Abstracts
Session 1 – Setting the scene.

Professor Berenice B Mendonca
Prof. Mendonca is a Full Professor of Endocrinology at the University of Sao Paulo, Brazil, where she is the Head of the Laboratory of Hormones and Molecular Genetics. Prof. Mendonca’s clinical research focuses on Developmental Endocrinology, mainly on Differences of Sex Development. She was the recipient of the 2009 Brazilian Society for Endocrinology and Metabolism Award and of the 2012 Latin American Society for Pediatric Endocrinology Award. She was the Clinical Endocrinology Trust Visiting Professor in the UK in 2012.

Guidelines for delivery of care to patients with DSD have changed a great deal since the mid 20th century and continue to evolve as knowledge about DSD develops. Stemming from Chicago meeting is the recognition of the importance of interdisciplinary teams to provide care to patients and their families, the value of molecular diagnostics, and need collaborations to study ways to optimize outcomes for affected people. What remains to be improved is understanding how to talk about DSD, as well as developing evidence-based mental health care, surgical interventions and fertility optimization.

In this symposium we will focus several important issues on DSD management such as recommendations for family-centered, interdisciplinary care including education for physicians, patients and caregivers, information sharing in DSD, training of physicians who care for patients with DSD, the best methodologies to improve treatment, controversies and improvement of hormonal and surgical treatment in CAH.

I hope that this symposium will improve a little more the clinical and surgical practice for treating DSD individuals.

Session 2 – The Impact of Collaborations.

Olaf Hiort, Division of Paediatric Endocrinology and Diabetes, Department of Paediatric and Adolescent Medicine, University of Lübeck, Lübeck, Germany

Olaf Hiort, M.D., Ph.D., is professor of paediatrics and lead of the Division of Paediatric Endocrinology and Diabetes at the University of Luebeck, Germany. He studied medicine at the University of Hamburg and at Tufts University School of Medicine, Boston. He completed his training in Paediatrics at the Medical School in Luebeck in 1998 and subspecialized afterwards in Neonatology, Laboratory Medicine for Paediatrics, and Paediatric Endocrinology and Diabetes. The main focus of his work is the pathophysiology and management of complex rare endocrine conditions. For this, he established several national and international networks and led both national and European collaborative projects, including the EU-funded COST Action DSDnet, which recently put forward numerous position papers on the topic. Currently he is the paediatric chair and co-coordinator of the European Reference Network (ERN) for rare endocrine conditions (Endo-ERN), which is aiming to re-structure the management of all rare and complex endocrine
conditions across the European Union in close collaboration with the European Society for Paediatric Endocrinology. Overall, he has been involved in more than 200 scientific papers and has edited several text books.

The Impact of European Collaboration

The European Collaboration in DSD-related research has a long-standing tradition, stemming from national networking activities and the development of the I-DSD registry through initiating efforts of a research grant by the European Society for Paediatric Endocrinology and further funding by the first European collaborative grant „EuroDSD“ from 2008-2011. Since then, further European collaborative efforts were granted with “DSDLife” and the “DSDnet”, which initiated the largest natural history survey of adults with DSD as well as widely acknowledged position papers on clinical management, genetic as well as endocrine diagnostic pathways, as well as surveys related to patient satisfaction and educational possibilities.

Currently, the European Union has established the European Reference Networks (ERN) for Rare Conditions, covering all known rare conditions, with the aim to establish an equality of health care for rare diseases amongst the member states of the EU and to promote research in the area. The ERN for Rare Endocrine Conditions (Endo-ERN) covers all endocrine conditions across the life-span in eight main thematic groups (MTG). Within the MTG7, Endo-ERN combines DSD with the conditions of hypogonadotropic hypogonadism, thus broadening the field to “sex development and maturation”. With this approach, a better acknowledgment of pubertal disorders is pursued and patient-related research can be promoted. A first workshop of MTG7 has been held and identified the need for future research. This includes the optimization of diagnosis and of patient management structures across Europe, as well the initiation of randomized clinical trials regarding hormone therapies, possibilities of induction of fertility, and elucidation of co-morbidities with aging. This will put DSD research in a true perspective across the life-span of affected people.

David E. Sandberg, Division of Pediatric Psychology and Susan B. Meister Child Health Evaluation and Research Center, Department of Pediatrics, University of Michigan Medical School, Ann Arbor, Michigan, USA

David E. Sandberg, PhD is a pediatric psychologist and clinical researcher whose research is closely linked to his clinical service; the psychosocial adaptation of persons born with disorders of sex development (DSD), and their families. He shares the role of principal investigator (with Eric Vilain, MD, PhD) of the DSD-Translational Research Network (DSD-TRN; National Institutes of Health, R01 HD093450). The 11-site, registry-based, clinical research network will standardize procedures in diagnosis and clinical decision-making and develop tools necessary to translate diagnostic and treatment protocols into clinical best practices. Dr. Sandberg’s other ongoing projects include: a study evaluating how patients with DSD, parents, healthcare specialists, and other stakeholders, differentially define optimal care and accept trade-offs in outcomes (NIH R01 HD086583); and a project designed to capture the pathways of care received by patients with DSD with a large integrated health care system (Kaiser Permanente) (NIH R01 HD092595). Dr. Sandberg was appointed in 2018 to the 3-member independent advisory board of the European Registries for Rare Endocrine Conditions (EuRRECa).
Impact of Collaboration in North America

A defining moment of our lives begins when we embark on a male or female path early in development; disruption of typical male or female development results in Disorders/Differences of Sex Development (DSD). The quality of life of people affected by DSD and their families is often threatened by uncertainty about what caused the condition, doubt over choices in care, the potential for chronic stress associated with anticipated or experienced stigma, and lifelong clinical care. To inform emerging best practices and, ultimately, the establishment of evidence-based clinical practice guidelines for the assessment and management of DSD, the DSD – Translational Research Network (or DSD-TRN) was launched in 2011 and remains funded by the National Institutes of Health.

The DSD-TRN is currently comprised of 11 sites located at pediatric medical centers across the United States. In collaboration with Accord Alliance, a nonprofit convener of DSD stakeholders, the DSD-TRN is designed to uncover genetic causes of DSD and examine connections between the genetics, medical and surgical decisions, and patient and family psychological adaptation. Requirements of DSD-TRN membership include the establishment of an interdisciplinary team, pursuit of a genetic causes of the DSD, and commitment to standardizing descriptions of the patient’s anatomic, endocrine and psychosocial phenotype. These requirements apply to all patients cared for longitudinally at member sites; only those patients and families providing consent have details of the medical record added to the DSD-TRN Registry and a blood sample added to the Biobank. The DSD-TRN serves as a platform for hypothesis-driven research, development of patient and family educational and shared decision making resources, and regular opportunities for collaborative learning through monthly web-based case presentations.

Reiko Horikawa – Abstract pending

Professor S. Faisal Ahmed

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After receiving the bulk of his training in Edinburgh and Cambridge, Professor Faisal Ahmed was appointed as a consultant in paediatric endocrinology at the Royal Hospital for Sick Children in Glasgow in 2000 and to the Samson Gemmell Chair of Child Health at the University of Glasgow in 2012. His clinical and research activities focus on rare conditions including sex development and skeletal development. Professor Ahmed leads on the Office for Rare Conditions in Glasgow, through which he coordinates the I-DSD and I-CAH
Registries and a new project, the European Registries for Rare Endocrine Conditions (EuRRECa), launched in 2018. He also serves as the Chair of the Science Committee of ESPE and the Chair of the e-health/ICT Work Package in the European Reference Network for Rare Endocrine Conditions (Endo-ERN). Professor Ahmed is an associate editor for Hormone Research in Paediatrics, a member of ESPE Council, medical advisor to CAH CLIMB, a trustee of the Glasgow Children’s Hospital Charity and the Brittle Bone Society and the treasurer to the Kelvin Valley Beekeeping Association. At I-DSD 2019, he will provide an update on the above registries and how they form a critical cornerstone of a successful professional and scientific network.

Oral communications 1 (OC1) Chairs: Martine Cools, Richard Auchus

Anu Bashamboo

Pathogenic variants in the fourth zinc finger domain of Wilms’ tumor 1 (WT1) gene are associated with 46,XX testicular/ovotesticular DSD.

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Background: 46,XX individuals with testicular Disorders/Differences of Sex Development (TDSD) or ovotesticular DSD (OTDSD), have virilized genitalia due to testosterone production associated with presence of testicular tissue. Many of these patients carry the testis-determining gene SRY, however most SRY-negative patients have an unknown etiology.

Methods: Exome sequencing was performed in 78 patients with 46,XX (O)TDSD of unknown etiology. The functional consequences of pathogenic variants in WT1 encoding Wilms’ Tumor 1 were investigated by in silico analysis, multiple in vitro assays and a mutant mouse model.

Results: Seven individuals (8.97%) with (O)TDSD carried heterozygous pathogenic variants in the 4th zinc finger (ZF4) of WT1 (p.Ser478Thrfs*17, p.Pro481Leufs*15, p.Lys491Glu, p.Arg495Gln (x4), p.Arg495Gly). In one family, a 46,XY sib had Meacham syndrome. The variants were de novo in 6 families (P value = 4.4x10^-6). Enrichment of WT1 missense/loss-of-function variants in 46,XX DSD is highly significant compared to control populations (P<1.8x10^-4). The introduction of WT1.p.Arg495Gly into a human granulosa cell line results in upregulation of endogenous Sertoli cell transcripts and XX mice carrying the p.Arg495Gly mutation display masculinization of the foetal gonads. The phenotype may be explained by the ability of the mutated, but not the wild-type, protein to physically interact with and sequester the key pro-ovary factor β-CATENIN.

Conclusion. Variants of WT1 specifically impacting ZF4 are a relatively common cause of SRY-negative 46,XX (O)TDSD. This expands the spectrum of phenotypes associated with WT1 mutations and shows that the WT1 protein lacking ZF4 can function as a pro-testis factor in an XX chromosomal context.

Rafael Loch Batista

The Mobile DNA at Work: L1 Retrotransposition in the 5’UTR of the Androgen Receptor Causing Partial Androgen Insensitivity Syndrome

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Context: Androgen Insensitivity Syndrome (AIS) is the most common cause of disorders of sex development in 46,XY individuals. That X-linked condition is usually caused by allelic variants in the androgen receptor (AR) gene. The AIS phenotype depends on the pathogenicity of the AR allelic variant, ranging from severe undervirilization (complete AIS - CAIS) to several degrees of external genitalia undervirilization (partial AIS - PAIS). While AR Exonic allelic variants are found in most cases of CAIS, this hold true for 30 - 50% of individuals with clinical and laboratorial PAIS. It suggest that factors outside AR exons might lead to PAIS phenotype. In its turn, most of human genome (about 50%) is composed by interspersed repeats what are derived from transposable elements, which are DNA sequences that have the ability to change their position within a genome. Collectively, they are named mobile DNA. Most of mobile DNA are retrotransposons which copy and insert themselves into a new genomic position using a RNA as intermediate in a process called retrotransposition. Genomic imbalance, exonation, gene disruption and reduced gene expression can result as consequence of their mobilization.

