CLINICAL APPROACH TO DISORDERS OF SEX DEVELOPMENT

Dr Vasana Kiridana
Consultant Paediatrician
Sirimavo Bandaranayake Children’s Hospital
Introduction

- Sex assignment at birth is instantaneous in the majority of infants
- Sexual ambiguity/ intersex is a very sensitive issue
- Avoid undue delay in sex assignment
Embryological, genetic & hormonal background

- Reproductive development is a highly integrated process
- Starts around 5 weeks of gestation and is complete 14, 15 years later when fertility is achieved at puberty
- Sexual abnormalities may present at different ages
Reproductive development

- Achieved in 4 stages:
  - Sex determination and differentiation (5 weeks)
  - Development of the fetal hypothalamic, gonadotrope axis (from 6 weeks gestation)
  - Postnatal reproductive and endocrine events from birth to 6 months
  - Puberty
Sex determination & sexual differentiation

- Phenotypic sex determination begins with chromosomal sex and follows a cascade
- Presence of Testis-determining factor on Y chromosome (SRY) guides the indifferent gonad to develop into a testis
- Absence/alteration of this region causes indifferent gonad to develop into an ovary
- Stabilization/ regression of internal ducts
Sex determination & sexual differentiation

Urogenital ridge

Bipotential gonad

ovary

Testis-determining genes

Chromosomal (genetic) sex

XY

XX

? Ovary-determining genes

Gonadal sex

Sertoli cells

MIS

Mullerian regression

Male sexual differentiation

Leydig cells

Testosterone, DHT

Gonadal steroids and peptides (T, DHT, AMH)

Phenotypic sex

Male sexual differentiation

Differentiation of internal & external genitalia

- When testicular tissue is absent, the fetus completes internal sex duct development and external phenotypic development of a female.

- When testicular tissue is present, testosterone and MIS are produced which guides development of male internal duct development and external male phenotype.
Ambiguous genitalia
a simple classification

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Masculinization of a female infant

- Congenital adrenal hyperplasia
- Fetal/maternal androgens

- CAH is the commonest cause
- A straightforward diagnosis to establish
- Autosomal recessive enzymatic defects at different steps of adrenal hormone synthesis
- Most result in cortisol deficiency → stimulates pituitary to produce more POMC & ACTH.
- Also stimulates adrenal hypertrophy & hyperplasia
Congenital adrenal hyperplasia
21 Hydroxylase deficiency

- Accounts for about 95% cases of CAH
- Enzyme deficiency causes inability to convert 17OHP $\rightarrow$ 11 Deoxycortisol $\rightarrow$ Cortisol deficiency
- 3 clinical forms
  - Salt wasting (classic)
  - Simple virilizing
  - Non classic
21 Hydroxylase deficiency

Salt-wasting in 75%: ↓ Na+, ↑ K+, ↑ renin.

- Cholesterol
  - 3β-hydroxyxysteroid dehydrogenase
    - 21-hydroxylase
  - Pregnenolone
    - 17α-hydroxylase 17, 20 lyase
    - 21-hydroxylase
  - Progesterone
    - 17-OH pregnenolone
    - 17-OH progesterone
  - Diagnosis
    - Deoxycorticosterone
    - Corticosterone
    - Aldosterone
    - Cortisol
    - Cortisone
    - Acute adrenal insufficiency

- Virilization PCOS
  - DHEAS
    - Androstenedione
      - Testosterone
        - Oestrone
        - Oestradiol
      - 5α-reductase
    - 17β-HSD
      - Dihydrotestosterone
        - Aromatase

21 Hydroxylase deficiency

- **Salt wasting type:**
  - Complete absence of the enzyme eliminates both glucocorticoid & mineralocorticoid synthesis
  - Virilization of the female fetus range from mild clitoromegaly, labioscrotal fusion and traversing of the urethra along the enlarged clitoris
  - Male infants generally appear normal

- **Simple virilizing:**
  - Virilized female infants with no salt wasting
  - Precocious puberty in males

- **Non classic:**
  - Mild form of CAH
  - Females present in adult hood with hirsutism, menstrual irregularities, reduced fertility
  - There may be no phenotypic manifestations at all
3 beta hydroxysteroid dehydrogenase deficiency

- A rare cause of glucocorticoid, mineralocorticoid & androgen deficiency
- Genetic female: clitoromegaly, mild virilization due to overproduction of DHEA
- Genetic male: small phallus with severe hypospadias due to inadequate androgens
- Clinical presentation- male & female genital ambiguity, salt wasting
17 alpha hydroxylase/ 17,20 lyase deficiency

