I-DSD 2017 Poster Presentations

P1 Infertility spectrum or consequence of DSD: a late diagnosis of PMDS. Mary García-Acero, Human Genetics Institute/Pontificia Universidade Javeriana, Bogota, Columbia

P2 A search for DSD among infertile men: is partial gonadal dysgenesis being overlooked? Andrea Trevas Maciel-Guerra, Department of Medical Genetics, State University of Campinas, SP, Brazil.

P3 A Reminder to Think Outside the Box: Use of Fertility Preservation Options for a Girl with an Unusual 45,X/46,XY DSD. Courtney Finlayson, Ann & Robert H. Lurie Children's Hospital of Chicago, USA

P4 Attitudes toward Disorders of Sex Development (DSD) Nomenclature Among Physicians, Genetic Counselors, and Mental Health Clinicians. Courtney Finlayson, Ann & Robert H. Lurie Children's Hospital of Chicago, USA

P5 Highlights on 1st Saudi International DSD Workshop Riyadh: DSD from Muslim traditional society. Ahmad AlShammari (presented by S Vallasciani), King Abdullah specialized Children Hospital, King Abdul Aziz Medical City, National Guard Health Affair, Riyadh, Saudi Arabia.

P6 Peer counseling – a contribution to DSD care. Wiebke Birnbaum, University Hospital Schleswig-Holstein, Campus Lübeck, Germany.

P7 An Audit of Psychological Services for Paediatric Populations with Disorders of Sex Development in Ireland. Claire Crowe, Endocrinology department, The National Children’s Hospital, Tallaght, Dublin, Ireland

P8 Sexual self-concept and sexual functioning in women with DSD: implications for clinical management. Arianne Dessens, Erasmus Medical Center Rotterdam-Sophia, dept. of Child and Adolescent Psychiatry and Psychology, Netherlands

P9 Increased Psychiatric morbidity in women with CAIS and Gonadal Dysgenesis. Hedvig Engberg, Karolinska Institute, Stockholm, Sweden

P10 The clinical coordinator of services for patients with Differences of Sex Development, an Australian first. Chloe Hanna, Royal Children’s Hospital, Victoria, Australia.

P11 Perception of Diagnosis in Adolescents with Disorders of Sex Development. T.P. Hemesath, PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA), Federal University of Rio Grande do Sul (UFRGS) - Brazil

P12 Sex Rearing and Parental Educational Strategies with Children with Disorders of Sex Development (DSD). T.P. Hemesath, PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA), Federal University of Rio Grande do Sul (UFRGS) - Brazil

P13 DSD online healthcare content: A process to evaluate quality of material. Kim Kennedy (presented by M Ernst), DSD Center/Cincinnati Children’s Hospital Medical Center, USA

P14 Holistic care of patients with CAIS through their lifespan. Zofia Kolesinska, Department of Paediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences, Poland.
Identifying controversies: Diverse sex development (dsd) within the HOOU (Hamburg Open Online University). **Ute Lampalzer**, Institute for Sex Research and Forensic Psychiatry, University Clinic Hamburg-Eppendorf, Germany

A Tale of Two Genetic Sisters: Considerations of Alternative Gender Assignments in Congenital Adrenal Hyperplasia with Markedly Virilized Genitalia. **Francisco Javier Blas Lopez**, Departments of Human Genetics and Pediatrics, UCLA School of Medicine, USA

What does it take to judge a minor’s ability to give informed consent in the field of DSD? **Louise Marshall**, Division of Experimental Paediatric Endocrinology, University of Lübeck, Germany.

Psychosocial Supports for Parents of Children with DSD. **Rowena Mortimer**, University of Melbourne, Australia

Identifying the resource needs of adolescents with DSDs. **Gina Tonkin-Hill**, Department of Gynaecology, Royal Children's Hospital, Melbourne, Australia.

Creation of an e-Resource Repository for Differences/Disorders of Sex Development (DSD): Collaboration between Clinicians and Advocates in the DSD Translational Research Network. **Meilan Marianne Rutter**, Division of Endocrinology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, USA

DSD information for doctors and healthcare workers from countryside. **Eduardo Corrêa Costa**, PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA), Federal University of Rio Grande do Sul (UFRGS) - Brazil.


Two-year-old child with progressive virilisation. **Eduardo Corrêa Costa**, PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA), Federal University of Rio Grande do Sul (UFRGS) - Brazil.

Prevalence and characteristics of patients with ovotesticular disorder of sex development in the Disorders / Differences of Sex Development Translational Research Network Registry. **Meilan Marianne Rutter**, Division of Endocrinology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, USA

45,X/46,XY mosaicisms in Male : Long - Term outcomes. A multicenter Italian - DSD Study Group survey. **Federico Baronio**, Department of Medical and Surgical Sciences, Pediatric Unit, Center for Rare Endocrine Conditions (CARENDO BO), S.Orsola - Malpighi University Hospital, Bologna, Italy

Bilateral Testicular Regression Syndrome: a cohort of 22 patients. **Cécile Brachet**, Pediatric Endocrinology, Hôpital Universitaire des Enfants Reine Fabiola, Brussels, Belgium

The sensitivity and specificity of the test with gonadoliberin in diagnosing of hypogonadotrophic hypogonadism in boys. **L. Brzezinski**, Department of pediatric endocrinology, Russian Medical Academy of Continuous Professional Education, Moscow, Russia
The COPENHAGEN Minipuberty Study – a cohort of infants and their parents. **Alexander S. Busch**, Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark

Leydig and Sertoli cells function in patients with genital ambiguity, 46,XY karyotype and normal testosterone production. **Guilherme Guaragna-Filho**, Interdisciplinary Group for Study of Sex Determination and Differentiation (GIEDDS) – School of Medicine – State University of Campinas (UNICAMP) – Campinas (SP) – Brazil.

Comparision between two inhibin B ELISA assays in 46,XY testicular DSD testicular with normal production of testosterone. **Guilherme Guaragna-Filho**, Interdisciplinary Group for Study of Sex Determination and Differentiation (GIEDDS) – School of Medicine – State University of Campinas (UNICAMP) – Campinas (SP) – Brazil.

Case report: Aromatase deficiency in two adult female siblings. **Justine Defreyne (presented by M Cools)**, Department of Endocrinology, Ghent University Hospital, Belgium.


Anogenital distance as a phenotypic signature through infancy: A longitudinal study of 689 children. **Katharina M Main**, Department of Growth and Reproduction and EDMaRC, Rigshospitalet, University of Copenhagen, Denmark.

A Single Centre Experience of Differences/Disorders in Sex Development (DSD) over 20 years. **Elim Man**, Genetics and Genomic Medicine, UCL GOS Institute for Child Health, London, UK; Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK.

Disorder of Sex Development. Research Project. **Renata Markosyan**, Endocrinology department, Yerevan State Medical University, Armenia.

Normal and dysregulated human fetal adrenal development and function: Establishment of a dynamic ex vivo culture model for congenital adrenal hyperplasia. **Cecilie Melau**, Department of Growth and Reproduction, University Hospital of Copenhagen (Rigshospitalet), Denmark.

17B-Hydroxysteroid Dehydrogenase Type 3 Deficiency: a multicenter Italian-DSD study group survey. **S. Meroni**, Department of Pediatrics, Endocrine Unit, Scientific Institute San Raffaele, Milan, Italy

45,X/46,XY Mosaicism: Clinical Characteristics and Follow-up Data. **Sukran Poyrazoglu**, Pediatric Endocrinology Unit, Istanbul Faculty of Medicine, Istanbul University, Turkey

46, XX testicular disorders of sex development: clinical and laboratory characteristics of patients. **Ekaterina Sannikova**, Russian Medical Academy of Continuous Professional Education, Russia

Long-term follow up in two cases with 46, XY DSD. **Elena Sukarova-Angelovska**, Department of Endocrinology and Genetics, University Children Hospital, Skopje, Macedonia.

High-Resolution Gene-Targeted Chromosome Microarray Analysis Uncovered Unreported Small Copy Number Variations Involving Genes Associated with Differences in Sex Development. **Ina**
E. Amarillo, Cytogenetics and Molecular Pathology Laboratory, Washington University in St Louis School of Medicine, USA

P42 Insights from targeted gene sequencing of a large international cohort of patients with Disorders of Sex Development. Katie Ayers, Murdoch Children’s Research Institute, Melbourne, VIC Australia and Department of Pediatrics, University of Melbourne, Melbourne, VIC Australia.

P43 Identification of Large Causative Genetic Variants via Next-Generation Genome Mapping. Hayk Barseghyan, Human Genetics, University of California, Los Angeles, USA

P44 A Mutation in WT1 (Wilms’ tumor suppressor 1) associated with 46,XX testicular DSD. Anu Bashamboo (presented by Caroline Eozenou), Human Developmental Genetics, Institut Pasteur, Paris, France

P45 Expanding The Molecular Diagnosis Of Androgen Insensitivity Syndrome. Rafael Loch Batista, Developmental Endocrinology Unit, HCFMUSP – Laboratory of Hormones and Molecular Genetics, São Paulo, Brazil.

P46 The transcriptional regulator CBX2 and ovarian function: a whole genome and whole transcriptome approach. Leila Bouazzi, Division of Endocrinology, Department of Medicine, University of Fribourg, Fribourg Cantonal Hospital, Germany.

P47 Karyotype phenotype correlation in patients with Turner syndrome. Hedi Claahsen-van der Grinten, Radboud University Medical Centre, Nijmegen, The Netherlands.

P48 Antenatally determined sesquiygosity in gender discordant monochorionic diamniotic twins (46,XX/46,XY): - postnatal clinical and gonadal phenotype. Louise Conwell, Department of Endocrinology and Diabetes, Lady Cilento Children’s Hospital, University of Queensland, Brisbane, Australia.

P49 46,XX Ovotesticular Disorder of Sex Development (DSD): - duplication of the XX SR region upstream of the critical testicular gene SOX9. Louise Conwell, Department of Endocrinology and Diabetes, Lady Cilento Children’s Hospital, University of Queensland, Brisbane, Australia.

P50 Genetic Diagnosis of Disorders/Differences of Sex Development (DSD): The DSD-Translational Research Network Experience. Emmanuèle C. Délot, Departments of Human Genetics and Pediatrics, Geffen School of Medicine, University of California, Los Angeles, CA, USA.

P51 Whole exome sequencing used to investigate target genes in individuals with 46,XY partial gonadal dysgenesis. Ana Paula dos Santos, Department of Medical Genetics, State University of Campinas, Brazil

P52 Analyses of the non-coding region of NR5A1 gene revealed five novel variations in eight patients with different phenotypes of 46,XY DSD. Helena Fabbri-Scallet, Center of Molecular Biology and Genetic Engineer, State University of Campinas, Brazil

P53 Mutation Spectrum in Patients with Differences of Sex Development analysed by Targeted Next-Generation Sequencing. Susanne Flieger, UKSH, University of Lübeck, Germany.
Exome Sequencing Reveals POLR3H Defect Associated With Primary Ovarian Failure In Two Unrelated Families. Monica Malheiros Franca, Hormone and Molecular Genetics Laboratory, University of Sao Paulo School of Medicine, Sao Paulo, SP, Brazil.

Delineation of phenotypic associations in Deciphering Developmental Disorders (DDD) Study participants with hypospadias. A retrospective review of clinical features. Gabriella Gazdagh, Academic Unit of Medical Genetics, University of Glasgow, UK

Diagnosing 46, XY Disorders of Sex Development (DSD) by using Targeted Massively Parallel Sequencing. Nathalia Lisboa Gomes, Developmental Endocrinology Unit, HCFMUSP – Laboratory of Hormones and Molecular Genetics, São Paulo, Brazil.

Identification of novel candidate genes for premature ovarian failure by investigating balanced chromosomal rearrangement breakpoints. Sylvie Jaillard, Cytogenetics and Cellular Biology Department, Rennes University Hospital, France.

Cohort of 13 Patients with 46,XX SRY negative testicular and ovotesticular variation of sex development: review of clinical findings, management and molecular studies. Sophie Lambert, Department of Pediatric Endocrinology, Centre de Référence des Maladies Endocriniennes Rares de la Croissance, Robert Debré Hospital, Paris, France.

Identification of 45,X/46,XY Mosaicism from a Gonad Biopsy on an Infant with 46,XY in Peripheral Blood with Proximal Hypospadias and Undescended Testicle. Eyby Leon, DSD-TRN, Genetics and Metabolism, Rare Disease Institute, Children’s National Health System. Washington, DC, USA

NR5A1 Mutations: Clinical, Endocrine and Genetic Features. A Survey by Italian DSD Study Group. Pietro Marchese, Adolescent Medicine, Division of Pediatrics, S. Chiara University Hospital, Pisa, Italy.

Targeted gene panel sequencing improves traditional molecular analysis in DSD. Idoia Martinez de LaPiscina, Endocrinology and Diabetes Research Group, BioCruces Health Research Institute, Hospital Universitario Cruces, Barakaldo, Spain.

Differential diagnosis of DSD, new progress of NRC activity in Egypt after application of next generation sequencing. Inas Mazen, Clinical Genetics & Endocrinology, National Research Centre, Cairo, Egypt

Genetic Characterization Of Patients DDS In Colombian Population. Adriana Patricia Rojas Moreno, Instituto de Genética Humana, Pontificia Universidad Javeriana, Colombia

Increased gene dosage: sex reversal in two patients with duplications of either DAX1 (46,XY gonadal dysgenesis) or the SOX9-regulatory region (46,XX testicular DSD). Stefan Riedl, Department of Pediatric Pulmology, Allergology and Endocrinology, Medical University of Vienna, Austria.

CBX2.2 mutation as novel cause for 46,XY Disorders of Sex Development. Patrick Sroll, Endocrinology, Department of Medicine, University of Fribourg, Germany

46, XX Disorder of sex development caused by a NR5A1 heterozygous mutation: a case report and literature review. Zhe Su, Shenzhen Children’s Hospital, Shenzhen, China.
P67  MAMLD1 deletions in three patients with proximal hypospadias. **Yolande van Bever**, Department of Clinical Genetics, DSD center Rotterdam, Erasmus University Medical Center, Rotterdam, Netherlands.

P68  Molecular genetic analysis to optimize the care pathway in individuals with disorders of sex development (DSD). **Emma Webb**, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism Birmingham Health Partners, UK.

P69  The role of a next generation sequencing panel in the diagnostic pathway in Disorders of Sex Development. **Emma Webb**, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism Birmingham Health Partners, UK.

P70  Does Genitography Outweigh Cystoscopy in Detecting Severity of Anomaly in Congenital Adrenal Hyperplasia? **Khaled Salah Abdullateef**, Pediatric surgery department, Cairo University Specialized pediatric hospital (CUSPH), Egypt

P71  Partial Urogenital Sinus Mobilization for Congenital Adrenal Hyperplasia: Twenty Patients Appraisal. **Khaled Salah Abdullateef**, Pediatric surgery department, Cairo University Specialized pediatric hospital (CUSPH), Egypt

P72  Establishment of a standardized anatomic evaluation tool for patients with disorders of sex development (DSD) within the DSD Translational Research Network. **Michael DiSandro**, University of California San Francisco Division of Pediatric Urology, USA

P73  Accuracy of pelvic MRI in the evaluation of internal genitalia and mullerian structures in patients with Disorders of Sex Development having at least one palpable gonad. **Linda Mahfouz El Nachar**, Department of Pediatric Endocrinology, Robert Debré Hospital, Assistance Publique-Hôpitaux de Paris, France

P74  Laparoscopically assisted vaginal pull through in four cases of congenital adrenal hyperplasia with high urogenital confluence: early results. **Ahmed Elham Fares**, Division of Pediatric surgery Fayoum University Hospital, Fayoum, Egypt

P75  Preponderance of ovarian tissue in a 47,XXY/46,XX patient with a predominant XXY cell line in the gonads. **Romina Grinspon**, Centro de Investigaciones Endocrinológicas ‘Dr. Cesar Bergadá’, Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina.

P76  Hypospadias Assessment Tool as proposed by the COST Action BM1303 “A Systematic Elucidation of DSD”. **Alexander Springer**, Department of Pediatric Surgery, Medical University of Vienna, Austria

P77  Clitoral Hoodplasty: A Novel Surgical Technique To Cover An Exposed Glans After Prior Genital Surgery In Women With DSD. **Katja Wolffenbuttel**, Department of Urology and Pediatric Urology, Erasmus Medical Center, Sophia Children’s Hospital, Rotterdam, Netherlands.
**P1: Infertility spectrum or consequence of DSD: a late diagnosis of PMDS**
García-Acero M1, Molina M1, Prieto JC1, Suárez-Obando F1, Gutiérrez A1, Moreno O1, Rojas A1

1. Human Genetics Institute, Pontificia Universidad Javeriana. Bogotá, Colombia
2. Department of Urology, Hospital Universitario San Ignacio. Bogotá, Colombia

**Introduction.** Fertility potential is one of the issues in gender assignment in individuals with disorders of sex development (DSD), also but infrequently is complaints as presentation of DDS in adult individuals. Persistent mullerian duct syndrome (PMDS) is commonly diagnosed during the prepubertal or pubertal age however we present a case of late diagnosis of PMDS in an adult.

**Case presentation.** A 33-year-old married man, son of consanguineous parents, was admitted to our clinic with complaints of infertility. During his physical examination, the patient was phenotypically male with bilateral cryptorchidism and normal penis length. His secondary sex characteristics were normal. An abdominal mass was palpated in the hypogastrium. The hormonal evaluation of the patient revealed hypergonadotropic hypogonadism. The patient’s karyotype analysis was 46,XY and fluorescence in situ hybridization (FISH) for the presence of SRY was positive. Diagnostic laparoscopy revealed multiloculated mass of 20 cm dependent of left gonadal tissue, right gonad not descended, and an appearance consistent with a uterus and fallopian tubes. Total excision of the mass, uterus with bilateral fallopian tubes and bilateral gonadectomy was performed.

Pathologic examination revealed pure seminomatous tumor and right teste was atrophic. Sections from both fallopian tubes showed congestion and fibrosis. No ovarian tissue was seen. Genetic studies in gonadal tissue showed karyotype 46XY, FISH-SRY positive and gene expression analysis of SRY using quantitative RT-PCR indicated normal values of mRNA.

**Discussion.** In DSD infertility is a frequent consequence if the proper hormonal management is not done or if the gonads are not repositioned. PMDS is a rare form of male pseudohermaphroditism, characterized by the presence of uterus and fallopian tubes owing the failure of mullerian duct regression in genetically normal males. They are infertile patients, since there is no spermatogenesis or this is very deficient due to the position of testis which also predisposes to the development of cancer as in this case.

Gene expression analysis of genes involved in sexual development using quantitative real-time RT-PCR, can explain the deregulation of the genes beyond the specific mutation and consequently respond to the phenotype of the manifested sexual disorder.
P2 A search for DSD among infertile men: is partial gonadal dysgenesis being overlooked?

Rafael Shiniti Yabiku, Mara Sanches Guaragna, Lizandra Maia de Sousa, Helena Fabbri-Scallet, Marcela Lopes de Souza, Cristiane Santos da Cruz Piveta, Taís Nitsch Mazzola, Pamela Pontes Henrique, Gil Guerra-Júnior, Maricilda Palandi de Mello, Andréa Trevas Maciel-Guerra

Abstract

Infertility affects 12% of the male population, and may be due to environmental or genetic factors. Among disorders of sex development (DSD), Klinefelter syndrome (KS) is most frequently associated with infertility; however, other DSD may lead to impaired spermatogenesis. DSD are usually recognized early due to genital ambiguity and (or) pubertal delay, but some patients may have a mild clinical picture and reach adulthood without investigation. Published studies on DSD usually refer to patients in the pediatric age range; on the other hand, studies on the etiology of male infertility usually do not include DSD in routine investigation, except for KS. The aim of this study was to verify the proportion of cases of male infertility that are due to DSD which remained undiagnosed up to adulthood. Our sample included 74 men aged over 20 years who were referred from 2010 to 2015 to our DSD service due to oligospernia (37 cases) or non-obstructive azoospermia (37 cases) of unknown etiology. Data regarding family history, genital abnormalities, testicular volume, hormonal measurements, cytogenetic and molecular studies were obtained from their medical files. In 10 cases (14%), a DSD was diagnosed in accordance with current classification (nine cases of KS and one of mild androgen insensitivity). Two patients had Yq microdeletions, and four presented NR5A1 variants of uncertain significance. In addition, a suggestive picture of DSD was found in one patient with a sex chromosome abnormality and ten 46,XY cases of unknown etiology, all with hypergonadotrophic hypogonadism and microorchidism. These cases deserve further investigation, including testicular biopsies, which could lead to the diagnosis of patients with partial gonadal dysgenesis and increase the contribution of DSD to the etiology of male infertility.
P3 A Reminder to Think Outside the Box:
Use of Fertility Preservation Options for a Girl with an Unusual 45,X/46,XY DSD

Courtney Finlayson1,6, Diane Chen2,3,6,7, Barbara Lockart4,6, Elizabeth Leeth6,10, Emilie K. Johnson5,8, Marybeth Madonna4,9, Earl Y. Cheng5,8, Elizabeth Yerkes5,8

Divisions of Endocrinology1, Adolescent Medicine2, Child & Adolescent Psychiatry3, Pediatric Surgery4,4, Urology5, Ann & Robert H. Lurie Children’s Hospital of Chicago, Departments of Pediatrics6, Psychiatry and Behavioral Health7, Urology8, Surgery9 and Center for Genetic Medicine10, Northwestern University Feinberg School of Medicine

Background: The Gender & Sex Development Program (GSDP) and Program in Fertility Preservation (FP) at Lurie Children’s work together to offer FP to youth with differences of sex development (DSD). An 11 year 5 month old girl presented to GSDP with Turner Syndrome (TS), 45,X/46,XY, diagnosed during a short stature evaluation at 4 years. At 9 years, pelvic ultrasound showed no uterus/gonads. At 11 years, MRI showed a uterus, well-defined left adnexal cyst, and a possible small follicle in a streak ovary. Estradiol was started 5 months before the initial GSDP visit with breast growth and menarche. The patient was referred to GSDP for consultation regarding gonadal germ cell cancer (GCC) risk.

Methods: Multidisciplinary care occurred at 6 visits over 10 months. Initially, the family reported distress from the child due to TS diagnosis because child had a strong desire for biological parenthood. Recent clitoral growth was noted. Physical exam: Tanner 3 breasts, Tanner 1 pubic hair, clitoris 2cm long, rugated labia majora, no posterior fusion, separate urethral and vaginal openings. After holding estradiol, laboratories: LH 6.45 uIU/mL, FSH 60 uIU/mL, anti-mullerian hormone 0.17 ng/mL, estradiol 3 pg/mL, testosterone 203 ng/dL. By 11 years 9 months, clitoris 4cm long, and voice had deepened. Thus, diagnosis was more consistent with mixed gonadal dysgenesis (MGD) than TS. Leuprolide was started to suppress further virilization, and two management options considered: 1) gonadectomy (the typical recommendation), and 2) continuing of leuprolide with gonadal monitoring. Surgeons discussed that surgery for clitoral reduction would be delayed until the patient could understand potential sexual health ramifications. Parents were very concerned about GCC risk, denied gender dysphoria, and desired gonadectomy. In psychosocial evaluation by the psychologist at 4 visits over 6 months, patient firmly asserted female gender identity and endorsed anxiety related to clitoromegaly, voice deepening, and her body “looking different” than peers. She demonstrated understanding of treatment options and expressed preference for gonadectomy to prevent further virilization and need for ongoing leuprolide injections. Counseling about fertility potential and FP was performed by the endocrinologist, urologist and FP nurse. Patient and family initially desired FP at the time of gonadectomy.

Results: Patient underwent gonadectomy at 12 years. Left gonad: streak gonad with well-developed fallopian tube, rudimentary wolffian remnants, no germ cells. Right gonad: dysgenetic testis with markedly decreased spermatogonia, no spermatogenesis. Testicular tissue cryopreservation was offered, with discussion of experimental nature and lack of current in vitro techniques to mature spermatogonia. Patient and family ultimately elected not to preserve and store tissue due to the experimental nature and non-traditional use of sperm for a female-identified individual.

Conclusion: This patient demonstrates an unusual presentation of MGD and the use of evolving FP options for patients who desire future biological parenthood.
P4 Attitudes Toward Disorders of Sex Development (DSD) Nomenclature Among Physicians, Genetic Counselors, and Mental Health Clinicians

Lauren Miller MS1, Elizabeth A. Leeth MS CGC1,7, Emilie K. Johnson MD MPH2, Ilina Rosoklija MPH2, Diane Chen PhD3,6,7, Sharon A. Aufox MS CGC1, Earl Y. Cheng MD2,5, Courtney Finlayson MD4,7

Center for Genetic Medicine1; Divisions of Urology2, Adolescent Medicine3, Endocrinology4, Ann & Robert H. Lurie Children’s Hospital of Chicago; Departments of Urology5, Psychiatry and Behavioral Sciences6, Pediatrics7 Northwestern University Feinberg School of Medicine

Introduction: In 2006 nomenclature for atypical sex development was updated and disorders of sex development (DSD) was introduced. Previous studies showed adoption of this term by pediatric endocrinologists (PE) in 60 European medical centers and in a survey of 15 neonatal healthcare professionals. There is other evidence, however, that DSD has not been universally accepted and adopted for use by clinicians and the affected individuals and community. Inconsistency in nomenclature may lead to confusion, affect clinician-patient communication and relationships, and interfere with the recommended multidisciplinary model for care. Among physicians, genetic counselors (GC), and licensed mental health clinicians, this study sought to: (1) determine diagnostic terms clinicians use with patients and families and (2) evaluate frequency of use and comfort with specific DSD terminology.

Methods: A web-based survey assessing use of terminology for DSD conditions was distributed to PEs, pediatric urologists, psychologists with a specified interest/expertise in DSD population, and GCs (prenatal and pediatric). A quantitative analysis was performed to assess differences among clinician use and comfort with specific terminology.

