What to do with ambiguous genitalia?

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Learning objectives

- Understand embryologic basis for some common reproductive tract anomalies
- Understand etiologies and diagnosis of disorders of sexual development (DSD)
- Learn a stepwise approach for evaluation of patient with ambiguous genitalia/disorders of sexual development (DSD)
Case report 1

- 38 yo P0010 s/p IVF and pre-implantation diagnosis (PGD) with embryo transfer of 2 blastocysts 46,XX and 46,XY

- Normal 1\textsuperscript{st} trimester aneuploidy screening, declined diagnostic testing

- 18 week anatomy scan: dichorionic twins, both appearing female

- What happened?
Case Report cont’d

- Possible causes
  - Lab error
  - Monozygotic twinning
  - Under-virilization of male fetus ➔ female phenotype
Case Report cont’d

- Next steps:
  - Patient declined invasive prenatal testing
  - cffDNA identified material from Y chromosome
  - Underwent amniocentesis (finally)
Case Report cont’d

- Karyotype: twin A 46,XX and twin B 46,XY
- Microarrays: no copy number variant detected
- Suspected sex reversal syndrome
  - FISH of SRY gene: no deletion
  - Androgen receptor gene sequenced with c.2461 G>T mutation detected

- Diagnosis: Androgen Insensitivity Syndrome
Sexual differentiation: major components

- Chromosomal sex (XY or XX)
- Gonadal sex (ovary or testes)
  - Male ductal system (Mesonephric (Wolffian))
  - Female ductal system (Paramesonephric (Mullerian))
- Phenotypic sex – determined by hormonal influence on internal and external genitalia
Development of Reproductive System

- Germ cells (ova and spermatogonia) migrate to genital ridge
- Genital ridge + germ cells ➔ testes and sperm or ovaries and eggs
Male development

- **Y chromosome**
  - SRY gene → testes determining factor (TDF) form testes
  - Anti-mullerian hormone (AMH)
  - Testosterone: reduced to dihyrotesterone (DHT) by 5 alpha reductase
Female development

- No Y chromosome (or deleted/muted SRY gene)
  - Silences SOX9 gene
  - Forms ovaries
- X chromosome + autosome + activated genetic pathways
Sex Determination

- Gonadal development directs differentiation of internal ducts/reproductive organs and external genitalia.
Differentiation of ductal system

- **Testosterone** → mesonephric ducts → male ductal system (epidydimus, vas deferens, etc)

- **AMH** → degeneration paramesonephric ducts

- **No testosterone:** mesonephric ducts degenerate

- **No AMH:** paramesonephric ducts develop (uterus, tubes, upper vagina)
Development of the external genitalia:

4-7 weeks: Genitalia undifferentiated

- Genital tubercle
- Urogenital fold
- Labioscrotal swelling
- Cloacal membrane
- Urorectal septum
- Urogenital membrane
- Anal membrane
Differentiation of external genitalia

- Males: influenced by DHT
- Urogenital folds fuse enclosing urethra
- Labioscrotal folds fuse forming scrotum

- Females: ?estrogens from placenta and ovaries
- Urogenital folds form labia minora
- Labioscrotal folds form labia majora
## Genes involved with gonadal development

**SRY:** expresses testes determining factor (TDF) – initiate testes formation

**SOX9:** *Insufficiency:* campomelic dysplasia and sex reversal in majority XYs:  
*Duplication:* only autosomal cause of sex reversal in XX

**SF-1/NR5A1 (steroidogenic factor 1):**  
*Mutations:* agonadism, adrenal hypoplasia/insufficiency, micropenis, XY sex reversal, etc

**DAX-1/NROB1:**  
*Mutations:* adrenal hypoplasia congenita  
*Duplications:* repress SRY and cause DSD with female phenotype in 46XY individual

**WT-1: Wilms tumor gene:**  
*Deletion of WT-1 gene and adjoining PAX6 gene:* **WAGR** syndrome  
**WWT-1 mutation:** Denys-Drash syndrome, Frasier syndrome
Typical genitalia on ultrasound

Male genitalia

Female genitalia

19 weeks

19 weeks

24 weeks

27 weeks
Typical ultrasound appearance of male genitalia

12-14 wks. Angle > 30 deg male: Angle < 10 deg female

14 wks. penile shaft

Bladder-rectal distance: Concave interface with bladd c/w male

19 wks penile shaft with scrotal sac

32 weeks see urethra extending to tip
Ultrasound appearance of female genitalia

19 wks 4 parallel lines of labia majora and minora

24 wks uterus and cervix with echogenic endometrium surrounded by hypoechoic myometrium with rectum posterior
Male fetus: 24 wks: posterior surface of bladder and anterior surface rectum. The interface measures 1 mm. Note flat (or concave) surface of bladder wall.