Objective: To identify the genetic etiology of AIS in a large multigenerational family with laboratorial and clinical phenotype of AIS (9 patients).

Methods: Analysis of whole exons of the AR gene including splicing sites regions was performed followed by sequencing of the 5’UTR region of the androgen receptor gene. Detailed phenotyping was performed at the time of initial diagnosis and long-term follow-up, and circulating levels of steroid gonadal hormones were measured in all affected individuals. Expression of AR was measured by RT-PCR using cultured fibroblasts. LINE-1 element was sequenced using specific primers for L1Hs. The sequences identified were analyzed using repeatmasker.

Results: The AR expression was severely reduced in all affected patients, despite their normal AR exonic sequencing. A complex defect (~1100 bp) was identified in the 5’UTR region of the AR in all affected individuals. In all cases it was inherited in hemizygosity. A very large insertion flanked by a small duplication was presented in the 5’UTR region of the AR gene (c.-268_-267). A Long PCR was able to sequence that insertion which was composed by 804 nt plus a long polyA tail (~200bp). The sequence was 99.7% similar to a Long Interspersed Nuclear Element 1 (LINE-1), a full-length retrotransposon sequence present at X chromosome (AC002980; Xq22.2). All structural hallmarks of retrotransposition were identified: the endonuclease site (c.-272_266 – 5’ TTTT/AA 3’) for first DNA cleavage, the target site duplication (c.-268_250; 18 bp flanking duplication) and the long polyA tail.

Conclusion: AIS is also a related-mobile DNA disease. The 5’UTR region of the AR gene has an endonuclease motif which was able to cleavage the DNA allowing LINE-1 insertion compromising the AR expression subsequently. The observation of LINE-1 insertion in the 5’UTR region of the AR gene expand the knowledge either about the role of 5’UTR region in the AR expression and the mechanisms for human LINE1-inherited diseases.

Salma Ali

Development Of An International Benchmark For Sick Day Episodes As A Core Clinical Outcome In People With Congenital Adrenal Hyperplasia

Background Congenital adrenal hyperplasia (CAH) is a rare condition characterised by adrenal insufficiency and a life-long risk of adrenal crises. There is a paucity of information on the epidemiology of acute adverse events in this population.

Objective To investigate the frequency, aetiology and consequences of acute adverse events attributed to adrenal insufficiency in CAH.

Methods A longitudinal analysis of patients with CAH in the International Congenital Adrenal Hyperplasia Registry (I-CAH registry, www.i-cah.org) which collects information on acute adverse events including sick day episodes and adrenal crises.

Results 509 patients (n= 478, 21-OHD) from 31 centres in 16 countries and a total of 3880 visits were evaluated. 261 patients (n=255, 21-OHD) had one or more sick day episodes (684 visits); of these, 215 (82%) were less than 18 years of age. 1034 sick day episodes were recorded in total, with 920 (89%) episodes recorded in those less than 18 years of age. The overall median number of sick day episodes for all centres per patient year was 3.0 for children (IQR 1.7-4.7) and 3.9 for adults (IQR 1.8-10.2) (p=0.26). The median duration of sick day episodes was 3 days (IQR 2.0-5.0) and 2 days (IQR 1.0-3.0) in children and adults respectively (p<0.05). During childhood, younger age and low hydrocortisone dose (mg/m²/day) were associated with a greater number of sick day episodes (p<0.01). Female sex was associated with higher rates of admission amongst both children and adults (p<0.01). Infectious illness was the most frequent event causing illness episodes and adrenal crises in both children (66%) and adults (23%). An adrenal crisis was reported in 37 (4%, 37/920) and 34 (30%, 34/114) sick day episodes amongst children and adults, respectively (P<0.05) and all adults required hospital admission.

Conclusions The real world data within the I-CAH registry are a valuable resource for studying a core clinical outcome that can be used as a benchmark for improving clinical care. Further work needs to be undertaken to understand the determinants of the observed variations in the occurrence of sick day episodes.

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Min Jeong Bag

Feminizing Genitoplasty in Patients with Disorder of Sex Development. Evaluation of Long–term Results of Anatomy, Genital Sensibility, Sexual Function and Satisfaction with Surgical Results.

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Background: Atypical genitalia are common in Disorder of Sex Development (DSD) and genitoplasty is part of the treatment of most of them. Reports of long-term surgical results are dissimilar, especially concerning Feminizing Genitoplasty (FG) and there are few publications about objective evaluation of genital sensibility and sexual function after FG. The aim of this study was to evaluate surgical results of a long-term cohort of DSD patients submitted to FG, regarding genital morphology and sensitivity, sexual function, surgical complications and patients’ satisfaction with FG.

Patients and Methods: We evaluated 60 DSD patients submitted to FG in a tertiary center. Congenital adrenal hyperplasia (CAH) accounted for 36 cases and 24 DSD were secondary to other etiologies. All the patients were submitted to FG, which meant only clitoral surgery in 11 cases, one-staged clitoral and urogenital sinus (UGS) repair in 48 and only UGS repair in 1 case. The mean time of follow-up was 13.8 ± 12 years. Patients were submitted to genital examination, quantitative sensorial testing of the genitalia and they completed questionnaires evaluating sexual function and satisfaction with FG. Clinical records were reviewed for postoperative complications. A control group was evaluated on regard to genital sensibility and sexual function. Results were analyzed in relation to age at surgery and surgical techniques. Associations between morphological and sensorial findings and sexual function were analyzed, and compared with the control group.

Results: Clitoral size was adequate in 93% of examined patients and in 4 cases the clitoris could not be found. Separated perineal orifices were found in 83% of patients with CAH and in 88% of patients with other etiology of DSD. Patients with persistence of UGS did not complain of any symptom due to the persistence. The genital sensibility of CAH patients was comparable to control group. The group of DSD due to other etiologies showed less sensitive clitoris, but other sensorial thresholds were similar to the control group. There was no difference of sensibility in the vaginal margin between groups of patients and control group. None of the CAH patients had sexual dysfunction; 3 patients with other etiology of DSD and 4 women of the control group had dysfunction and the same affected domains. Postoperative complications were found in 17% cases of CAH and in 26% patients with other etiology of DSD. Most of the patients were satisfied with the results of FG. Most of them agreed that FG should be done at childhood and disagreed with doing it at adulthood.

Conclusions: Anatomical results of FG were satisfactory in our cohort of patients. Overall genital sensibility was not affected by surgery. Sexual function in active patients was comparable to a control group. Patients were satisfied with FG and they preferred to be operated early in life.

Martine Cools

Growth, pubertal course and long-term outcome of 46, XY boys born with atypical genitalia and low birthweight.

Authors:
Introduction: Boys born small for gestational age (SGA) often have undermasculinized genitalia. Little is known about the pubertal development and gonadal function on a longer-term in this specific group of males.

Aims: To determine the (pubertal) development and long-term urological and endocrine outcome of undermasculinized boys born SGA compared to undervirilized boys born appropriate for gestational age (AGA).

Methods: Clinical data were retrieved from the I-DSD Registry on boys with non-specific 46, XY DSD who were aged ≥2 years at the time of the study. Statistical analyses included: Pearson Chi-Square, Fisher’s Exact, unpaired Student t-test, Mann-Whitney U test and Shapiro-Wilk test, as appropriate.

Results: Data of 179 cases (115 SGA, 64 AGA) from twelve centers were included. At 2 years of age, 31/104 SGA boys (29.8%) had incomplete or absent catch-up growth. Sufficient catch-up growth was even less likely in cases with comorbidities, birth length or weight ≤3SD or preterm birth (p=0.019, 0.017 and 0.030, respectively). Eight SGA cases had received growth hormone therapy. At last assessment, both SD-scores for height and weight were lower in SGA boys (both p<0.001) at a median age of 8.0 and 7.7 years for SGA and AGA, respectively. Delayed neuromotor development was present in 19.6% of SGA boys as compared to 1.9% of AGA boys (p=0.001). The number of reinterventions for hypospadias repair was similar in both groups, with a median of 1 (IQR: 2; p=0.836). At last assessment, nearly all cases had an external masculinization score of 12/12, with residual hypospadias being the most frequent cause of lower scores in both groups. Postnatal or childhood treatment to stimulate penile growth was reported to have a good clinical effect in 38/42 (90.5%) and 14/15 (93.3%) of SGA and AGA cases, respectively. LH levels during minipuberty were higher in SGA boys, with lower peak testosterone levels post stimulation (p=0.037 and 0.040 respectively). The majority of cases had a spontaneous onset and uneventful course of puberty. At the end of puberty, no difference in sex hormone levels was observed between SGA and AGA boys.

Conclusions: About one-third of boys with non-specific XY DSD who have SGA show insufficient catch-up growth. The urological outcome and effect of treatments to increase penile size was similar between SGA and AGA cases. Our data suggest a dysfunction of infantile Leydig cells in SGA boys, which does not seem to persist in adult-type Leydig cells. Alternatively, alteration of the hypothalamic-pituitary-gonadal axis during infancy may underlie the hormonal changes found in SGA boys.

Funding: The I-DSD Registry was initially developed with support from the UK MRC (G1100236), EUFP7 (201444) and European Society of Paediatric Endocrinology (ESPE). Lloyd Tack is supported by a research fellowship from ESPE, research grants from the Belgian Society Pediatrics, the Belgian Society for Pediatric Endocrinology and Diabetology and Flanders Research Foundation (FWO). Martine Cools is supported by a Senior Clinical Investigator grant from the FWO.
A Nationwide Study Of The Prevalence & Initial Management Of Atypical Genitalia & Delayed Sex Assignment In The Newborn

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Background: The prevalence of atypical genitalia and the time taken to assign sex in such cases remains unclear. Provision of optimum healthcare during this period requires a clear understanding of the occurrence of atypical genitalia.

Methods: Prospective electronic survey of clinical members of managed clinical networks in Scotland between 2013 and 2018 seeking notification of term neonates requiring specialist input for atypical genitalia and who were then followed up to the age of 3 months. Cross-verification of the notification process was performed through regional genetics laboratories using karyotype as a surrogate marker to identify additional newborns with atypical genitalia.

