FEMALE AND MALE INFERTILITY
GENETIC CAUSES

Susana Fernandes
Genetics Department
Faculty of Medicine of Porto

Introduction

- Research on genetic causes of male and female infertility rapidly expanded in the last years, following the development of in vitro fertilization techniques.
- Genetic tests are available to explore the cause of the infertility and assess the risk of a given couple to transmit its genetic characteristics. Possibility to take an informed decision when choosing for ART.
- The genetic work-up of the infertile couple has become good practice for an appropriate diagnosis, including PND and PGD.

Infertility affects 10 -15% of couples

- Female factor
- Male Factor
- Both factors and unexplained
### Genetic causes of infertility

- Chromosomal abnormalities
- X-linked disorders
- Monogenic disorders

### Female
- Turner syndrome - 45,X
- POF syndrome

### Male
- Klinefelter syndrome - 47,XXY
- Y chromosome microdeletions (Yq11.2)
- *CFTR* mutations – CAVD (7q.31.2)

### Translocations, inversions and deletions

### Chromosomal abnormalities in infertile couples

**Translocations, inversions and deletions**

- **Numerical abnormalities** - Aneuploidies of the sexual chromosomes (1%)
  - (47,XYY; 47,XXX - generally fertile)

- **Structural abnormalities** - Reciprocal translocations (0.6%)
  - Robertsonian translocations (0.2%)
  - Inversions (0.1%)
  - Deletions (0.1%)
### Chromosomal abnormalities

**Turner syndrome - 45,X**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-disjunction during oogenesis</td>
<td>Monosomy 45,X – 50%</td>
</tr>
<tr>
<td>Incidence: 1/2500 female births</td>
<td>Mosaicism 45,X/46,XX – 20%</td>
</tr>
<tr>
<td>Clinical features: <strong>Severe phenotype:</strong> Short stature, POF, Primary amenorrhea, Low estrogen levels</td>
<td>Isochromosome 46,X,i(Xq) – 15%</td>
</tr>
<tr>
<td>Clinical features: <strong>Mild phenotype:</strong> Secondary amenorrhea, Sterility</td>
<td>46,X,r(X), 46,X,del(Xp), etc – 15%</td>
</tr>
</tbody>
</table>

Spontaneous pregnancy only occurs in 2-4% of TS women

Spontaneous abortion or perinatal death

Pregnancies with children with chromosomal abnormalities

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### X-linked disorders

**POF syndrome**

- Deletions on X chromosome
  - Xq26qter
- Fragile X carriers
<table>
<thead>
<tr>
<th>X-linked disorders</th>
</tr>
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<tbody>
<tr>
<td><strong>POF syndrome</strong></td>
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**Premature Ovarian Failure – X-Fragile**

CGG repeat in \( FMR1 \) gene
and methylation silencing the gene

\( FMR1 \geq 200 \) CGG repeats – full mutation
\( 50-200 \) CGG repeats – premutation

Female carries of \( FMR1 \) premutation
• increased risk of POF
• ~25% subclinical ovarian dysfunction
• ~20% menses cessation before 40ties

<table>
<thead>
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<tr>
<td><strong>Klinefelter syndrome - 47,XXY</strong></td>
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Non-disjunction in gametes
Incidence: 1/500 men
Mostly diagnosed after puberty

Clinical features: Long limbs, large hands and feet
Gynecomastia
Small testis
Hypogonadism hypergonadotropic
Azoospermia

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- Successful sperm recovery in KS patients range from 44%
  (16-60%) - ICSI candidates
- Embryos with slightly increased risk of aneuploidies for sexual chromosomes (1/40) and other trisomies
- PGD could be performed
Chromosomal abnormalities

**Y chromosome microdeletions**

- Deletions are too small to be detected by karyotyping

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**Chromosomal abnormalities**

**Y chromosome microdeletions**

1976 - Tiepolo e Zuffardi  
1996 - Vogt

(Yq11.2)  
AZFa  
AZFb  
AZFc  

Region more frequently deleted

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**Y chromosome**

<table>
<thead>
<tr>
<th>SRY</th>
<th>Yq11.21</th>
<th>Yq11.22</th>
<th>Yq11.23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AZFa (1 Mb)</td>
<td>USP9Y, DBY, UTY</td>
<td>USP9Y, DBY, UTY</td>
</tr>
<tr>
<td></td>
<td>AZFb (6.2 Mb)</td>
<td>SMCY, EEF1AY, PRY, XXY, RBMY, TTY5, TTY6</td>
<td>SMCY, EEF1AY, PRY, XXY, RBMY, TTY5, TTY6</td>
</tr>
<tr>
<td></td>
<td>AZFc (3.5 Mb)</td>
<td>BPY2, CDY1, CSPG4LY, DAZ, GOLGA2LY, TTY3, TTY4</td>
<td>BPY2, CDY1, CSPG4LY, DAZ, GOLGA2LY, TTY3, TTY4</td>
</tr>
</tbody>
</table>

Microdeletions in Yq11 (AZFa, AZFb, AZFc) are the most frequent genetic cause of male infertility after KS

Testicular histology associated to AZF deletions

- SCOS (5%)
- MA (10-16%)
- Oligo, HP, MA and SCOS (60%)
Chromosomal abnormalities

Y chromosome microdeletions

- Deletions are too small to be detected by karyotyping
- Deletions caused by intrachromosomal recombination events between homologous repetitive sequences