Female fetus: 25 wks: shows uterus between calibers with a rectovesical interspace of 6.7 mm. Note convex indentation into posterior aspect bladder wall.
Disorders of Sexual development (DSD): Classification

**Previous**

- Female pseudohermaphrodite
- Male pseudohermaphrodite
- True hermaphrodite
- XX male
- XY sex reversal

**Revised**

- 46 XX DSD
- 46 XY DSD
- Ovotesticular DSD
- 46 XX testicular DSD
- 46 XY complete gonadal dysgenesis

2006 LWPES and ESPE proposed changes
Other ways of classifying DSD

- Virilized XX
- Under-virilized XY
- Mixed sex chromosome pattern
- Also
  - Gonadal differentiation and chromosomal disorders
  - Syndromes associated with ambiguous genitalia
Types of DSDs

**46 XX virilized female**

- Maternal conditions
  - CAH
  - Adrenal/ovarian tumors
  - Exogenous progestins/androgens
- Maternal and fetal condition
  - Aromatase deficiency

**46 XY under-virilized male**

- Androgen insensitivity
- 5 alpha reductase deficiency
- Defects of testosterone synthesis
- Leydig cell hypoplasia
- Persistent mullerian duct syndrome
### Types of DSDs

#### Gonadal differentiation and chromosomal disorders
- 46 XY gonadal dysgenesis (streak gonads)
- Ovotesticular DSD (both testicular and ovarian tissue present)

#### Syndromes with ambiguous genitalia
- Turner syndrome
- Campomelic dysplasia
- Denys-Drash syndrome
- Frasier syndrome
- WAGR syndrome
- Robinow syndrome
Case 2: 32 yo P1001 presents at 20 weeks for routine anatomy scan
How to approach ambiguous genitalia

- Stepwise approach
  - Describe abnormality
  - Decide if isolated or non-isolated
  - Pregnancy history
  - Family history
  - Lab work
Step 1: Describe genital abnormalities: Incomplete masculinization male genitalia

- Absent, short or abnormal shape of phallus
- Absent or bifid scrotum
- Absent/undescended testes later 3rd trimester
- Discordance between external genitalia and internal organs
Measurements of penile length

- **Micropenis**: penile length < 5th or < 10th % ile
  - Johnson and Maxwell 2000
    - 95 fetuses 16-38 wks
    - Tip to base at scrotum
  - Pinette 2012
    - Tip to base of shaft 17 – 41 wks
  - Danon et al 2012
    - Measured penile width and length in 100 fetuses
    - Also correlated with EFW % ile

Step 1: Describe genital abnormalities: masculinization *female* genitalia

- Enlarged phallic structure
- Abnormal/fused labia with identified uterus or relatively large rectovesical distance

![Ultrasound image of cliteromegaly in coronal plane](image-url)
Step 2: Decide if isolated or non-isolated

### Non-isolated causes

#### Chromosome abnormalities
- Trisomy 13,18
- Triploidy
- deletions

#### Single gene disorders
- Fraser syndrome (AR)
- Smith – Lemli – Opitz syndrome (AR)
- Fryns syndrome (AR)
- Carpenter syndrome (AR)
- McKusick – Kaufman syndrome (AR)
- Robinow syndrome (AR and AD)
- Noonan syndrome (AD)
- CHARGE syndrome (AD)
- Cornelia de Lange (AD)
- ATRX syndrome (X-linked)
- Aarskog syndrome (X-linked)

#### Associations
- **CHARGE** association (Coloboma, Heart, choanal Atresia, Retarded growth, Genital, Ear abn)
- **EEC** association (Epispadias, bladder Exstrophy, Cloacal extrophy,
- **MURCS** association  
  - MUllerian, Renal, Cervicothoracic, Somite

#### Imprinting disorders
- Prader – Willi syndrome
Step 2: Decide if isolated or non-isolated

Isolated causes

• Multifactorial condition or single gene disorder
  • CAH
  • 5 alpha reductase
  • SRY gene mutation
  • Androgen insensitivity
Step 3: pregnancy history

- Maternal diseases resulting in hirsutism
- Maternal exposure to androgens
- Teratogens
- Check 2nd trimester DS screen
  - low unconjugated estriol
    - SLOS, CAH, placental aromatase deficiency, trisomy 18
  - ↑AFP, ↑hCG, ↑PAPP-A (plac’l insuff) – hypospadias in males
Step 4: family history

- h/o genital or other abnormalities, recurrent miscarriage, stillbirth, mental retardation,
- consanguinity
- h/o infertile/amenorrheic females – androgen insensitivity
- h/o unexplained infant death – salt wasting CAH
Step 5: lab work