Results: 81 neonates who satisfied the reporting criteria were identified through the clinicians and the laboratories providing a birth prevalence of 1 in 3,378 term births in Scotland that received specialist input for atypical genitalia. Of the 77 cases that completed the 3 month follow up, 49 (64%) presented within 24 hours of birth. Age at presentation ranged from birth to 28 days. Although the age at sex assignment ranged from birth to 14 days, in 51 of 77 infants (66%), sex assignment occurred at birth. Only 1 case was reassigned and had a different sex at 3 months. Of the 59 infants with a karyotype with a Y-chromosome, 55 (93%) were assigned a male sex and the remainder female. During the first three months, specialist input from a neonatologist or a pediatrician, endocrinologist, surgeon and psychologist was reported in 74 (96%), 58 (75%), 50 (65%) and 9 (12%), respectively.

Conclusions: Atypical genitalia requiring specialist input within the first month of life is rare in term newborns and in only a third of these cases, sex assignment is delayed beyond birth. This study provides new clinical benchmarks for comparing and improving the delivery of care in centers that manage these complex conditions.

Martine Cools

Endocrine and reproductive outcome of men born with various degrees of hypospadias
Introduction: Limited, small-scale studies have revealed that men with proximal hypospadias (HS) or with other signs of undermasculinisation (i.e. complex HS) are at risk of reduced fertility and/or impaired testicular hormone synthesis. However, the extent of this phenomenon and if milder forms of isolated HS are also affected, remains unclear.

Aims: To explore reproductive hormones and semen quality of young men (16-21 years old) born with all forms of non-syndromic HS in comparison to healthy controls.

Methodology: Cross-sectional assessment was performed at Ghent University Hospital and Wien Medical University (ongoing). Blood sampling was done between 8:00 and 9:00 for total and free testosterone, LH, FSH and Inhibin B measurement. Participants were asked to give two semen samples for a spermiogram, according to the WHO 2010 criteria. Statistical analysis was performed using IBM SPSS© 25.0 using an unpaired Student t-test or Mann Whitney-U test as appropriate.

Results: A total of 153 HS (108 distal, 45 proximal) and 42 controls have currently entered the study. No differences in free and total testosterone and DHT levels were found between distal or proximal HS, or between isolated or complex HS, as compared to controls. FSH levels were higher and Inhibin B levels lower in complex HS as compared to isolated HS and controls (FSH: p=0.011 and p=0.005; Inhibin B p=0.001 and p=0.008, respectively). Azoospermia was found in 6 (4.3%) HS. Oligozoospermia was present in 24 (17.3%) HS and 1 (2.4%) control. According to the WHO 2010 criteria, 60/139 (43.2%) HS had a normal spermiogram as compared to 24/42 (57.1%) controls. In controls, mild asthenozoospermia and teratozoospermia were the most common causes of abnormalities (figure). No difference in semen concentration was found between distal and proximal HS (p=0.557). However, both groups had lower sperm concentrations as compared to controls (distal: p=0.022; proximal: p=0.040). Men born with complex HS had lower semen concentration as compared to men who had isolated HS and controls (p=0.007 and p<0.001, respectively).

Conclusion: In our cohort, over 20% of men born with HS have reduced semen quality. In contrast to previous studies, no difference in semen concentration was found between proximal and distal HS. However, complex HS was associated with lower semen concentrations. No difference in testosterone or LH levels was found between HS and controls.
Introduction: Although the practice of gonadectomy in conditions affecting sex development has undergone intense scrutiny, objective knowledge regarding the indications and timing of gonadectomy is lacking.

Methods: The International Registry for Disorders of Sex Development (I-DSD Registry) was interrogated for clinical information regarding the diagnosis, karyotype, sex of rearing and timing of gonadectomy in all those cases that were over the age of 16 years at the time of search and who had a disorder of androgen action or synthesis, gonadal dysgenesis or a non-specific disorder.

Results: Of the 3,618 cases in January 2019, 757 (21%) met the inclusion criteria and data regarding gonadectomy status were available in 656 (87%) from 44 participating centres. Of these, 226 (34%) with a median age of 24 years (range 17, 72) were registered as male and 430 (66%) with a median age of 26 years (16, 90) were registered as female. Of the 656 cases, gonadectomy was performed in 373 (57%) cases and in these cases the karyotype was 46, XY in 329 (88%); 46, XX in 7 (2%); 45, X/46, XY in 26 (7%); 46, XX/46, XY in 5 (1%); 47, XXY in 2 (0.5%) and other in 4 (1%). Females were more likely to undergo gonadectomy (n=338, p<0.0001) and the most common diagnoses in those who had gonadectomy were CAIS (n=123, 28%) and complete gonadal dysgenesis (n=88, 24%). Females and males had gonadectomy at a median age of 14 yrs (range 0.3, 68) and 5 yrs (0.1, 54), respectively.
duals had gonadectomy performed in 1 vs 3 years, p=0.03). There was a trend towards an increasing interval between age of presentation and gonadectomy was shorter for females than males (median 1 vs 3 years, p=0.03). There was a trend towards an increasing interval between age of presentation and gonadectomy in those born after 1990 compared to those born before (median interval 1 vs 2 years, p=0.1).

**Conclusions:** Not only does the rate of gonadectomy vary according to underlying diagnosis and sex of rearing, it also seems that there is a clear discrepancy between the age at presentation and age at gonadectomy. As a marker of clinical care this requires further exploration.

Christa E Flück

**Broad phenotypes of disorders/differences of sex development in MAMLD1 patients through oligogenic disease.**

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Disorders/differences of sex development (DSD) are the result of a discordance between chromosomal, gonadal and genital sex. DSD may be due to mutations in any gene involved in sex determination and development in general, as well as gonadal and/or genital development specifically. MAMLD1 is a recognized DSD gene, but its role is controversial. We previously tested 9 MAMLD1 variants (9 46,XY DSD patients with broad phenotypes) and all mutants (except truncated L210X) presented transcriptional activity and protein expression similar to wild-type. We hypothesized that MAMLD1 variants may not be sufficient to explain the 46,XY-DSD (broad) phenotype, and that we should search for additional hits.

Therefore we performed whole-exome sequencing in seven of these 46,XY DSD patients and in one 46,XX patient with ovarian insufficiency, who all carried MAMLD1 variants. Data were
filtered by an algorithm including MAMLD1- and DSD-related gene lists. Fifty-five potentially-deleterious variants in 41 genes were identified. Variants were reported in genes associated with hypospadias, cryptorchidism, micropenis and female sex development. Patients carried additional 1-16 variants (in 1-16 genes). Network analysis revealed that 23 of the identified genes/proteins interacted with MAMLD1 (Figure). Thus, our study shows that the broad phenotypes of individual DSD might involve multiple genetic variations contributing towards the complex network of sexual development.

**Figure** - Interaction network of DSD- and MAMLD1-related genes identified in DSD individuals harboring genetic variants in MAMLD1.

**Ken McElreavey**

**Mutations in the DEAH-box RNA Helicase DHX37 are a frequent cause of 46,XY gonadal dysgenesis and 46,XY testicular regression syndrome.**


From the Human Developmental Genetics Unit, Institut Pasteur, Paris, France (K.ME., C.E., T.M., J.M-T., D.H., A.B.); Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark (A.J,); School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong (D.S.T., R.J); Genetics and Genomic Medicine, UCL GOS Institute of Child Health (F.B., J.C.A.) and Reproductive Medicine Unit, Institute for Women’s Health (G.S.C.) both UCL, London, UK; Mammalian Genetics Unit, Medical Research Council Harwell Institute, Oxfordshire, UK (N.W., R.G.G.K., A.G.); Sorbonne Université, Maladies Génétiques d’Expression Pédiatrique, F-75012 Paris, France (M.P., J.P.S); Genetics Department, National Research Center, Cairo, Egypt (I.M.); Endocrinology et
XY individuals with Disorders/Differences of Sex development (DSD) are characterized by reduced androgenization caused, in some children, by gonadal dysgenesis or, more rarely, testis regression during early fetal development. The genetic etiology for most patients with 46,XY gonadal dysgenesis and for all patients with testicular regression syndrome (TRS) is unknown. Identification of novel genes involved in DSD is crucial for providing an accurate clinical diagnosis, aiding patient management and understanding the biological processes involved. We performed exome and/or Sanger sequencing in 145 individuals with 46,XY DSD of unknown etiology gonadal dysgenesis (n=81), TRS (n=16), boys with penoscrotal hypospadias (n=33) or anorchia (n=15). Thirteen children carried heterozygous missense mutations involving the RNA helicase DHX37, which is essential for ribosome biogenesis in yeast. Enrichment of rare/novel DHX37 missense mutations in 46,XY DSD is highly significant compared to controls ($P$ value = 5.8x10^{-10}). Five mutations are de novo ($P$ value = 1.5x10^{-5}). Twelve mutations are grouped in two highly conserved functional domains and are predicted to disrupt biological function. Mutations were specifically associated with gonadal dysgenesis (9/81, 11%) and TRS (4/16, 25%). Consistent with a role in early testis development, DHX37 is expressed specifically in somatic cells of the developing human and mouse testis. DHX37 mutations are a relatively common cause of an autosomal dominant form of 46,XY DSD, which includes both gonadal dysgenesis and TRS, showing that these conditions are part of a clinical spectrum. This raises the possibility that some forms of DSD may be a ribosomopathy.

**Session 3 – Lecture**

**Amy Wisniewski**

Amy Wisniewski is a Research Professor in the Department of Psychology at Oklahoma State University. She received her BA in Neuroscience from Oberlin College and her PhD in Experimental Psychology from The Johns Hopkins University. Amy then completed a postdoctoral fellowship in Behavioral Endocrinology with Dr. Claude Migeon in Pediatric Endocrinology at The Johns Hopkins School of Medicine. Amy’s research interests include gender development and surgical outcomes in people affected by DSD.

A Tribute to Claude Migeon: Family-Centered, Interdisciplinary Care for People with DSD and
Dr. Claude Migeon made several important contributions to the treatment of people with DSD, including but not limited to endocrine care during childhood. For example, in the early 21st Century Dr. Migeon documented patient dissatisfaction with medical knowledge, as well as their desire to meet others with similar medical histories. He then developed some of the first Internet-based educational material about sex differentiation and DSD. Dr. Migeon's long-term studies of adults with 46,XY and 46,XX DSD continue to inform current research aimed at establishing evidence-based, medical and surgical procedures for improving outcomes for people with DSD. Finally, Dr. Migeon's dedication to training fellows throughout the world was a precursor to the international collaborations highlighted by participants of I-DSD.