Results: The survey was completed by 286 clinicians (8.9% response rate). Most reported using just the diagnosis (81%) and disorder of sex development (73%) to explain a diagnosis to patients and families. Few clinicians reported using intersex (17%) and only urologists and prenatal GCs used hermaphrodite (2.8%). Table 1 represents the comfort and frequency of use of terms for all participants. The use of specific terms varied significantly based on clinician specialty, sex, patient volume, length of practice, and practice setting. Hermaphrodite and intersex were referenced more often in the media, whereas, disorder of sex development and DSD were referenced more in case conference and the literature.

Table 1. Comfort and Frequency of Term Use

<table>
<thead>
<tr>
<th>Term Used to Describe:</th>
<th>Most Comfortable</th>
<th>Most Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Genitalia</td>
<td>Genital difference</td>
<td>Ambiguous genitalia</td>
</tr>
<tr>
<td>Genital Structures</td>
<td>Penis</td>
<td>Penis</td>
</tr>
<tr>
<td>Non Typical</td>
<td>Variation</td>
<td>Difference</td>
</tr>
<tr>
<td>DSD Diagnosis</td>
<td>Disorder of sex development</td>
<td>Disorder of sex development</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Least Comfortable</th>
<th>Least Often</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical Genitalia</td>
<td>Neutral genitalia/ Nonspecific genitalia</td>
</tr>
<tr>
<td>Genital Structures</td>
<td>Clitorphallus</td>
</tr>
<tr>
<td>Non Typical</td>
<td>Divergent</td>
</tr>
<tr>
<td>DSD Diagnosis</td>
<td>Sex development atypia</td>
</tr>
</tbody>
</table>

Conclusion: Our study confirms that a broad range of providers most often use disorder of sex development terminology. However, inconsistencies remain in preference and use of terms describing other aspects of DSD. While provider preference varies, our results suggest that words such as difference and variation, would be acceptable replacements for the term disorder. Disorder of sex development nomenclature is most frequently used by the medical community, but this term is not usually presented to the public by the media. Comparison to preferred terminology among affected individuals and advocates is needed to identify discrepancies among major stakeholders and work toward universally accepted nomenclature.

Acknowledgment: Special thanks to the Disorder of Sex Development-Translational Research Network, Pediatric Endocrine Society, Society of Pediatric Urology, Society of Pediatric Psychology, and National Society of Genetic Counselors for their participation and to Arlene Baratz, MD and Georgiann Davis, PhD for assistance in creation of the survey.
P5 Highlights on 1st Saudi International DSD Workshop Riyadh: DSD from Muslim traditional society.

A. AlShammari1, S Vallasciani2, A. Alsagheir3

1 Consultant, Pediatric Urology Division, King Abdullah specialized Children Hospital, King Abdul Aziz Medical City, National Guard Health Affair, Riyadh, Saudi Arabia
2 Consultant, Pediatric Urology Division, Urology Department, King Faisal Specialist Hospital and Research Centre and Alfaisal University, College of Medicine, Riyadh, Saudi Arabia
3 Consultant, Pediatric Endocrinology Division, Pediatrics Department, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Background
DSD are conditions where multidisciplinary approach has become the standard owing to its multiple areas of impact. Beside the medical aspects (surgical, endocrinological, genetical, psychological), social issues have important effects on the DSD individuals. The GCC countries (Gulf Cooperation Council) in general, and Saudi Arabia in particular, are areas where collective-driven social model under Muslim religion is present in terms of a conservative culture where religious and social influences play a major role in management decision of those individuals. Aim of this presentation is to present the highlights of the 1st Saudi International Workshop on DSD.

Materials and methods
International Speakers from north and south Europe as well as from United Kingdom, local specialists on the field from all the geographical areas of the country participated to the Workshop. The meeting consisted of state of the art lectures, local and international experience, round table discussion, case presentations and interactive sessions with onsite attendance and off site attendance via active live web link.

Results
In this specific geographical area, the “Traditional culture” plays a major role in the daily life behavior and action by individual. This term, however, must not be interpreted as a tie to the past denying any evolution, but as a foundational value that the society desires to preserve.

In this environment, the Society and the Religion are closely related and the decisions are made based more on the collectivity interests rather than on individual interests. It would be a matter of discussion among the society the limits whether the collective interest can interfere with an individual interest. In addition, this is a male dominant society.

Religious laws and commandments are under the umbrella of Shahria law. Regarding DSD conditions, the general statement available is: genotypically females (including high undervirilized individuals) should be raised as a female while genotypically males (including virilized individuals) should be raised as a male. In rare conditions where mixed genotype and indifferent phenotype is present, they are discussed individually and medical advice is taken into consideration. There are no specific statements on what is allowed and what is prohibited for rare conditions and particular situations.

In a society where the gender of the newborn has to be announced immediately or at least at the time of discharge from the hospital this socio-cultural-religious issues are nowadays affecting the management of DSD individuals in terms of sex assignment since the future social roles and expectancies for males and females are different.

Actually in Saudi Arabia, there is only one Center dedicated to Sex assignment while in most of the other Centers in the Country, the management is done only by Surgeons and Endocrinologists. In general, the psychological support is not available in regular basis. By the same way, no registries are present.

Discussion
As the result of the Meeting, a working force on DSD is being developed for multidisciplinary discussion under the Muslim rules in a collective-driven society to give the patients and families the most suitable recommendations for the future of the children. Patient and families associations of DSD seem difficult to be built at this stage.
P6 Semi-professional Peercounseling – a contribution to DSD care

Background:

Differences of Sex Development (DSD) pose a challenge for concerned persons and their families. It is not only the rareness of the diagnose but also the aspect of shame involving very personal topics such as gender identity, sexual functioning and self-esteem.

Parents want to protect their child from discrimination and often cannot imagine their intersex child to grow up without surgical cosmetic procedures. Interdisciplinary care can lay the basis for a good understanding of the biomedical facts and psychological counselling can address personal needs. Yet it is of special value for patients to get advice from other concerned persons/ families that have met the same challenges and have developed coping strategies. Semi-professionell peercounseling can serve as a building block in the process of acceptance and coping.

Methods:

Through the Kindness for Kids Health Care award in 2016 we had the possibility to finance semi-professional peer-counselling for patients seen in our outpatient center in Lübeck. Organized and coordinated by the self-advocacy group "Intersexuelle Menschen e.V" a curriculum for a nonbiased advisory service in DSD had been developed and implemented. Both parents and concerned persons had been trained for peercounselling in consideration of medical knowledge, counseling techniques, self-disclosure and self-protection.

Peercounseling was offered to all DSD patients and their families in our center through our patient navigator. The advisory service was conducted either at our clinic or at a chosen location in a tandem constellation. This allows an exchange between parents and concerned people at the same time.

Results:

In the beginning the sole offer of peer advisory service was declined in many cases. But when we introduced peercounseling as a special requirement in the decision-making process it was better accepted. We saw that two groups of care seekers predominantly used the new service. One group were the parents of DSD children when decisions on interventions had to be made. The other group represented grown-up people with a DSD diagnose with a clear wish for a better understanding and coping experiences. The cohort of teenagers and young patients <25 years appeared cautious to the offer to get into contact with other concerned persons especially if they are not the same age.

All patients and families stated that the peercounseling was helpful to manage their needs.

Conclusions:

Peercounseling is helpful before medical interventions such as surgical procedures or hormone treatment and for the prospect of understanding and dealing with personal concerns in adult life. It has to be introduced and explained by a member of the interdisciplinary team respecting a possible refusal. Teenagers and young adults have to be addressed more appropriately. In this sensitive developmental phase young patients become aware of their special condition or have been diagnosed in the first place. Thus a major part might still be to involved with resulting consequences to be open for an exchange.
Background: Disorders of Sex Development (DSD) have an important impact on the psychological wellbeing of the children and their families living with these conditions. A diagnosis of DSD can be associated with psychological distress, as families grapple with complex medical information, and making decisions that have longstanding medical and psychological implications whilst coping with an uncertainty about the future. Research has shown that families report greatest distress during the time of diagnosis and subsequent surgical or medical interventions. They also experience stress in disclosing the condition to others and in engaging in normative everyday parenting activities such as seeking care providers for their children. Although parents of children with DSD report a high need for psychological support half of these parents reported that the psychological support provided is not adequate. The Chicago Consensus in 2005 stated that psychological care should be an integral part of management of DSD. This audit was conducted to assess the current psychological service offered to children with DSD in Ireland in order to inform service development for children and their families.

Methods: An audit of all children with a diagnosis of DSD across the leading paediatric hospitals in Ireland was conducted to investigate children and families’ access to psychological services.

Results: The audit investigated the number of referrals to psychology, the timing of referrals, availability of services, obstacles to accessing psychological intervention, engagement with psychology, types of consultation offered and outcomes of psychological interventions for children with DSD and their families. The results of the audit will be presented.

Discussion: Reflections on the current status of psychological services for children with DSD and their families in Ireland are discussed.
P8 Sexual self-concept and sexual functioning in women with DSD: implications for clinical management

1Nina Callens PhD, 2Nita G.M. de Neve-Enthoven MSc, 3Maaike van Kuyk MSc, 3Jet H. van Kuppenveld, 2Jan van der Ende MSc, 2Stenvert Drop MD, PhD, 4Peggy T. Cohen-Kettenis PhD, 2Arianne B. Dessens PhD

1Ghent University, Ghent, Belgium 2Erasmus Medical Center Rotterdam-Sophia Netherlands, 3Radboud University Medical Center-Amalia Children’s Hospital Nijmegen, Netherlands, 4VUmc Genderteam, Amsterdam, Netherlands Correspondence to: a.b.dessens@erasmusmc.nl

Background: Many women with DSD report sexual difficulties. While previous research mainly explored the relationship with genital surgery, sexological models of sexual dysfunction suggest an integral role of sexual self-concept (cognitions about oneself regarding one’s sexuality). In women with DSD, the role of sexual self-concept in relation to sexuality outcomes has received little attention yet.

Research Question: Does sexual self-concept predict sexual problems in women with DSD?

Method: Study group: 76 Dutch women with DSD (aged 17-60 years, median age 26), 589 control women (aged 18-68 years, median age 23). Measures: Questionnaires on sexual self-concept (Women’s Sexual Self-Concept Scale -WSSCS), sexual functioning and distress (FSFI, FSDS-R), satisfaction with body and genital image (BIS). For this study, we translated the original WSSCS and validated the instrument for Dutch speaking Belgian and Dutch women. Statistics: Principal component analysis to validate the Dutch translation of WSSCS. Mann-Whitney U, Kruskal-Wallis tests, multiple regression analyses.

Results: The three-factor structure of the Dutch WSSCS corresponded largely to the original version, capturing three dimensions describing (1) women’s active role in sexuality, labeled ‘Agentic Sexuality’ (AS) (2) sexual anxiety and coercion, labeled ‘Negative Associations’ (NA) and (3) faithfulness to a partner, (re)labeled ‘Loyalty’. Women with DSD reported a less active role in sexuality (AS) than control women (p < .001); no differences between groups were found on the Negative Associations (p=.533) and Loyalty scales (p=.941). Genital virilization at birth did not affect women’s responses on the WSSCS. Compared to controls, more women with DSD reported dissatisfaction with their external genital image (p < .001), but were equally satisfied with other sex-specific body characteristics (p=.229) and general body image (p=.196). Women with DSD who perceived their external genitals more negatively, evaluated their sexuality more negatively as well (p < .001).

Conclusions: Women with DSD reported a less sexually active role and experienced more sexual distress than women without DSD. Women with DSD who seek help for sexual problems might benefit from exploring their sexual self-schema and a cognitive approach in sex therapy.

Keywords: Sexual self-concept, DSD, Female sexual distress and dysfunction, Body Image

Funding: FWO Flanders (NC), Swart – van Essen Foundation (NdN-E), Edli Foundation (AD).
Background: Knowledge about mental health outcomes is important to optimize health among women with disorders of sex development (DSD). The aim of this study was to estimate if the prevalence of psychiatric morbidity in adult women diagnosed with complete androgen insensitivity syndrome (CAIS), 46,XY Gonadal Dysgenesis (46,XY GD) and 46,XX Gonadal Dysgenesis (46,XX GD), age-matched population controls.

Method: This study was conducted at the Karolinska University Hospital, Stockholm, Sweden, and includes 33 women with DSD (20 CAIS, 6 46,XY GD, 7 46, XX GD) and 61 population-derived controls. Psychiatric morbidity was assessed using the Mini International Neuropsychiatric Interview plus (MINI+). Results are presented as p-values and estimated risks (OR, 95% CI) of psychiatric conditions among women with DSD in comparison with age-matched population derived controls.

Results: Twenty-eight of the 33 women (85%) fulfilled the criteria for at least one psychiatric disorder according to the MINI+, with depression and anxiety disorders as most common. This was significantly higher compared to population controls (52%) (OR 5.1, 95% CI 1.7-14.9).

Conclusion: Increased psychiatric morbidity in women with CAIS and Gonadal Dysgenesis highlights the need of psychiatric screening among these patients.
At the Royal Children’s Hospital (RCH), Melbourne, Australia, there are estimated to be 460 patients who have a Difference of Sex Development (DSD). On a yearly basis, this represents 20 new diagnoses in infancy and 10 patients presenting in late childhood or adolescence per year. The best practice model of care for patients with complex problems is a multidisciplinary team (MDT). At RCH, DSD patients have care provided by departments of urology, endocrinology, gynaecology, genetics, social work and bioethics, with research input from the Murdoch Children’s Research Institute (MCRI). Research findings and patient feedback identify that supports and resources for patients and their families have been limited.

In 2015/2016 the RCH employed a DSD clinical coordinator to manage the care provided to patients with DSD by the MDT of clinicians from RCH. This first year has seen the development of the role with the identification of systems for the clinical coordination needs of patients, families, and the MDT clinical team. The key elements of this role have been to:

1. Facilitate MDT meetings
2. Liaise with other medical departments and services including input from pathology, genetics, mental health services and other community services
3. Ensure timely provision of information, support and referral of families of newly diagnosed infants, children or adolescents
4. Provide support, education, and the opportunity to participate in peer-support groups as patient and family needs change over time
5. Ensure timely age appropriate referrals to the appropriate clinicians and services.
6. Establish and maintain a database to ensure that patients and their families are not lost to follow-up
7. Liaise with state governments regarding patient resources
8. Participate in the development of research identifying patient needs and appropriate resources

The MDT DSD Meeting held monthly is a patient review forum for clinicians to identify best care plans for DSD patients as well as a forum for the teams to review DSD management, ensure interdisciplinary communication, and identify gaps in RCH processes. Representatives at this meeting include the two tertiary children’s hospitals in Victoria as well as international participation from Singapore and Malaysia. Before the position of clinical co-coordinator, less than half this number participated. These relationships have enabled better communication between departments and transparency between services. Consequently, the provision of standardised care is facilitated.

The clinical coordinator has provided support to over 100 patients and families, 23 new patients presenting with variations in their genitals and 8 adolescents newly diagnosed due to differences in secondary sex characteristics, as well as patients with DSD already engaged in RCH care. This role has established productive links with peer support groups to promote patient support, self-efficacy, and patient resources. The clinical coordinator has identified and re-engaged a number of patients that were lost to follow-up.

For patients and their families, having access to a consistent, ongoing point of contact at the hospital, psychosocial support and relevant resources is highly beneficial and aims to increase their quality of life and overall wellbeing.
Disorders of Sex Development (DSD) are congenital malformations that promote undifferentiated sexual anatomy in newborn children. The present study aimed to investigate how patients diagnosed with DSD, in puberty and in the beginning of adolescence, understand this disease. Six adolescents with DSD, between the ages of 11 and 15 years old, participated in the study. The means of data collection and analysis were a semi-directed structured interview and content analysis, respectively. The patients have a partial understanding of the complexities of the disease and its multifactorial character. Patients show difficulties in subjectifying the technical information about the diagnosis, the DSD etiology and the influence of the disease in their development.

**Keywords:** genital disorders, information, adolescence
P12 Sex Rearing and Parental Educational Strategies with Children with Disorders of Sex Development (DSD)

T.P. Hemesath¹; E.C. COSTA²; B.B. Soll³.
PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA)
Federal University of Rio Grande do Sul (UFRGS) - Brazil

The Disorders of Sex Development (DSD) are congenital malformations characterized by promoting anatomically undifferentiated genital in newborn children. This paper is investigated how the parents of patients diagnosed with DSD, in late childhood, establishing educational strategies for this condition. It is a qualitative study, with four participants, three mothers and a father of children with DSD, in ages between 8 and 13 years old. A semi-structured interview and the Content Analysis were, respectively, used as methods of data collection and data analysis. The results indicate that parents make use of overprotective educational strategies towards the disease of their children, and they are exposed to an important confounding factor in understanding what concerns the formation of gender identity of these children, thus causing an authoring environment permeated by ambivalent behaviors.

Keywords: Disorders of Sex Development, Educational Strategies, Children
P13 DSD online healthcare content: A process to evaluate quality of material.

K. Kennedya, M. M. Ernstb, D. Chen, c, d, C. Foleye, T. Jewel, A. Sajwanif, D.E. Sandbergf and in collaboration with the DSD-TRN Psychosocial Workgroup and Accord Alliance

aCincinnati Children’s Hospital Medical Center, Department of Urology; bCincinnati Children’s Hospital Medical Center, Department of Pediatrics; Ann & Robert H. Lurie Children’s Hospital of Chicago, Division of Adolescent Medicine and Department of Child & Adolescent Psychiatry; cNorthwestern University Feinberg School of Medicine, Departments of Pediatrics and Psychiatry & Behavioral Sciences; dHofstra Northwell School of Medicine; eOberlin College; fUniversity of Michigan Medical School, Department of Pediatrics

Background: The DSD-TRN is a US National Institutes of Health-sponsored clinical research network established with a primary goal of delivering standardized, high-quality care to patients with Disorders/Differences of Sex Development (DSD) and their families. The DSD-TRN has invested in making high quality DSD educational/informational resources available to patients, their families and the general public to promote patient-centered care. Online websites are a primary source for patients and families seeking health information, and can vary dramatically in quality. A number of validated assessment tools are available to evaluate the quality of health-related web information. These tools measure key features of online health information including understandability (i.e., reading level; clear presentation of material), accuracy and comprehensiveness of treatment options. As part of a quality improvement project targeting the online health content provided by DSD-TRN member sites, a systematic evaluation process was developed to assess web-based health information and identify areas for improvement. A subcommittee of the DSD-TRN psychosocial workgroup (representing 4 of the 10 member sites) reviewed the literature and identified validated health information quality assessment tools to systematically evaluate information across a range of domains. The application of this systematic evaluation process to one exemplar website will be presented.

Methods: DSD-related search terms identified via expert consensus were used as search queries on the institutional website of one DSD-TRN participating site to identify all webpages with DSD-related content. All original online material was evaluated using 3 validated tools: The reading level of the material was assessed using the Simple Measure of Gobbledygook (SMOG), a validated and frequently-used scoring system in which all the words containing > 3 syllables are counted within 3 10-sentence passages. The clarity of presentation of the material was assessed using the Patient Education Materials Assessment Tool (PEMAT), a validated evaluation system developed by the US Agency for Healthcare Research and Quality, which has been applied to information about an array of health conditions. The PEMAT assesses domains including clarity and focus of content, word choice and style, organization of material, layout and design, and whether specific actions users can take are clearly identified. The comprehensiveness of the material was assessed using the DISCERN tool, a validated instrument for assessing the transparency of the source material as well as whether a full range of treatment choices are comprehensively presented with both risks and benefits of each treatment option discussed (including the possibility of no-treatment).

Results. The search within the selected DSD-TRN member website identified webpages related to the DSD Center and health topics including “disorders of sex development,” “androgen insensitivity syndrome,” “congenital adrenal hyperplasia,” “mixed gonadal dysgenesis,” “hypospadias/chordee” and “feminizing genitoplasty.” Across webpages, SMOG scores were generally above the 12th grade readability level. PEMAT scores were typically in the 60th percentiles. The DISCERN analysis revealed shortcomings in overall quality of information provided.

Conclusions: Knowing that patients and families continue to take greater responsibility for their own health care needs, and continue to seek knowledge about health and treatment options via technology, it is imperative that institutions are vigilant in providing high quality, readable, understandable, and actionable information for all stakeholders. This is particularly critical for health conditions in which there may be divergent stakeholder perspectives on best practices and a range of viable treatment options exist. The systematic evaluation of online health materials across multiple health literacy domains can identify areas of improvement for institutions creating web-based content for patients and families.
P14 Holistic care of patients with CAIS through their lifespan.

Authors in alphabetical order: Z. Kolesinska $^{1,2}$, A. Chodecka $^{2,3}$, K. Kapczuk $^{2,4}$, M. Niedziela $^{1,2}$

Affiliations:
$^{1}$Department of Paediatric Endocrinology and Rheumatology, 2$^{nd}$ Chair of Paediatrics, Poznan University of Medical Sciences, Poland
$^{2}$Karol Jonscher’s Clinical Hospital, Poznan University of Medical Sciences, Poland
$^{3}$Department of Social and Clinical Sexology, Institute of Psychology, Adam Mickiewicz University, Poznan, Poland
$^{4}$Department of Perinatology and Gynaecology, Poznan University of Medical Sciences, Poland

Background: The birth of a child with differences in sex development is a very stressful event especially for parents, but also for health care providers. As gender constitutes one of the basic categories that regulate human relationships, lack of parental knowledge about differences in sex development (DSD) and possible difficulties in sex assignment strengthens it even more. Additionally, the Polish socio-culture context still regards the gender as a dichotomy construct. On the other side, medical help based on multidisciplinary team (MDT) work is emerging while struggling for developing medical standards and guidelines that would be helpful in the legal necessity of early birth assignment, proper medical and psychological management without overmedicalisation.

Objectives: Therefore, we present an attempt to provide holistic care by a newly formed MDT to a group of 8 patients diagnosed with complete androgen insensitivity syndrome (CAIS) at different age.

Results:
Neonatal period and Childhood (2 cases):
The medical care was sought because of labioscrotal swellings containing gonads. The management was primarily focused on proper diagnosis, that may be challenging if genetic analysis of AR gene is not a part of routine diagnostic procedure. If assessment of AMH is available, it might be helpful in making diagnosis. Being aware that minipuberty is absent in patients with CAIS, assessment of baseline testosterone is not indicative, thus long-lasting hCG test constitutes the alternative for AMH evaluation. In the differential diagnosis 5alpha-reductase deficiency and 17beta-hydroxysteroid dehydrogenase type 3 deficiency should be excluded. In the light of new findings, surgical correction of inguinal hernias with gonadal preservation was advocated. Psychological care for parents required psychological assessment (fear, guilt, sadness), formulation of their needs (gaining knowledge on DSD and on how to share it with a child and others, support groups) and providing support.

Adolescence (4 cases):
The cause of referral was primary amenorrhea. Normal breast development, sparse pubic hair, no uterus, specific hormonal profile and 46,XY karyotype guided the health care providers towards the diagnosis. If the puberty was completed the question of further gonadal preservation emerged. Vaginal enlargement procedures were not required. During psychological management patient often declared anger, sadness, fear of peer review. When asked to define their needs, they looked for different possibilities and ways of disclosure and they underlined the need of sharing their situation with other similar adolescents.

Adulthood (2 cases):
Patients addressed the gynaecologist due to difficulties in sexual intercourse. If the gonads had been preserved the problem of suitable and reasonable follow up appeared. However traditional hormonal replacement therapy after gonadectomy may not be sufficient in terms of overcoming loss of libido and menopausal symptoms.

Conclusions: Although providing care to patients with DSD needs elaboration of systematized medical care and transparent guidelines, the individual management is crucial. Therefore, anticipation of different needs in different moments of life will certainly ease facing up to medical and psychological challenges.
The birth of a child with an intersex condition or diverse sex development (dsd) still confronts parents, but also experts in the field, with a variety of challenges. Many questions come up for which there are no easy answers. As gender development and later gender identity are not predictable in most dsd, a difficult decision concerns the child’s gender in which it shall initially grow up. Moreover, there are scientific controversies concerning irreversible elective medical interventions. So far, there is no comprehensive German online platform that imparts knowledge around these topics from different perspectives and disciplines, and also addresses open questions and gaps in knowledge.

The aim of the project presented is to conceptualize an online platform for a broad target group 1) by involving and addressing medical students, parents, persons concerned and experts in the field (e.g. medical doctors and psychologists), 2) opening new, rather positive than deficit-oriented views on diverse sex development, and 3) providing information in an understandable manner while also emphasizing the challenges related to complexity, uncertainty and ambiguity.

This paper outlines the conceptualization process of the blog intersex-kontrovers.blogs.unihamburg.de. The blog comprises video interviews, illustrations, links and short texts from various perspectives, such as from psychology, support groups, the arts, medical ethics, law, different medical disciplines (e.g. endocrinology, pediatrics, urology) and sex research.

Preliminary results of the accompanying research will be presented: In a self-constructed questionnaire, persons concerned, parents and other experts were asked which topics and open questions regarding intersex they considered most important and controversial. N = 25 adults (age range: 32-80 years) participated in this pilot assessment: 5 parents, 6 adult persons affected and 14 experts in the field. The findings indicate the following topics being conceived as highly controversial: 1) conflicts between human rights and medical possibilities, 2) clinical diagnostics and treatment after birth and in puberty, 3) rights concerning marriage/civil partnership and 4) finding an appropriate, respectful language.

Based on these results, which resemble findings from an earlier online study (Schweizer et al., 2016), structured interviews with individual representatives of the different target groups will be conducted to find out more details about each controversy mentioned. Furthermore, the contents of the blog (e.g. vignettes on ethical conflicts) will be tested in medical teachings, and a systematic evaluation will be carried out.
A Tale of Two Genetic Sisters: Considerations of Alternative Gender Assignments in Congenital Adrenal Hyperplasia with Markedly Virilized Genitalia:

F.J. Blas and E. Vilain

Departments of Human Genetics and Pediatrics, UCLA School of Medicine

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of sex development (DSD) often leading to genital ambiguity. When the diagnosis of CAH is made in the first few months of life, it has traditionally been recommended that the gender of rearing, regardless of the degree of virilization of the genitalia, be female. The arguments favoring a female gender assignment are mainly centered around the preservation of fertility, because of the presence of potentially functional ovaries and uterus. In addition, this recommendation relies on outcome data that a female gender identity can be expected in the vast majority of the cases. However, recent data showed that gender dysphoria is experienced by approximately 5% of (assigned) girls with CAH, irrespective of the Prader stage of their genitalia at birth.