- Chromosomal analysis and microarray
- Depending on US findings: Amniotic fluid tests for
  - Fetal DNA
    - 21 hydroxylase deficiency
    - 5 alpha reductase deficiency
    - SRY mutation
  - 17 hydroxyprogesterone, testosterone, androstenedione, 11 deoxycortisone, 7 dehydrocholesterol
Step 6: Compiling information and differential diagnosis

- Non-isolated DSD:
  - Fetal chromosome abnormalities
  - Single gene disorders
  - Sporadic conditions (cloacal dysgenesis, omphalocele-extrophy of the bladder-imperforate anus-spine abnormality (OEIS))

- Majority DSD have isolated genital abnormalities
  - Amniocentesis for chromosomal analysis and hormonal studies
Let’s try it

Let’s say you know karyotype
Ultrasound findings in 46XX DSD

- Enlarged phallus (clitoris)
- Redundant/incompletely separated or fused labia majora
46 XX DSD:
Most cases due to exposure to high levels androgens

• **Extrinsic**
  • Maternal consumption/production androgens
  • Maternal luteoma or theca-lutein cysts

• **Intrinsic**
  • CAH (>90% of cases of 46XX DSD) or placental aromatase deficiency

• **Other causes**
  • SRY translocation (dx with FISH)
  • SOX9 duplication (dx with FISH)
  • Ovotesticular DSD
Let’s say you know karyotype 46XY.
XY DSD

AMBIGUOUS GENITALIA

HYPOSPADIAS & downward urine stream

MICROPENIS

BIFID SCROTUM
46 XY DSDs

- Impaired production of testosterone
- Impaired peripheral action of these hormones
- Decreased conversion of testosterone to DHT
XY DSD work-up/differential dx

- **Determine karyotype**
  - confirm XY
  - Check for SRY gene (and if normal)

- **If don’t have chromosomes look for testes**
  - **Absence of testes**
    - SRY gene mutation/deletion
    - DAX-1 duplication or SF-1 mutation
    - Sex chromosome abnormalities (45X/46XY mosaicism)
    - Testicular regression syndromes

- **Testes present**
  - *Abnormal production of testosterone* due to lack of luteinizing hormone or lack of response to pituitary hormones

*Franasiak et al Obstet Gynecol 2015;125:383–6*
Abnormal synthesis of testosterone due to enzyme deficiencies
Abnormal conversion of testosterone into DHT in external genitalia (5 alpha reductase deficiency)
Abnormal/lack of response to testosterone (complete/partial androgen insensitivity syndrome)

Will not detect these if fetal karyotype is not known
An approach toward the differential diagnosis of fetal disorders of sex development based on US finding and chromosome sex

**Non-isolated**

- **Chromosome abnormalities**
  - Female rectovesical interspace or uterus present
    - 1. Disorders of testicular development
    - 2. Persistent Mullerian duct syndrome
  - Male rectovesical interspace or uterus not seen
    - 1. Disorder androgen synthesis
    - 2. Disorder androgen action
    - 3. Congenital hypogonadotropic hypogonadism
    - 4. Vanishing testes syndromes
    - 5. Isolated hypospadias

- **Single gene disorders/associations**
  - Female rectovesical interspace or uterus present
    - 1. Disorder gonadal development
  - Male rectovesical interspace or uterus not seen
    - 1. Mullerian agenesis/hypoplasia
    - 2. Uterine abnormalities

**Isolated**

- **XY**
  - Female rectovesical interspace or uterus present
    - 1. Disorder androgen synthesis
    - 2. Disorder androgen action
    - 3. Congenital hypogonadotropic hypogonadism
    - 4. Vanishing testes syndromes
    - 5. Isolated hypospadias

- **XX**
  - Female rectovesical interspace or uterus present
    - 1. Disorder gonadal development
  - Male rectovesical interspace or uterus not seen
    - 1. Androgen excess
      - CAH
      - feto-placental aromatase deficiency
      - maternal virilizing tumors or androgen medications
    - 2. Uterine abnormalities
Management

- Multidisciplinary approach
- Findings at delivery may differ from prenatal US
- Options of pregnancy continuation/termination
- Maternal RX
  - Resection maternal androgen-secreting tumor
  - Maternal dexamethasone for XX DSD CAH
Delivery and Postnatal w/u

- No indication for c/s
- Cord blood for possible hormone studies, DNA analysis, electrolytes
- Neonatal imaging for presence of gonads, uterus and/or vaginal
- Inspection for undescended testes
- Assess adrenal glands for hypertrophy/hyperplasia
Thank you for your attention!!

whatever happened to our sexual relations?

I don't know. I don't even think we got a card from them this year.

Hanukkah