Symposium Day 2 (Friday, 5th July 2019)

Session 4 – Improving Diagnostic Outcomes in DSD

Ken McElreavey, Institut Pasteur Paris, France

Ken McElreavey has been working on the genetics of human sex-determination and human population genetics for 30 years at the Institut Pasteur in Paris, France where he is the head of the Human Developmental Genetics Unit since 2002. He has over 200 publications in human reproductive biology. In the early 1990s he proposed the “Z” hypothesis of mammalian sex-determination, which predicted that the male and female sex-determining pathways are mutually antagonistic. In the late 1990s and early 2000s his team made major contributions in human population studies including using genetics to understand human population histories, trace ancient human migrations (including the out-of-Africa hypothesis), define the role of the Y chromosome in human infertility and identify selection acting on the human genome. He was awarded the Annandale Memorial Medal by the Asiatic Society for his contribution to research in human anthropology. His group has a strong background in human medical genetics and have involved in several major discoveries including the findings that CDON and ROBO1 mutations cause anomalies of pituitary development and function, and that common FILLAGRIN variants predispose individuals to eczema, allergy and asthma. His team has made major discoveries in the genetic basis of both XX and XY DSD. In the gene discovery program, they were pioneers in determining that mutations in WT1 cause Frasier syndrome as well as 46,XX DSD. They demonstrated, for the first time, that mutations in the genes GATA4, TSPYL1, FOG2, SOX8, NR2F2, DMRT1, ZNRF3 and PBX1 cause DSD. They were the first to discover that genes that are associated DSD, such as NR5A1 and SOX8, can also be an important cause of unexplained male as well as female infertility. The unit currently focuses on using human cellular reprogramming to model different forms of DSD and specific DSD mutations. The
gene discovery program continues with a large genome sequencing project aimed at identifying all genetic causes of DSD.

Dr McElreavey is the organiser of the International Genomics and Community Genetics training program, which has trained over 500 international fellows in the last 15 years. Dr McElreavey has been given numerous awards and honours including Honorary Professor, Capital Medical University, Beijing; Prix Evelyne Barre for infertility research; Grand prize of the French National Academy of Medicine and the Henning-Andersen Prize for Research in Pediatric Endocrinology. He has participated in numerous taskforces, advisory committees, and international consortium including the Royal College of Obstetrics & Gynaecology (UK), Taskforce on Reproductive Genetics; Advisor to Finnish Academy of Science; the International Working Party on DSD Evaluation; the EU COST Action BM1303 DSDNet and EuroDSD.

The strengths and pitfalls of genetics

The genetic analysis of individuals with DSD has taken a more prominent position in recent years due to changes in sequencing technologies and the associated reduction in the overall cost in screening large numbers of genes reliably for pathogenic variants in a relatively short space of time. Over 150 genes are now known to cause either syndromic or non-syndromic DSD and as more individuals undergo sequencing for either the exome or the entire genome, this figure is likely to continue to increase. This is having several major impacts. It is leading to the recognition of new genes and pathways that when disrupted can cause DSD. As well resulting in a striking improvement in the diagnostic yield, this also gives insights into the underlying biology of the developing and the adult gonad. The other major impact of high throughput sequencing has been the remarkable broadening of the phenotypic spectrum associated with well-characterized genes that can cause DSD. This includes, for genes such as NR5A1 and SOX8, both male and female infertility in individuals with apparently normal gonad development. For many years the genes involved in either 46,XY or 46,XX DSD were considered to be distinct, however genomic analysis is changing this concept as data emerge to show that variants in some genes such as NR5A1 and WT1 can cause DSD in both XY and XX individuals. The benefits of determining a genetic etiology cannot be underestimated. Screening of neonate and young children with DSD reveal genetic variants that not only cause DSD but may have an impact on the development of other organ systems or result in the development of disease later in life. A classic example of this is the pathogenic variants associated with WT1. However, unbiased sequencing approaches are expanding the phenotypic spectrum associated with other genes known to cause DSD.

The pitfalls of current genome analyses are varied. It includes our inability, in many circumstances, to understand what the genetic data is actually telling us. Even for genes that are very well studied, such as NR5A1, we lack the ability to predict the phenotype based solely on the profile of variants in the gene. On other occasions, there can be a
tendency to be seduced by the narrative potential of human genomic variation that can lead to over-interpretation of the genetic findings and thus an incorrect molecular etiology.

In this presentation, I will give an overview of our current understanding of the genetic causes of DSD, identify problematic areas in the interpretation of genetic data and what steps we could take in order to resolve these issues.

Richard Auchus

Dr. Auchus is the James A. Shayman and Andrea S. Kevrick Professor of Translational Medicine at the University of Michigan and the Section Head of Endocrinology and Metabolism at the Ann Arbor VA Hospital. Dr. Auchus and his group are active in research projects ranging from basic chemical principles of steroid biosynthetic enzymes and steroid mass spectrometry to clinical trials and translational investigation in disorders of the pituitary, adrenal, ovaries, and testes that cause hypertension, infertility, and obesity. His clinical interests also focus on pituitary, adrenal, and reproductive diseases that involve disorders of steroid production, and he is particularly interested in the care of adults with genetic disorders of steroid biosynthesis and action.

The strengths and pitfalls of biochemistry to diagnose DSDs

Background: The presence of genital ambiguity and disorder of sex development (DSD) in a newborn has implications beyond sex assignment, such as adrenal insufficiency, increased risk of gonadal malignancy, infertility, and maldevelopment of internal organs. Failure to diagnose the underlying condition can have life-threatening consequences.

Objective: To review the strengths and pitfalls of biochemical methods to diagnose the etiology of a child with DSD, with a focus on mass spectrometry profiling of serum and urine for adrenal-derived steroids.

Results: This presentation will review traditional and modern methods of biochemical testing for diagnosis of DSD. In particular, recent data demonstrate use of non-traditional adrenal-derived steroids in distinguishing similar conditions. The presentation will also review approaches to circumvent periods of gonadal quiescence.

Conclusion: The field has moved beyond measuring traditional analytes such as testosterone and 17-hydroxyprogesterone alone in individual assays. Mass spectrometry profiling and attention to ordinarily minor steroids produced via secondary pathways offer the potential to quickly and unambiguously diagnose a range of DSDs using small quantities of blood or urine.

Christa E. Flück

Christa Flück is Professor of Pediatrics and Head of the Division of Pediatric Endocrinology, Diabetology and Metabolism at the University Children’s Hospital Bern, Switzerland. Her research focus is on steroid hormones including their regulation at the molecular level, biochemistry and human disorders. She is PI of the Swiss DSD Cohort study and the SF1next
study, both supported by I-DSD. Currently she is also the coordinator of the DSD working group of ESPE.

**Improving Diagnostic Outcomes in DSD by Combining Biochemistry and Genetics**

Diagnosing disorders/differences of sex development can be a challenge. Workup including both biochemical and genetic methods is recommended. Depending on clinical phenotype different methods may be chosen to reveal a specific diagnosis. Yet in 20-50% of DSD patients diagnosis remains unsolved at the molecular level. By contrast imaging studies and assessment of laboratory parameters of the hypothalamus-pituitary-gonadal axis (at birth, minipuberty or latest after expected age of onset of puberty) will reveal results of direct consequences concerning postnatal sexual development, function and fertility even without specific molecular diagnosis.

**Oral communications 2 (OC2) Chairs: Alicia Belgorosky, Olaf Hiort**

Tulay Guran

**PPP2R3C Gene Variants Cause Syndromic 46,XY Gonadal Dysgenesis and Impaired Spermatogenesis in Humans**

Tulay Guran¹, Gozde Yesil², Serap Turan³, Zeynep Atay³, Emine Bozkurtlar⁴, AghaRza Aghayev⁵, Sinem Gul⁶, Ilker Tinay⁷, Basak Aru⁸, Sema Arslan⁹, M.Kutay Koroglu¹⁰, Feriha Ercan¹⁰, Gulderen Y. Demirel¹⁰, Funda S. Eren⁴, Betul Karademir⁹, Abdullah Bereket¹.

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2. Bezm-i Alem University, School of Medicine, Department of Genetics, Istanbul, Turkey
3. Medipol University, School of Medicine, Department of Paediatric Endocrinology and Diabetes, Istanbul, Turkey
4. Marmara University, School of Medicine, Department of Pathology, Istanbul, Turkey
5. Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul University, Istanbul, Turkey
6. Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey
7. Marmara University, School of Medicine, Department of Urology, Istanbul, Turkey
8. Yeditepe University, Faculty of Medicine, Department of Immunology, Istanbul, Turkey
9. Marmara University, School of Medicine, Department of Biochemistry, Genetic and Metabolic Diseases Research and Investigation Center, Istanbul, Turkey
10. Marmara University, School of Medicine, Department of Histology and Embryology, Istanbul, Turkey
Context: Most of the knowledge on the factors involved in human sexual development stems from studies of rare cases with disorders of sex development. Here, we have described a novel 46, XY complete gonadal dysgenesis syndrome caused by homozygous variants in PPP2R3C gene. This gene encodes B gamma regulatory subunit of the protein phosphatase 2A (PP2A), which is a serine/threonine phosphatase involved in the phospho-regulation processes of most mammalian cell types. PPP2R3C gene is most abundantly expressed in testis in humans, while its function was hitherto unknown.

Patients and Methods: Four girls from four unrelated families with 46, XY complete gonadal dysgenesis were studied using exome or Sanger sequencing of PPP2R3C gene. In total, 4 patients and their heterozygous parents were investigated for clinical, laboratory, immunohistochemical and molecular characteristics.

Results: We have identified 3 different homozygous PPP2R3C variants, c.308T>C (p.L103P), c.578T>C (p.L193S) and c.1049T>C (p.F350S), in 4 girls with 46, XY complete gonadal dysgenesis. Patients also manifested a unique syndrome of extragonadal anomalies, including typical facial gestalt, low birth weight, myopathy, rod and cone dystrophy, anal atresia, omphalocele, sensorineural hearing loss, dry and scaly skin, skeletal abnormalities, renal agenesis, and neuromotor delay. We have shown a decreased SOX9-Phospho protein expression in the dysgenic gonads of the patients with homozygous PPP2R3C variants suggesting impaired SOX9 signaling in the pathogenesis of gonadal dysgenesis. Heterozygous males presented with abnormal sperm morphology and impaired fertility.

Conclusion: Our findings suggest that PPP2R3C protein is involved in the ontogeny of multiple organs, especially critical for testis development and spermatogenesis. PPPR3C provides insight into pathophysiology, as well as emerging as a potential therapeutic target for male infertility.