AZFc locus in Yq11

The palindromic structure stabilizes the AZFc genes function
AZFc deletion

b2/b4 Intrachromosomal recombination

Complete AZFc deletion 3.5 Mb
All gene copies are deleted

Kuroda-Kawaguchi et al. 2001

AZFb and AZFc deletion

Intrachromosomal recombination


Chromosomal abnormalities
Y chromosome microdeletions

AZFb deletion patterns of our patients suggestive of a putative critical region responsible for the initiation of human spermatogenesis

Costa P et al. 2008
AZFa deletion

Chromosomal abnormalities
Y chromosome microdeletions

- Deletions are too small to be detected by karyotyping
- Deletions caused by intrachromosomal recombination events between homologous repetitive sequences
- Deletions on Y chromosome detected by multiplex-PCR

AZF microdeletions

STS – Specific Tagged Sequences

Multiplex 1: GYS
- sY254
- sY134
- sY142
- sY152

Multiplex 2: DAZ1
- sY14
- sY154
- sY142

Multiplex 3: USP9Y
- TSPY
- TSPY
- TSPY
- TSPY

7% azoospermia and 4% severe oligozoospermia
Y chromosome microdeletions

AZF microdeletions frequencies in 3002 infertile males

<table>
<thead>
<tr>
<th>Karyotype</th>
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<tbody>
<tr>
<td>198 abnormal (86 – 47,XXY)</td>
<td></td>
</tr>
<tr>
<td>288 unknown</td>
<td></td>
</tr>
<tr>
<td>2714 normal</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Secretory Azoospermia</th>
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</thead>
<tbody>
<tr>
<td>6.9% (56/816)</td>
<td></td>
</tr>
<tr>
<td>AZFa</td>
<td>6</td>
</tr>
<tr>
<td>AZFa+b</td>
<td>3</td>
</tr>
<tr>
<td>AZFb</td>
<td>1</td>
</tr>
<tr>
<td>AZFb+c</td>
<td>10</td>
</tr>
<tr>
<td>AZFc</td>
<td>36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oligozoospermia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8% (20/528)</td>
<td></td>
</tr>
<tr>
<td>AZFb</td>
<td>1</td>
</tr>
<tr>
<td>AZFb partial</td>
<td>2</td>
</tr>
<tr>
<td>AZFc</td>
<td>17</td>
</tr>
</tbody>
</table>

With ART, AZFc microdeletions are transmitted to male offspring!

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Monogenic disorders

CFTR mutations - CAVD

About 98% of males affected with CF are infertile

Clinical features related with infertility

male: atrophy, fibrose or congenital absence of vas deferens
female: reduced fertility, thick dehydrated mucus in the cervix

Congenital Absence of Vas Deferens (CAVD)

1-2% male infertility, 6% obstructive azoospermia

Mutations (>1300) in CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator)
### CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator)

- Monogenic disease, most common autosomic recessive disorder
- Affects 1:2500 new-born (0.04%)
- 1:25 (4%) asymptomatic carriers (1 mutation) in Caucasians
- CFTR gene identified in 1989
- Maps on chromosome 7q3.1.2
- Chloride channel regulated by cAMP

### Monogenic disorders

#### CFTR mutations - CAVD

**Analysis of (TG)m(T)n haplotypes of the IVS8 acceptor splice site**

- Intron 8 of CFTR gene – polyT sequence (5, 7 or 9 Timines)
- mRNA variants with exon 9 absent
- CFTR non-functional protein
- The analysis of this polymorphism is used for CABV molecular diagnosis

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**CFTR mutations - CAVD**

**Initial screening – 1st Step**

- Search for 31 most frequent CF mutations
- Analysis of the (TG)m(T)n haplotypes of the IVS8 acceptor splice site

- 2 mutations (CF or CFTR-RD)
  - 0-1 mutation CF or CFTR-RD
    - Screening for rare mutations – 2nd Step
      - DGGE/dHPLC/Sequencing

- 1 CF + 1 CF/CFTR-RD
  - 0-1 CF/CFTR-RD mutation
    - CF/CFTR-RD + CF7 mutation
      - Screening of CFTR rearrangements – 3rd Step
        - Quantitative fluorescent PCR (QFM_PCR)
Initial Screening

45 CAVD patients

- 2 mutations: 55.6%
- 1 mutation: 33.3%
- No mutations: 11.1%

Extensive CFTR gene analysis

- 2 mutations: 91.1%
- 1 mutation: 8.9%
- No mutations: 0%

Rearrangements analysis

- 2 mutations: 93.3%
- 1 mutation: 6.7%
- No mutations: 0%

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Monogenic disorders

CFTR mutations - CAVD

Prevalence of CFTR mutations in infertile male with CAVD is 100% with at least 1 mutation (after complete study of CFTR gene)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence</th>
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</thead>
<tbody>
<tr>
<td>T5 allele</td>
<td>31.1%</td>
</tr>
<tr>
<td>DeltaF508</td>
<td>23.3%</td>
</tr>
<tr>
<td>R334W</td>
<td>6.7%</td>
</tr>
<tr>
<td>R117H</td>
<td>4.4%</td>
</tr>
<tr>
<td>G556A</td>
<td>4.4%</td>
</tr>
<tr>
<td>R668C</td>
<td>4.4%</td>
</tr>
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</table>

Indications for CFTR mutations before ART:
- Obstructive azoospermia

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TAKING-HOME Message

Molecular diagnosis for female infertility
- Karyotype
- \(FMR1\) gene analysis

Molecular diagnosis for male infertility
- Karyotype
- \(Y\) chromosome microdeletions in non-obstructive azoospermia and severe oligozoospermia
- CFTR mutations in obstructive azoospermia