- Rare
- Reduced synthesis of androgens
- Mild symptoms of glucocorticoid and mineralocorticoid deficiency

Typical presentation:
- Teenage female with sexual infantilism, failing to undergo puberty. Phenotypically normal at birth
- Males have absent or incomplete development of external genitalia
- Over production of DOC causes Na retention, hypertension, hypokalaemia, suppress plasma renin activity & Aldosterone secretion
11 beta hydroxylase deficiency

- Rare
- Enzyme deficiency leads to inability to convert DOC to Aldosterone
- High concentrations of DOC causes hypertension
- Sexual ambiguity is seen in female babies
Lipoid adrenal hyperplasia (congenital adrenal hypoplasia)

- Most severe disorder of steroid hormone synthesis
- The lesion prevents conversion of cholesterol to pregnenolone
- Absence of all steroids
- Male pseudohermaphroditism
- Salt wasting
Maternal androgens

- Foetus is generally protected from maternal androgens by placental aromatase
- Causes virilization of the female infant in the presence of placental aromatase deficiency
The undermasculinized male

- 3 broad categories
  - Defects in testis determination
  - Defects in androgen biosynthesis
  - Defects in androgen action
Defects in testis determination

- Complete or partial gonadal dysgenesis is the end result.
- Normal development and function of Sertoli & Leydig cells are essential for hormone-mediated sex differentiation of internal & external genitalia in the male.
- Streak gonads are completely undifferentiated.
- When both gonads are streak, phenotype is female – “complete sex reversal with no ambiguity.”
Defects in testis determination

- Partial forms of gonadal dygenesis causes sexual ambiguity
- Mixed gonadal dysgenesis – 45X/46XY mosaicism. The degree of sex reversal is determined by the proportion of X and XY cell lines
- Syndromes with gonadal dysgenesis – Denys Drash, Frasier
Defects in androgen biosynthesis

- Fetal leydig cell androgen synthesis is dependent on placental hCG initially and LH thereafter.
- Mutations in LHR gene causes severe under masculinization.
- Investigations will show:
  - Low Testosterone, high LH
  - No Testosterone response to hCG stimulation
  - Histology – absent leydig cells
Enzyme deficiencies in Testosterone synthesis

- **17 beta hydroxysteroid dehydrogenase deficiency**
  - Ambiguity range from complete sex reversal to varying degrees of hypospadias
  - Low Testosterone, normal AMH
  - hCG stimulation
    - Testos: Androstenidione ratio < 0.8

- **5 alpha Reductase deficiency**
  - Fusion of labio scrotal folds is dependent on DHT. Testosterone has a mild effect. Causes partial fusion
  - Testosterone, Androstenidione, ratio are all normal
Defects in androgen action

- 46XY with normal Androgen levels defines tissue specific resistance to the action of androgens
- Total resistance causes Complete Androgen Insensitivity Syndrome with complete XY sex reversal (CAIS/ Testicular Feminization)
- Some response to hormones results in Partial Androgen Insensitivity Syndrome (PAIS-manifest as mild clitoromagaly, true ambiguity, hypospadias or impaired fertility in an otherwise normal male
Androgen insensitivity

elevated LH & Testosterone levels, androgens are aromatized to estrogens causing breast development

- **CAIS**
  - Normal female phenotype at puberty
  - Scanty pubic & axillary hair
  - Primary amenorrhea
  - Inguinal herniae in infancy

- **PAIS**
  - More male phenotype
  - Gynecomastia at puberty
  - Breast cancer common
Ovotesticular DSD (true hermaphroditism)

- Presence of both testicular and ovarian tissue which are well differentiated.
- 46XX is the most frequent karyotype, majority having normal external male genitalia. Testis are small & firm, height below average, gynaecomastia is common.
- Majority are SRY gene positive due to X-Y chromosomal interchange during paternal meiosis.
Sex chromosome abnormalities

- Klinefelter syndrome (47XXY)
  - affects around 1:600 males
  - Primary hypogonadism with small testis
  - Fibrosis of seminiferous tubules causing infertility
  - Tall stature with long legs
  - Behavioural & psychological problems
  - Gynecomastia at puberty
Investigations

- The aim is to arrive at a functional diagnosis to allow early sex assignment.
- Local protocols should be determined by the available facilities & practice.
- Clinical assessment.

**Idealized male:** XY karyotype with penile length 2.5-4.5 cm, normal position of the urethral meatus, testes in the scrotum.