Vincent Harley

FGF9 missense variants affect testis development

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⁵Murdoch Children’s Research Institute, Flemington Rd, Parkville VIC, Australia 3052
Male sex determination requires FGF9 to maintain SOX9 expression in the developing embryonic testes. FGF9 binds to and activates FGFRs, and FGF9 activity the result of an equilibrium between FGF9 dimer which can’t bind receptor, and FGF9 monomer which can. The FGFR2c missense mutation C342S occurred in a patient with a rare syndromic form of XY gonadal dysgenesis we termed CSR (craniosynostosis and XY sex reversal (Bagheri-Fam et al., HMG 2015). Given that FGFR2c is the receptor of FGF9 (Bagheri-Fam et al., Endo 2017), we asked whether FGF9 mutations affect testis development. The FGF9 missense mutation S99N was identified in an XY male patient with synostosis. While no reproductive defects were reported in this patient and his family, we analysed mice bearing the Fgf9S99N mutation. Fetal testes from XY Fgf9S99N/S99N mice showed partial gonadal sex reversal (ovo-testes) and we also observed ovo-testes in a second synostosis model, Fgf9N143T/N143T.

DSD panel sequencing of a DSD patient with isolated XY gonadal dysgenesis identified the heterozygous missense variant, FGF9D195N/+ . Fetal gonads from XY Fgf9D195N/- mice generated by CRISPR/Cas9 showed XY sex reversal. Unique among FGF9 mutants studied, purified recombinant FGF9-D195N reduces dimerization without affecting receptor binding. Both D195N and S99N mutant proteins showed dose-dependent loss in the ability to stimulate Sertoli cell proliferation in vitro. Together, our findings suggest a biological function for FGF9 during testicular development that requires both FGF9 dimer formation and receptor binding. Our studies also suggest that defective FGF9 signalling mutations can affect testis and/or bone development, and contribute to isolated and syndromic forms of 46, XY gonadal dysgenesis.

Angela Lucas-Herald

Vascular Dysfunction In Boys With Hypospadias

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2- Developmental Endocrinology Research Group, School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow, UK.
3- Department of Paediatric Surgery, Royal Hospital for Children, Glasgow, UK.

Background: Hypospadias in boys may be associated with insufficient androgen exposure during the masculinisation programming window in utero. Testosterone is a vasoactive hormone and accordingly we tested whether vascular function is altered in boys with hypospadias.

Methods: Small arteries were dissected from excess foreskin tissue from boys undergoing hypospadias repair (cases) or circumcision (controls). Vascular contractility was assessed by
wire myography in response to sex hormones and differences in canonical and non-canonical sex hormone signaling were investigated in vascular smooth muscle cells (VSMCs) obtained from the foreskin tissue.

**Results:** 20 boys with hypospadias and 29 age-matched controls were enrolled in this study (median age 1.9 (range 1.3, 12.2) years). There were 8 (40%) cases of distal, 5 (25%) of midshaft and 7 (35%) of proximal hypospadias. There were no differences in clinical cardiometabolic or biochemical parameters between the groups. Arteries from cases demonstrated increased vasoconstriction versus controls (Emax: 137.9 vs 66.3, p<0.001). Exposure of vessels to testosterone (Emax: 66.3 to 124.6 p<0.001) and oestradiol (Emax: 66.3 to 90.7, p<0.01) increased vasoconstriction in controls only. Vascular hypercontractility in cases was associated with reduced endothelium-dependent and endothelium-independent vasorelaxation, which was improved by testosterone (p<0.05) and oestradiol (endothelium dependent only, p<0.05). There was no difference in mRNA expression of the AR and GPRC6A between cases and controls but cases had higher expression of ESR1 (2.7 fold), ESR2 (2.6 fold) and GPR30 (2.9 fold). Expression of Nox5, a major ROS-generating oxidase in vascular cells, was also higher in cases (2.6 fold, p<0.05). VSMC superoxide anion (5.3 fold) production and H2O2 (3.3 fold) levels was higher in cases compared to controls (p<0.05). The increased contraction in arteries from boys with hypospadias reduced with the ROS scavenger N-acetylcysteine (Emax: 137.9 vs 89.1, p=0.04) and with the Nox5 inhibitor melittin (Emax: 137.9 vs 62.9, p<0.0001).

**Conclusions:** Small arteries from boys with hypospadias exhibit increased vascular contractility and decreased vasorelaxation in response to androgen and oestrogen respectively. This is ROS dependent and mediated via Nox5. Our novel findings suggest that hypospadias, a marker of androgen deficiency, is associated with sex hormone-induced vascular dysfunction.

ALH is funded by the British Heart Foundation Centre of Research Excellence Award RE/13/5/30177

**Martine Cools**

**Long-term urological and psychosexual outcome of men born with hypospadias**

L. Tack¹, E Van Hoecke², A Springer³, S. Riedl⁴, U. Tonnhofer³, M. Hiess³, J. Weninger³, E Van Laecke⁶ P Hoebeke⁶, AF Spinoit⁶ and M. Cools¹

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2 Department of Pediatric Psychology, Ghent University Hospital, Ghent, Belgium
3 Department of Pediatric Surgery, Medical University of Vienna, Vienna, Austria
4 Department of Pediatric Pulmonology, Allergology and Endocrinology, Medical University of Vienna, Vienna, Austria
5 Department of Pediatrics, St Anna Children´s Hospital, Medical University of Vienna, Vienna, Austria
**Introduction:** According to EAU's guidelines, hypospadias (HS) repair is best performed between 6 and 18 months of age. Little is known about the long-term patient satisfaction or urological outcome following HS surgery.

**Aims:** To examine the psychosexual and urological outcome of young adult men (16-21 years old) born with all forms of non-syndromic HS as compared to healthy controls, as well as patient and parental satisfaction following HS surgery.

**Methodology:** Cross-sectional assessment in Ghent University Hospital and Vienna Medical University. Participants filled in five questionnaires: the Decision Regret Scale (DRS), Penile Perception Score, Sexual Quality of Life – Male, International Index of Erectile Function and a custom-made questionnaire. The DRS and custom-made questionnaires were also completed by the participants’ parents. Urological examinations included: uroflow, postmictional and testicular ultrasound and genital examination. IBM SPSS© 25.0 was used to analyze the data: using a Pearson correlation, unpaired student t-test, Mann Whitney-U test or chi-square test, as appropriate.

**Results:** Results are presented in Table 1. At time of analysis, 153 HS (108 distal, 45 proximal) and 42 healthy controls had participated. The diagnosis of HS was challenging for most parents and gonadal hormone production, sexuality and fertility remain causes of concern on the longer term. The number of surgical interventions was linked to regret of both participants and parents.

For patients, penile length was the most common cause of genital dissatisfaction. For physicians, suboptimal esthetic outcome was characterized by excessive scar tissue, extreme curvature in erection and/or fistulae. Erectile disfunction was more frequent in HS than controls, but few patients met criteria for sexual disfunction.

Abnormal uroflows were found in approximately one-third of proximal and distal HS, requiring cystoscopy or surgical intervention in some cases. High grade varicocele was four times more prevalent in HS as compared to controls (p=0,018). Testicular ultrasound was similar in HS and controls, with bilateral smaller testicular volumes in complex HS.

**Conclusion:** Very few patients regret having had HS surgery in childhood. Patients and physicians value outcome of HS surgery according to different criteria. We found a high rate of varicocele post HS surgery of unclear origin so far. Our data highlight the need for postpubertal revision of HS cases as long-term complications may occur that require surgical intervention at some times. In some cases, psychosexual counseling may be recommended.

**Table 1. Summary of results**

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<th>A. Questionnaires</th>
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<tr>
<td>Custom parents</td>
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<td>Shocked by HS: at birth 95/150 (63,3%) - on the long-term: 19/150 (12,7%)</td>
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<td>Worries about testicular function: 71/150 (distal: 45,7%; prox: 51,1%)</td>
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<tr>
<td>Custom participants</td>
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<td>Regretted that their parents decided for them on having the HS repair: 3/153 (2,0%)</td>
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<td>Wished they never had the repair: 2/153 (1,3%)</td>
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<td>DRS</td>
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<td>PPS</td>
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<td>IIEF-5</td>
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**B. Urological**

| Suboptimal esthetic outcome | Distal: $16/108$ (14.8%) | Prox: $16/45$ (35.5%) |
| Varicocele grade II or higher | HS: $31/153$ HS (20.3%) | Controls: $2/42$ (4.8%) |
| Abnormal uroflow | **Plateau**<br>HS: $34/149$ (22.8%)<br>Distal: 21.9%; Prox: 25.0% | **Staccato**<br>HS: $2/149$ (1.3%) | **Residue**<br>Distal: $5/103$ (4.9%)<br>Prox: $2/45$ (4.4%) |
| Testicular ultrasound | **Microlithiasis**<br>$(p=0.777)$<br>HS: $13/153$ (8.5%)<br>Controls: $3/42$ (7.1%) | **Mean volume**<br>HS/controls<br>Right: $12.3mL/12.7mL$, $p=0.547$<br>Left: $11.9mL/12.1mL$, $p=0.740$ | **Mean volume**<br>Complex/isolated HS<br>Right: $9.5mL/12.6mL$, $p=0.002$<br>Left: $7.9mL/12.3mL$, $p<0.001$ |

HS: hypospadias; Prox: proximal hypospadias; DRS: Decision Regret Scale; PPS: Penile Perception Score; SQoL-M: Sexual Quality of Life – Male; IIEF-5: International Index of Erectile Function.

**Anna Biason-Lauber**

**Whole Exome Sequencing in a cohort of 94 DSD patients**

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\(^3\) Department of Genetic Medicine & Development, Faculty of Medicine, University of Geneva, Geneva, 1200, Switzerland
Approximately one out of 4'500 newborns is diagnosed with Disorders/Differences of Sex Development (DSD). Children born with DSD and their families face considerable challenges, potentially including surgical intervention and gender assignment, as well as associated complications such as infertility and predisposition to gonadal tumors. Causative genetic variants are currently identified only in about 50% of the affected patients, partly due to a lack of knowledge concerning the complete gene and protein pathways involved in sex development.

We used whole exome sequencing (WES) on 94 DSD patients in order to identify genes and variants implicated in DSD. For 26 patients causative genetic variants in previously known DSD genes could be identified. We also identified 40 potential candidate genes previously not linked to DSD, based on the number of patients carrying variants, the similarity of the phenotype, the pathogenicity prediction and their expression in tissues important for sex development (e.g. gonads and pituitary), like NPAP1 and PDZD2. WES allowed us to identify new genes potentially involved in DSD, advancing our understanding of human sex development and our capacity to accurately diagnose, support and treat patients and their families.