The purpose of this study was to analyze the decision-making process of gender assignment by the medical team and the family in a case of two genetic females with markedly virilized CAH. The family was given both general information about CAH, and data specific to the patients, including the genetic findings, showing mutations consistent with classic CAH, and endoscopy, showing a urogenital sinus with a high level of confluence in one case and low level in the other. For both children with CAH, the parents were initially presented with the options of female and male gender assignment, but elected to not choose any assignment, and to raise their child in a “neutral” gender. However, it became rapidly clear that the family was considering the older child (currently 3 years of age) as a boy, and the younger (currently 2 years of age) as a girl, as evidenced by manner of dress, hair style and gendered use of pronouns. The interactions, between the members of the multidisciplinary clinical team (Urologist, Geneticist, Endocrinologist, Child Psychiatrist, Social Worker, Genetic Counselor) and between the clinicians and the family were tape recorded (after informed consent) and analyzed. The family has been followed up for over three years with recording and analysis of all interactions.

We review the factors to be considered for gender assignment, and discuss management strategies that promote the best possible quality of life across the lifespan for patients with CAH with markedly virilized genitalia.
P17 What does it take to judge a minor’s ability to give informed consent in the field of DSD?

Guidelines for DSD treatment demand that the concerned person must be heard and put at first when it comes to medical interventions or a challenging decision making process. The newborn and young child fully depends on the parents’ ability to act in its best interest. The adolescent and young adult must be stepwise empowered to overtake responsibility for his own care.

Certain diagnoses pose a great challenge at the beginning of puberty when serious physical changes might even lead to a change of gender role without intervention. The possibility of such a transition is strongly disturbing and a hardly comprehensible option for the teenager and his family. What does it take to elucidate this complex situation and to develop a treatment plan that brings the teenager’s opinion in balance with ethical considerations, possible long term outcomes and not least the law. Criteria that assess the teenager’s maturation, the understanding of the complexity of the subject and long term consequences need to be found.

Methods

We present an interdisciplinary approach to reach informed consent of adolescent DSD patients in a decision shaping phase of sex development. Six patients at the age of 11 to 16 were invited separately to an inpatient evaluation program (Siblings with 5 ARD, one case of 17-ß-HSD deficiency, two patients with partial gonadal dysgenesis, one 46-XX-male CAH).

Patient and parents were stepwise trained in the underlying biomedical condition using self-developed teaching material by the endocrinologist and the pediatric surgeon. The psychologist assessed patient’s maturation, self-concept and gender identity applying adapted questionnaires, interviews and projective instruments. GnRH-analogues were administered to gain time for the decisionmaking process. Peer counseling was part of the process in two families.

Results

The inpatient admission set an appropriate frame for the patient to learn about his condition and to reflect on important time windows in care. All adolescents stated at first that they wish to remain in their accustomed gender role. The idea of transformation released discomfort and distress in all teenagers. Reactions spread from social withdrawal, sadness to aggression. Yet a 15-year-old Kurdish patient (5 ARD) who first asserted his conviction to remain in the female role through went transformation to male gender role emphasizing the stability of an intersexual self-concept. His sister however required to remain in the female gender role. One female teenager with partial gonadal dysgenesis (16) expressed her wish for clitoris reduction to remove signs of virilization after a thoroughly counseling process. A twelve-year-old 46, XX-male-CAH strongly demanded the removal of his female internal genitalia and male hormone replacement therapy to continue male development.

Conclusions

In order to empower minors to give informed consent the consistency of their will and degree of maturation needs to be assessed and reevaluated repeatedly. There is a need for semi standardized procedures and criteria that allow evidence for valid patient’s statements. Adolescents should understand this course of action not as a mistrust of their statement, but as a mean of responsibility toward their personal and legal protection. Potential change of personal judgement due to psychological maturation needs to be taken into account. Good documentation is paramount to accompany the process of decision making over time.
Parents of children with Differences of Sex Development (DSD) demonstrate higher rates of anxiety, depression and stress than the parents of unaffected children. Parenting behaviours may also differ, and studies have shown increased risk for parenting stress, parental overprotection and perceived child vulnerability in parents of children with DSD. It is widely accepted that psychosocial supports for parents are a cornerstone of care; however there is little information available on how and when such supports should be provided. This study aims to sensitively measure qualitative and quantitative aspects of the services provided and support needs of parents, and thus inform the provision of future services.

Parents of youth currently aged 1.0-20.0 years who are presently receiving, or have previously received, treatment in relation to their DSD at the Royal Children’s Hospital were invited to participate in an online survey. Participants were asked questions on the types of medical and surgical interventions recommended and received, and their needs for psychosocial support in these contexts. They were also asked about their preferences for information and emotional supports (including medical and peer supports), their understanding of their child’s condition, their opinions on disclosure to their child and others, and subjective feelings of concern for different domains of their child’s life.

Preliminary data is not yet available for analysis. Recruitment commenced on 01/03/2017 and will continue until 15/05/2017. This study is being undertaken as part of a post-graduate medical research project, and analyses will be completed for thesis submission in June 2017. We anticipate that this study will provide further insights into specific psychosocial support needs of parents of children and adolescents with DSD and their preferences for future services.
P19 Identifying the resource needs of adolescents with DSDs


¹ University of Melbourne, Parkville, Victoria, Australia
² Royal Children’s Hospital, Parkville, Victoria, Australia
³ Murdoch Children’s Research Institute, Parkville, Victoria, Australia
⁴ Walter and Eliza Hall Institute, Parkville, Victoria, Australia

Adolescents with differences of sex development (DSDs) often have complex medical and psychological care needs and require age-appropriate resources. We aim to describe the results of a qualitative online survey exploring adolescents’ with DSDs need for information and support.

We contacted young people aged 14–30 with DSD diagnoses according to the 2006 consensus statement. Patients were identified from a DSD database of people who had attended the Royal Children’s Hospital (RCH), Melbourne, Australia or the private practice of clinicians linked to RCH. Contact has been attempted with over 200 young people. Respondents are representative of diagnoses including congenital adrenal hyperplasia, androgen insensitivity syndrome, bladder extrophy, cloacal anomalies, gonadal dysgenesis, Turner syndrome, premature ovarian insufficiency, vaginal agenesis and hypospadias.

We will collect and analyse respondents’ perceptions of the availability, access and adequacy of information and support they received since their diagnosis. Sources of information and support included specialist care, general practitioner care, family, friends, peer support groups, written pamphlets and online. Respondents are asked what additional resources they felt would improve their understanding of their diagnosis, examinations, treatments, body diversity, gender, sexuality, relationships, fertility, physical and psychosocial well-being.

The results from the survey analysis will be presented. We anticipate our results will have a direct practical application in the future development of resources for adolescents with DSDs.
P20 Creation of an e-Resource Repository for Differences/Disorders of Sex Development (DSD): Collaboration between Clinicians and Advocates in the DSD Translational Research Network

M.M. Rutter1,2, M. Muscarella1,3, J. Green1,3, G. Indig1,3, A. von Klan1,3, K. Kennedy1,2, E. Rosen3, T. Mazur4, D.E. Sandberg1,5, in collaboration with the DSD Translational Research Network

1DSD Translational Research Network (including Advocacy Advisory Network); 2DSD Center, Cincinnati Children’s Hospital Medical Center and University of Cincinnati, Cincinnati, USA; 3Accord Alliance; 4University at Buffalo, Buffalo, USA; 5University of Michigan, Ann Arbor, USA

Background
Families and individuals affected by Differences/Disorders of Sex Development (DSD) need information and education about their condition, as well as emotional and peer support, to reduce stress and social isolation, participate in patient- and family-centered shared and informed decision-making, and optimize health and quality of life. Barriers to receiving information and support include attitudes and awareness by health providers, biases and relationships between patient and medical communities, quality of educational resources, and the sensitive nature of DSD.

Objective
To create an e-resource repository for people affected by DSD and DSD providers, by collaboration between affected individuals and advocates, and interdisciplinary DSD health providers and scientists.

Methods
The project was a collaboration between advocates, health providers and scientists who were members of the DSD Translational Research Network (DSD-TRN), a network of 10 DSD teams in the US committed to optimizing and standardizing care in DSD. The e-resource repository was developed in three stages: 1) creation of the initial repository by the project team (workgroup of three advocates and one physician), 2) evaluation and feedback of the resources by interdisciplinary teams in the DSD-TRN (a survey by the DSD-TRN Psychosocial workgroup), and 3) achieving consensus. Twitter-like descriptions were written, and resources were categorized by target age, audience, and specific condition.

Results
Forty resources, including educational and informational, peer support and advocacy groups, young adult- and clinician-oriented resources were reviewed and categorized. Seven of 10 centers responded to the survey. Awareness and familiarity about the resources varied between centers from complete lack to full awareness, and teams provided feedback regarding which resources to include. A consensus was achieved when opinions differed, and 30 resources were eventually included in the repository. The repository will be available to the public online and as a printable brochure.

Conclusions
This e-resource repository was created to increase awareness and access to resources that provide information, education and support for those affected by DSD. It represents collaboration and communication between advocates and health providers, an important shift towards transforming care in DSD. As knowledge about DSD grows, it is hoped that the repository will continue to evolve.
DSD information for doctors and healthcare workers from countryside

E.C. Costa¹; C.G. Molina-Bastos²; R.N. Umpierre³; T.P. Hemesath⁴; L.P. de Paula⁵; J.C. Leite⁶; C. Carvalho⁷.


PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA) Federal University of Rio Grande do Sul (UFRGS) - Brazil

**Introduction:** Telessaude is a Project from Epidemiology Post-graduation Program of the Federal University (UFRGS) from the South of Brazil. The principal aim of this Project is provide specific information about health for doctors and healthcare workers to increase assistance. It’s an online platform.

**Report:** Our DSD team support Telessaude to create two questions that were refer to that public. Frist one was about which physical exam findings should suggest DSD in a newborn. After the question we described all findings that indicate when we should start an investigation. It also describes where is the place to refer patients and how to refer. (www.ufrgs.br/telessauders/perguntas/psgenitalia-ambigua/). The second question was when is the right moment to do the civil registration in a newborn with DSD. After this question we emphasized importance to postpone the civil registration until we finished complete investigation (www.ufrgs.br/telessauders/perguntas/ps-ads/). It’s important to say in Brazil family needs civil registration for different reasons, not only legal reasons but there are some social ones too. We also developed a refer protocol to help doctors correct way to refer those patients as quickly they could do.

**Comments:** It’s important to provide information about such specific disease that could easily be identified with physical exam, besides we could prevent social and legal issues if we suggest to postpone civil registration. Refer protocol should be created to help healthcare workers to define best way to do that.
Leydig_cell_hypoplasia_case_report

E.C. Costa; R. Riveiro; L.P. de Paula; J.C. Leite; C. Carvalho; T.P. Hemesath.


PADS – DSD program – Hospital de Clínicas de Porto Alegre (HCPA)
Federal University of Rio Grande do Sul (UFRGS) - Brazil

Introduction: The Leydig cell hypoplasia is a rare form of 46,XY DSD with autosomal inheritance pattern. An inadequate fetal testicular Leydig cell differentiation leads to an incomplete virilisation in such patients. The aim of this paper is to report a case.

Case Report: A newborn was refer to us after 16 days from birth, he presented an undifferentiated external genitalia and hypoglycaemia at birth. Physical examination had shown a micropenis with hypospadias and impalpable gonads. Also had a non-topic anus. Blood analysis was 17 - hidroxiprogesterone: 880ng/dl, total testosterone: 0.41ng/ml, sodium: 138mEq/l, potassium: 5.0mEq/l, progesterone: 1.78ng/ml, LH: 0.5mUI/ml, ACTH: 42pg/ml, cortisol: 5.6microg/ml, androstenedione: 2ng/ml at 19 days from birth. After those results was proposed a stimulation test with hCG, LH:4.4mUI/ml, androstenedione: 1.5ng/ml, total testosterone: 0.35ng/ml and DHT: 49pg/ml previous to the test and total testosterone: 1.02ng/ml, DHT: 104pg/ml and androstenedione: 104pg/ml after test. Karyotype was 46,XY. The ultrasound didn’t show mullerian reminiscent ducts in the abdomen, and shown both gonads on the inguinal channel with testicle appearance. Laparoscopy confirmed the absence of mullerian reminiscent ducts and testicular biopsy was performed. Pathology showed Leydig cell hypoplasia that was confirmed by imuno-histoquemistry test (figures).

Comments: As related in literature after stimulation test with hCG these patient didn’t have total testosterone and androgen precursor increasement. Although pathology and imuno-histoquemistry analysis confirmed the proposed diagnose. Unfortunately in our hospital we don’t have specific molecular analysis for this disease.
Two-year-old child with progressive virilisation

E.C. Costa; C. Carvalho; T.P. Hemesath; R. Riveiro; L.P. de Paula; J.C. Leite.


PADS – DSD program – Hospital de Clinicas de Porto Alegre (HCPA)
Federal University of Rio Grande do Sul (UFRGS) - Brazil

Introduction: The need for molecular confirmation is increasingly present in the investigation of DSDs, being crucial to a better sex designation and genetic counselling.

Case Report: Phenotypically normal newborn of a non-consanguineous couple, female-sex designated, in a small suburb town in South Brazil. Phallus increase was noticed with 2 months of age, and family pediatrician reassured normality with expectant management until 1y-old, when patient was referenced to a pediatric surgeon. Pelvic US had been performed, showing no uterus or ovaries. Also a karyotype was performed - 46,XY, with PCR to SRY detected. Inguinal US images suggested testicles bilaterally. The child was then referred to tertiary university hospital. Our group evaluated with hormonal dosing, reviewed image exams and performed psychological advice. Physical examination had shown virilised genitalia with palpable inguinal gonads (figures). A hCG test was performed and testosterone/dihydrotestosterone ratio was higher than 50 – highly suggestive of 5-alfa-reductase deficiency. Family well insured of the female designation and well informed about future infertility. After psychological evaluation that guarantees family’s comprehension about possible long-term evaluation, team and family agreed to maintain as a girl. So gonadectomy was performed when 3y-old, as well as clitoroplasty and labiaplasty (figure) - AP studies showed testicles and cavernosa bodies. DNA study was performed and revealed an androgen receptor gene mutation - c.2667C>T (Genes studied: SRD5A2).

Comments: Besides being a silent mutation, as no amino acid change occurs, it is a pathogenic mutation due to interfering in splicing process. It was described elsewhere as related to partial androgens insensitivity syndrome, which is now the diagnosis hypothesis. Nevertheless the syndrome was diagnosed after a sex identity was formed, the family will benefit for genetic counseling, because it is an autosomal recessive disease.
P24 Prevalence and Characteristics of Patients with Ovotesticular Disorder of Sex Development in the Disorders/Differences of Sex Development Translational Research Network Registry

M.M. Rutter1,2, L. Hornung1, M.M. Ernst1,2, L. Breech1,2, P. Reddy1,2, K. Kennedy1,2, T. Schafer-Kalkhoff1,2, H. Hoefgen1,2, B. Vanderbrink1,2, C. Sheldon1, J. Howell1, R. Hopkin1,2, H. Saal1,2, J. Johnson1,2, A.H. Antommaria1,2, D.E. Sandberg1,3, E. Vilain1,4, E. Delot1,4, in collaboration with the DSD Translational Research Network.

1DSD Translational Research Network, 2Cincinnati Children’s Hospital Medical Center and University of Cincinnati, 3University of Michigan, 4University of California Los Angeles School of Medicine, USA

Background: Ovotesticular disorder of sex development (OT DSD) is a rare DSD for which many issues remain unresolved, including molecular etiologies, gender determination, and endocrine and surgical care. Outcome data, including psychosocial functioning, is lacking in part because multicenter studies are hampered by the absence of standardized measures for DSD.

Objectives: To assess prevalence and characteristics of patients with OT DSD in the DSD Translational Research Network (DSD-TRN) Registry.

Methods: We reviewed patients enrolled in the DSD-TRN Registry who had a diagnosis of OT DSD. The DSD-TRN is a network of 10 teams in the U.S. committed to optimizing and standardizing care in DSD. Age, race, ethnicity, genetic, sex, endocrine, pathologic, anatomic, and surgical data, and psychosocial risk (assessed by the Psychosocial Assessment Tool) at time of enrolment are reported.

Results: Eleven patients, mean age 7.8 ± 5.9 years, had a diagnosis of OT DSD, giving a prevalence of 4% in the registry. Most were Caucasian (64%) and non-Hispanic/Latino (82%). Most had a 46,XX karyotype. Five (45%) had a genetic diagnosis, including SRY translocation, SOX9 duplication, and sex chromosome mosaicism. Three were in puberty (two male), and one was on testosterone therapy. Hormone concentrations reflected gonadal status and age. Most (73%) had undergone genitourinary surgery (prior to referral to their TRN team in 55%), including gonadectomy (64%). With limited exception, families reported supportive resources and relatively low overall psychosocial risk.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (years)</th>
<th>Karyotype</th>
<th>Sex assigned at birth</th>
<th>Sex legal</th>
<th>Right Gonad</th>
<th>Left Gonad</th>
<th>Uterus Present</th>
<th>PSY PAT Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>46,XX</td>
<td>Male</td>
<td>Male</td>
<td>Testis</td>
<td>Ovotestis*</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
<td>46,XX</td>
<td>Female</td>
<td>Female</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Low</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>46,XX</td>
<td>Female</td>
<td>Female</td>
<td>Ovotestis*</td>
<td>Ovotestis*</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>4</td>
<td>3.4</td>
<td>46,XY</td>
<td>Female</td>
<td>Female</td>
<td>Dysgenetic</td>
<td>Ovotestis</td>
<td>Yes</td>
<td>High</td>
</tr>
<tr>
<td>5</td>
<td>3.9</td>
<td>-</td>
<td>Not assigned</td>
<td>Female</td>
<td>Ovary</td>
<td>Testis</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>6</td>
<td>5.4</td>
<td>46,XX</td>
<td>Male</td>
<td>Female</td>
<td>Ovotestis</td>
<td>Ovotestis</td>
<td>No</td>
<td>Low</td>
</tr>
<tr>
<td>7</td>
<td>10.1</td>
<td>46,XX</td>
<td>Male</td>
<td>Male</td>
<td>Ovotestis</td>
<td>Ovotestis</td>
<td>No</td>
<td>Medium</td>
</tr>
<tr>
<td>8</td>
<td>12.9</td>
<td>-</td>
<td>Female</td>
<td>Female</td>
<td>Streak</td>
<td>Ovotestis</td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td>9</td>
<td>14.1</td>
<td>Mosaic</td>
<td>Male</td>
<td>Male</td>
<td>Ovary</td>
<td>Testis</td>
<td>Yes*</td>
<td>Low</td>
</tr>
<tr>
<td>10</td>
<td>15.4</td>
<td>46,XX</td>
<td>Male</td>
<td>Female</td>
<td>Ovotestis</td>
<td>Ovary</td>
<td>Yes**</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>15.5</td>
<td>Mosaic</td>
<td>Male</td>
<td>Male</td>
<td>Testis (US)</td>
<td>Ovotestis</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

PSY, Psychosocial; PAT, Psychosocial Assessment Tool; *Gonadoblastoma; NA, Not assessed; “-“, Not available; US, Ultrasound (otherwise pathologic diagnoses for gonads); *Right remnant; **Duplication with left remnant

Conclusions: Prevalence of OT DSD in the DSD-TRN Registry is in keeping with some published reports. However, a genetic diagnosis was established in a high proportion of patients. Ongoing prospective, interdisciplinary, multicenter collaboration with standardization of physical and psychosocial assessments is important for optimizing decision-making and care, and ultimately health and well-being, in patients with OT DSD.
**P25: 45,X/46,XY mosaicisms in Male: Long-Term outcomes. A multicenter Italian-DSD Study Group survey.**

F.Baronio a, S.Bertelloni b, S.Pedicelli c, G.Russo a, C. Bizzarri c, M.Cappa c, S.Ciccone c, E.Dati f, L.Ghizzoni g, P.Grammatico h, N.A. Greggio i, L.Mazzanti i, S.Meroni i, R.Ortolano j, M.Salerno i, S.Scommegna i, M.R. Stancampiano a, G. Ubertini c, A.Balsamo a, On behalf of It-DSD Study Group, Multidisciplinary National Network, Italy

a Department of Medical and Surgical Sciences, Pediatric Unit, Center for Rare Endocrine Conditions (CARENDO BO), S.Orsola-Malpighi University Hospital, Bologna; b Adolescent Medicine Unit, Division of Pediatrics, S.Chiara Hospital, University of Pisa, Pisa; c Hospital of Empoli, Empoli; c Unit of Endocrinology and Diabetes, Bambino Gesù Children's Hospital, Rome; d Department of Pediatrics, Endocrine Unit, Scientific Institute San Raffaele, Milan; e Department of Pediatrics, Endocrine Unit, “Bufalini” Hospital, Cesena; f Department of Medical Sciences, Division of Endocrinology, Diabetes, and Metabolism, University of Turin, Turin; g Sapienza University and h) San Camillo-Forlanini Hospital, Rome; i Pediatric and Adolescent Endocrinology Unit, Padua University Hospital, Padua; j Department of Translational Medical Sciences, Pediatric Endocrine Unit, University of Naples Federico II, Naples; on behalf of It-DSD Study Group, Multidisciplinary National Network; ITALY;

**Background:** People carrying 45,X/46,XY mosaicism and variants may present with large phenotypic differences. In patients with a main male phenotype, impaired genital and auxological development have been reported, but few data are available on long-term outcomes. **Objective:** To describe the long-term outcomes (i.e. gonadal function, adult height and co-morbidities) in a group of 24 males with 45,X/46,XY mosaicism and variants, recruited within a study project developed by the Italian “It-DSD Study Group” (www.gruppodistudio-it-dsd.org/). **Methods:** Multicenter retrospective study by specific developed electronic data-sheet. Only post-pubertal patients who reached adult or near adult (1 case) height were included. **Results:** In total, 24 males from 9 Italian Centers were enrolled [mean age at the last evaluation 23.3 years (range 13.5–70 years)]. Thirty-seven percent (9/24) of the patients presented a “classical” 45,X/46,XY karyotype in blood (7/24) or gonadal tissue (2/24); 63% showed an abnormal structure or duplication of the Y chromosome. External masculinization score (EMS) at time of diagnosis was 8.0 (range 2–12). Six patients (25.0%) had a score of 12 (complete external virilization). Nineteen patients (79.1%) entered puberty spontaneously. Seven (29.1%) had received testosterone treatment. Eight out of 17 untreated patients (47.0%) presented signs of declined testicular function at the end of puberty (increased levels of FSH and low levels of testosterone). Thirteen patients (54.1%) had received growth hormone (GH) treatment, started at an age of mean 10.9 (range 6-13.9 years), for a mean period of 5.6 years (range 1.9 - 9 years) at a dose of 20-40 mcg/kg/d. Whole group final height was 155.8 cm (range 140.0–167.9; mean Delta FH-TH -2.7 SDS, range -1.2 to -4.8 SDS), with no significant difference between GH Treated 158.3 ± 1.487 vs. GH Not treated 152.9 ± 2.895 cases (p=0.0951) . Five patients (20.8%) had renal disease/abnormalities and 1 (4.1%) had congenital cardiac malformations. One patient (4.1%) had a gonadal tumor and apparently no one had precursor lesions. **Conclusions:** In summary, most of 45,X/46,XY children born with ambiguous genitalia and raised as boys entered puberty spontaneously, but they showed an altered pubertal course. All of them, independently from genital phenotype, have adult short stature and did not benefit significantly from GH therapy, at least at the used doses. Several patients were affected by renal/urinary abnormalities, which must be taken into consideration for a holistic management. The prevalence of gonadal neoplasia in situ or tumors appears to be low in this series, although the histology was available in a limited number of cases. The multidisciplinary-multicentric structure of the It-DSD Study Group permitted to recruit a relatively large number of Italian 45,X/46,XY mosaicism cases; it may represent a national base for collaborative studies in the field of sexual development and an opportunity - in collaboration with official Societies – for the European Reference Network for rare endocrine conditions (ENDO-ERN).
**P26 Bilateral Testicular Regression Syndrome: a cohort of 22 patients**

_C. Brachet¹, E. Boros¹, S. Luyckx², K. Khelif², S Tenoutasse², C. Heinrichs¹_

¹Paediatric Endocrinology Unit and ²Paediatric Urology Unit

Hôpital Universitaire Des Enfants Reine Fabiola U.L.B.

Avenue J.J. Crocq,15

1020 Brussels, Belgium

Aim: Clinical description of a cohort of patients with Testicular Regression Syndrome. More specifically, we aim to look for associated clinical features, review diagnostic procedures and response to treatment.

Methods: Retrospective study of the clinical files of 22 patients presenting with bilateral Testicular regression syndrome followed-up in the Paediatric Endocrinology Unit of HUDERF.

Results: 3/21 patients are from consanguineous families. 10/22 patients have at least one parent originating from Maghreb. 21/22 are born after spontaneous pregnancies. 2/19 patients are born SGA. 2/22 have a family history of cryptorchidism or unilateral testicular absence.

Only 1 boy from consanguineous parents had associated clinical features (hydronephrosis, PDA and dental anomalies).

The absence of testicular tissue was ascertained by exploratory surgery in 10 patients, hCG stimulation test without exploratory surgery in 3 patients and AMH and gonadotropin dosage without surgery in 9 patients (all born after 2008).

13/22 patients had a stretched penile length inferior to 30 mm and received testosterone treatment to increase penile length in infancy. Penile length increased from 26.9 ± 6 mm to 40.3 ± 8 mm after depot Testosterone (Sustanon *) treatment 25 mg IM monthly for a total of three injections except in one patient who had severe micropenis (13mm penile length) and required a total of six 50 mg monthly injections to normalise penile length.

