FEMALE AND MALE INFERTILITY GENETIC CAUSES

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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	Male
• Turner syndrome - 45,X	• Klinefelter syndrome - 47,XXY
• POF syndrome	• 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

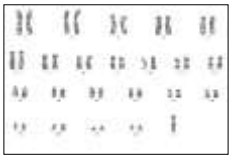
Turner syndrome - 45,X

Non-disjunction during oogenesis
Incidence: 1/2500 female births
Clinical features: Severe phenotype: Short stature
POF
Primary amenorrhea
Low estrogen levels
Mild phenotype: Secondary amenorrhea
Sterility

~25% not diagnosed until adolescence

Chromosomal abnormalities

Turner syndrome - 45,X



Monosomy 45,X – 50%
Mosaicism 45,X/46,XX – 20%
Isochromosome 46,X,i(Xq) – 15%
46,X,r(X), 46,X,del(Xp), etc – 15%

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

⇩

Spontaneous abortion or perinatal death
Pregnancies with children with chromosomal abnormalities

X-linked disorders

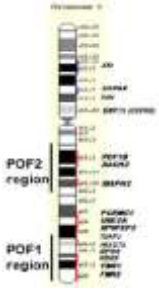
POF syndrome

Premature Ovarian Failure

✓Deletions on X chromosome

- Xq26qter -

✓Fragile X carriers



X-linked disorders

POF syndrome

Premature Ovarian Failure – X-Fragile

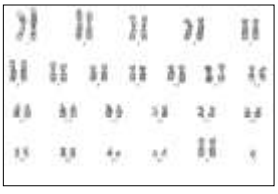
CGG repeat in *FMR1* gene
and methylation silencing the gene

FMR1 >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

Chromosomal abnormalities

Klinefelter syndrome - 47,XXY



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

Chromosomal abnormalities

Klinefelter syndrome - 47,XXY

- ✓ 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

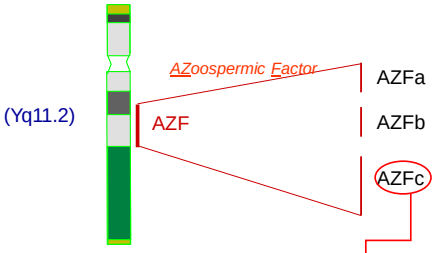
✓ Deletions are too small to be detected by karyotyping

Chromosomal abnormalities

Y chromosome microdeletions

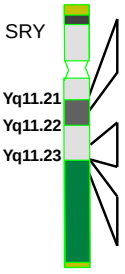
1976 - Tiepolo e Zuffardi

1996 - Vogt



Region more frequently deleted

Y chromosome



AZFa (1Mb) *USP9Y DBY UTY*

AZFb (6.2Mb) *SMCY, EIF1AY PRY, XKRY RBMY,TTY5,TTY6*

AZFc (3.5Mb) *BPY2, CDY1, CSPG4LY DAZ, GOLGA2LY TTY3, TTY4*

Testicular histology associated to AZF deletions

SCOS (5%)

MA (10-16%)

Oligo, HP, MA and SCOS (60%)

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

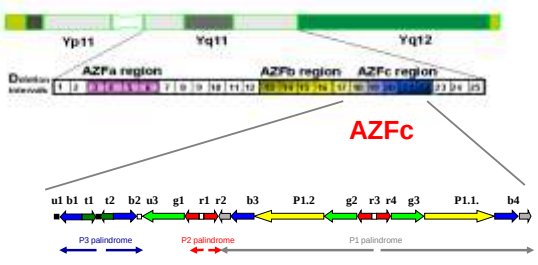
Chromosomal abnormalities

Y chromosome microdeletions

✓ Deletions are too small to be detected by karyotyping

✓ Deletions caused by intrachromosomal recombination events between homologous repetitive sequences