Peter Vogt

Gonadoblastoma Y (GBY) genes, DDX3Y and TSPY, are differentially expressed in the germ cells of DSD-46,XY individuals with CAIS and Swyer syndrome; these studies reveal UTY as third GBY candidate gene


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Background

Women with a Y chromosome in their karyotype (46,XY) are suffering from some disorder of gonad development (DSD-XY group). Clinically, these individuals can display complete Androgen- Insensitivity-Syndrome (CAIS) or Complete Gonadal Dysgenesis (CGD) also called Swyer syndrome. Females with CGD display an early disruption of their sex determination pathway, i.e. SRY or SOX9, respectively, WT4 or RSPO1 are not functioning resulting in early germ cell loss due to accelerate apoptosis, respectively, causing their neoplastic conversion due to extended OCT3/4 expression phase and increased expression of the Y genes of the Gonadoblastoma Y (GBY) locus, DDX3Y and TSPY (Vogt et al. 2019). Females with CAIS display a late disruption of their sexual gonad development: SRY and SOX9 are functioning for male sex determination and only later complete insensitivity of their Androgenreceptor (AR) for binding androgens, testosterone, dihydrotestosterone (T and DHT) results in female phenotype. Their gonads have formed testicular cords with aberrant male germ cells.
arrested at fetal spermatogonia phase. However, their testis tubules are not descended in a scrotum, but are located intra-abdominal or inguinal and their germ cells have often lost their state of pluripotency. Consequently, the risk of CAIS individuals to develop some germ cell tumour is significantly lower than that of CGD individuals although with the same karyotype (Cools et al. 2017).

**Results**
A clinical data base of 150 DSD-XY individuals has been established and bilateral gonadal tissue samples have been taken from 30 individuals after written consent. Immuno- histochemical expression analysis of the proteins encoded by the GBY candidate genes, DDX3Y and TSPY, in the germ cells of these gonads was compared with that of UTY, the putatively third GBY candidate gene because also expressed in fetal male germ cells like DDX3Y and TSPY proteins. We found a comparable expression pattern of all three Y genes in CGD tissue samples, but not in CAIS tissue samples. Germ cell tumour development (dysgerminoma, seminoma) was only found in germ cells with nuclear UTY expression, mostly in parallel with OCT3/4 expression, i.e., in germ cells with state of pluripotency.

**Conclusions**
Data presented illustrate that the expression profile of the GBY locus in the aberrant germ cells of CAIS and CGD individuals is different and its intensity variable depending on their state of pluripotency. To mark their risk of malignancy, analysis of OCT3/4 expression might be probably sufficient. Most interesting, nuclear expression of UTY, encoding a functional H3K27-Demethylase often corresponded to that of OCT3/4 in the same germ cells. This suggests that the structure of chromatin in the aberrant germ cells is changing during tumour development. The UTY gene can therefore probably be considered as third functional gonadoblastoma susceptibility gene.

**References**
- Vogt PH. et al. (2019) Gonadoblastoma Y locus genes expressed in germ cells of individuals with dysgenetic gonads and a Y chromosome in their karyotypes include DDX3Y and TSPY. Hum Reprod. doi:10.1093/humrep/dez004.

**Rafael Loch Batista**

**Clinical, Hormonal, Psychosexual Aspects, and Genetic Characteristics of Androgen Insensitivity Syndrome in a Androgen Insensitivity Cohort: Long Term Outcomes and Novel Androgen Receptor Gene Allelic Variants**
Introduction: Androgen Insensitivity Syndrome (AIS) is the most common cause of Disorders of Sexual Development (DSD) in 46,XY individuals. It is a X-linked genetic disease caused by allelic variants in the Androgen Receptor Gene (Xq11-12), causing 3 different phenotypes according to the degree of undervirilization: Complete (CAIS), Partial (PAIS) and Mild (MAIS). Despite well characterized, the molecular diagnosis is obtained only in 28–50% of PAIS. There are few studies about psychosexual development and long term outcomes in AIS in the literature.

Methods: Patients with clinical suspicious of AIS (familial history, atypical genitalia, primary amenorrhea and/or inguinal hernia) performed hormonal serum measurements (LH, FSH, estradiol, testosterone) and molecular sequencing of AR gene, including all exonic region (8 exons) and 5’UTR region. Psychosexual variables (gender identity, gender role and sexual orientation) were evaluated by questionnaires. Gender identity was also evaluated by psychological test (HTP test).

Results: We present a cohort composed by 64 individuals: CAIS (n=26) and PAIS (n=38), from 46 different families (24 PAIS; 22 CAIS). Molecular diagnosis was obtained in 96% of CAIS and in 87% of PAIS families. There are 9 novel AR allelic variants (4 em CAIS – c.384_385delGA; p.L713P; c.1769-1G>C and c.1314_1315delCT and 5 em PAIS – p.L839F; p.P914L; p.M781R; p.D865E and p.W719G). Hormonal data (LH, FSH, basal testosterone, basal estrogen and LH/testosterone) were similar between CAIS and PAIS. Final height and weight were similar between PAIS and CAIS. Inguinal hernia was the first clinical presentation in 35% of CAIS. In PAIS, 20 (52%) were assigned female at birth and 18 (48%) were assigned as male. In PAIS, there was a significant difference between median (Sinnecker scale) of external genitalia virilization and sex assignment (p<0.01). Gender identity at adulthood, gender role at childhood and sexual orientation were in agreement with sex assignment in almost cases of PAIS (males and females) and CAIS. No cases of gender change were observed. Fertility desire was frequent in all AIS individuals, specially in PAIS males (100% desire fatherhood).

Conclusion: Hormonal data, including LH and testosterone levels are similar between PAIS and CAIS regardless of AR allelic variant. Inguinal hernia is common in AIS and it should call attention to the possibility of AIS diagnosis. Sex assignment was related with the degree of external genitalia appearance in PAIS. Psychosexual development in AIS is directly related with sex assignment Psychosexual variables, including gender identity, gender role and sexual orientation are in agreement with sex assignment and no gender change was observed in this cohort.

Min Jeong Bag

URETHRAL STRICTURE IN THE LONG-TERM FOLLOW-UP OF PATIENTS WITH DSD SUBMITTED TO HYPOSPADIAS REPAIR

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Purpose
Urethral stricture is a common complication of hypospadias repair. Patients with DSD may present with hypospadias, which in most of the cases are proximal and therefore with higher risk of complications. The aim of this study was to evaluate the incidence of US in the long-term follow-up of DSD patients submitted to this procedure.

Material and methods
We reviewed 65 DSD patients submitted to proximal hypospadias repair, regarding the presence of urethral stricture and its management. Surgeries were performed between 1965 and 2006 and a two-staged repair was performed in 95.5% of the cases. Non-parametrical analysis was done when comparing groups of patients with and without US.

Results
Urethral stricture was found in 15 (23%) patients, all of them were primarily submitted to a two-staged surgery. The median time between stages in the group with urethral stricture was 8 (5-24) months, which did not significantly differ from the group without stricture. The median age at the first stage was 13 (1-34) years in the group with urethral stricture, and 5 (1-47) years in the group without this complication, this difference was is statistically significant. Three (20%) patients with urethral stricture presented obstructive symptoms for the first time 20 years after surgery.

Successful treatment of urethral stricture was achieved with dilatations in 8 patients (53.3%), while 2 (13.3%) needed endoscopic urethrotomy and 5 (33.5%) required surgical correction, one of them (6.7%) underwent a transplantation of an acellular matrix after many failed surgeries.

Conclusions
Urethral stricture is a common complication in hypospadias repair and it can be symptomatic for the first time after decades. It is mostly found in cases operated at older age. Urethral dilatation should be the first option for treatment, considering surgical urethral reconstruction in complex cases.

Tania Bachega

Reproductive Outcomes in Females with Virilizing Forms of Congenital Adrenal Hyperplasia

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Unidade de Adrenal, Laboratório de Hormônios e Genética Molecular – LIM42, Disciplina de Endocrinologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo

Introduction: Subfertility rate has been observed in women with classical forms of CAH, being inversely correlated with the severity of enzymatic defects. It has been discussed that psychosexual behaviors, genital abnormalities, chronic anovulation and/or androgen exposure could corroborate for this finding. However, there are scarce data in the literature regarding reproductive outcomes in women with virilizing forms of CAH.
Objective: To evaluate the reproductive outcomes in women with classical forms of CAH.

Patients: Eighty four adult CAH females with mean age of 33±11yrs were analyzed: 79 with 21-hydroxylase deficiency (21OH) and 4 with 11β-hydroxylase (11OH) were retrospectively analyzed. The hormonal diagnosis of all patients were confirmed by molecular analysis.

Methods: Data regarding Prader score, age at first genitoplasty, sexual activity, sexual orientation, desire of fertility, fecundity rate and pregnancy conditions were evaluated. In the statistical analysis, the t test and Chi-square test were used.

Results: Twenty seven percent of patients have never had sexual activity, due to fear of hurting or shame of her genitalia. Homosexual and bisexual orientations were identified in 15% and 1.2% of patients, respectively; only 38% of patients were engaged in stable relationships. Desire of fertility was expressed by 26/84 (31%) of patients, significantly lower than our reference population (76%, p<0.05), the clinical form presented a significant influence in this outcome (28% of salt wasters-SW versus 67% of simple virilizers). There were no differences in mean Prader score as well as in the age at feminizing genitoplasty (3.4±3yrs in both groups) between patients with and without desire of fertility. Fertility was achieved in 21/26 patients, without ovulation inductions; the mean time to conceive was 2.2±4 yrs. Two patients were submitted to in vitro fertilization. The mean age of the first pregnancy in our cohort was 29.8±6.4yr and in the normal population was 21yrs (p<0.05).

Full-term pregnancy occurred in 20/21 cases, one 11OHD patient had premature labor due to hypertension. Gestational diabetes was observed in only one case. The glucocorticoid doses remained stable during pregnancy and were increased in 8% of patients in the last trimester; fludrocortisone was administered only to the SW patients. No patient had adrenal crises during gestation and all of them received stress glucocorticoid doses during delivery.

All patients, except one, were submitted to cesarean section. The fecundity rate was 1.3 child/CAH patient, lower than the normal population (1.7, p<0.05).

Conclusion: we observed that the low fertility rate in CAH females is mainly secondary due to lower desire to conceive, these data may result of psychosexual behavior. Regarding the fertility rate considering only those women who wanted to get pregnant, most of them succeed spontaneously with low rate of comorbidities.

Salma Ali

A Health-Related Quality of Life Tool for Parents of Young Children With Disorders of Sex Development
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**Background** Disorders of sex development (DSD) may be associated with adverse psychosocial and psychosexual outcomes in adults. However, there is a paucity of information on health-related quality of life outcomes in parents and young children with DSD.

**Objective** To evaluate the use of parent-reported outcome (PRO) questionnaires that can be routinely used in the outpatient setting to assess the impact of DSD on parents and children.