Conclusion: Bilateral Testicular Regression Syndrome is a rare endocrine condition that can nowadays be easily ascertained by undetectable AMH serum level. More than half of the patients present with undervirilisation which is amenable to penile length normalisation with only modest doses of depot Testosterone. In our experience, most young boys did not desire testicular prosthesis insertion. A long-term follow-up study of this cohort is planned to inquire about ongoing medical follow-up, testicular prosthesis insertion/satisfaction, disease knowledge by the patient.
P27 The sensitivity and specificity of the test with gonadoliberin in diagnosing of hypogonadotropic hypogonadism in boys.

O. Latyshev, L. Brzezinski, L. Samsonova, E. Kiseleva, G. Okminyan, E. Kasatkina
Russian Medical Academy of Continuous Professional Education, Moscow

Objective. We examined the sensitivity and specificity test with gonadoliberin (Diferelin 0,1 mg) in diagnosing of hypogonadotropic hypogonadism in boys.

Materials and methods. The study included 25 boys with delayed puberty. We evaluated anthropometric indicators, bone age, testicular volume, testosterone, inhibin b, antimullerian hormone, LH, FSH. We conducted a test with gonadoliberin, it was positive when LH increased more than 9 mU/l. All patients were examined after 2 years (second medical visit). We evaluated the testicular volume. The start of puberty was determined by the testicular volume on ultrasound (≥3 cm³). The patients were divided into two groups: the first group had ≥3cm³ Median/Me 6,5 [5,5;7,7], there was a group with constitutional delay of puberty CDP (n=20) and the second group had <3cm³ Me0,5[0,4;0,8], there was a group with hypogonadotropic hypogonadism (n=5).

Results. At the first visit all patients had the same height (Me Ht-SDS -1,1 vs-0,1, p=0,7), weight (Me SDS BMI 0,2 vs 1,4, p=0,2), bone age (Me SDS -1,8 vs 0,3, p=0,09) and antimullerian hormone (Me 24,1 vs 22,4 pg/ml, p=0,4), testosterone (Me 1,2 vs 0,9 nmol/l, p=0,2). However, in boys with CDP, testicular volumes was much more (Me 1,3 vs 0,4 cm³, p=0,004 ) and hormones were significantly higher, such as, LH (Me 1,1 vs 0,1 mIU/ml, p=0,006), FSH (Me 2,9 vs 0,2 lU/l, p=0,001), inhibin b (Me 129,5 vs 31,3 pg/ml, p=0,0008) than in boys with hypogonadotropic hypogonadism.

In the first group of patients with CDP 90% boys (18/20) had a true positive result in test with diphereline (0,1 mg). In the second group of patients with hypogonadotropic hypogonadism 100% boys (5/5) had a true negative result in this test. Moreover, boys in the second group had clinical and laboratory symptomatic, which often combine with hypogonadotropic hypogonadism: one boy had the defect of the gene Prop-1, one boy- the Prader-Willie syndrome, two boys- the anosmia, one boy – the hyposmia.

Conclusion. The test with gonadoliberin (Diferelin 0,1 mg) has a sensitivity of 90% and a specificity of 100% in diagnosing of hypogonadotropic hypogonadism in boys. We need to continue to study the testicular volume, inhibin b, LH, FSH in diagnosing of hypogonadotropic hypogonadism in boys.
P28 The COPENHAGEN Minipuberty Study – a cohort of infants and their parents

A.S. Busch1,2,*, M.L. Johansen1,2,*, N. Kolby1,2, C.P. Hagen1,2, A. Juul1,2

1Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark
2International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Denmark

*to be considered as joint first authors

INTRODUCTION: Minipuberty is a term used to describe this transient activation of the hypothalamic–pituitary–gonadal axis (HPG) axis in early life. The biological rationale behind infants reaching adult levels of reproductive hormones is not known, nor is the exact timing of the peak or the factors determining the subsequent hormonal suppression. Data suggests that minipuberty may serve as a diagnostic window for endocrine disorders. However, no study to date has directly compared healthy infants with infants under suspicion of having or having an endocrine disorder, e.g., Disorders of sex development (DSD).

OBJECTIVES AND HYPOTHESIS:

The aim of this project is compare normal minipuberty with minipuberty in patients with DSD. We suggest that early postnatal activation of the HPG hormone axis (minipuberty) reflects adult reproductive function. This is the first study to evaluate the marked dynamic changes of the HPG axis in early human life by serial blood and urine sampling and clinical examinations in a large cohort of healthy infants. We will investigate genetic factors involved in regulation of minipuberty in both boys and girls, and evaluate the epigenetic profile at birth as well as changes throughout minipuberty.

METHODS:

In this prospective, longitudinal cohort we aim to recruit 200 healthy infants and their parents. Furthermore, all children under suspicion of or with a confirmed DSD diagnosis under the age of 6 months referred to our outpatient clinic will be invited to participate along with their parents. Healthy infants will be examined 6 times in the one-year follow up period. Clinical examinations of all children (healthy and DSD) will include: anthropometrics, pubertal staging, classification of external genitalia, measurement of ano-genital distance (AGD) as well as blood- and urine sampling. Examinations will be performed as follows:

STATUS AND PERSPECTIVES: The cohort recruitment has commenced in autumn 2016. By spring 2017 a third of the expected participants have been recruited. Knowledge of the genetic and epigenetic control mechanisms of minipuberty will aid the understanding of reproductive physiology and in particular DSD pathology.
Introduction: Since normal male sexual differentiation involves a greater number of genetically determined events compared to the female one, Disorders of Sex Development (DSD) with genital ambiguity and 46,XY karyotype present a greater complexity for its etiological definition. Partial Androgen Insensitivity Syndrome (PAIS) may present clinical manifestations indistinguishable, mainly, in relation to 5α-reductase Deficiency (5AR2D). Anti-Mullerian Hormone (AMH) levels have been shown to be of great value in the evaluation of these patients. However, other Leydig and Sertoli cell markers were not evaluated in this group of patients. Objectives: To evaluate the function of Leydig and Sertoli cells in patients with genital ambiguity, 46,XY karyotype and normal testosterone (T) secretion. Methods: 35 patients were included, 8 with PAIS, 8 with 5AR2D and 19 idiopathic from the Outpatient Clinic of the GIEDDS-UNICAMP. All of them had already been evaluated at diagnosis and had normal T secretion. Patients with PAIS and 5AR2D had a confirmed molecular diagnosis. For the control group, 42 male healthy individuals were included. All patients underwent a further evaluation of basal levels of LH, FSH, AMH, Inhibin B and INSL3, and T and dihydrotestosterone (DHT) basal and after human chorionic gonadotropin stimulation, when necessary. Results: The age at the evaluation was not statistically different between the cases and controls group (p = 0.595). T (p = 0.122), DHT (p = 0.485), T/DHT (p = 0.989), LH (p = 0.169) and FSH (p = 0.320) did not differ among the three groups of 46,XY DSD. AMH was inversely proportional to age, with a moderate correlation, both in the total group of cases (r = -0.68, p < 0.0001) and in the control group (r = -0.83; p < 0.0001) with levels significantly lower in the total group of cases when compared to the control group (p = 0.031). Inhibin B levels were also significantly lower in the total group of cases compared to the control group (p < 0.001) and in 5AR2D subgroup in relation to PAIS and idiopathic subgroups, and in the idiopathic one when compared to the control group. On the other hand, INSL3 levels were significantly higher in the total group of cases when compared to controls (p = 0.003). Conclusions: Serum levels of AMH, inhibin B and INSL3 were generally no different among the three subgroups of 46,XY DSD with normal T secretion, but inhibin B levels were lower in patients with 5AR2D and idiopathic subgroups when compared to the controls. Our study was the first to evaluate the other major testicular hormones only in patients with 46,XY DSD with normal T secretion. It was also the first to show that patients with 5AR2D have lower serum inhibin B levels.

P30 Comparison between two inhibin B ELISA assays in 46,XY testicular DSD with normal production of testosterone.

G. Guaragna-Filho¹, A. R. Calixto², G. B. De Paula¹, L. C. De Oliveira³, A. M. Morcillo⁴, M. P. De Mello⁵, A. T. Maciel-Guerra¹, G. Guerra-Junior¹

¹ Interdisciplinary Group of Study of Sex Determination and Differentiation (GIEDDS), School of Medicine (FCM), State University of Campinas (UNICAMP), Campinas, Brazil
² Laboratory of Metabolism and Diabetes Investigation (LIMED), UNICAMP, Campinas, Brazil
³ Laboratory of Physiology - Hospital de Clínicas, UNICAMP, Campinas, Brazil
⁴ Department of Pediatrics, School of Medicine (FCM), UNICAMP, Campinas, Brazil
⁵ Center of Molecular Biology and Genetic Engineering (CBMEG), UNICAMP, Campinas, Brazil

Abstract

Introduction: Inhibin B is a hormone produced by the Sertoli cells which can provide important information for the investigation of the Disorders of Sex Development (DSD) 46,XY. Objective: To compare two ELISA assays for dosage of serum inhibin B in patients with DDS 46, XY with normal testosterone production. Methods: Twenty-nine patients with DDS 46, XY and normal testosterone production (Partial Androgen Insensitivity Syndrome n = 8; 5α-Reductase Deficiency n = 7 and Idiopathic 46,XY DSD n = 14) were included. Molecular analysis of Androgen Receptor (AR) and SRD5A2 genes were performed in all patients and NR5A1 in the Idiopathic group. Dosages of Inhibin B were performed by two second generation ELISA assays (Beckman-Coulter e AnshLabs). Assays were compared using the Interclass Correlation Coefficient (ICC) and the Bland-Altman method. Results: A ICC of 0.915 (95% CI: 0.828 - 0.959) was found, however it was observed a discrepancy between the trials which is more evident in the higher values when analysed by the Bland-Altman method. Conclusion: It is recommended to perform the inhibin B dosages always using the same ELISA kit when several evaluations are required for the same patient.
P31 Case report: Aromatase deficiency in two adult female siblings

1 Department of Endocrinology, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium;
2 Center for Medical Genetics, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium;
3 Center for Sexology and Gender, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium;
4 Department of Pediatric Endocrinology, Pediatrics and Genetics Research Unit, Ghent University Hospital and Ghent
University, De Pintelaan 185, 9000 Ghent, Belgium.

INTRODUCTION
Aromatase deficiency (AD) results from an inactivating mutation in the aromatase gene, leading to absent or decreased
conversion of androgens (testosterone and androstenedione) to estrogens (estriol and estrone). The aromatase enzyme
protects the fetus against the virilizing action of fetal adrenal androgens. In addition, aromatase plays a role in the central
nervous system, in programming the brain during fetal and neonatal life for noncyclic hypothalamic GnRH function and gender
identity. Few cases with AD have been reported, of which only four are adult women.

METHODS
We report genetic, clinical, biochemical and bone mineral density findings in two adult sisters, 39 and 45 years old with a
genetically confirmed diagnosis of AD in adulthood.

RESULTS
Both sisters, 46, XX, had virilized genitalia (Prader V, EMS 6/12) at birth; the oldest was first assigned male, but was reassigned
female based upon presence of ovaries and uterus. They both recall start and course of puberty as unremarkable, including
occurrence of regular menses. At the age of 39, the youngest sister had experienced an ovarian torsion. Subsequently,
both sisters were referred to our DSD center for endocrine work-up at ages 39 and 45 years. At physical examination, they had
typical breasts, which had developed at the age of 11.5 and 12 years, and they had had cyclic bleeding through the urethral
opening. There were no signs of hirsutism. Menopause occurred at the age of 39 in the oldest sister and 35 in the youngest.
The oldest sister was 165cm tall, BMI was 25.5kg/m². The youngest sister was 168cm tall, with a BMI of 24.5kg/m². Calculated
target height was 158cm ± 8.5cm. Laboratory analysis (table 1) showed menopausal levels of estradiol, follicle stimulating
hormone, luteinizing hormone and anti-Mullarian hormone, low levels of 17-hydroxy progesterone and normal levels of
testosterone, dehydroepiandrosterone sulfate and D4 androstenedione. Cholesterol levels were slightly elevated in both
sisters. Areal bone mineral density (aBMD), assessed by DEXA scanning, showed a decreased aBMD at the level of the lumbar
spine in the oldest sister (Z-score -2.2) and a normal aBDM at the level of the spine, femur and femoral neck in the youngest
sister.

Sequencing of the CYP19A1 gene revealed a novel homozygous substitution c.1124G>A leading to a missense variant
p.Arg375His, predicted to be deleterious. General biochemistry and data on cardiovascular risk profile and bone densitometry
results are summarized in Table 1.

CONCLUSION
Due to long-term exposure to increased androgens and decreased estrogens, adult women with AD are at risk of developing
polycystic ovaries or metabolic syndrome. AD has also been associated with low bone mass. We here describe two sisters with
AD who were severely virilized at birth but who had, apart from early menopause, apparently no medical problems,
underscoring the variability of the phenotype in adulthood.

| Table 1: Biochemical Findings of Subjects with CYP19A1 Mutations |
|---------------------------------|-----------------|-----------------|
| Estradiol (E2) (<138)            | <25.0 ng/L      | <25.0 ng/L      |
| Testosterone (8.4-48.1)          | 21.54 ng/dL     | 30.81 ng/dL     |
| Follicle stimulating hormone (>26)| 92 U/L           | 130 U/L         |
| Luteinizing hormone (8 – 59)     | 61 U/L           | 25 U/L          |
| 17 Hydroxy progesterone (0.1-1.98)| <0.2 mg/mL       | <0.2 mg/mL      |
| Anti-Mullerian hormone (<2.96)   | 0.045 µg/L       | <0.03 µg/L      |
| Dehydroepiandrosterone sulfate (DHEAS) (60.9 – 337) | 260.1 µg/dL | 272.1 µg/dL |
| D4 androstenedione (13 – 82)     | 79.7 ng/dL       | 61.3 ng/dL      |
| Total cholesterol (147 - 253)    | 255 mg/dL        | 240 mg/dL       |
| HDL cholesterol (20 - 35)        | 27.8 %           | 25.6 %          |
| LDL cholesterol (80 - 191)       | 165 mg/dL        | 160 mg/dL       |
| Triglycerides (47 – 209)         | 97 mg/dL         | 97 mg/dL        |
P32 Sexual dysgenesis disorder in the structure of delayed puberty in females

Objectives. To study the structure of sexual dysgenesis disorder in females with delayed puberty.

Methods. 30 girls, 14.9 ± 0.7 years, with delayed puberty were examined. Entry criteria were the absence of secondary sex characteristics at 13 and/or if the menarche failed to occur by the age of 15.

Puberty stage according to Tanner, anthropomorphic and genitometric parameters, bone age, follicle – stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estradiol levels in blood serum, results of genetic and histological examination.

Results. Depending on the levels of gonadotrophic hormones patients were divided into two groups: high (46.7%, 14/30; Me LH 24.3 mIU/ml; Me FSH 65.85 mIU/ml) and normal/low level (53.3%, 16/30; Me LH 3.43 mIU/ml; Me FSH 3.68 mIU/ml).

The reasons to visit a doctor in group with hypergonadotropic hypogonadism were the absence of secondary sex characteristics in 71.4 % (10/14) of patients and primary amenorrhea in 28.6% (4/14, р = 0.0176).

The girls with hypergonadotropic hypogonadism had female external genitalia, the Mullerian duct derivatives (Me of uterus size 2.28 cm³, 0.64 cm³ ÷ 8.4 cm³). The gonadals were not visualized of 57.14% (8/14). Me Tanner breast development stage 1,0 (1.0 ÷ 3.0), Me Tanner stages of development of pubic hair 1,0 (1.0 ÷ 5.0).

Pathological growth retardation had 35.7% (5/14) of females, delayed bone age occurred 43% (6/14) of patients.

Sex hormones levels in blood serum: Me of estradiol 13.75 pmol/l (2.0 pmol/l ÷ 82.9 pmol/l) and Me of testosterone 0.48 nmol/l (0.17 nmol/l ÷ 5.2 nmol/l).

Chromosome analysis was not conducted in one case of hypergonadotropic hypogonadism, in other cases 46.2% (6/13) of female patients had monosomy X, which corresponds to chromosome disorder of sexual dysgenesis – Turner syndrome, 30.8% (4/13) had karyotype 46, XY and 23% (3/13) 46, XX, allowing to diagnose pure gonadal dysgenesis in 53.8 % (7/13) of patients.

Two patients with karyotype 46, XY were performed a bilateral gonadectomy. In one case gonadoblastoma was diagnosed, in the other - dysgerminoma in combination with gonadoblastoma. The stage of sexual development according to Tanner of both girls is B3 P3, levels in blood serum of testosterone is 4.6 nmol/l and 5.2 nmol/l, of estradiol is 36.65 pg/ml and 44.81 pg/ml correspondingly.

Conclusion. Sexual dysgenesis disorder in the structure of the delay puberty in females is presented by the following variants: Turner syndrome and pure gonadal dysgenesis with karyotype 46, XY and 46, XX (46.2% vs 53.8%). High risk of neoplastic transformation in patients with pure gonadal dysgenesis with karyotype 46, XY identifies the need for bilateral gonadectomy. Estrogen-dependent signs of pubertal in patients with gonadoblastoma can mask gonadal dysgenesis and contribute to a later diagnosis of this pathology.
P33 Anogenital distance as a phenotypic signature through infancy: A longitudinal study of 689 children

Authors: L Priskorn, JH Petersen, N Jørgensen, HB Kyhl, MS Andersen, KM Main, AM Andersson, NE Skakkebæk and TK Jensen

Affiliations:
1Department of Growth and Reproduction and EDMaRC, Rigshospitalet, University of Copenhagen, DK-2100 Copenhagen, Denmark
2Department of Biostatistics, University of Copenhagen, DK-2100 Copenhagen, Denmark.
3Odense University Hospital, Hans Christian Andersen Children’s Hospital, DK-5000 Odense, Denmark
4Odense Patient data Explorative Network (OPEN), Odense University Hospital, DK-5000 Odense, Denmark
5Endocrinological Department, Odense University Hospital, DK-5000 Odense, Denmark
6Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, DK-5000 Odense, Denmark

Presenting author: Katharina M Main

ABSTRACT

Background: Anogenital distance (AGD) has been suggested to represent a phenotypic signature reflecting in utero androgen action. However, it is not known whether an individual’s AGD at birth correlates to AGD later in life.

Methods: In 689 children we measured AGD from the anus to the scrotum (AGDas) and the penis (AGDap) in 407 boys, and to the posterior fourchette (AGDaf) and the clitoris (AGDac) in 282 girls. Each measure was repeated three times at 3 and 18 months and a subgroup of children were furthermore examined by two different examiners. We assessed age related changes and reproducibility of measurements by calculating paired intra-class correlation coefficients and variance components.

Results: AGD was almost twice as long in boys compared to girls. For all AGD measures, an increase was observed between the two examinations and AGD correlated within the child. A large proportion of the observed variation in AGD was due to true differences between the children (62% for AGDas, 40% for AGDap, 30% for AGDaf and 21% for AGDac), and measurement error due to between- and within-examiner variation was low.

Conclusions: Our study showed that AGD measurements were well reproduced within and between examiners when the child was measured in the same position. Measures of AGD within a child correlated well during infancy, especially in boys and particularly for AGD measured as the distance between anus and scrotum. A planned follow-up of our cohort through childhood and puberty will reveal whether AGD represents a phenotypic signature throughout life.
A Single Centre Experience of Differences/Disorders in Sex Development (DSD) over 20 years

Elim MAN, GOSH DSD MDT, John C ACHERMANN

Introduction: Differences/Disorders in Sex Development (DSD) represent a diverse range of conditions that can present at various stages of life. A multidisciplinary team (MDT) approach is required to reach a specific diagnosis and management plan, but few large single centre studies of the range and prevalence of diagnoses have been undertaken.

Method: Medical records of all children with DSD discussed at a single MDT between 1-January-1996 and 31-December-2015 (n=580) were retrospectively reviewed to evaluate the referral patterns and diagnoses; clinical features, biochemical data and genetic analyses were considered.

Results: A total of 271 (1996-2005) and 309 (2006-2015) children were discussed in two respective decades. The relative proportions of DSD categories were similar across two decades (Sex chromosome DSD [SCDSD]: 8.1%/12.0%; 46,XX DSD: 29.2%/25.9%; 46,XY DSD: 62.7%/62.1% in 1996-2005 and 2006-2015 respectively). Overall, 300/580 of children were discussed in their first year of life, with an increase in the number and proportion of children referred earlier in the second decade (2006-2015) (185 versus 115; 59.8% versus 42.4%). Specific diagnoses among children discussed in their first year of life for SCDSD (n=30) included 45,X/46,XY and X variants (n=21), 47,XXY variants and children with 46,XX/46,XY chimerism (n=3). Most children with 46,XX DSD (n=87) had congenital adrenal hyperplasia (CAH) (n=58), the rest having ovotesticular DSD (n=4), isolated clitoromegaly or cloacal anomalies. Among 46,XY DSD (n=183), specific molecular diagnoses were reached in 39 children (including disease-causing variants in SF1, WT1, SOX9, STAR, HSD3B2, HSD17B3, AR, and SRD5A2). Most (n=112) of the others were 46,XY boys with severe hypospadias and often associated features including intra-uterine growth restriction, and/or renal, cardiac or syndromic anomalies. Relatively few 46,XY females presented in infancy (30/109), but a specific molecular diagnosis due to pathogenic genetic variants was reached in 20/23 46,XY females born within the last 10 years.

Conclusion: Children with a heterogeneous range of conditions were referred to the DSD MDT at our centre. Understanding the relative prevalence of those conditions is very valuable. Molecular analysis is useful in reaching a specific diagnosis and directing management, but the underlying pathophysiology in most 46,XY children with severe hypospadias currently remains unknown, and could include genetic, epigenetic, and/or environmental factors.
Disorders of sex development (DSD) are a group of congenital developmental disorders in which the chromosomal, gonadal, or anatomical sex is atypical. The clinical diagnosis and management of DSD are difficult and complex because of the various aetiology and diverse manifestation. The project “Genetics of Human Disorders of Sexual Development” is funded by Swiss National Science Foundation and fulfilled by the University of Geneva Medical School (Switzerland), the Medical Centers from Armenia, Poland and Ukraine. The goal is to identify mutations underlying unresolved DSD phenotypes – in novel DSD genes, or regulatory regions that lead to atypical gene expression. Identification of new genes involved in human sex determination and differentiation is carried out through exome sequencing and CGH microarray in parallel. Armenian partner is participating in all stages preceding the exome sequencing: clinical data collecting (caryotype, family history, physical examination, ultrasound, hormonal status, surgery, histology), caryotyping, DNA samplings (proband, parents, siblings), SRY gene deletion detecting and SRY, SOX9, WT1, SF1, LHX9, RSPO1, FOXL2, WNT4, DMRT1, DMRT2 genes Sanger sequencing.

During 4 years Armenian part collected 28 DNA samples from 28 DSD cases – 5 patients with 46,XY complete sex reversal and ovarian development, 13 patients with 46XY partial gonadal disgenesis, 4 patients with 46XY ovotestes, 5 of 46,XX DSD male (two of them are twins), and 1 patient with 45X(inv9)P13q13 with testes development. We also collected DNA samples from parents. We expect the research will provide the opportunity to develop new genetic tests for DSD diagnosis and to improve understanding of the molecular mechanisms of ovarian and testicular differentiation. The results on the cohort will be discussed in details.

SCOPES 2013-2017: Joint Research Projects
Normal and dysregulated human fetal adrenal development and function: Establishment of a dynamic ex vivo culture model for congenital adrenal hyperplasia

C. Melau1,2, J.E. Nielsen1,2, H. Frederiksen1,2, AM. Andersson1,2, R.T. Mitchell3, A. Juul1,2, A. Jørgensen1,2

1Department of Growth and Reproduction, University Hospital of Copenhagen (Rigshospitalet), Denmark. 2International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, Denmark. 3MRC Centre for Reproductive Health, The Queen’s Medical Research Institute, University of Edinburgh, UK.

The human adrenal cortex has important endocrine functions already during early fetal development resulting in severe effects when adrenal steroidogenesis is dysregulated as seen in congenital adrenal hyperplasia (CAH). Despite this, early human fetal adrenal steroid metabolite profiles and levels of androgen secretion have not yet been completely characterized. Therefore, the aim of this project is to increase the understanding of adrenal function in human fetal development by systematically characterizing the expression pattern of key steroidogenic enzymes and the levels of androgens, glucocorticoids and mineralocorticoids produced in human fetal 1st and 2nd trimester adrenals. Secondly, we aim to establish a novel ex vivo culture model allowing functional studies to examine how alterations in steroidogenesis manifest within human fetal adrenals. Upon establishment of the ex vivo culture approach we will develop an adrenal CAH model by conducting lentivirus mediated knock-down of the CYP21A1 gene, which is mutated in more than 90% of CAH cases.

The initial characterisation of human fetal adrenals is enabled by the available human fetal adrenal tissue from 1st and 2nd trimester stored in the biobanks in Copenhagen and Edinburgh, respectively. This material will be used to determine the expression level and pattern of steroidogenic enzymes by qPCR and immunohistochemistry (IHC) as well as the tissue levels of androgens, glucocorticoids and mineralocorticoids measured by LC-MS/MS. Furthermore, our well-established ongoing collection of human fetal adrenal tissue and the ex vivo culture approach, which has previously successfully been used to culture human fetal testes and ovaries, will be used to establish a novel human fetal adrenal ex vivo culture model. The adrenal tissue will be divided into 1 mm^3 tissue fragments and set-up in ‘hanging drop’ cultures for up to two weeks. Endpoints include collection of tissue for morphology assessment, IHC or qPCR as well as collection of media throughout the experimental period to determine steroid secretion.

