800 cases. Conclusions: Our preliminary data showed a higher rate than expected of underdiagnosed KS compared to the Italian population that is made up of about 27.000.000 male subjects. This result, even if partial, raises the question of the true prevalence of KS, at least in Italy. In the European Northern countries national patients' register data have been used for statistical purposes for 50 years, providing the opportunity to collect significant results. Furthermore, many registers can be linked. The register system itself has great impact on how statistical data are generated and can be a powerful tool to clarify the un-answered questions, especially in the study of rare diseases.

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