**Methods** Previously validated DSD-specific and generic PRO items were combined to develop a Parent Self-Report questionnaire and a Parent Proxy-Report questionnaire for children under 7 years of age. Questionnaires were completed by parents of 95 children attending DSD and Endocrine clinics at one tertiary paediatric hospital in Scotland.

**Results** 146 questionnaires were completed. Mothers of children with DSD reported greater Future Concerns (median SDS -0.28; range -2.14, 1.73) than mothers of children with other Endocrine conditions (SDS 1.17; -2.00, 1.73) (p<0.001). Similarly, fathers of children with DSD had greater Future Concerns (SDS -1.60; -4.21, 1.00) compared with fathers of children with other Endocrine conditions (SDS 0.48; -2.13, 1.52) (p<0.01). Mothers of children with DSD experienced greater distress (SDS -0.06; -1.18, 1.47) when Talking to Others about their child’s condition, compared with mothers of children with other Endocrine conditions (SDS 0.78; -0.83, 1.47) (p=0.03). Compared to reference data, mothers of children with DSD felt overwhelmed with information from healthcare professionals (p=0.02) and fathers of children with DSD had less stress associated with Clinic visits, managing their child’s Medication, and experienced fewer symptoms of Depression and Anger, (p<0.05).

**Conclusion** The use of brief PRO tools in parents and young children with DSD is an acceptable practice and can be routinely used in the outpatient setting to assess and monitor parent and patient needs. DSD was associated with greater parental concerns over the child’s future than other Endocrine conditions and highlights opportunities for targeted intervention.

**Session 5 - Improving Health Outcomes in Sex Chromosome Disorders**

**Dr TCJ Sas, MD PhD, Pediatric Endocrinologist**

*Erasmus Medical Center-Sophia Children’s Hospital and Diabeter Rotterdam, The Netherlands*

Theo Sas studied medicine at the University of Rotterdam (1987 -1994). He performed clinical research at the department of Pediatric Endocrinology of the Sophia’s Children Hospital in Rotterdam and defended his PhD thesis about growth hormone therapy in girls with Turner syndrome and SGA children in 1999. Then, he did his residency in pediatrics in the Sophia Children Hospital in Rotterdam (2000-2005). In 2005 he started with his clinical fellowship in pediatric endocrinology in the Juliana Children’s Hospital in The Hague and at the Leiden University Medical Center. In 2008-2009, he went to the Armand Trousseau Hospital in Paris for a year of research on molecular medicine, epigenetics and growth. After working for several years as a Pediatric Endocrinologist at the Albert Schweitzer Hospital in Dordrecht, presently he works in the Erasmus Medical Center - Sophia Children’s Hospital and in the national diabetes
treatment and research center Diabeter in Rotterdam in the Netherlands. His main research area is Turner syndrome, growth hormone treatment and diabetes mellitus. He is the Secretary of the Board of the Turner Working group of the ESPE. He is one of the authors of the “Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting.”

**Turner Syndrome**

In 2016, an International Turner Syndrome Meeting was organized in Cincinnati in USA leading to a document Clinical Practice Guidelines for the Care of Girls and Women with Turner Syndrome in 2017. That paper gives the summary of the evidence and expert opinions about several aspects of the treatment and follow-up of individuals with Turner syndrome, with the goal to improve health outcome. In this presentation, the main conclusions and recommendations will be reported and discussed. In addition, questions which have to be answered in the future will be addressed.

**Claus Højbjerg Gravholt**  
Medical doctor, Phd, Dr. med sci., Professor. Specialist in adult endocrinology and internal medicine.

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 rare sex chromosome abnormalities for the last 20 years. I have performed clinical, genetic, epidemiological and experimental studies. I have published more than 200 original publications and review papers. I am an active participant in the international Turner and Klinefelter syndrome research community, as well as national and international research societies. Recently I headed the development of new international guidelines on Turner syndrome, together with Philippe Backeljauw. My main research activities are in the endocrinology of rare syndromes, focusing on GH, IGFs, androgens and estrogens, and on diabetes and metabolism, epidemiology, cardiology, and genomics.  
In our clinic we care for more than 700 persons with Turner and Klinefelter syndrome, as well as other groups of chromosome abnormalities and disorders of sex development.

**Klinefelter syndrome**

Klinefelter syndrome (KS; 47,XXY) is a sex chromosome aneuploidy with a prevalence of 150 per 100,000 newborn boys. The gain of a supernumerary X chromosome affects the majority of patients with KS at multiple organ levels and consequently both morbidity and mortality is raised. KS continue to pose significant diagnostic challenges, as many patients are still misdiagnosed, or remain undiagnosed. In fact, as few as 25% of KS patients are accurately diagnosed, and most of these diagnoses are not made until adulthood. Classic characteristics of KS include small testes, infertility, hypergonadotropic hypogonadism, and cognitive impairment. However, the pathophysiology behind KS is not well understood,
although genetic effects are also thought to play a role. For example, recent developments in genetics and genomics point to a fundamental change in our understanding of KS, with global epigenetic and RNA expression changes playing a central role for the phenotype. KS is also associated with more general health markers, including higher morbidity and mortality rates, and lower socio-economic status (which likely affects both morbidity and mortality). In addition, hypogonadism is associated with greater risk of metabolic syndrome, type 2 diabetes, cardiovascular disease, breast cancer, and extragonadal germ cell tumors. Medical treatment typically focuses on testosterone replacement therapy (TRT), although future studies need to evaluate the effects of TRT on metabolic risk and neurocognitive outcomes. Many males with KS will in addition also benefit support during schooling and also neurocognitive therapy during early adulthood. Criminality seems to be increased and preventive measure ought to be considered. Here, I will present a comprehensive, interdisciplinary examination of recent developments in genetic, endocrine and neurocognitive science. I will present recommendations for improving clinical practice, including neonatal KS screening programs, and a multidisciplinary approach to KS treatment from childhood until senescence.

Martine Cools

Pediatric Endocrinology, Ghent University and University Hospital Ghent, Belgium

Martine Cools is full professor and head of the Paediatric Endocrinology and Diabetology Unit at the Ghent University Hospital. Supported by a Senior Clinical Investigator Grant from the Flanders Research Foundation, Martine Cools coordinates the clinical and research activities of the Ghent DSD center. Her research focuses on gonadal development, clinical management and cancer risk in DSD. Other research areas are dimorphic effects of sex steroids and transgender care. Martine Cools is deputy chair of the ESPE Science Committee and paediatric chair of the Research and Science work package of Endo-ERN. She has been actively involved in several collaborative European research projects related to DSD (FP7, Sober, COST, EuRRECa). She supervised 8 PhD students and (co)authored > 100 original papers, invited reviews and book chapters, mainly in the field of DSD and endocrine treatment of transgender youth. She received the ESPE Young Investigator Award in 2010.

Improving health outcomes in sex chromosome disorders: 45,X/46,XY

Clear guidelines have been published for growth hormone treatment and for the medical follow-up of girls with Turner syndrome, both for those who have monosomy for X and for those who have mosaic karyotypes. Individuals with 45,X/46,XY chromosomes can be raised as girls or as boys. In this session, we will investigate if according to the international literature, a similar follow-up schedule is advised for this group of children. Data from our group with regard to growth, cardiac, gonadal and other health outcomes will also be discussed.
Lunch session “Challenging case discussions in DSD”

Heidi Claahsen & Bernice Mendona
Moderators David Sandberg, Rudolph Rey

1. 46 XX CAH male (Berenice de Mendonça)
2. Prenatal 5 alpha reductase deficiency (Hedi Claahsen – van der Grinten)

Main questions/challenge: what is the role and the responsibility of the health care provider?

Abstracts:

1. 46 XX CAH male (Berenice de Mendonça)

A.S. 20 days of life
Child was born at term after normal gestation without complications and was registered in the male social sex. The couple had 2 daughters and the mother became pregnant again because she had lost a male newborn child due to dehydration and wanted to have a male child. The delivery was cesarean section and the mother did tubal ligation. At 15 days the child presented a salt loss crisis and was referred to our hospital. In our service, CAH due to 21-hydroxylase deficiency was diagnosed and the parents were instructed to change the social registry to female. After several discussions and dialogs with the psychologist and multidisciplinary team, the parents decided to maintain the male social sex. When argued with them about the advantages of female social sex, the father replied: “I know he would be a girl but God made a boy for us!”
The male social sex was maintained and the boy was treated with hydrocortisone and fludrocortisone during childhood and at 5 years the patient underwent bilateral oophorectomy and hysterectomy. At 18 years old, testicular prostheses were placed in the scrotum.

At adult age he is been treated with low dose dexamethasone (0.25 mg) fludrocortisone (100 ug) and with testosterone cypionate 200 mg twice a month. He was followed by the psychologist during treatment and at 25 years of age he has a male identity and behavior although still dependent of financial support from parents.

2. Prenatal 5 alpha reductase deficiency (Hedi Claahsen – van der Grinten)

Non consanguine, muslim parents were referred to the DSD centre because of ambiguous genitalia detected at 20 weeks of gestation. Further evaluation showed ambiguous genitalia but no other abnormalities. Parents expressed their wish to terminate pregnancy but after extensive counselling by our team parents decided to continue pregnancy and a healthy child was born at GA of 40 weeks. The neonate had ambiguous genitalia and was assigned as male because of the presence of 46 XY chromosomes, testes located within the labioscrotal walls, absence of uterus and male AMH and testosterone levels. Based on the biochemical profile the diagnosis 5 alpha reductase deficiency was made and confirmed by mutation analysis. Both parents are carriers of the pathogenic mutation. Genital surgery
was not performed. Parents were coached by an experienced psychologist and counselled by a geneticist. The parents expressed their feelings of being happy with their boy but they feel a strong burden due to his different looking genitalia. One year later the mother contacted our centre because she was pregnant (GA 9 weeks). The parents requested prenatal diagnostics. In the case of a 46 XY karyotype the plan to terminate pregnancy.

Session 6 - Congenital Adrenal Hyperplasia

Roxana Marino
Hopital de Pediatria JP Garrahan

Roxana Marino graduated in biochemistry (1997) at the University of Buenos Aires and obtained a postgraduate specialist degree in Endocrinology (2008). Her research has been focused on molecular alterations in 46,XX and 46,XY DSD patients with 28 indexed publications. Since 2001 she has been Head of the Diagnostic Molecular Biology Laboratory of the Endocrinology Department, Hospital de Pediatria Garrahan, Buenos Aires, Argentina.

Diagnoses of CAH

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from the deficiency of one of the enzymes involved in cortisol biosynthesis. In more than 90% of cases, CAH is secondary to deleterious mutations in the CYP21A2 gene leading to 21-hydroxylase deficiency (21-OHD).