The initial experiments indicate that ex vivo culture of human fetal adrenal is possible and our current work to establish and validate this model includes optimization of culture media composition, evaluation of tissue morphology as well as detection of markers for proliferation (Ki67 and BrdU incorporation) and apoptosis (cleaved PARP and cleaved caspase 3). Secretion of androgens, glucocorticoids and mineralocorticoids will be determined by LC-MS/MS in culture media collected throughout the experimental period. As an initial proof of principle that steroidogenesis can be manipulated in this ex vivo model androgen secretion will be stimulated by addition of ACTH and forskolin to the media, while abiraterone will be used as an inhibitor.
**17B-HYDROXYSTEROID DEHYDROGENASE TYPE 3 DEFICIENCY: A MULTICENTER ITALIAN-DSD STUDY GROUP SURVEY**

S. Meroni\(^a\), S. Bertelloni\(^b\), R. di Mase\(^c\), A. Balsamo\(^d\), L. Balazzetti\(^d\), F. Baldinotti\(^b\), M. Salerno\(^c\), P. Marchese\(^b\), M.R. Stancampiano\(^c\), C. Ungaro\(^c\), G. Russo\(^a\), On behalf of It-DSD Study Group, Multidisciplinary National Network, Italy

\(^a\) Department of Pediatrics, Endocrine Unit, Scientific Institute San Raffaele, Milan; \(^b\) Adolescent Medicine Unit, Division of Pediatrics, S. Chiara Hospital, University of Pisa, Pisa; \(^c\) Department of Translational Medical Sciences, Pediatric Endocrine Unit, University of Naples Federico II, Naples; \(^d\) Department of Medical and Surgical Sciences, Pediatric Unit, Center for Rare Endocrine Conditions (CARENDO BO) S. Orsola-Malpighi University Hospital, Bologna; on behalf of It-DSD Study Group, Multidisciplinary National Network, Italy.

**Introduction:** 17β-hydroxysteroid dehydrogenase type 3 (17βHSD3) deficiency is a very rare autosomal recessive DSD in Western countries. It’s due to impaired testicular conversion of androstenedione (A) to testosterone (T) because of mutations in 17βHSD3 gene on chromosome 9. 46,XY homozygotes or compound heterozygotes have testes, normally developed Wolffian duct derivatives, but undervirilization of external genitalia. The diagnosis can be suspected if the T/A ratio is < 0.8. The risk of misdiagnosis is especially problematic because the clinical findings in 17βHSD3 deficiency may mimic androgen insensitivity syndrome in childhood and 5a-reductase deficiency in puberty. **Materials and methods:** multicenter retrospective analysis by specific developed electronic data-sheet of clinical history, endocrine and genetics evaluation and sex assignment of 11 Italian patients with diagnosis of 17β-HSD3 deficiency, recruited from 2003 to 2016 within a study project of the Italian “It-DSD Study Group”.

**Results:** a total of 11 children with 17β-HSD3 deficiency were recruited from 4 centers. Patients have been diagnosed with 17β-HSD3 deficiency from pregnancy (discordance between 46,XY karyotype and ultrasound appearance of female external genitalia; 18%) to childhood (inguinal hernia with testes ± clitoridomegaly; 55%) and adolescence (virilization; 27%). Endocrine data before gonadectomy (T/A ratio on baseline or after hCG stimulation during infancy) was available for 7 cases: they are informative in all but one cases with mean T/A ratio 0.32 (range 0.16-0.55). In pubertal patients increased LH ± FSH values at baseline were seen, indicating an impairment of the pituitary regulatory control by the gonadal hormones and/or testicular damage. Imaging studies revealed the absence of Mullerian structures and the presence of abdominal/inguinal/labial testis. In all patients female sex was assigned before diagnosis. In 9 subjects female sex was confirmed and gonadectomy was performed in infancy or at puberty; in 2 patient the psychological evaluation is still ongoing. In 2 patients, identified at puberty, temporary GnRH analog treatment was started to suppress androgen secretion and to provide time to confirm or change birth sex assignment. Histological reports of the testes from 4 subjects were available and showed testicular tissue in 3 cases (in 1 case with microcalcification) and sex cord- stromal tumor of the left testicle in 1 case. Molecular analysis of 17β-HSD3 gene was available for 9 cases and confirmed the diagnosis in all but one cases. In this patient molecular analysis showed only heterozygosis for IVS325+4 A>T mutation, despite a clinical history (virilization at puberty) and a T/A ratio (0.45) strongly suggestive for 17βHSD3 deficiency. **Conclusion:** in our country the frequency of 17βHSD3 deficiency seems to be extremely rare, but it is often misdiagnosed because it is clinically indistinguishable from other forms of 46,XY DSD. It may present from pregnancy to puberty for different clinical issues: suspicion should be present for any female who presents inguinal hernia or mild clitoridomegaly in infancy or early childhood as well as virilization at puberty. The growing use of prenatal investigations leads to an increasing possibility to detect early diseases, such as DSD, that might otherwise be missed until adolescence. In most patients the correct diagnosis can be achieved by the T/A ratio and can be confirmed by genetic investigations. Female sex was assigned to the majority of Italian 46,XY children with 17βHSD3 deficiency. The It-DSD Study Group may represent a national base for collaborative studies in the field of sexual development and an opportunity - in collaboration with official Societies – for the European Reference Network for rare endocrine conditions (ENDO-ERN).
P38 45,X/46,XY Mosaicism: Clinical Characteristics and Follow-up Data

S. Poyrazoglu, F. Bas, R. Bundak, F. Darendeliler

Istanbul University, Istanbul Faculty of Medicine, Pediatric Endocrinology Unit

Background: 45,X/46,XY mosaicism is associated with a broad spectrum of phenotypes, ranging from partial virilization and ambiguous genitalia at birth to patients with a completely male or female phenotype. Turner syndrome stigmata and associated anomalies could be found in these patients.

Aim: To evaluate clinical presenting symptom and follow-up data of patients with 45,X/46,XY karyotype.

Patients and Methods: Thirty patients with 45,X/46,XY mosaicism were reviewed retrospectively. The mean age at diagnosis was 5.7±6.3 yrs (range 0.03–16.7). Their presenting symptoms were ambiguous genitalia (n=16), bilateral undescended testis (n=3), hirsutism and amenorrhea (n=1), amenorrhea (n=2), and short stature (n=7). One patient was diagnosed by prenatal amniocentesis; karyotype was confirmed after birth. Turner stigmata were found in 8 patients. Müllerian structures were identified in 28 patients on ultrasound. Sixteen children were reared as female, thirteen were reared as male and one year old patient was undefined. Four patients (10%) had cardiac anomalies, two had renal anomalies (5%), two had celiac disease (5%), two had Hashimoto thyroiditis (5%) and one patient had bilateral conductive hearing loss. Three patients had gonadoblastoma. Growth hormone (GH) treatment was initiated six patients (5 F, 1 M) at a mean age of 12.9±0.8 yrs (range 12.0–14.1). Mean height SDS at the initiation of GH treatment of 6 patients was -4.3±1.7. Four patients reached a mean adult height of -3.4±2.6 on GH treatment.

Conclusion: Although main presenting symptom of 45X/46XY mosaicism is ambiguous genitalia in early ages, significant number of patients with different symptoms could be diagnosed in older ages. Besides follow-up for gonadal tumors, patients with 45,X/46,XY mosaicism require a clinical evaluation similar to that performed in Turner syndrome and growth velocity must be routinely followed up for GH treatment.
P39 46, XX testicular disorders of sex development: clinical and laboratory characteristics of patients

E. Sannikova, O. Latyshev, L. Samsonova, E. Kiseleva, G. Okminyan, E. Kasatkina
Russian Medical Academy of Continuous Professional Education, Moscow

Objective: To study clinical and laboratory characteristics of patients with 46,XX testicular disorders of sex development (DSD).

Subjects and methods: It was included 4 patients with diagnosis XX male syndrome. All children were assessed height, structure of the external genitalia using a specially devised scoring system (external masculinization score, EMS, range 0-12), internal genitalia by ultrasound examination, hormonal research in mini-puberty (n=2) and puberty (n=2), semen analysis were also assessed (n=1). A gonadal biopsy was performed (n=1).

Results: all patients with 46, XX testicular DSD were divided into two groups, SRY-positive (50%, 2/4) and SRY-negative (50%, 2/4), according to the presence or absence of the SRY gene, which is located in the Y chromosome.

All patients with SRY-positive variant had normal phenotypes at birth (EMS 12) and are diagnosed in 12 years, because of mismatch between gonad size (the total volume of the gonads are 2.8 and 1.2ml) and sexual development stage, gynecomastia. One patient (50%, 1/2) had a short stature (height -2.2 SD score). Ultrasonography of the pelvis (n=2) revealed a prostate. In both cases were subclinical hypergonadotropic hypogonadism - partial (testosterone 10.9 nmol/L, LH 7.49 mU/L, FSH 33.9 mU/L) and total (testosterone 15.7 nmol/L, LH 24.6 mU/L, FSH 46.8 mU/L). Also AMH (70 and 0.5 ng/ml) didn’t correspond to the stage of sexual development both, while inhibin B was in normal range patient with partial hypogonadism (133.1 vs 5.5 pg/ml). Semen analysis of the ejaculate indicated oligoastenoteratozoospermia, nekrozoospermia.

Patients with ambiguous genitalia were SRY-negative, diagnoses were identified in 8 and 9 month. In the first case, patient had penile hypospadias (EMS 11), in the second case had the combination of penoscrotal hypospadias with unilateral cryptorchidism with palpable gonad (EMS 6). Pelvic ultrasound (n=2): processus vaginalis of urogenital sinus, no evidence of Mullerian duct structure. AMH content (50.1 and 70 ng/ml) and inhibin B (64.8 pg/ml) didn’t meet the age. Stimulated human chorionic gonadotropin ΔT were 5.53 and 9.86 nmol/L.

Research of the DMRT1, SOX9, RSPO1, WNT4, NR0B1, SF-1 genes for patient with EMS 6, detected no mutations. Gonadal biopsy: histology of fetal testis, without evidence of Leydig cells.

Conclusion: The group of patients with 46, XX testicular DSD was heterogenous in the clinical picture: from incomplete masculinization with microorchidism and gynecomastia to ambiguous genitalia with hypospadias, cryptorchidism in combination with short stature, hypergonadotropic hypogonadism, azoospermia. SRY-negative XX-male represents the greatest difficulty in diagnosis. The mismatch between gonad size and sexual development stage, gynecomastia, short stature determines the need for a cytogenetic study.
P40 Long-term follow up in two cases with 46, XY DSD

E. Sukarova Angelovska E*, M. Kocova*, G Ilieva*, Georgieva S#, Ivanova D

*Department of Endocrinology and Genetics, University Children Hospital, Skopje, Macedonia
#University clinics of gynecology and obstetrics, Skopje, Macedonia

XY disorders of sexual development encompass etiologically heterogeneous group of patients including gonadal dysgenesis, and defects in androgen action and synthesis. The phenotypic spectrum of external genitalia, gonads and development of Wolfian and Mulerian duct derivatives varies in all patients. A set of hormonal and genetic tests are needed to perform as soon as possible in order to make a decision about gender identity.

We describe different approach in two patients with XY DSD where complete testicular feminization was present. The first patient has been evaluated due to the lack of pubertal signs at 15 years of age. She had female external genitalia with gonads in the lower part of the abdomen and hormonal test confirming androgen insensitivity. Gonadectomy was performed confirming testicular tissue and gonadoblastoma bilaterally. Estrogen therapy was initiated after chemotherapy. The second child was evaluated shortly after birth since the existence of bilateral palpable gonad in the inguinal. Testosterone level was normal for the age, some of the tests were not able to perform at that time. After consulting with the parents the gonadectomy was performed in early childhood confirming normal testicular tissue. At pubertal age estrogen treatment was initiated. Both patients had apparent female orientation. The first patient had 3SDS above the predicted height, and the second where gonadectomy was performed early stayed 2SDS below the predicted height.

Consensus guidelines about the timing of the gonadectomy in complete testicular feminization are dependent of the cause and are constantly reviewed. Final height has rarely been evaluated in the studies of patients with XY DSD with variable timing of the gonadectomy. However it should be take into the consideration having in mind the influence of androgens on the final height.
Chromosome microarray analysis (CMA) has accumulated a wealth of copy number variations (CNVs) associated with different genetic disorders including differences in sex development (DSD). However, a significant portion of CMA data, the 1-50 kb genomic window (“small CNVs”), remains underrepresented in genomic databases and literature. We conducted a retrospective high-resolution (1 kb) CMA focusing on this underinvestigated genomic gap and targeted ~334 genes related to sex development (“DSD genes) including 83 genes of the fibroblast growth factor pathway. About 55% of such genes were smaller than 50 kb. We uncovered isolated or combinations of small, rare, and recurrent or overlapping CNVs as small as 1 kb in ≥2 patients with similar clinical findings including those with previously reported normal clinical CMA. Detailed analysis of these high-resolution data revealed salient genomic and epigenomic profiles; structural and functional domains, CpG islands, repeat elements, active transcription or repression sites, and regulatory regions. Integration of these genomic data with DNA methylation, histone modification and RNA expression profiles in normal testis and ovary suggests spatiotemporal and tissue-specific gene regulation. This study highlights a DSD-specific and gene-targeted CMA approach that uncovered previously unanalyzed or unreported small genes and CNVs, contributing to the growing resources for small CNV map and facilitating the narrowing of the genomic gap for identifying candidate genes or regions in DSD. This high-resolution analysis approach could improve the diagnostic utility of CMA not only in DSD but in other clinical populations as well. These integrated data provide a better genomic-epigenomic landscape of DSD and more opportunities for downstream research.
Disorders of sex development (DSD) represent a major pediatric concern and clinical management of these conditions can be difficult. Uncertainty about a child’s gender can be traumatic for the individual and their family and may carry profound psychological and reproductive consequences. Most often the underlying cause of DSD is a variant in a gene or genes regulating gonadal/genital or steroidogenic pathways. Providing a molecular diagnosis for patients with a DSD and their families can serve multiple purposes: naming the underlying cause contributes to acceptance, reduces stigma or blame, and provides crucial clues and guidance for clinical management, including information on the malignancy risks associated with some types of DSD. A diagnosis is integral to genetic counseling and family planning and yet it has been found that as few as 13% of patients with a DSD will receive a clinical molecular genetic diagnosis.

To address this we have developed a massively parallel sequencing targeted DSD gene panel which allows us to sequence 64 known diagnostic DSD genes and 1000 candidate genes simultaneously. Using this we have analyzed DNA from the largest reported international cohort of patients with DSD (278 patients with 46,XY DSD and 48 with 46,XX DSD). We found variants in a total of 28 diagnostic genes highlighting the genetic spectrum of this disorder. Sequencing revealed 93 previously unreported DSD gene variants. Overall, we identified a likely genetic diagnosis in 43% of patients with 46,XY DSD. In patients with 46,XY disorders of androgen synthesis and action the genetic diagnosis rate reached 60%. Surprisingly, little difference in diagnostic rate was observed between singletons and trios. In many cases our findings are informative as to the likely cause of the DSD, which will facilitate clinical management. We will discuss the clinical utility of this targeted DSD genetic screen and how we are using additional approaches to fill the diagnostic gaps that still exist.
Identification of Large Causative Genetic Variants via Next-Generation Genome Mapping

Hayk Barseghyan, Wilson Tang, Miguel Almalvez, Eva Segura, Emmanuèle C. Délot, Eric Vilain
Department of Human Genetics, University of California, Los Angeles, USA

Novel genomic technologies such as chromosomal microarrays, exome sequencing, and DSD-targeted panels have revolutionized the diagnostic process in DSD care, uncovering new genetic etiologies and progressively establishing genetic diagnosis as a first-approach diagnostic tool. In spite of the diagnostic success of next-generation sequencing, about half of the patients with DSD remain without a firm diagnosis. Next-generation sequencing has the capability of reliably identifying single nucleotide variants (SNPs), as well as small insertions and deletions (INDELs). However, due to its innate methodology, which relies on generation of short DNA reads, this platform cannot identify large structural variants (SV) such as insertions, deletions, inversions and translocations. While these currently represent a rare cause of DSD, they might be an under-recognized cause due to the lack of available diagnostic technology.

In order to overcome these limitations and provide genetic diagnosis for patients still undiagnosed after exome sequencing, we used novel Genome-Mapping technology, combined with Whole Genome Sequencing (WGS). Irys genome mapping technology relies on imaging of fluorescently labeled native-state long DNA molecules (up to 1Mb in size) in nanochannel arrays for genome assembly. This allows the detection of large structural variants (SVs).

First, to ascertain the SV detection capability of the Bionano Irys system, we investigated a series of patients diagnosed with Duchenne Muscular Dystrophy who were known to carry a large deletion, insertion, or inversion in the Dystrophin gene. We were able to successfully identify the pathogenic SVs ranging in size from 10 kb to 5.1 Mb, also greatly refining the location of breakpoints compared to traditional methods. We were also able to determine carrier status in the mothers of affected boys, demonstrating that the technology can distinguish between heterozygous and hemizygous status of the SVs.

On average Bionano Genome Mapping identified 1300 insertions, 700 deletions, 50 inversions and 20 translocations per genome. A control database containing SV data for 144 healthy individuals was used to filter out common variants, greatly reducing the number of variants to investigate manually. This method was applied to a pilot group of 17 DSD genomes. While no SV affecting a known DSD gene was identified in this small cohort (as expected), we use these data to build a genome-wide map of SVs in DSD genomes to identify new candidate regions.

In parallel, WGS was performed on 100 trios of a range of undiagnosed 46,XX and 46, XY DSD conditions. The diagnostic ability of WGS in known DSD genes, which is expected to be higher than that of exome sequencing, will be reported for this new cohort. The combination of genome sequencing and genome mapping data should further enhance our ability to detect novel complex etiologies for DSD, including combinations of SNVs and SVs in known and novel regions of the genome.

The authors thank Emilie Douine, Richard Wang, Hane Lee, Stanley Nelson (UCLA) for access to the Duchenne patient samples, the clinicians who submitted samples to the DSD-TRN Biobank, and the patients who enrolled into the study. WGS sequencing was sponsored in part by a grant from the Gabriella Miller Kids First Pediatric Research Program to EV. Authors were supported in part by the NICHD RO1 grant that allowed the creation of the DSD-Translational Research Network (DSD-TRN).
A Mutation in WT1 (Wilms' tumor suppressor 1) associated with 46,XX testicular DSD

C. Eozenou, L. Fusee, A. Elaidy, K. McElreavey, I. Mazen, A. Bashamboo,

1Human Developmental Genetics, Institut Pasteur, Paris, France. 2National Research Centre, Cairo, Egypt.

46,XX DSD (Disorder of Sex Development) includes individuals with ovotestes (ovotesticular DSD (OTDSD)) or testes (testicular DSD (TDSD)). Most individuals with 46,XX TDSD carry the SRY gene. However, our understanding of the molecular causes of TDSD and OTDSD remains incomplete. Recently, using exome sequencing we and others have identified a recurrent missense mutation in NR5A1 associated with 46,XX TDSD/OTDSD. Here, using a similar approach we identified a novel and de novo missense mutation of the WT1 gene in a case of 46,XX TDSD. The boy of Egyptian origin presented with male external genitalia, dysgenic testis, microcephaly and a small uterus. Kidney function was normal. The DNA of the boy and his parents was exome sequenced. Possible pathogenic mutations were confirmed by Sanger sequencing. Transient gene expression assays and protein-protein interaction studies were performed to show the effect of the mutation on the biological activity of the WT1 protein. The 46,XX boy lacked the SRY gene in DNA from peripheral blood and testis tissue. Array CGH indicated normal ploidy. Exome sequencing identified a de novo missense mutation of a highly conserved arginine residue in the fourth zinc-finger of WT1 (p.Arg495Gly). This mutation was absent from all public databases and an in-house panel of 300 ancestry-matched controls. Transient gene expression assays and protein-protein interaction studies showed that the mutant WT1 protein abnormally regulated/interacted with genes/proteins involved in both male and female gonadal development. More interestingly, in the in-vitro conditions, the mutation altered the transcriptome of granulosa cells to resemble that of Sertoli cells. These data are strongly in favour of the WT1 mutation being responsible for the development of testis in this 46,XX boy.

Mutations in WT1 have been previously reported in anomalies of testis formation in 46,XY individuals. This is the first time that a mutation in WT1, has been identified associated with 46,XX TDSD. This raises the intriguing possibility that specific mutations in WT1 may be associated with testis formation in 46,XX chromosomal context in a similar manner to the recurrent p.R92W mutation of NR5A1.
EXPANDING THE MOLECULAR DIAGNOSIS OF ANDROGEN INSENSITIVITY SYNDROME

RL Batista, AS Rodrigues, MY Nishi, JLO Madeira, JM Silva, LR Carvalho, NL Gomes, JAD Faria Jr, EMF Costa, SDomenice, BB Mendonca.

Developmental Endocrinology Unit, HCFMUSP – Laboratory of Hormones and Molecular Genetics / LIM42 – São Paulo / Brazil

Introduction: Androgen Insensitivity Syndrome (AIS) is a common cause of 46, XY DSD. Mutations in the androgen receptor (AR) gene has been identified in more than 90% of the cases with complete phenotype (CAIS) but only about 30-50% of cases with partial phenotype (PAIS).

Objectives: To expand the molecular diagnosis of AIS.

Methods: We sequenced the exonic regions and the proximal intronic region (splicing site) in 63 patients with AIS (PAIS=33 patients / 21 families and CAIS = 30 patients / 20 families) with clinical diagnosis of AIS (gynecomastia, presence of inguinal hernia, elevated LH and testosterone levels). In patients without exonic AR mutations, we sequenced the 5'UTR region (including the promoter region). All mutations were submitted to “in silico” analysis: ExAC, mutation taster, Polyphen 2, Mut Assess, HSF3 and FSPLICE. The new mutations were confirmed by functional studies: AR expression (fibroblast culture, in the case of 5'UTR mutation) and fibroblasts AR protein analyses (for splicing alterations). The RNA secondary structure of AR short tandem repeats alterations were predicted by ViennaRNAfold. The AR protein tertiary structure from the wild type and mutant were predicted by I-TASSER server.

Results: By exonic AR sequencing, non-synonymous mutations were identified in 88% of the CAIS and 67% of the PAIS. In exonic AR region, two novel “CTG” insertions (p.Ser57_Leu58insLeu and p.Ser57_Leu58insLeuLeuLeu) were identified in two unrelated PAIS patients. These insertions results from DNA replication slippage and both of them are able to alter the secondary structure of RNA and tertiary structure of the AR protein. We identified a novel synonymous mutation in the AR NTD domain (p.S510S) in two unrelated families with CAIS leading to a exonic splicing alteration which causes a shorter AR protein (p.M508Vfs12*). Microsatellite analysis of the X chromosome in these two families demonstrates a founder effect of the p.S510S variant. In another PAIS family with 9 affected members, a large insertion (c.-285ins1200pb) in the AR promoter region was identified. The AR protein expression was significantly reduced in the mutant (p <.001).

Conclusions: We identified the first synonymous mutation related to CAIS and its founder effect. We found the first insertion in the AR promoter causing PAIS. Novel CTG tandem repeats related to DNA replication slippage and PAIS phenotype were found in two unrelated PAIS patients. This pattern of DNA alteration has been already described in poly-repeat conditions, as Kennedy disease. With this approach, we were able to identify molecular deffects in 88% of PAIS and 100% of CAIS.
The transcriptional regulator CBX2 and ovarian function: a whole genome and whole transcriptome approach

Leila Bouazzi¹, Patrick Sproll¹, Eid Wassim¹, Anna Biason-Lauber¹,²

¹Division of Endocrinology, Department of Medicine, University of Fribourg, ²Fribourg Cantonal Hospital, 1700 Fribourg, Switzerland

It is now well established that CBX2 is indispensable for testis development in human and mice. Conversely, the role of this polycomb in the ovary remains largely undefined. Addressing this issue, we conducted a comprehensive and unbiased genome-wide analysis based DamID and RNA-seq strategies to identify CBX2-candidate responsive genes in the ovary. We identified thousands of genes, but we focused on gonads-specific genes reportedly involved in sex development and DSD. Functional enrichment analysis revealed CBX2 involvement in several molecular pathways counting the developmental processes. Notably, we found out that CBX2.1 and CBX2.2 are ahead genes contributing to folliculogenesis and steroidogenesis (i.e. ESR1, NRG1, PTGER2, TGFβ, BMP2, FSHR, STARD6 and NTRK1/2). In addition, we identified CBX2-related genes involved in PCOS like AMH, RSPO2 and DKK1 as for genes inculpated in POF (POF1B, AKR1C1, BMP15 and HOXA13) and pituitary deficiency hormone (i.e. LHX4 and KISS1). Besides having a sex determining role, CBX2 isoforms protect granulosa cells from uncontrolled growth by affecting oncogenic markers like FZD7, TGFα, AMIGO2 and RSPO3. Consistent with this, our study figured out a fundamental role of CBX2 in the molecular ovarian circuit and eventually clarified the exact rank of this polycomb in the female regulatory events.
P47 Karyotype phenotype correlation in patients with Turner syndrome.


1. Afdeling Kinderendocrinologie, Amalia Kinderziekenhuis, Radboud Universitair Medisch Centrum, Nijmegen, The Netherlands
2. Department for Health Evidence, Radboud Universitair Medisch Centrum, Nijmegen, The Netherlands
3. Afdeling Interne Geneeskunde, Radboud Universitair Medisch Centrum, Nijmegen, The Netherlands
4. Unite d’Endocrinologie, Genetique et Gynecologie medicale, Hopital des Enfants, Toulouse, France
5. Department of Paediatric Endocrinology and Diabetology, Charite Universitasmedizin, Berlin, Germany

Abstract

Objective: To identify associations between karyotype, phenotype and gonadal function in patients with Turner syndrome.

Methods: This study was part of the DSD life study (www.dsd-life.eu). We evaluated the different karyotypes and compared these with the age of diagnosis, dysmorphic features, FSH at diagnosis, spontaneous puberty, puberty induction and cardial/renal involvement (bicuspid aortic valve (BAV), coarctation of the aorta (COA) and horseshoe kidney (HK)). Median (min-max) was used to describe baseline characteristics and associations between phenotype and karyotype were analysed using different parametric and non-parametric tests. A p-value < 0.05 was considered statistically significant.