**Idealized female:** XX karyotype with clitoral size 0.5-0.85 cm, normal female reproductive tract.
Clinical assessment

- Family history, prenatal exposure to reproductive teratogens
- Examination of the ext. genitalia should record the following:
  - Phallus – size, chordee, micropenis/ clitoromegaly
  - Site of urethral opening
  - External orifices in the perineum
  - Development of labioscrotal folds – bifid scrotum/ fused labia, rugosity, pigmentation
  - Whether gonads are palpable & position

- Prader score
Laboratory investigations

- Genetic sex: FISH analysis for Barr bodies
- Karyotype to confirm FISH results, analysis of a sufficient no. of mitoses to exclude mosaicism
- Karyotype 46XX → 17OHP > 300nmol/L with uterus in USS confirms 21 hydroxylase deficiency
- Ancillary biochemical tests to identify salt loosers
- Synacthen (ACTH) stimulation test in premature infants and when rarer CAH is suspected
Laboratory investigations

- Measurement of urinary steroid metabolites by gas chromatography
- Establishing location & function of testes in the XY or XY/XO infant with hCG stimulation test. Can be coupled with imaging & laparoscopy to identify the gonadal site and histology. Pre & post hCG blood samples analyzed for Testosterone, Androstenidione & DHT. Concomitant 24 hr urinary steroid analysis can be performed
- Testosterone : Androstenidione ratio
  - > 0.8 – Androgen insensitivity
  - Normal – 5 alpha reductase deficiency
  - <0.8 – 17 beta hydroxysteroid dehydrogenase deficiency
Laboratory investigations

- Sertoli cell function – measuring AMH, Inhibin B. both proteins are elevated during infancy. AMH levels fall during puberty in response to Testosterone
  - Androgen insensitivity – ↑AMH
  - Gonadal dysgenesis – ↓AMH
  - Anorchia – AMH undetectable
- Imaging with US and MRI to delineate the internal genital anatomy
- Histology to provide exact details of gonads by laparoscopy
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<th>Laboratory findings</th>
<th>Likely diagnosis</th>
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<td>Masculinization of female infant, salt wasting</td>
<td>↑17-OHP before &amp; after ACTH, ↑serum Androgens, ↑urine 17-ketosteroids, suppression of androgens with glucocort treatment, ↑ ACTH &amp; PRA</td>
<td>21-OHD</td>
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<td>Male &amp; female pseudohermaphroditism, salt wasting</td>
<td>↑ACTH, PRA, ▲ 5/▲ 4 steroids, ▲ 5 steroids before &amp; after ACTH, ↓ 17-OHP</td>
<td>3 Beta -HSD</td>
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<td>Masculinization of female infant, postnatal virilization in males &amp; females</td>
<td>↑11-deoxycortisol before &amp; after ACTH, ↑ACTH &amp; ↓ PRA, hypokalaemia, ↑ serum Androgens &amp; 17 ketosteroids, suppression of androgens with glucocort treatment</td>
<td>11 Beta OHD</td>
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<td>Male pseudohermaphroditism, female sex infantilism, hypertension</td>
<td>↑DOC, low 17 alpha hydroxylated steroids and poor response to ACTH, poor response to hCG, hypokalaemia, ↑ACTH &amp; ↓ PRA</td>
<td>17 Alpha-OHD</td>
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Summary of clinical evaluation

Ambiguous genitalia

Karyotype

XX

17OHP, serum Androgens

high

21 OHD

↑ACTH, PRA, K

11 beta OHD

↓ACTH, ↓PRA, ↓K

low

Urinary 17/4 ketosteroid ratio

3 beta HSD

↑ACTH, ↑PRA, ↑K

17alphaOH

↓ACTH, ↓PRA, ↓K

XY

Testosterone, LH, hCG stimulation test, gonadal histology

↑LH, ↓T, T:A < 0.8

17 beta OH

↑LH, T, T:A - N

5 alpha RD

↓T, ↑H, streak gonads

CAIS/PAIS

Gonadal dysgenesis

↑LH, ↓T, streak gonads

↑ACTH, PRA, K

Genetic defect in androgen synthesis

↑LH, T, no T response

→ ↑LH, T, T:A - N

17OHP, serum Androgens

Androgens high

N/H

21 OHD

ACTH, PRA, K

11 beta OHD

Urinary 17/4 ketosteroid ratio

3 beta HSD

Genetic defect in androgen synthesis

↓ACTH, ↓PRA, ↓K

T, LH, streak gonads

Gonadal dysgenesis
Thank you