The region containing the CYP21 genes is duplicated and contains several other very closely linked, sometimes overlapping genes, that are very complex to genotype. Approximately 25% of CYP21A2 gene mutations are large gene macrodeletions generated by unequal meiotic crossing over. The CYP21A2 gene is partially overlapped by the TNXB gene, encoding an extracellular matrix protein called Tenascin-X (TNX). TNX plays a role in collagen deposition by dermal fibroblasts and is expressed in the dermis and connective tissue of the heart and skeletal muscles. TNXB deficiency is associated with the Ehlers-Danlos syndrome (ED) phenotype.

The contiguous gene deletion syndrome, CAH-X, was reported in 8.5% of CAH patients with a TNXA/TNXB chimera (Morissette et al 2015). There are three TNXA/TNXB chimeras (CH1, CH2, CH3) that differ in the junction site, described in monoallelic or biallelic forms. Recently, at our laboratory, copy number variations and genetic status of the TNXB gene were analysed in 58 CAH patients with CYP21A2 deletion to determine the frequency of TNXB alterations in our population. Seventy-five percent of alleles were found to carry a contiguous deletion that extended into the TNXB gene. Of 58 patients studied, the monoallelic form was found in 38 (65%), while 4 patients were found to have a biallelic form (7%). These patients have joint hypermobility and a spectrum of other comorbidities associated with their connective tissue disorder, including chronic arthralgia, joint subluxations, hernias, and cardiac defects with a severe phenotype in biallelic forms.

In addition, other forms of CAH are discussed, in particular 3βHSD deficiency, in which patients present with less genital virilization at birth, in contrast to those with 21-OH and 11-OH deficiencies. The absence of adrenal 3βHSD activity precludes elevation of
intraadrenal 17OHP and the production of backdoor-derived DHT and 11-oxygenated androgens, which would be the major source of virilising androgens in other forms of CAH.

Tania Bachega

Hedi L. Claahsen – van der Grinten , MD., PhD, Associate Professor, is paediatric endocrinologist and head of the department of pediatric endocrinology at the Radboud University Nijmegen Medical Centre, the Netherlands.

She graduated as MD in 1995 and followed her paediatric training at the Radboud University Nijmegen Medical Centre. In 2002 she started her training in Paediatric Endocrinology at the Department of Paediatric Endocrinology of the Radboud University Nijmegen Medical Centre, The Netherlands. She was registered as paediatric endocrinologist in January 2007 and graduated as PhD in 2007 with the thesis entitled “Adrenal rest tumours in congenital adrenal hyperplasia”.

Her research line focuses on different aspects of congenital adrenal hyperplasia (CAH) and other forms of disorders/differences of sex development (DSD), with emphasis on the etiology of testicular adrenal rest tumours (TART). She is chair of the national congenital adrenal hyperplasia working group and founding member and member of the steering committee of the Radboud Adrenal Centre, Nijmegen that serves as an expertise centre for the diagnosis and treatment of adrenal diseases, both in children and adults. Furthermore, she is founding member and leading coordinator of the Radboud DSD expert centre, Nijmegen. Since 2015 she is member of the medical ethical comite of the Radboudumc. She is HCP representative of the European reference network ENDO – ERN.

Critical Outcomes in classic CAH

Since the introduction of glucocorticoid and mineralocorticoid replacement therapy in the 1950s classic congenital adrenal hyperplasia (CAH) is no longer a potentially life threatening condition. As a result of successful steroid hormone replacement regimens and careful follow up, CAH changed into a well treatable chronic condition. Consequently, long term health consequences, co-morbidities and quality of life became more important. Both CAH related factors, e.g. elevated androgen levels and chronic exposure to elevated steroid hormone precursor levels, and side effects of long term treatment with glucocorticoids and/or mineralocorticoids play a role in the current and future health status and quality of life of CAH patients. Long-term cardiovascular consequences include increased BMI, increased body fat, increased blood pressure levels and insulin resistance. Furthermore, impairment of gonadal function is a serious complication that already can occur during puberty. It can result in menstrual disturbances in females, and hypogonadism and infertility in both male and female patients. In males, gonadal dysfunction can be caused by primary gonadal failure due to testicular adrenal rest tumours (TART), and by secondary gonadal failure due to poor hormonal control. In females, gonadal dysfunction can result from an
overproduction of adrenal androgens and progestins, and rarely from ovarian adrenal rest tumours. Furthermore, polycystic ovarian syndrome has been described in CAH. Therefore, optimal monitoring and long term follow up is necessary in all patients with classic CAH.

Session 7 - Improving Surgical Outcomes

Maria Helena Palma Sircili MD, PhD
Post graduated at The School of Medicine of The University of São Paulo, São Paulo-S.P. Brazil 2000-2009
2003 Master degree in feminizing genitoplasty
2009 PhD in masculinizing genitoplasty
Since 1999 working with Disorders of Sexual Development’s patients. Since 2010, assistant doctor of Urology and Endocrinology at the Hospital of Clinics of the University of São Paulo.
Since 2016 doctoral advisor division of Urology at School of Medicine of The University of São Paulo, São Paulo-S.P. Brazil

Female Genitoplasty
A large number of procedures have been develop to reconstruct female genitalia in DSD patients and an individualized approach is recommended due to the diversity of diagnosis and the great anatomical variability in genital atypia. The techniques for feminizing genitoplasty evolved with time to achieve better cosmetically appearance and normally genital function, but it is still challenge concerning type of surgery, need for reoperation and genital function in adult life.
Long-term surgical outcomes of a large cohort of patients are rarely reported in literature. The Hospital of Clinics of the school of Medicine of the University of São Paulo, concentrates a large number of patients with DSD treated by a specialized multidisciplinary team. These patients are followed since childhood until adult life. We will also present our surgical and functional results of our female patients submitted to feminizing genitoplasty since 1960 and their long-term outcomes.

Francisco Tibor Dénes, M.D., Ph.D.
Associate Professor of Urology, Chief, Pediatric Urology Unit / Chief, DSD and Transgender Unit, Division of Urology, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo

Masculinizing surgery
In cases of genital atypia due to undervirilization of XY patients or overvirilization of XX patients with male identity, masculinizing genitoplasty is recommended. Preoperative physical examination, as well as genetic, hormonal and image studies are required to define
diagnosis and evaluate the presence, morphology and functionality of the internal and external genital organs. The aim of masculinization is to improve the size and aspect of the phallus in order to allow micturition in a standing position and future penetrative sexual activity. Orchidopexy and removal of contradictory genital structures ensure normal reproduction when possible and prevent genitourinary complications such as infection, urinary incontinence and retention and gonadal malignancy.

Objectively, when normal but ectopic testes are present, either laparoscopic or open orchidopexy is to be performed, according to the original testicular position. When present, contradictory (ovarian) tissue or gonads must be removed, together with residual müllerian structures, such as tubes, uterus and the vaginal component of the urogenital sinus. Care must be taken to prevent damage to wolffian structures such as seminal vesicles and vas deferens.

The external genitalia must be treated in the same way as in hypospadias, with rectification of the penile shaft (phalloplasty) and elongation of the urethra to the tip of the glans (urethroplasty) in simultaneous or sequential procedures. When present, separate labioscrotal folds, bifid scrotum and penoscrotal transposition must also be corrected. Many surgical techniques can be employed according to the external genital configuration, as well as to the surgeon’s expertise. Despite usually satisfactory results, early and late complications may occur, such as persistent penile curvature and urethral stricture or fistula, that often require reoperation.

Ignacio Bergadá, Division of Endocrinology at the Hospital de Niños R.Gutierrez

Ignacio Bergadá is the chief of the Division of Endocrinology at the Hospital de Niños R.Gutierrez in Buenos Aires (Argentina) since 2009. He graduated as a physician from the University of Buenos Aires in 1980, and received his degrees in pediatrics in 1988 and pediatric endocrinology in 2000 from the Argentinian Society of Pediatrics. From 1982 to 1986, Ignacio Bergadá worked as a resident at the Hospital de Niños R.Gutierrez in Buenos Aires (2 years general pediatrics and 2 years pediatric endocrinology). From 1986 to 1988, he was a clinical and research fellow at the Department of Endocrinology & Metabolism, Montreal Children's Hospital, McGill University, Montreal (Canada). He then worked since 2001 as a research physician (Gobierno de la Ciudad de Buenos Aires). He published 70 papers in international peer-reviewed journals (referenced on PubMed) and contributed to 16 book chapters. He received a scholarship for his fellowship at the McGill University in Montreal (1986-1988) and was awarded four times for his work.

Postponing surgery in CAH: Nothing is ever black and white

Congenital Adrenal Hyperplasia (CAH) is the most common cause of sexual ambiguity. When prompt neonatal diagnosis and properly managed, 95% will have a female gender identity and usually sexual activity is preserved. However, the debate over the timing of surgical repair in girls with CAH is a matter of concern, especially by patient advocacy groups mostly composed of unhappy adults who were operated at early pediatric ages. This led to
statements that “in the absence of imminent dangers to patient’s live or health, gender variant conditions must be managed with the least invasive means available and respect for each patient’s autonomy”.

It is important to make clear that CAH is one specific subtype of DSD, but according to the disease itself, it should not be compared with other forms of DSD in which outcomes are less well defined. In addition, there are no randomized controlled studies of either the best age or the best methods for feminizing surgery.

We undertook a survey of different pediatric endocrinologists and urologist around the globe to explore their policy regarding their attitudes on surgical approach in CAH girls.

Results regarding the age of surgery for short and long urogenital sinus, surgical approach for clitoromegaly and the documented or not judicial rules regarding time of surgery in atypical genitalia in patients with CAH will be presented.

Finally, intimacy is still a major issue and the psychologic aspects of these girls are influenced by cultural, religious and personal beliefs as well as for their impaired genital self-image. Many of these factors may lead to social withdrawal. Therefore, any radical change in medical concepts should be based on known risks, outcomes and good quality of evidence.

Early surgery according to most recent manuscripts clearly maintains a space in the treatment of girls with CAH. All surgical approaches with available options and evidence should be discussed with parents.

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Auditing outcomes of “DSD” surgery.

DSD encompasses a range of conditions where the indications for surgery are complex and the benefit to the individual patient can be variable. Despite this, the vast majority of patients and their families wish to proceed with surgery. Therefore, it is our duty to know what the management options are and the risks and benefits of any intervention we may
suggest. Collaborative working and prospective audit have improved the management and outcome in many surgical conditions. What are the challenges of this approach in DSD surgery and how can we overcome them.

Session 8 - This house believes that it is time to discard the term ‘DSD’

Arguments for - Eric Vilain
Arguments against – Andy Greenfield