Results: Information on 328 patients with TS (median 28 (15-73) years) was available. Participants had a karyotype of monosomy 45X (47%), mosaicism 45X/46XX (10%), karyotype with isochromosome (19%) or other karyotype (25%). The peak age of diagnosis was in infancy and at the age of 10-11 years. Patients with a monosomy 45X were more frequently diagnosed during infancy compared to patients with other karyotypes. Besides, the median age (median 10 (0-61) years) at diagnosis was lower in this patient group (median 10 (0-61) versus median 11 (0-53) years; p=0.02). Patients with a monosomy 45X had significantly more dysmorphic features (p<0.0001), BAV (p=0.006) and COA (p=0.002) compared to the other karyotypes. No associations with HK were found. Levels of FSH at diagnosis in girls (median 23 (0-289) U/L) were higher in patients with 45X monosomy (p=0.01). 27% of the women had a history of spontaneous puberty, this was more common in women with a 45X/46XX mosaicism (p<0.0001) than in women with a monosomy 45X (p<0.0001).

Conclusion: These results show that the clinical signs of TS are more severe in patients with monosomy 45X. Adequate prediction of the clinical phenotype is important for the personalized follow-up of patients. Despite the more severe features in monosomy 45X, the median age of diagnosis is only slightly lower compared to patients with other karyotypes, which suggests opportunities for improvement.
Antenatally determined sesquizygosity in gender discordant monochorionic diamniotic twins (46,XX/46,XY): postnatal clinical and gonadal phenotype.

L.S. Conwell¹,², G.E. Phillips²,³, A. Nandini⁴, P.A. Borzi²,⁵, M.T. Gabbett²,⁶

1. Department of Endocrinology and Diabetes, Lady Cilento Children’s Hospital, Children’s Health Queensland, Brisbane, Australia
2. Faculty of Medicine, University of Queensland, Brisbane, Australia
3. Anatomical Pathology, Health Support Queensland, Department of Health, Queensland Government, Lady Cilento Children’s Hospital and Royal Brisbane and Women’s Hospital, Brisbane, Australia
4. Cytogenetics Department, Pathology Queensland, Brisbane, Australia
5. Department of Surgery, Lady Cilento Children’s Hospital, Children’s Health Queensland, Brisbane, Australia
6. Genetic Health Queensland, Royal Brisbane and Women’s Hospital, Queensland, Brisbane, Australia

Sesquizygosis is an exceptional intermediate between mono- and dizygotic twinning. A spontaneously conceived twin pregnancy had monochorionic, diamniotic placentaion from the 6 week ultrasound (US) (confirmed postnatally). From 14 weeks gestation, US showed phenotypic gender discordance in otherwise structurally normal twins with male (twin A) and female (twin B) phenotypes.

FISH / SNP karyotyping on mid-trimester amniotic fluid showed twin A 50:50 XX/XY and twin B 90:10 XX/XY. Similar ratios were later confirmed in cord tissue.

Genotyping indicated (i) maternally identical, sharing half their paternal genome, hence three-quarter identical or sesquizygotic twins (ii) parthenogenetic activation of the haploid oocyte as the initial step in the aetiology.

Objective:- Describe the postnatal clinical and gonadal phenotype.

Methods:- Postnatal clinical and US assessments; FSH, AMH, Inhibin B levels; gonad biopsies with histopathology, immunohistochemistry and cytogenetics.

Results:- Postnatal assessments confirmed twin A to be phenotypically male and twin B female with accordant gender of rearing.

The female had granulosa cell compromise suggested by FSH, AMH, Inhibin B at age 4.5 and 16 months. Bilateral intra-abdominal gonad biopsies at age 16 months showed dysgenesis with ovarian type stroma, abnormal primordial follicles and no seminiferous tubules. There were islands of cells, some cystic, with partly cribriform architecture, the largest consistent with gonadoblastoma. On immunohistochemistry, these islands of cells were positive for OCT-4, c-KIT, PLAP and Inhibin B. Ki-67 (proliferation marker) was positive in 2-10% of these cells. Cytogenetics (FISH) identified XY in 2.8-8.5% of cells, but confined to those islands of cells. The gonads were removed (no invasive germ cell tumour).

The male had robust Sertoli cell markers at age 4.5 months. Bilateral intra-scrotal gonad biopsies at 25 months showed normal testes for age (OCT-4, c-KIT negative). Cytogenetics (FISH): 50% and 40% of cells XY in the gonads.

Conclusions:- This adds to the one prior report¹ in which postnatal genotyping suggested sesquizygotic twins, 46,XX/46,XY but with phenotypic differences and no immunohistochemistry reported.

46,XX Ovotesticular Disorder of Sex Development (DSD):- duplication of the XX SR region upstream of the critical testicular gene SOX9.

L.S. Conwell1,2, S. Statthís2,3, A. Franklin3, P.A. Borzi2,4, A. Nandini5, H.T. Aung5, G.E. Phillips2,6, T. Ohnesorg7, K.L. Ayers7,8, A.H. Sinclair7,8

1. Department of Endocrinology and Diabetes, Lady Cilento Children’s Hospital, Children’s Health Queensland, Brisbane, Australia
2. Faculty of Medicine, University of Queensland, Brisbane, Australia
3. Child and Youth Mental Health Service, Children’s Health Queensland, Brisbane, Australia
4. Department of Paediatric Surgery, Lady Cilento Children’s Hospital, Children’s Health Queensland, Brisbane, Australia
5. Cytogenetics Department, Pathology Queensland, Brisbane, Australia
6. Anatomical Pathology, Health Support Queensland, Department of Health, Queensland Government, Lady Cilento Children's Hospital and Royal Brisbane and Women's Hospital, Brisbane, Australia
7. Murdoch Children’s Research Institute, Melbourne, Victoria, Australia
8. Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

A term baby had a 2cm phallus (mild chordee), perineal urogenital opening, bifid fusion of labioscrotal folds containing palpable gonads and no other dysmorphic features. Karyotype was 46,XX (no Y chromosome). FISH was negative for SRY and Y centromere probes. Sinugram indicated a large prostatic utricle. Surgical assessment on day 11: no fallopian tubes or uterus; vas deferens, vessels entered the inguinal rings; gonads macroscopically were bipolar ovotestes, histologically ovarian and testicular tissue in upper and lower poles. A male gender of rearing was decided. A 2-stage penile reconstruction and urethroplasty was performed.

Gender confusion, rather than dysphoria, was apparent from 4 years, amidst complex psychosocial circumstances.

Breast tissue developed prior to 8 years with Tanner stage 1 pubic hair and genitalia. Leuprorelin testing was pubertal. Ultrasound indicated symmetrical, prepubertal sized gonads. α-FP and β-HCG were low. At 9 years (height 8th percentile, bone age advanced for a male), puberty is suppressed with depo leuprorelin. Gender identity will continue to be assessed.

Objective:- Genetic analysis of this patient with SRY-negative 46, XX ovotesticular DSD.

Methods:- Genomic DNA analysed (i) Targeted Massively Parallel Sequencing (MPS) DSD panel (64 diagnostic genes including SOX9) (ii) Single-nucleotide polymorphism (SNP) array analysis (iii) Multiplex Ligation-dependent Probe Amplification (MLPA) - known DSD genes, including SOX9 and upstream regulatory regions.

Results:- No causative variations were identified by the MPS DSD panel. SNP array detected a heterozygous interstitial duplication at 17q24.3, 519-623Kb upstream of SOX9.

MLPA confirmed (i) SRY-negative 46,XX DSD (ii) a duplication upstream of SOX9. This covers the XX SR / RevSex (XX sex reversal) region, but does not extend to XY SR at the 5’ end, nor the TESCO (testis-specific enhancer of SOX9 core), or the SOX9 gene at the 3’ end.

Conclusions:- The SOX9 enhancer duplication likely led to high levels of SOX9 in the developing ovary, causing ovotestes. An increased risk of germ cell cancer is not expected. The testicular / ovarian components may be hormonally active. Ongoing assessment of gender identity will be crucial to guide management.
Genetic Diagnosis of Disorders/Differences of Sex Development (DSD): The DSD-Translational Research Network Experience

Emmanuèle C. Délot¹, Jeanette C. Papp¹, the DSD-TRN Genetics Workgroup*, David E. Sandberg², Eric Vilain¹.
¹University of California, Los Angeles, USA; ²University of Michigan, Ann Arbor, USA

The DSD Consensus Conference called for the establishment of an infrastructure for collaborative interdisciplinary clinical practice and research, to integrate scientific understanding of DSD with real-time standardization and improvement in clinical practice. In response, the DSD-Translational Research Network (DSD-TRN) was created, the first such North American infrastructure, a network of 4 (now expanded to 13) US research and clinical sites and a central registry, with the collaboration of patient advocates convened by Accord Alliance. To address variability within and across medical, surgical, and behavioral health aspects of care, the DSD-TRN is dedicated to scientific discovery and the standardization of diagnostic and treatment protocols. A critical aspect of this standardization is a commitment to an early and comprehensive diagnostic process (including genetic), associated with extensive standardized phenotyping and psychosocial screening and support of patients and families.

A known genetic etiology currently explains about half of DSD conditions. An accurate diagnosis is critical to predicting the occurrence of life-threatening crises (such as in salt-wasting forms of CAH), response to Hormone Replacement Therapy, eventual gender, fertility, recurrence risk, cancer risk, and patient empowerment toward optimal long-term health-related quality-of-life outcomes. Genomic technologies, chromosomal microarrays and next-generation sequencing, are revolutionizing the approach to DSD diagnosis. With exome sequencing and DSD-specific panels now routinely available in the clinical realm, DSD-TRN best practice guidelines recommend early, comprehensive genetic testing as a means to improve the path to an accurate diagnosis and optimized clinical management. Although many next-generation sequencing platforms are being developed around the world, implementation is facing multiple hurdles from clinicians’ habits, institutional constraints, and insurance coverage. A significant hurdle to the full adherence of clinical teams to DSD-TRN guidelines is the current lack of integration of the standardized clinical forms into the various electronic medical record systems. Time allocated to research (e.g., registry data entry) is also severely limited at most sites for lack of funding supporting this new effort of development and implementation of best practices. In spite of these hurdles, genetic information for half the enrolled patients is already available in the DSD-TRN.

Longitudinal data collection in the registry and monthly clinical activity reports allow tracking of the effort, success, completion, and timeline of the diagnostic process for each clinical team. Relative diagnostic efficacies of genetic testing methods, frequencies of the different DSD conditions, and rates of definitive genetic diagnosis were analyzed. Analysis of data for 144 probands at 9 clinical sites showed that genetic diagnostic efforts by DSD-TRN teams were rewarded by a substantial increase in patients with a firm diagnosis: from 24% to 46%. The percentage of diagnosed patients showed great site-to-site variability. The rate of successful diagnosis can however be vastly improved by increased adherence to DSD-TRN diagnostic guidelines: for almost all (97%) of the patients who remain without a diagnosis, currently available diagnostic methods (such as trio exome sequencing and chromosomal microarray) have not been exhausted.

*The Genetics Workgroup that created the standardized forms included Emmanuèle Délot, Michelle Fox, Wayne Grody, Hane Lee, Jeanette C. Papp, Eric Vilain (UCLA), Catherine Keegan (U. Michigan), Linda Ramsdell (Seattle Children’s Hospital), and Janet Green (Accord Alliance). The group now also includes Hayk Barseghyan, Naghmeh Dorrani (UCLA), Lauren Mohnach (UM), Margaret Pearson (Phoenix Children’s Hospital), Jullianne Diaz, Eyby Leon (National Children’s Hospital), Robert Hopkin, Jodie Johnson, Howard Saal, (Cincinnati Children’s Hospital), Ina Amarillo (Washington U., St Louis), Margaret
Adam (Seattle Children's Hospital).
Whole exome sequencing used to investigate target genes in individuals with 46,XY partial gonadal dysgenesis

Ana Paula dos Santos MSc\textsuperscript{1,2}, Juliana Gabriel Ribeiro de Andrade, PhD\textsuperscript{3}, Helena Fabbri-Scallet MSc\textsuperscript{2}, Gil Guerra-Junior PhD\textsuperscript{3,4}, Maricilda Palandi de Mello, PhD\textsuperscript{2,3}, Andréa Trevas Maciel Guerra, PhD\textsuperscript{1,3}
\textsuperscript{1}Department of Medical Genetics, State University of Campinas (UNICAMP), SP, Brazil
\textsuperscript{2}Molecular Biology and Genetic Engineering Center, State University of Campinas (UNICAMP), SP, Brazil
\textsuperscript{3}Interdisciplinary Group for the Study of Sex Determination and Differentiation (GIEDDS), State University of Campinas (UNICAMP), SP, Brazil
\textsuperscript{4}Department of Pediatrics, State University of Campinas (UNICAMP), SP, Brazil

46,XY partial gonadal dysgenesis (PGD) is a rare and usually sporadic condition. This non-syndromic form of testicular dysgenesis is included in the group of 46,XY Disorders of Sex Development (46,XY DSD) and is characterized by genital ambiguity due to variable degrees of testicular dysgenesis in individuals with a 46,XY karyotype. Mutations have been described in \textit{NR5A1} and other genes participating in gonadal differentiation such as \textit{SRY}, \textit{WT1} and \textit{SOX9}; however, the etiology of most cases remains unknown. The aim of this study was to search for variants in 42 target genes related to testicular and ovarian differentiation in patients with PGD. Whole exome sequencing was performed for a total of 8 unrelated patients. The sequences were aligned to the human reference genome (GRCh37/hg19). Analysis of target genes revealed six variants in four patients. The first one was the c.877G>A transition within exon 5 of \textit{NR5A1}, leading to p.D293N heterozygous mutation, which had already been described in homozygosis in a family including patients with XY PGD, XY complete gonadal dysgenesis (CGD) and XX CGD. In that family, heterozygous relatives were phenotypically normal. The second variant was a likely pathogenic mutation in \textit{DMRT3}, the c.1291G>C transversion within exon 2, leading to the novel p.E431Q heterozygous mutation. Both variants were inherited from the phenotypically normal mothers. Three variants were identified in one patient, two already described within \textit{HHAT} (rs528513055) and \textit{WWOX} (rs193001955) genes, and the novel p.P583R in \textit{SIX4} gene. The last variant was identified in \textit{IGF1R} gene (rs141802822), it was inherited from the phenotypically normal father. No other variant with potential pathogenicity among the 42 target genes were identified in the remaining four patients. Our results show that targeted exome can be effective to detect pathogenic variants in patients with XY PGD, although a comprehensive exome analysis is still necessary to define the etiology of this disorder in a high proportion of cases. (Fapesp 2015/04763-4).
Analyses of the non-coding region of NR5A1 gene revealed five novel variations in eight patients with different phenotypes of 46,XY DSD

H. Fabbri-Scallet¹, R. Werner², G. Guerra-Juínor³, A.T. Maciel-Guerra³, L.M. de Sousa¹, J.G.R. Andrade⁴, O. Hiort² & M.P. de Mello¹

¹Center of Molecular Biology and Genetic Engineer, State University of Campinas, Brazil; ²Department of Paediatric and Adolescent Medicine, Universität zu Lübeck, Germany; ³Medical Genetics Department, State University of Campinas, Brazil; ⁴Paediatric Department, State University of Campinas, Brazil.

Disorders of Sex Development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. Mutations in the NR5A1 gene, which encodes the transcription factor SF-1, are responsible for different phenotypes of DSD. Direct sequencing of the proximal promoter region and the 5'-UTR of NR5A1, revealed five novel variations in eight patients. According the reference sequence NM_004959.4, three variations were located in the upstream promoter region. The c.-413G>A and the c.-207C>A SNV were identified in one allele of a patient with 46,XY Partial Gonadal Dysgenesis (GD) and the c.-762C>T SNV was found in an idiopathic 46,XY DSD patient. Two further variations were found in the 5'-UTR of non-coding exon 1, the SNV c.-133G>A (n.55G>A) was found in a patient with 46,XY Partial GD and the recurrent c.-156_139dup (n.32_49dup) was identified in five unrelated patients, one with 46,XY Partial GD, one with idiopathic 46,XY DSD and three referred to the service for male infertility. Three elements that are essential for NR5A1 promoter activity are localized within the -61 bp upstream of the beginning of the non-coding exon 1. Within the proximal promoter and exon 1, 14 CpG sites were recognized. They are responsible for inactivating the promoter when methylated, controlling the SF1 expression during embryonic development, in adult tissues, and in cell lines. Therefore, mutations in this region may lead to deregulation of protein expression, by modifying the recognition of cofactors to this region. Modifications in conserved motifs were tested through in silico tools. In some cases transcription factor binding sites were lost after mutations. For either c.-133G>A or c.-413G>A an SP1-binding site was suppressed by the mutation, and for c.-156_139dup a new SP1-binding site was created. For c.-762C>T and c.-207C>A, the native SP1-binding site was maintained, however sites for NF-muE1, Egr-1 and NR-1 were suppressed. The loss of a transcription factor binding site might lead to a change of NR5A1 transcription or may affect its mRNA stability and/or the translation efficiency. However functional studies, that are currently being performed, are necessary to confirm either hypothesis. Since five not yet annotated nucleotide variations have been identified in association with DSD in 10% of our cohort (8/73 patients) we would like to highlight the importance of studying NR5A1 promoter region.

Financial support: FAPESP #2013/05603-5
Mutation Spectrum in Patients with Differences of Sex Development analysed by Targeted Next-Generation Sequencing

S. Flieger, B. Brix, I. Parenti, B. Reiz, F. J. Kaiser, O. Hiort, R. Werner

Sektion für Experimentelle Pädiatrische Endokrinologie und Diabetologie, Klinik für Kinder- und Jugendmedizin, Universität zu Lübeck, Marie-Curie-Straße, 23562 Lübeck, Germany
Sektion für Funktionelle Genetik am Institut für Humangenetik, Universität zu Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany
Institut für Kardiogenetik, Universität zu Lübeck, Maria-Goeppert-Str. 1, 23562 Lübeck, Germany

The term Differences of Sex Development (DSD) comprises a diversity of clinical phenotypes that exhibits a genetic heterogeneity with poor genotype-phenotype correlation. Patients with a variant in the same gene can present different clinical features. Therefore currently only a small proportion of these patients receive an accurate molecular genetic diagnosis.

We developed a customized AmpliSeq 46,XY next generation sequencing (NGS) gene panel including 83 genes. Some of the genes were already described to be mutated in patients with 46,XY DSD, while others were selected as interesting candidate based on functional investigations in human or animal model systems. 1205 amplicons were designed in two libraries, covering 96.5% of target regions. Until know we examined 60 samples and 4 controls within 14 panel experiments. The clinical well characterized patients presented a phenotypic range from severe hypospadias to complete gonadal dysgenesis.

We identified two new homozygous mutations in HSD17B3, two compound heterozygous mutations in SRD5A2 and one in DHH. We could also find a pathogenic variant in NR5A1 in a patient with gonadal dysgenesis. Four rare variants were found in GATA4 in four patients with hypospadias. We could also find a likely pathogenic mutation in WWOX, two in ZFPM2 and MAP3K1. Three patients had variants of uncertain significance, two mutations in MAML1 and one mutation in MID1 and CHD7. Four patients with known mutations were included as controls and all their mutations could be re-identified. All in all we were able to identify new potential relevant and rare mutations in 17 of 60 patients (28.3%). Two patients had more than one rare DSD-associated mutation.

A genetic diagnosis using traditional tools can only be made in cases with characteristic phenotypes or unique laboratory findings. The NGS panel sequencing allows a targeted mutational screening of many genes simultaneously in a short time with a high sensitivity, less cost and a small amount of DNA. Important functional analysis will follow to proof the impact of the new variants.
P54 EXOME SEQUENCING REVEALS POLR3H DEFECT ASSOCIATED WITH PRIMARY OVARIAN FAILURE IN TWO UNRELATED FAMILIES

M. Franca1; A. Lerario1; M. Funari1; M. Nishi1; E. Fontenele2, S. Domenice1, AC. Latronico1, A.Jorge1,3, B. Mendonca1.

1 Laboratory of Hormone and Molecular Genetics (LIM42), University of Sao Paulo School of Medicine, Sao Paulo, Brazil.
2 Endocrinology and Diabetes Center of Walter Cantidio University Hospital, Federal University of Ceará, Fortaleza, CE, Brazil.
3 Laboratory of Endocrinology Genetics (LIM25), University of Sao Paulo School of Medicine, Sao Paulo, Brazil.

Abstract: Primary ovarian failure (POF), characterized by amenorrhea, hypoestrogenism, and elevated gonadotropin levels in women under the age of 40, is a common cause of infertility. Several genetic alterations have been associated to POF, however in most of the patients the etiology of this disorder remains unknown. Our aim was to identify new genes implicated in the etiology of POF using Whole-Exome Sequencing (WES). We studied 11 familial cases (22 affected women) all of them with primary amenorrhea. In family 1, DNA of two affected daughters and their mother was available. In the second family the DNA of two affected sisters, one unaffected sister and their parents was analyzed. The parents of Family 2 are second cousins, suggesting a recessive mode of inheritance. Exons and splice sites were captured with the Agilent SureSelectXT Human Exon V5 Kit, and 2 × 100 bp paired-end. WES was performed on the Illumina HiSeq 2500. The mean coverage of the captured regions was > 50x in all samples. The raw data was aligned to the reference genome (hg19 assembly) with BWA. Variant calling was performed with Freebayes and annotated with ANNOVAR. Sanger Sequencing was used to confirm Exome Sequencing variants and to evaluate 200 fertile women controls for putative identified damaging variants. The novel homozygous missense variant (c.149A>G; p.D50G) in POLR3H, the gene encoding Polymerase (RNA) III (DNA directed) polypeptide H, was identified in all 4 affected women from both families. This protein is ubiquitously expressed and it is highly expressed in ovarian tissue. POLR3H catalyzes the transcription of DNA into RNA. The parents were heterozygous for this variant and the unaffected sister did not carry the variant, consistent with perfect segregation in autosomal recessive mode of inheritance. The c.149A>G POLR3H variant was not present in the public available databases 1000Genomes, 6500ESP and ExAC. Additionally, the c.149A>G variant is predicted to be deleterious according in silico prediction sites - Polyphen, Mutation Assessor, SIFT, Mutation Taster. Finally, this POLR3H variant was not identified in 400 alleles from fertile Brazilian women used as normal control. In conclusion, we identified a novel homozygous variant in POLR3H in two unrelated families with POF. These findings support that defects in POLR3H gene is a novel genetic cause of POF, thus expanding the molecular pathways of the regulation of ovarian function.

Nothing to disclosure: MMF, AL, MF, MN, EF, SD, ACL, AJ, BBM

Sources of Research Support: MMF is supported by FAPESP 2014/12413-0; FAPESP Temático 2013/02162-8 awarded to BBM.
Delineation of phenotypic associations in Deciphering Developmental Disorders (DDD) Study participants with hypospadias. A retrospective review of clinical features.

Gabriella Gazdaghi, Ruth McGowan, S. Faisal Ahmed, DDD Study, Edward S. Tobias

School of Medicine, Dentistry & Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow

West of Scotland Regional Genetics Service, Laboratory Medicine Building, Queen Elizabeth University Hospital, Glasgow

Developmental Endocrinology Research Group, Royal Hospital For Children, University of Glasgow

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge

Background: Disorders of sex development (DSD) are a group of rare conditions characterised by abnormalities of the genital system. In an initial phenotype analysis we have reviewed the clinical features commonly associated with hypospadias in individuals recruited to the UK-wide DDD study of children with an undiagnosed developmental disorder.

Objective: To report associated developmental, ophthalmic and cardiovascular abnormalities observed in the DDD cohort of patients with hypospadias.

Method: Retrospective review of anonymised phenotype data of DDD participants manifesting any form of hypospadias.

Results: All DDD participants with at least one human phenotype ontology (HPO) term within the disease category ‘Abnormalities of the genital system’ were identified and included in our cohort. Of the 13,632 DDD recruits, 1,092 (8%) individuals were selected for inclusion. Of 247 (23%) participants with hypospadias, one was reported as both penoscrotal and coronal and another participant was reported as both penoscrotal and perineal hypospadias. Of the 27 observed disease categories within the DDD study, developmental, ophthalmic and cardiovascular phenotypes were particularly common. 217 developmental delay phenotypes were recorded in the 247 patients with hypospadias. Many (107 of 217, 49%) of these phenotypes were global delays, but intellectual disability and cognitive impairment (46 of 217, 21%) as well as speech delays (49 of 217, 23%) were also frequently described. With reference to ophthalmic abnormalities and, in particular, those of the periorbital region (218), abnormalities of the palpebral fissures (36 of 218, 16%) were commonly described. In addition, hypertelorism was observed in 35 (16%) of the 218 ophthalmic phenotypes and abnormalities of the eyebrow in 23 (10%), ptosis in 17 (8%) and epicanthus in 15 (7%). 128 cardiovascular phenotypes were recorded, of which 33 (26%) were ventricular septal defects, 25 atrial septal defects (19%) and 11 patent ductus arteriosus (9%). Additionally, abnormalities of the hands (122, 6%), mouth (111, 5%) and face (109, 5%) were recurring features identified in the hypospadias cohort.

Conclusion: Developmental, ophthalmic and cardiovascular abnormalities are frequently described in association with hypospadias in children with developmental disorders. The recognition of phenotypic associations with hypospadias might alert clinicians to the possible presence of important undetected additional clinical problems and thus further improve clinical management.
P56 Diagnosing 46, XY Disorders of Sex Development (DSD) by using Targeted Massively Parallel Sequencing

NL Gomes (1); AM Lerario (1,2); MM França (1); MY Nishi (1); MF Funari (1); Costa EMF (1); JAD Faria Junior (1); RL Batista (1); S Domenice (1); BB Mendonca (1)
(1) Unidade de Endocrinologia do Desenvolvimento/ LIM42/SELA, Hospital das Clínicas. SP/ Brasil (2) Division of Endocrinology and Diabetes, University of Michigan. Ann Arbor, USA

Background: An accurate genetic diagnosis for 46, XY DSD is important to clear prognosis to families and for genetic counseling. However, few patients with gonadal dysgenesis (GD) obtain a molecular diagnosis by Sanger sequencing, given the complexity of the genetic defects underlying 46,XY DSD.

Objective: To investigate the underlying genetic etiology of 46, XY DSD patients of unknown genetic etiology by using targeted massively parallel sequencing (TMPS).