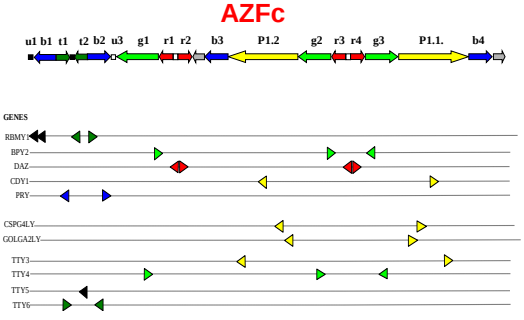
AZFc locus in Yq11



Amplicons – Repetitive units with 99.9% homology

Chromosomal abnormalities

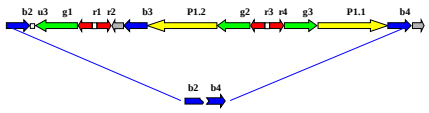
Y chromosome microdeletions



The palindromic structure stabilizes the AZFc genes function

AZFc deletion

b2/b4 Intrachromosomal recombination



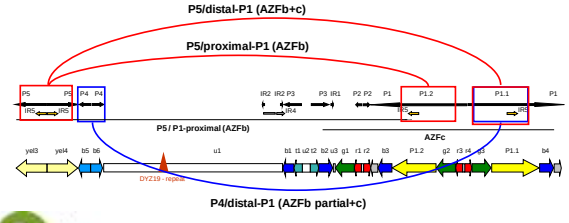
Complete AZFc deletion
3.5 Mb

All gene copies are deleted

Kuroda-Kawaguchi *et al.* 2001

AZFb and AZFc deletion

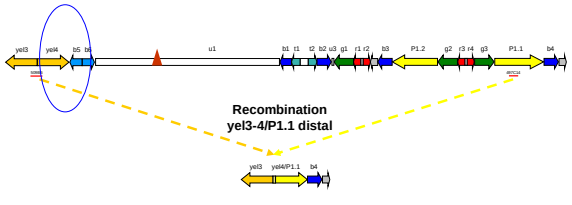
Intrachromosomal recombination



Repping *et al.* 2002, Costa P *et al.* 2008

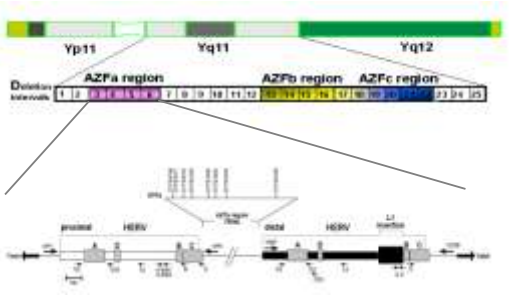
Chromosomal abnormalities
Y chromosome microdeletions

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



Kamp *et al.* 2000, Sun *et al.* 2000

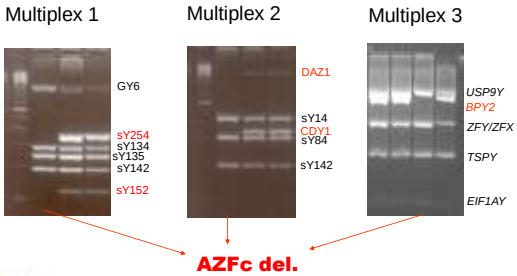
Chromosomal abnormalities

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



AZFc del.

7% azoospermia and 4% severe oligozoospermia

Chromosomal abnormalities

Y chromosome microdeletions

AZF microdeletions frequencies in 3002 infertile males

- Karyotype
  - 198 abnormal (86 – 47,XXY)
  - 288 unknown
  - 2714 normal
- Secretory Azoospermia 6.9% (56/816)
  - AZFa 6
  - AZFa+b 3
  - AZFb 1
  - AZFb+c 10
  - AZFc 36
- Oligozoospermia 3.8% (20/528)
  - AZFb 1
  - AZFb partial 2
  - AZFc 17

With ART, AZFc microdeletions are transmitted to male offspring!

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 could have prognostic value for patients undergoing ART

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

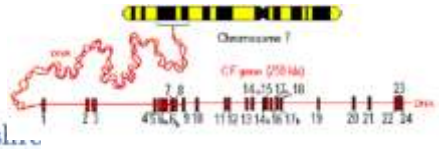
Congenital Absence of Vas Deferens (CAVD)

1-2% male infertility, 6% obstructive azoospermia

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

CFTR gene(Cystic Fibrosis Transmembrane Conductance Regulator)

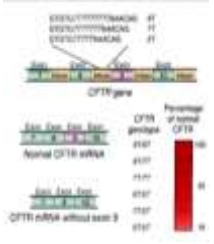
- ✓ Monogenic disease, most common autosomic recessive disorder
- ✓ Affects 1:2500 new-born (0.04%)
- ✓ 1:25 (4%) asymptomatic carriers (1 mutation) in Caucasians
- ✓ CFTR gene identified in 1989
- ✓ Maps on chromosome 7q3.1.2
- ✓ Chloride channel regulated by cAMP



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

Chillon *et al.*,1995

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

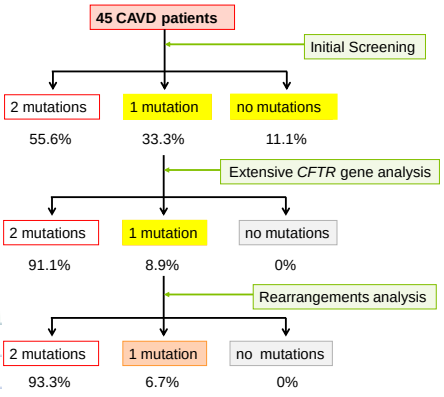
1 CF + 1 CF/CFTR-RD
2 CFTR-RD mutations

0-1 CF/CFTR-RD mutation
CF/CFTR-RD + CF? mutation

Screening of *CFTR* rearrangements – 3th Step

Quantitative fluorescent PCR (QFM_PCR)

CFTR mutations - CAVD



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 C*FTR* gene)

T5 allele	31.1
DeltaF508	23.3
R334W	6.7
R117H	4.4
G576A	4.4
R688C	4.4

Indications for CFTR mutations before ART:

- ✓Obstructive azoospermia

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