Materials and Methods: We studied thirty-nine 46,XY DSD patients: 26 patients with GD, 12 patients with unknown DSD etiology (UDSD) due to previous gonadectomy or an inconclusive hormone profile and one patient with 17-hydroxylase deficiency heterozygous for a CYP17A1 mutation. All GD patients were previously screened for NR5A1 and/or SRY mutations and UDSD patients for AR mutations by Sanger. We designed an amplicon-based capture panel of 63 genes related to DSD for targeted sequencing. Sequencing was performed in the Illumina MiSEQ platform. Paired-end reads were aligned to the hg19 assembly of the human genome with BWA-MEM. Variants were called and annotated with Platypus and ANNOVAR, respectively. Analysis of copy number variation was performed with CONTRA.

Results: TMPS identified a total of 9 variants, including 6 pathogenic and 2 likely pathogenic variants in 3 GD patients, 4 UDSD patients and in the patient with 17-hydroxylase deficiency. Two previously known variants (p.R245W in SR5A2 and p.A203V in HSD17B3) and seven novel variants were identified, including six missense variants (p.A340V; p.T29R; p.H24Q in NR5A1; p.P407R in GATA4; p.L335P in DHH; p.T81M in HSD17B3) and a duplication of exons 1-2 in CYP17A1 gene found in the patient with 17-hydroxylase deficiency (which was confirmed by MLPA). All these variants are localized in conserved regions of the genes and absent or in very low frequency in population databases. At least 6 prediction site tools classified them as deleterious.

Conclusion: Our TMPS approach was able to identify a likely causal variant in 20% (8/39) of this cohort 46, XY DSD patients. Considering that these patients had been previously screened for possible causative variants, these findings support that NGS-based approaches lead to improvements in molecular diagnosis of 46, XY DSD and potentially will be incorporated into clinical practice in a near future as the first-tier diagnostic tool.
Identification of novel candidate genes for premature ovarian failure by investigating balanced chromosomal rearrangement breakpoints

M. Beaumont\textsuperscript{1-2}, C. Ravel\textsuperscript{2-3}, L. Akloul\textsuperscript{4}, E. Launay\textsuperscript{1}, M. Mehrjouy\textsuperscript{5}, G. Jouve\textsuperscript{2}, M. Domin\textsuperscript{6}, N. Tommerup\textsuperscript{5}, S. Odent\textsuperscript{4}, M.A. Belaud-Rotureau\textsuperscript{1-2}, S. Jaillard\textsuperscript{1-2}

1- CHU Rennes, Service de Cytogénétique et Biologie Cellulaire, F-35033 Rennes, France
2- INSERM U1085-IRSET, Université de Rennes 1, F-35042 Rennes, France
3- CHU Rennes, Service de Biologie de la Reproduction CECOS, F-35033 Rennes, France
4- CHU Rennes, Service de Génétique Clinique, CLAD Ouest, F-35033 Rennes, France
5- Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark, International Breakpoint Mapping Consortium (IBMC)
6- CHU Rennes, Département de Gynécologie Obstétrique et Reproduction Humaine, F-35033 Rennes, France

Premature ovarian failure (POF) is characterized by the occurrence amenorrhea before the age of 40 associated with high follicle stimulating hormone (FSH) levels and low estradiol levels. It is supposed that the pathogenesis of POF is strongly under the control of genetic factors, and that it is a multifactorial complex disease. Identification of a causative genetic alteration has become a new challenge due to the possibility of preservation of female fertility. Nevertheless, the task appears difficult due to the polygenic nature of POF. Patients with POF may harbor multiple genetic variants which justify investigation of POF by next generation sequencing (NGS). NGS is also a powerful tool for characterizing and mapping balanced chromosomal rearrangements at the nucleotide level. We investigate by FISH and NGS the breakpoints of balanced chromosomal pericentric inversions bore by two patients with POF: a \textit{de novo} chromosome 9 inversion with breakpoints at 9p21.3 and 9q31.1 and a familial chromosome 2 inversion with breakpoints at 2p23.1 and 2q21.1. Novel candidate genes potentially involved in reproductive phenotype thus in POF were identified. On chromosome 9, \textit{ELAVL2} located at the breakpoint on 9p21.3, is implicated in efficient production of fully grown and meiotically competent metaphase II oocytes. On chromosome 2, \textit{SRD5A2}, which encodes the steroid 5-alpha reductase 2, is located in the vicinity of the breakpoint on 2p23.1. Its level of expression has been linked to polycystic ovarian syndrome and fertility. Although other genetic factors may take part to the occurrence of POF, the analysis of the breakpoints of two balanced inversions have deciphered novel candidate genes for POF. Further studies are needed to make these gene reliable genetic factors for the ovarian deficiency.
Cohort of 13 Patients with 46,XX SRY negative testicular and ovotesticular variation of sex development: review of clinical findings, management and molecular studies

Sophie Lambert (1), Capucine Hyon (2,3), Claire Bouvattier (4), Matthieu Peycelon (5,9), Laurence Dumeige (1,6), Michel Peuchmaur (7), Julie Léger (1,8,9), Dominique Simon (1), Annabel Paye-Jaouen (5), Jean-Pierre Siffroi (2,3), Jean Claude Carel (1,8,9), Ken McElreavey (10), Alaa El Ghoneimi (5,9), Laetitia Martinerie (1,6,9)

Department of Pediatric Endocrinology, Centre de Référence des Maladies Endocrinienes Rares de la Croissance, Robert Debré Hospital, Assistance publique-Hôpitaux de Paris, Paris, F-75019 Paris France
(2) AP-HP, Hopitaux Universitaires Est Parisien, Hôpital Trousseau, Service de Génétique et d’Embryologie médicales, Paris, France
(3) UPMC Univ Paris 06, UFR de Médecine Pierre et Marie Curie, Paris, France
(4) Pediatric Endocrinology Department, Bicêtre Hospital, Paris-Sud University, Kremlin Bicêtre, 94270 Paris, France
(5) Department of urology and visceral surgery, Robert Debré hospital, Assistance publique-Hôpitaux de Paris, Paris, France
(6) Institut National de la Santé et de la Recherche Médicale (Inserm), Unité 1185, F-94276 Le Kremlin Bicêtre, France
(7) Pathology Department, Robert Debré Hospital, Assistance publique-Hôpitaux de Paris, Paris, France
(8) Institut National de la Santé et de la Recherche Médicale (Inserm), Unité 1141, DHU Protect, F-75019 Paris, France
(9) Paris Diderot University, Sorbonne Paris Cité, F-75019 Paris, France
(10) Institut Pasteur, Human Developmental Genetics, Paris, France

Background: 46,XX testicular and ovotesticular disorders of sex development (TDSD and OTDSD) represent a very rare and unique cause of DSD where testicular tissue develops in absence of a Y chromosome. The most frequent underlying mechanism identified is a translocation of the SRY gene (~20% of the cases), but several new genes and pathways involved in the development of testicular tissue have recently been identified. To date, very few studies have described the phenotype, the clinical and surgical management and investigated the genetic aspects of 46,XX SRY- TDSD and OTDSD patients.

Methods: The records of all 46,XX SRY negative TDSD and OTDSD patients, followed between 1994 and 2015 in the Endocrine Clinics of two French centers were retrospectively reviewed and completed by a prospective genetic evaluation using SNP-array and whole exome sequencing.

Results: Among the eleven patients with 46,XX OTDSD and two patients with 46,XX TDSD included, most (9/13) were seen in the neonatal period. Sex of rearing was male for six patients and female for seven, while the clinical presentation varied, with an external masculinization score from 1 to 10. Ovotestes/testes were found bilateral for 50% of the patients and unilateral for the others (with a contralateral ovary). Two girls were successfully treated with GnRH analog therapy to avoid virilisation during minipuberty. Genital surgery preserved appropriate gonadal tissue in the majority of cases. Spontaneous puberty occurred in two girls and one boy, while two boys required hormonal induction of puberty. One of the girls conceived spontaneously and had an uneventful pregnancy. The DNA of twelve patients was analyzed by SNP-array and a whole-exome sequencing was performed on five patients. Genetic analyses did not find any pathological variation in previously identified genes or candidate genes.

Conclusion: This study presents a well-characterized population of patients with 46,XX, SRY negative TDSD and OTDSD. Molecular diagnosis for these patients is currently missing which suggests the existence of defects in other genes or DNA regulatory sequences involved in gonadal determination.
Identification of 45,X/46,XY Mosaicism from a Gonad Biopsy on an Infant with 46,XY in Peripheral Blood with Proximal Hypospadias and Undescended Testicle.

E. Leon¹; A. Hill²; H. Pohl³.

Genetics¹; Pathology²; Urology³, Children’s National Health System. Washington, DC, United States.

45,X/46,XY mosaicism is associated with a broad spectrum of phenotypes ranging from apparently normal male development to individuals with incomplete sexual differentiation and clinical signs of Turner syndrome in both males and females. The most common presentation among individuals with a 45,X/46,XY karyotype is sexual ambiguity, accounting for approximately 60% of cases, while the least common category of 45,X/46,XY patients consists of those with bilaterally descended testes, found in 11-12%.

We present an infant boy who was initially seen in the Urology clinic for unilateral undescended testicle and proximal hypospadias at 1 month of age. On exam he had a left descended testicle, which was slightly hypertrophied in size subjectively. The right testis was non-palpable. He had a 2.5cm phallus with a hooded foreskin and hypospadias of the proximal shaft associated with a small amount of chordee that tethered his scrotal skin up to the level of the meatus. He also had a superficial sacral dimple. At 2 months of age, pelvic and scrotal ultrasounds showed a left testis with normal architecture, echotexture, and dimensions of 0.6 x 1.2 x 1 cm, with a total volume of 0.5 mL. A right gonad was identified in the pelvis just to the right of the midline with approximate dimensions of 0.9 x 1.1 x 0.4 for a volume of 0.2 mL. It was difficult to ascertain flow because of the location and it being among peristalsing bowel loops. His spine and renal ultrasounds were normal.

A laparoscopic orchidopexy with hypospadias repair at 7 months was switched to diagnostic laparoscopy with laparoscopic biopsies after noticing absence of left vas deferens with mildly dysplastic appearing testicle, right ovarian tissue with fallopian tube and hemi-uterus, and a right intra-abdominal dysplastic testicle tissue. Pathology showed testicular tissue with germ cell hypoplasia, streak ovary with small “gonadoblastoma-like” structures without germ cells, and connective tissue without obvious Mullerian or Wolffian structures respectively. Cytogenetic analysis of the streak ovary revealed 45,X[24]/46,XY[6] mosaicism. Genetics was consulted and physical exam was completely normal besides known genital findings. His growth parameters at almost 8 months of age were: weight 50th percentile, length 25th percentile and head circumference 75th percentile. Echocardiogram was normal. At 10 months of age he had a laparoscopic right gonadectomy, removal of hemi-uterus and fallopian tube, as well as hypospadias repair and release of chordee without complications. Pathology report showed fallopian remnant and fibrovascular tissue. At age 2, he had excision of sebaceous penile inclusion cyst without complications. At that time, his height was in the 6th percentile and weight was between 50th to 75th percentiles, showing short stature for parental height. He did not have any problems voiding.

45,X/46,XY mosaicism constitutes one of the most common causes of Disorders of Sex Development (DSD). To our knowledge, this is the first patient reported with 45,X/46,XY phenotype that has a normal karyotype on peripheral blood. This case not only emphasizes the need of diagnostic laparoscopy in young children where imaging evaluation is limited, but also the importance of laparoscopic biopsies of intra-abdominal gonads that have abnormal morphology along with cytogenetic studies. The comprehensive diagnostic DSD work-up can be better achieved with a multidisciplinary approach that will ultimately benefit the patient’s anticipatory guidance and care.
NR5A1 Mutations: Clinical, Endocrine and Genetic Features. A Survey by Italian DSD Study Group


Introduction. NR5A1 (nuclear receptor subfamily 5 group A member 1) is a transcriptional regulator of genes involved in adrenal and gonadal development and function. In humans, NR5A1 mutations have been reported as an emerging cause of 46,XY disorders of sex development (DSD). The clinical, endocrine and genetic features of a large series of Italian 46,XY DSD individuals with NR5A1 mutations are reported.

Materials and methods. Multicenter retrospective study by specific developed electronic data-sheet was done within the Italian DSD Study Group (It-DSD; www.gruppodistudio-it-dsd.org). All subjects followed in participating It-DSD centres with 46,XY DSD and bearing NR5A1 pathogenic mutations were included.

Results. A total of 23 children with NR5A1 deficiency were recruited from 7 centres. Median age at diagnosis was 2.9 years (range 1 month – 22.3 years). At first observation, the phenotype ranged from female with mild clitoromegaly and/or inguinal hernia containing gonads to male with micropenis (mean Prader score 2.5). NR5A1 deficiency was not the first diagnosis in all but one cases. Mean birth weight was 3,676 g (range 2,750 – 4,750). At birth, assigned sex was female in 15 newborns (65%) and male in 7 ones (30%); sex of rearing was not assigned in 1 baby. During follow-up, sex reassignment was done in 3 children (2 girls to male sex; 1 boy to female sex); female sex was assigned to the baby with no sex assignment at birth. Gonadal surgery was performed in 12 girls (gonads were maintained in 3 females and in all the males). Levels of gonadal hormones ranged from undetectable to normal age-related male values. Any significant relationship among testosterone, AMH, inhibin B and Prader score was not found. Biochemical evidence of adrenal insufficiency was not detected at diagnosis or during follow-up in all but one patient carried a homozygous NR5A1 alteration. NR5A1 mutations were widespread in all exons of NR5A1 gene; genotype-phenotype correlation was not found. NR5A1 deficiency was also detected in the mother of one girl, reporting premature ovarian insufficiency.

Conclusions: Present data showed that female sex was assigned to the majority of Italian 46,XY children with NR5A1 deficiency. Wrong preliminary diagnosis was made in the large majority of the cases, likely due to the poor knowledge of this relatively new cause of 46,XY DSD. Before sex assignment adequate endocrine and genetic investigations should be done to perform a proper diagnosis and to evaluate gonadal function. The multidisciplinary-multicentric structure of the It-DSD Study Group permitted to collect a relatively large sample with the rare NR5A1 gene mutations, allowing to better define the clinical spectrum of this DSD and its management. The It-DSD Study Group may represent a national base for collaborative studies in the field of sexual development and an opportunity - in collaboration with official Societies – for the European Reference Network for rare endocrine conditions (ENDO-ERN).
P61 Targeted gene panel sequencing improves traditional molecular analysis in DSD.

AUTHORS
I. Martínez de LaPiscina1, A. Aguayo1, G. Pérez- Nanclares1, A. Vela1,2, A. Rodriguez1,2
I. Esteva3, M. Alonso4, MC. Fernandez5, L. Forga6, L. Castaño1,2.
1 Endocrinology and Diabetes Research Group, BioCruces Health Research Institute, Hospital Universitario Cruces, UPV/EHU, CIBERER, CIBERDERM, Barakaldo, Spain.
2 Pediatric Endocrinology Service, Hospital Universitario Cruces, Barakaldo, Spain.
3 Endocrinology Service, HRU de Malaga, Malaga, Spain.
4 Pediatric Service, Hospital Universitario Ramon y Cajal, Madrid, Spain.
5 Pediatric Endocrinology Service, Hospital Universitario Basurto, Bilbao, Spain.
6 Endocrinology Service, Complejo hospitalario Navarra, Pamplona, Spain.

BACKGROUND
Disorders of sex development (DSD) are defined as a congenital condition in which the chromosomal, gonadal or phenotypic sex is atypical. The wide extent of phenotypic variation that result from this unusual development may range from hypospadias, ambiguous genitalia and complete XX or XY reversal.

Improvements in the precise diagnosis and clinical management for DSD have been facilitated in recent years by the increasing availability of genetic techniques, such as next-generation sequencing (NGS).

The present study was performed to investigate the genetic etiology of historic patients with DSD using a targeted gene panel in order to establish a genotype/phenotype correlation.

PATIENTS AND METHODS
We analyzed DNA from 45 historic patients with 46XY DSD, 12 with 46XX DSD and 1 presenting with 47XY karyotype in a targeted gene panel using Ion Ampliseq (ThermoFisher Scientific) technology. All the patients were negative in previous genetic studies. Variants were verified by Sanger sequencing and in silico analysis were performed.

RESULTS
We identified known pathogenic mutations in 5 patients in the MAMLD1, NR5A1, SRD5A2, CYP17A1 and LHCGR genes. We also identified seven novel sequence variants in the GATA4, NR5A1, LHCGR, MAP3K1, MAMLD1 and ATRX genes, which 5 were classified as likely pathogenic, and the remaining two were categorized as uncertain significance.

CONCLUSIONS
We were able to identify a likely genetic diagnosis in 15% of historic DSD patients using a candidate gene panel. Targeted gene panel sequencing is an efficient tool to improve the diagnosis of DSD.
P62 Differential diagnosis of DSD, new progress of NRC activity in Egypt after application of next generation sequencing.


Prof of clinical genetics & Endocrinology, Department of clinical Genetics, Human Genetics & genome Research Division, National Research Center.

Abstract

Disorders of sex development (DSD) are congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical.

276 patients were referred to the genetic endocrinology clinic at NRC with different DSD presenting features. All cases with ambiguous genitalia, primary amenorrhea or undescended testis were subjected to full clinical and genital examination, cytogenetic study in 50 metaphases, hormonal assay of Testosterone and its precursors & DHT in 46, XY patients, FISH analysis and molecular study of AR, SRD5A2, NR5A1, 17BHSD genes were done when indicated. Exome sequencing was done in cases of idiopathic 46, XY DSD.

Sex chromosomal disorders were detected in 76 patients and included different types of numerical and structural sex chromosomal abnormalities.

Seven patients presenting with primary infertility and azoospermia had 46,XX testicular DSD; Autosomal abnormalities were detected in four patients with multiple congenital anomalies associated with DSD. 82 patients were diagnosed with 46,XY DSD. Sequencing analysis of SRD5A2 gene showed G34R mutation in three patients confirming the gene founder effect, one patient had Q56R mutation, two patients showed homozygous Y91H mutation and three showed G196S, while 4 patients showed compound heterozygous mutations (A207D + L89V), and (G196S + L89V).

Molecular analysis of AR gene showed a novel mutation c.2731del TC, that led to frame-shift and premature stop codon in one patient, while four patients showed previously reported mutations.

Mutations in HSD17B3 were detected in 5 patients, 6 novel mutations were determined in 4 patients. Two patients showed compound heterozygous mutations, while 8 families had probands with homozygous mutations. The molecular analysis of NR5A1 gene showed a novel mutation in a 46,XY gonadal dysgenesis patient, who had shown also MAP3K gene mutation, suggesting digenic inheritance.

A mutation in HHAT gene was found in another female patient with 46,XY gonadal dysgenesis.

WT1 mutation was also detected in a patient with 46,XY gonadal dysgenesis. A mutation in AMHR was detected in a 46,XY patient with undescended testis; while a novel mutation was found in CYP17A1 gene in two sisters with 46,XY DSD.

This study enlarge the scope of both unusual cytogenetic, monogenic mutations and emphasize the application of new technique for accurate diagnosis and treatment for better genetic counseling.
Introduction
Disorders of Sex Development (DSD) involve atypical clinical conditions related to sex assignment. One of the determining factors of this phenotype is the genetic component, represented by the presence of chromosomal abnormalities, in particular of the sex chromosomes, or by alteration of genes involved with the embryonic development of the sexual organs. Successful sexual differentiation depends on fine genetic control in sexual and autosomes genes. These genes are responsible for the tissue differentiation of different organs and the proper production and functioning of stereidogenic enzymes. Any alteration in one of these actors will result in an inadequate formation of the internal and / or external genital organs leading to the expression of a phenotype of sexual development disorder in the affected individual. Although the genes involved in the activation of the testicular or ovarian differentiation pathways are known; in some cases this information is not sufficient to explain the particular phenotype of a patient. The objective of this study was to genetically characterize 15 patients with DDS evaluated by the transdisciplinary DDS group of the Hospital Universitario San Ignacio (2016-2017).

Methodology: For the chromosomal analysis, high resolution cell cultures for G and R banding and FISH were performed with a specific probe to evaluate the presence or absence of the SRY gene. To evaluate alterations in the number of copies of the NR0B1, SOX9, SRY, WNT4 and NR5A1 genes MLPA (Multiplex Ligation-dependent Probe Amplification) analyzes were performed using the SALSA MLPA P185-C1 Intersex MRC-Holland kit.

Results: Fifteen patients with disorders of sexual development were analyzed, 8 were assigned male sex and 7 were assigned female sex; The clinical characteristics of these patients included hypospadias (47%), complete gonadal dysgenesis (20%), presence of ovotestis tissue (7%), gonadal regression (7%), androgen synthesis defect Receptor defect or HAM (7%). 100% of the patients were analyzed by conventional cytogenetics and FISH for SRY. Likewise, MLPA analysis was performed for the NR0B1, SOX9, SRY, WNT4 and NR5A genes.
Increased gene dosage: sex reversal in two patients with duplications of either DAX1 (46,XY gonadal dysgenesis) or the SOX9-regulatory region (46,XX-testicular DSD)

S. Riedl1,2, D.A. Ertl1, A. Raimann1, G Häusler1, U. Tonnhofer3, A. Springer3

1Department of Pediatric Pulmology, Allergology and Endocrinology, Medical University of Vienna, Austria

2St Anna Children’s Hospital, Medical University of Vienna

3Division of Pediatric Surgery, Department of Surgery, Medical University of Vienna

Objectives

Duplications of “Dosage sensitive sex reversal adrenal hypoplasia congenita critical region” (DAX1; Xp21) constitute a rare cause of 46,XY-DSD due to suppression of the genetic testicular pathway by increased DAX1 gene dosage. Analogously, duplications of a regulatory element upstream of SOX9 (17q24) have been identified in 46,XX SRY-negative individuals with testicular DSD. We observed two such patients.

Methods

Clinical data collection, biochemical investigations and genetics testing (Karyotyping, CGH-Array, MLPA) were performed using routine techniques/procedures.

Results

19-year-old patient 1 (188cm/88kg, phenotypically female) was referred to our department because of secondary amenorrhea. She was mentally retarded and received antipsychotic treatment due to a severe behavioural disorder. In addition, she had dyslipidemia, hepatic steatosis and mild type 2 diabetes (HbA1c 7.1%). Karyotype was 46,XY. Hormone tests showed primary gonadal insufficiency, AMH <0.08ng/mL. Pelvic MRI revealed a hypoplastic uterus and irregular gonadal structures with a right-sided cyst. CGH-array analysis showed a 9.5 Mbp duplication in Xp [Xp22.11p21.1(23,917,544-33,382,057)x2], including DAX1. 9-year-old patient 2 was referred to our surgical department for right undescended testis (inguinal). He had had surgery for hypospadias in early childhood (Syria). Karyotype was 46,XX. On MRI no Mullerian structures could be visualized, AMH lying in the male range. HCG test (5000 IU/sqm) showed a diminished testosterone rise (0.3ng/mL). MLPA revealed a 1 Mbp duplication upstream of SOX9 that contains SOX9-regulatory elements [17q24.3(68,694,434-69,773,309)x3].

Conclusions

Increased DAX1 or SOX9 gene dosage may lead to suppression of predetermined gonadal differentiation resulting in 46,XY-DSD with gonadal dysgenesis or 46,XX testicular DSD, respectively. Associated psychomental symptoms in our DAX1-duplication patient are attributable to additionally duplicated genes whereas metabolic syndrome was probably drug-induced (atypical antipsychotics).
P65 CBX2.2 mutation as novel cause for 46,XY Disorders of Sex Development

P. Sproll#, W. Eid#, C. Gomes§, B. Mendonca§, E. Costa§, A. Biason-Lauber#

# University of Fribourg, Dept of Medicine, Division of Endocrinology, Switzerland
§ University of Sao Paulo, Medical School, Brazil

patricksproll@unifr.ch

Introduction:
Sexual differentiation during embryonic development is one of the defining moments of human life. The chromatin regulator CBX2.1 has previously been identified as essential for human male development. However, less is known about the second isoform CBX2.2. We set to elucidate the role of CBX2.2, taking advantage of two distinct mutations in two unrelated 46,XY DSD patients with female phenotype and dysgenetic gonads.

Methods:
Whole Exome Sequencing has been performed, to exclude mutations on other genes implicated in Disorders of Sex Development. To identify CBX2.2 targets, we performed DNA adenine methyltransferase and next generation sequencing in human testicular cells and analyzed the data with Pathway Studio and Gene Ontology enrichment. The expression pattern of potential candidate genes has been validated using qRT-PCR under overexpression of either WT or mutant CBX2.2.

Results:
No rare malignant mutations have been found on other genes besides CBX2.2 through Whole Exome Sequencing. We could identify over 1900 direct binding targets of CBX2.2. Six were selected based on their known role in sex development: EMX2, MAK, HOXA13, WDR77, TWIST1 and BNC2. We validated the influence of WT and both mutant CBX2.2 on these targets using qRT-PCR. In particular, WT CBX2.2 increased the expression of EMX2, whereas the mutated CBX2.2 proteins were inactive.

Conclusion:
It is intriguing to hypothesize that, at least in part, mutations in CBX2.2 impairing EMX2 expression causes gonadal dysgenesis in 46,XY individuals, similarly to EMX2 haploinsufficiency. This study shows the importance of CBX2.2 and identifies several of its partners, broadening our understanding of sex development and disorder of sex development.
P66 46, XX Disorder of sex development caused by a NR5A1 heterozygous mutation: a case report and literature review


Corresponding author: Su Zhe, Email: Su_zhe@126.com

【Abstract】 Objectives To summarize the clinical manifestations of 46,XX disorder of sex development(DSD) caused by a NR5A1 heterozygous mutation. Methods The first case of 46,XX DSD caused by a NR5A1 heterozygous mutation in China was reported with a review of 11 similar cases in the literatures from July 2016. Results The 5.6-year-old child raised as female was born with ambiguous genitalia. After clinical evaluations, diagnosis of 46, XX DSD was made with absent of uterus. The gonads located in the inguinal region (left) and intra-abdomen (right). They both were found to be ovotestis by pathologic examination. SRY gene was negative. A heterozygous de novo mutation c.274C>T (p.Arg92Trp) in the accessory DNA-binding region of NR5A1 gene was found in the child. It was the same mutation with other 10 cases in literatures and heterozygous mutation of the other one case was reported to be c.275G>A (p.Arg92Trp) . Our case showed similar presentations with the reported cases assigned to be females. The possible underling mechanism might relate to impairment of the binding between the mutant protein and target DNA which might lead to decreased inhibition of the male developmental pathway through downregulation of female antitestis genes. Conclusions This was the first case of 46,XX DSD caused by a NR5A1 heterozygous mutation in China. She had similar presentations as reported.

【Key words】 46,XX; Disorders of sex development; NR5A1 gene; SF-1; Mutation
**P67 MAMLD1 deletions in three patients with proximal hypospadias**

Y. van Bever¹, L.J.C.M. van Zutven¹, S. Demirdas¹, W.Oostdijk³, K.P.Wolffenbuttel²,⁴, A. de Klein¹, B. Eussen, M.M. van Veghel-Plandsoen¹, J.J. Saris⁵ en E.Oussoren⁵

¹Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, the Netherlands
²DSD center, Erasmus University Medical Center, Rotterdam, the Netherlands
³Department of Pediatrics, Leiden University Medical Centre, Leiden, the Netherlands
⁴Department of Pediatric Urology, Erasmus University Medical Center, the Netherlands
⁵Department of Pediatrics, Center for Lysosomal and Metabolic Diseases, Erasmus University Medical Center, Rotterdam, the Netherlands.

Hypospadias is a congenital malformation that has a prevalence of 4-43:10,000. Distal or (sub)glandular hypospadias is far more common than proximal hypospadias. In most cases of isolated distal hypospadias inheritance is multifactorial, while the more proximal anomalies are part of the spectrum of Disorders of Sex Development (DSD) and can have a variety of genetic defects. **MAMLD1** is one of the genes associated with hypospadias, although functional studies in mouse KO models do not support this as the male knockout mice do not show hypospadias and have normal fertility¹²³. Patients with a contiguous syndrome involving the **MTM1** gene and **MAMLD1** have been described as well as patients with deletions encompassing **IDS** and **MAMLD1** not showing hypospadias.

We present the findings in two sibs with proximal hypospadias and a very small deletion of **MAMLD1** confirmed with MAQ assay and a third patient with Hunter syndrome and hypospadias carrying a deletion encompassing the **IDS** gene, which extends to the first exon of transcript 1 of **MAMLD1**.

²Camats N, Fernández-Cancio M, Audi L, Mullis PE, Moreno F et al. CE. Human **MAMLD1** gene variations seem not sufficient to explain a 46,XY DSD phenotype. PLoS ONE 2015: 10 (11):1-20
³Miyado M, Nakamura M, Miyado K, Morohashi K-I, Sano S et al. Mamld1 deficiency significantly reduces mRNA expression levels of multiple genes expressed in mouse fetal Leydig cells, but permits normal genital and reproductive development. Endocri 2012:153(12)6033-6040
The role of a next generation sequencing panel in the diagnostic pathway in Disorders of Sex Development

Webb EA1,2, Hughes LA3, Allen S3, Cole T3, Reed J4, Drinkall, ND3, Chandran H5, McCarthy L5, Kirk JMW1, Krone NP1,6.

1 Department of Endocrinology & Diabetes, Birmingham Children’s Hospital, Steelhouse Lane, Birmingham, B4 6NH, United Kingdom
2 Institute of Metabolism and Systems Research, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism Birmingham Health Partners, Birmingham, UK
3 West Midlands Regional Genetics Service, Birmingham Women’s NHS Foundation Trust, Birmingham, B15 2TG
4 Department of Clinical Psychology, Birmingham Children’s Hospital, Steelhouse Lane, Birmingham, B4 6NH, United Kingdom
5 Department of Urology, Birmingham Children’s Hospital, Steelhouse Lane, Birmingham, B4 6NH, United Kingdom
6 Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield, Sheffield Children’s Hospital, UK

Objectives
To highlight how advances in genomic medicine change clinical management of individuals with disorders of sex development (DSD).

Results
Patient 1 presented at birth with proximal peno-scrotal hypospadias, micropenis, partial bifid scrotum and no palpable gonads. Initial hyponatraemia (128mmol/L) quickly resolved with low dose sodium supplements. Urinalysis was normal. Karyotype was 46,XY, cortisol peak to synacthen stimulation was normal and baseline testosterone low (1.3nmol/L). Following review at the multidisciplinary DSD clinic laparoscopy was planned to locate the gonads and clonal sequencing using a custom designed TruSeq amplicon panel covering 31 genes associated with DSD performed.

Aged 4 months pathogenic WT1 mutation was identified (c.1087A>T p.(Arg363*)). Urgent USS pelvis identified a heterogeneous lesion in the left kidney, On biopsy diffuse mesangial sclerosis was identified. The patient went on to have a left nephrectomy and chemotherapy for Wilms tumour aged 9 months.

Patient 2 had genetics performed as part of investigation for her right sided diaphragmatic hernia and motor delay aged 2 years; karyotype was 46,XY. She has normal external female genitalia. Baseline and hCG stimulated testosterone were undetectable. She was reviewed in the DSD clinic prior to the development of the DSD gene panel. USS pelvis aged 3 years showed a rudimentary uterus, possible right ovary and hyperechogenic kidneys. Following review at the multidisciplinary DSD clinic aged 4 years targeted WT1 screening was performed in view of her diaphragmatic hernia and echogenic kidneys. A heterozygous c.1228+5G>A pathogenic splice site mutation in intron 9 of WT1 was identified. Aged 5 years she had excision of a left streak gonad and a right gonadoblastoma.

Conclusions
These cases highlight the value of targeted sequencing panels in achieving an early diagnosis in children with DSD. Children with WT1 mutations are at significant risk of malignancy as well as renal failure and benefit from regular clinical assessment. If the DSD panel had been available at the time patient 2 was first seen in the DSD clinic her WT1 mutation may have been identified several years earlier and she may not have developed a gonadoblastoma. In our clinical practice, we have therefore introduced NGS analysis at an early stage of our clinical pathway to enable timely personalized medicine delivery.
P69 Molecular genetic analysis to optimize the care pathway in individuals with disorders of sex development (DSD)

Webb EA1, 2, Hughes LA3, Allen S3, Fews G3, Chandran H4, McCarthy L4, Kirk JMW1, Krone NP1, 5, Cole T3

1 Department of Endocrinology & Diabetes, Birmingham Children’s Hospital, Steelhouse Lane, Birmingham, B4 6NH, United Kingdom
2 Institute of Metabolism and Systems Research, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism Birmingham Health Partners, Birmingham, UK
3 West Midlands Regional Genetics Service, Birmingham Women’s NHS Foundation Trust, Birmingham, B15 2TG
4 Department of Urology, Birmingham Children’s Hospital, Steelhouse Lane, Birmingham, B4 6NH, United Kingdom
5 Academic Unit of Child Health, Department of Oncology & Metabolism, University of Sheffield, Sheffield Children’s Hospital, UK

Background: The classic first-line diagnostic pathway in individuals with disorders of sex development (DSD) consists of clinical examination, biochemical investigations and karyotype determination. Once a presumptive diagnosis has been made targeted molecular genetic analysis candidate genes is consequently performed in a stratified manner.

Method: A 30 gene DSD panel was designed using Illumina software Design Studio. TruSight One technology was used to screen 30 DSD related genes in 75 patients who presented with DSD of unknown aetiology (Table 1).

Results: We identified a diagnosis in 24 individuals (32%). Pathogenic mutations in AR, SRD5A2, HSD17B3, NR5A1, AMH, AMHR2, WT1, LHCGR & MAMLD1 were found. Four previously unreported mutations were identified. 35 individuals had no evidence of a mutation and in 16 patients we identified variants of unknown significance. Genetic analysis impacted significantly on patient management. For example, in a child with 46,XY DSD the identification of an AR mutation led to the previously planned surgical intervention being cancelled. The confirmation of a WT1 mutation in a 4 month old baby ensured early referral for Wilms tumour screening.

Conclusion: Prior to Next Generation Sequencing (NGS), genetic tests were only available for a few genes associated with DSD, which required laborious and lengthy sequential testing. We have demonstrated that a NGS strategy can improve the molecular diagnosis of DSDs. This approach is, however, not without challenges as a significant number of variant of unknown significance are detected. Genetic testing in complex multigenic conditions such as DSD enables accurate early diagnosis leading to early instigation of personalised patient management. We therefore suggest the early use of genetic testing in conjunction with clinical and biochemical assessment.

<table>
<thead>
<tr>
<th>Disorders of Testicular Development</th>
<th>Disorders of Hormone synthesis or action</th>
<th>Disorders of Ovarian Development</th>
<th>46, XX DSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1 (11p13)</td>
<td>DHCR7 (11q12-q13)</td>
<td>SRY (Yp11.3)</td>
<td>HSD3B2 (1p13)</td>
</tr>
<tr>
<td>CBX2 (17q25)</td>
<td>LHCGR (2p21)</td>
<td>SOX9 (17q24)</td>
<td>POR (7q11.2)</td>
</tr>
<tr>
<td>NR5A1/SF1 (9q33)</td>
<td>StAR (8p11.2)</td>
<td>RSPO1 (1p34.3)</td>
<td>CYP11B1 (8q21-q22)</td>
</tr>
<tr>
<td>SRY (YP11.3)</td>
<td>CYP11A1 (15q23-24)</td>
<td>WNT4 (1p35)</td>
<td>CYP19A1 (15q21)</td>
</tr>
<tr>
<td>SOX9 (17q24-q25)</td>
<td>HSD3B2 (1p13.1)</td>
<td></td>
<td>Glucocorticoid receptor (5q31)</td>
</tr>
<tr>
<td>DHH (12q13.1)</td>
<td>CYP17A1 (10q24.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARX (Xp22.13)</td>
<td>POR (7q11.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSPYL1 (6q22-23)</td>
<td>CYB5A (18q23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAMLD1 (Xq28)</td>
<td>HSD17B3 (9q22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMRT1 (9p24.3)</td>
<td>SRD5A2 (2p23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATRX (Xq13.3)</td>
<td>AR (Xq11-q12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NROB1/DAX1 (Xp21.3)</td>
<td>AMH (19p13.3-p13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNT4 (1p35)</td>
<td>AMHR2 (12q13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table listing the genes covered by our panel.
Does Genitography Outweigh Cystoscopy in Detecting Severity of Anomaly in Congenital Adrenal Hyperplasia?


*Division of Pediatric surgery, Cairo University Specialized pediatric Hospital (CUSPH), Cairo, Egypt.
**Division of Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU), Cairo University Specialized pediatric Hospital (CUSPH), Cairo, Egypt.

**Purpose:** We present our experience in performing genitography under general anesthesia for girls with congenital adrenal hyperplasia giving particular attention to confluence depth, preconfluence urethral length and vaginal diameter. We question the ability of cystoscopy as a routine popular investigation in measuring depth of urethro-vaginal confluence.

**Materials and Methods:** Data is presented in a prospective preoperative evaluation of 10 girls with congenital adrenal hyperplasia who underwent feminizing genitoplasty. Patients were generally anesthetized and lied in left lateral decubitus after placing radio-opaque marker of known length at the site of future vagina. Depth of confluence of vagina with urethra was measured in millimeters.

**Results:** Ten patients were preoperatively assessed by combined genitography and cystoscopy. Depth of confluence was <15 mm in 3 patients while cystoscopy showed long common channel in one of them giving false impression of a high anomaly. Depth of confluence in 4 patients was >15 mm and <25 mm. Three patients had a confluence depth of >25 mm. Anomaly level was confirmed with intraoperative surgical results.

**Conclusions:** Depth of urethro-vaginal confluence from perineal planned site of vagina detected precisely using genitography is more accurate than length of urogenital sinus and distance from vaginal orifice to external meatus that are detected by cystoscopy. This is crucial in preoperative plan of surgical management.

**Key Words:** Urogenital Sinus; Adrenal Hyperplasia, Genitography; Cystoscopy; Confluence.


*Division of Pediatric surgery Cairo University Specialized pediatric Hospital (CUSPH), Cairo, Egypt.
**Division of Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU) Cairo university, Cairo, Egypt.

Purpose: We present our experience using partial urogenital sinus mobilization in girls with congenital adrenal hyperplasia, with particular attention to vaginal caliber, vaginal exteriorization and clitoral cosmetic appearance.

Materials and Methods: Data is presented in a prospective evaluation of 20 girls with congenital adrenal hyperplasia who underwent feminizing genitoplasty using urogenital sinus mobilization with preservation of pubourethral ligaments during a 3-year period. Depth of confluence of vagina with urethra determined by genitography done under general anesthesia and degree of external genitalia virilization defined according to Prader classification, were evaluated before reconstruction. At follow up patients were examined under general anesthesia for evaluation of overall external genitalia cosmesis and calibrating the vagina.

Results: Patient age at operation ranged from 14 to 62 months (median 24 months), with a mean follow up of 23.5 months (3 to 66). Degree of virilization was Prader type II in 2 children (10%), type III in 14 children (70%), type IV in 4 (20%). Confluence depth was 20 mm or less in 18 children. Cosmetic results were good in 18 patients (90%) and satisfactory in 2 (10%). The vaginal and urethral openings were separate and identified at the surface of the vestibule in 17 girls (85%). Adequate caliber of the mobilized vagina was achieved in 19 patients (95%).

Conclusions: Fair cosmetic appearance with partially hidden small clitoral glans and adequate exteriorization of the vaginal and urethral openings is achieved in most children with urogenital sinus treated with partial urogenital sinus mobilization.

Key Words: Urogenital Procedures; Adrenal Hyperplasia, Congenital; Urethra; Vagina.
The use of a comprehensive standardized anatomic intake form for patients with a DSD has been shown to be feasible within the DSD-TRN, and thus standardization of anatomic findings should be possible for all patients with a DSD. Filling out the forms completely and correctly has been shown to be difficult and will need to be addressed before such standardized forms are used nationally.
P73 Accuracy of pelvic MRI in the evaluation of internal genitalia and mullerian structures in patients with Disorders of Sex Development having at least one palpable gonad

L. Mahfouz El Nachar (1), D. Rekik (2), N. De Roux (3), M. Peycelon (4), J. Léger (1,3,5), A. Paye-Jaouen (4), M. Alison (2), A. El Ghoneimi (4), JC. Carel (1,3,5), L. Martinerie (1,5)

(1) Department of Pediatric Endocrinology, Centre de Référence des Maladies Endocriniennes Rares de la Croissance, Robert Debré Hospital, Assistance Publique-Hôpitaux de Paris, F-75019 Paris France
(2) Department of pediatric Radiology, Robert Debré hospital, Assistance Publique-Hôpitaux de Paris, France
(3) Institut National de la Santé et de la Recherche Médicale (Inserm), Unité 1141, DHU Protect, F-75019 Paris, France
(4) Department of visceral surgery, Robert Debré hospital, Assistance Publique-Hôpitaux de Paris, France
(5) Paris Diderot University, Sorbonne Paris Cité, F-75019 Paris, France

Background: Patients with disorders of sex development (DSD) require multidisciplinary management for etiology identification and gender assignment. Identification of mullerian structures is a crucial step of the evaluation process. Ultrasonography remains the first-line imaging modality to delineate mullerian structures; while the importance of pelvic magnetic resonance imaging is insufficiently studied to date.

Objective: To evaluate the diagnostic accuracy of pelvic MRI in the assessment of internal genitalia in DSD patients with at least one palpable gonad at diagnosis.

Design, setting and participants: Retrospective comparative single-center study (2008-2014) of DSD patients with at least one palpable gonad, who underwent pelvic MRI and surgical management. Clinical, biological and cytogenetic data were evaluated. A radiologist reviewed imaging blindly. Pelvic MRI findings were compared to ultrasound and per-operative cystoscopy whenever performed.

Results: Forty-six patients were included: 46, XY (n=41), 45, X/46, XY (n=3), 46, XX ovotestis (n=1) and 46, XX testicular DSD (n=1). Only one patient was raised as female. Thirty patients (65,2%) were seen during the neonatal period (2 ± 2,1 days), with severe hypospadias (95,6% of cases), and mean genital bud length of 20,1 ± 5,8mm. Urethroplasty was performed at 15,1 ± 4,1 months. Pelvic ultrasound and per-operative cystoscopy were done in 89,1% and 28,2% of patients, respectively. Pelvic MRI findings were concordant with ultrasound in identifying cavities with a retro-urethral origin (vagina or utricle) and uterine structures in 83% and 100% respectively. When comparing to per-operative cystoscopy, sensitivities of pelvic MRI and US were equal (80%) in identifying uterus with a specificity calculated at 100% in both cases; while sensitivities to identify vagina or large utricle were estimated at 86,7% and 80% for pelvic MRI and ultrasonography respectively.

Conclusion: Pelvic MRI evaluation for internal genitalia appears complementary to ultrasonography in the neonatal period only for vagina/ large utricle cavities. Thus, cost effectiveness of this expensive technique in evaluating DSD patients should be reviewed to reduce costs on public health.

Keywords: Disorder of Sexual Development, mullerian structures, pelvic MRI, gender assignment.
P74 Laparoscopically assisted vaginal pull through in four cases of congenital adrenal hyperplasia with high urogenital confluence: early results

A.E. Fares*, K.S. Abdullateef*
*Division of Pediatric surgery Fayoum University Hospital, Fayoum, Egypt.
**Division of Pediatric surgery Cairo University Specialized Pediatric Hospital (CUSPH), Cairo, Egypt.

Purpose: Surgical management of the high urogenital sinus is challenging. Separation of the vagina from the urogenital sinus is the most challenging portion of the operation. Presence of short urethra is a contraindication for urogenital sinus mobilization as this will jeopardize urinary continence.

In this study we report our initial results with four cases of high urogenital sinus treated by laparoscopically assisted vaginal pull through technique.

Materials and Methods:

This study included four girls undergoing laparoscopically assisted vaginal pull through. All have the diagnosis of congenital adrenal hyperplasia with high urogenital sinus.

The surgical technique was performed in supine position with the camera at the umbilicus and two working ports in the right and left iliac fossae. Another port for retraction of the urinary bladder was inserted in the supra pubic area. Mobilization of the vagina was initiated by hook cautery.

We performed this mobilization of the vagina till the confluence became visible and vaginal size become tapered at its junction with the urethra. Then the connection is sutured or clipped then divided. The tract for pullthrough is created from the perineum and a clamp is passed from down to the peritoneal cavity. The vagina is grasped and pulled outside then sutured to the skin.

Results: The four patients were preoperatively assessed by genitography. All had short urethra above the urogenital confluence 15 mm or less. They had laparoscopically assisted vaginal pull through. Mobilization of the vagina has been possible on all cases attempted without injuries to the near structures in the pelvis. Dilatation started 2 weeks postoperative and showed patency and good diameter of the pulled vagina.

Conclusions:
The laparoscopically assisted vaginal pull through approach provides optimal exposure, facilitates vaginal dissection and separation from the urethra, avoids injuries to the urinary structures. It also allows reconstruction of the vagina without tension. Early results showed good vaginal size and that urinary functions are not impaired.

Key Words: vaginal pull through; congenital adrenal hyperplasia, laparoscopy; high urogenital confluence
Title Preponderance of ovarian tissue in a 47,XXY/46,XX patient with a predominant XXY cell line in the gonads

Authors: R. Grinspon1, G. Bastida2, M. Venara3, M. Podesta4, M. Podesta H5, R. Castera5, S. Avila6, R. Campaña5, C De Carli5, M. Mac Donell6, S. Diaz7, M. Drut8, R. Rey1, I. Bergadá1

1Centro de Investigaciones Endocrinoles ‘Dr. Cesar Bergadá’ (CEDIE), CONICET-FEI-División de Endocrinología, Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina; 2Servicio de Endocrinología, Hospital Provincial Neuquén, Dr E Castro Rendón, Neuquén, Argentina; 3Unidad de Urología, Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina; 4Servicio Genética, Hospital Provincial Neuquén, Dr E Castro Rendón, Neuquén, Argentina; 5Unidad de Cirugía Pediátrica, Hospital Provincial Neuquén, Dr E Castro Rendón, Neuquén, Argentina; 6Servicio Anatomía Patológica, Hospital Provincial Neuquén, Dr E Castro Rendón, Neuquén, Argentina; 7Servicio de Pediatría, Hospital Provincial Neuquén, Dr E Castro Rendón, Neuquén, Argentina; 8Centro Consultor en Patología Perinatal y Pediátrica, La Plata, Argentina.

Background Clitoromegaly in a newborn or infant girl may be a manifestation of excess virilization in an XX patient, owing to abnormally elevated adrenal or gonadal androgen secretion, or a mild virilization in patients carrying an XY cell lineage, due to testicular dysgenesis or to specific defects in androgen synthesis or action.

Case presentation: A female patient was referred to us for clitoromegaly at 1.1 years of age. She had a moderately enlarged clitoris (14 mm x 8 mm) with normal vulvae and separated urethral and vaginal orifices. No gonads were palpable. Ultrasonography showed the existence of a uterus, and a Fallopian tube with a structure compatible with gonad only in the right side.

Congenital adrenal hyperplasia was ruled out based on normal 17OHP (0.10 ng/ml; NR 0.10-0.73). Basal serum steroid levels were uninformative (testosterone <10 ng/dl and estradiol <10 pg/mL), and elevated gonadotrophins with FSH predominance, LH 0.60 IU/L (NR 0.10-0.30), FSH 11.51 IU/L (NR 0.57-7.50). AMH was in the upper range of the female normal reference (45 pmol/l; NR 7-55) and hCG-stimulated testosterone was higher than expected for a girl (79 ng/dl), suggesting the presence of scarce testicular tissue. Peripheral blood karyotype was 47,XXY[34]/46,XX[18]. Ovotesticular DSD was suspected.

Laparoscopy confirmed the presence of uterus and Fallopian tubes. Unexpectedly, histology of both gonadal biopsies exclusively showed the presence of bilateral ovaries with abundant primordial and primary follicles (Figure 1a). Fluorescence in situ hybridization (FISH) for the centromeric regions of the X (DXZ1) and Y (DYZ3) chromosomes from both gonads described a clear predominance of XXY cell lines; XXY[74%]/XX[26%] in the right gonad and XXY[65%]/XX[35%] in the left gonad (Figure 1b).

Conclusion and Discussion: We report the unexpected finding of a clear preponderance of ovarian tissue in a 47,XXY/46,XX patient with a predominant XXY cell line in gonadal cells. On the other hand, this case poses a clinical dilemma regarding the decision whether or not to perform gonadectomy, owing to the presence of a Y chromosome. Although tumor risk is low in ovotesticular DSD with a 46,XX karyotype, scarce information is available in patients with ovotesticular DSD with a Y chromosome.
P76 Hypospadias Assessment Tool as proposed by the COST Action BM1303 “A Systematic Elucidation of DSD”


Background
Reconstructive surgery has always been a substantial part in the care of DSD. It is a basic surgical principle that continuous reevaluation and assessment of surgical outcome has a major impact on future clinical practice. Here we present the Hypospadias Assessment Tool (HAT) as proposed by the COST Action BM1303 “A Systematic Elucidation of DSD”.

Materials and methods
A user-friendly, short, efficient and non-time-consuming assessment tool has been designed which collects preoperative severity of hypospadias, primary surgical management, and postoperative objective outcome assessment. Assessment included: complication rates, redo surgeries, cosmetic outcome, functional outcome and optional items (psychology, genetics). Optional non-operated patients can also be registered in HAT. In this feasibility study 30 patients have been enrolled by different surgeons.

Results
Median age at time of surgery was 1.5 years (0.5-16yrs). Follow-up period was 1.5yrs. Remarks by the users of the tool: Collecting data and using the HAT is feasible and user-friendly. Using objective cosmetic assessment tools (HOPE, v.d. Toorn, 2013) is time consuming. In staged repair and/or difficult cases it is difficult to register all surgical steps and to define an end point. Using objective tools does not prevent bias and/or promote/enhance/replace surgical honesty. Long-term feasibility of HAT is still to be proven. For specific surgical research questions HAT does not replace prospective randomized trials.

Discussion
The HAT is a standardized long-term follow-up tool. It can be used as institutional data base as well as an international hypospadias register once available in the I-DSD registry. Follow-up and adequate counseling of hypospadias patients up to adult life is necessary, although demanding. HAT may help to give a more accurate estimation of the long-term outcome of hypospadias surgery.
P 77 CLITORAL HOODPLASTY: A NOVEL SURGICAL TECHNIQUE TO COVER AN EXPOSED GLANS AFTER PRIOR GENITAL SURGERY IN WOMEN WITH DSD

Katja Wolffbuttel, Department of Urology and Pediatric Urology, Erasmus Medical Center, Sophia Children’s Hospital, Rotterdam, Netherlands.

AIM

Poor cosmetic and functional outcome of early feminizing surgery has been reported before, resulting in greater awareness for restrictive use of feminizing surgery in childhood. Another unfavorable but underreported result is an exposed clitoral glans devoid of clitoral hood covering. We report the early results of our novel surgical technique to cover a bothersome exposed glans in women with DSD after previous feminizing surgery.

PATIENTS AND METHODS

Between 2012 and 2015 we treated 6 patients (average age 13.5 years, range 4-21 years) with an exposed clitoral glans after previous feminizing surgery for congenital adrenal hyperplasia (CAH). Indication for surgery was discomfort and/or dissatisfaction with the cosmetic appearance secondary to glans exposure. Our patients were treated with a newly developed technique, clitoral hoodplasty (CH-plasty). This procedure can be summarized as a modified V-Y skinplasty, resulting in a natural looking, multilayered clitoral hood. CH-plasty was done as a single procedure in 2/6 and was part of a larger surgical reconstruction in 4/6 patients.

RESULTS

Postoperatively the glans was completely covered in 5/6 patients and partially in 1/6. One adult and sexual active patient with preoperative clitoral hypersensitivity and pain reported complete resolution of symptoms, and expressed her satisfaction on a written questionnaire 9 months after CH-plasty.

CONCLUSION

Clitoral hoodplasty provides simple yet effective relief for women with cosmetic concerns or clitoral discomfort after previous feminizing surgery. As prevention is better than cure we propose to meticulously balance the pros and cons of early feminizing surgery in girls with CAH, and preserve the preputial hood in those where clitoroplasty in childhood is performed.