PRE-CONGRESS COURSE 6

SIG Reproductive Genetics
"Sex chromosomes in human reproduction: towards understanding their basic function"

CONTENTS

PRE-CONGRESS COURSE 6 - PROGRAMME

SIG Reproductive Genetics

Sex chromosomes in human reproduction: towards understanding their basic function

Course co-ordinators: P.H. Vogt (D), S. Viville (F) & K. Sermon (B)

Course description: This course aims to provide an overview of our current understanding of the function of X and Y chromosomes, in human reproduction in general and gametogenesis in particular. Why and how have they evolved, thereby accumulating functional key elements for associated genetic control mechanisms? After having presented a basic and molecular insight into the genomic evolution and conservation of both sex chromosomes, their abnormalities leading to interference in meiosis and inadequate germ cell production will be presented. For the male, Klinefelter syndrome is chosen as an example, while for the female the Turner syndrome will be discussed. Finally, X and Y genes with a clear role in human folliculogenesis and spermatogenesis, will be discussed with a focus on their impact in assisted reproductive technologies.

Target audience: Geneticists with an interest in reproduction, particularly those involved in genetic screening in infertile males; clinical embryologists with a daily experience in the consequences of male infertility, necessitating ART e.g. ICSI; andrologists wishing to brush up their basic knowledge on the genetic causes of male infertility; ART specialists confronted with female infertility of genetic causes.

Programme

09.00 - 09.30:	Genomic structure and genes of the X and Y chromosomes: evolution and future - ***S. Repping (NL)***
09.30 - 09.45:	*Discussion*
09.45 - 10.15:	X chromosome activity in gametogenesis and the strange phenomenon of sex chromosome silencing during male meiosis -***P. De Boer (NL)***
10.15 - 10.30:	*Discussion*
10.30 - 11.00:	***Coffee break***
11.00 - 11.30:	Elucidating the functions of mammalian Y chromosome in spermatogenesis - ***P. Burgoyne (UK)***
11.30 - 11.45:	*Discussion*
11.45 - 12.15:	Klinefelter syndrome: genetic variability and consequences for spermatogenesis ***– F. Vidal (E)***
12.15 - 12.30:	Discussion
12.30 - 13.30:	***Lunch***
13.30 – 14.00:	Turner syndrome and deletions in the X chromosome: genetic variability - ***P. Scriven (UK)***

14.00 – 14.15: *Discussion*

14.15 – 14.45 X genes with a role in gametogenesis – ***A. Rajkovic (US)***
14.45 – 15.00: *Discussion*

15.00 - 15.30: ***Coffee break***

15.30 - 16.00: Y genes interfering with gametogenesis. - ***P.H. Vogt (D)***
16.00 - 16.15: *Discussion*

16.15 - 16.30: Closing remarks ***– S. Viville (FR)***

Genomic structure and genes of the X and Y chromosomes: *evolution and future*

Sjoerd Repping, PhD

Layout of course

- Sex determination
- Evolution of human X and Y chromosomes
- Current contents
- The future
- Conclusion

Sex determination – XY/XX

- XY male
- XX female

Nature, May 2008

Sex determination – ZW/WW

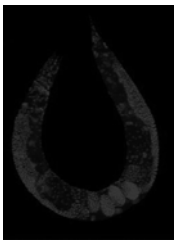
- ZW female
- WW male

Sex determination – XO/XX

- XO male
- XX female

Sex determination – XO/XX

- XO male
- XX hermaphrodite



Sex determination – haploid/diploid

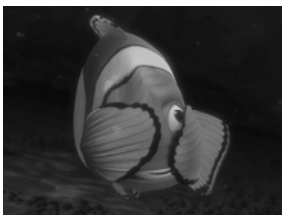
- Haploid male
- Diploid female

Sex determination – other

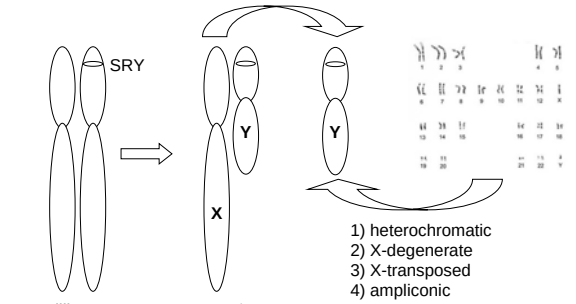
- Temperature

Sex determination – other

- Sequential hermaphroditism



Evolution



300 million years ago

present

1) heterochromatic
2) X-degenerate
3) X-transposed
4) ampliconic

Current contents – X chromosome


The DNA sequence of the human X chromosome

Ross, et al., Nature (2005)

Current contents – X chromosome

- Structure

Ross, et al., Nature (2005)

Current contents – X chromosome

- Genes

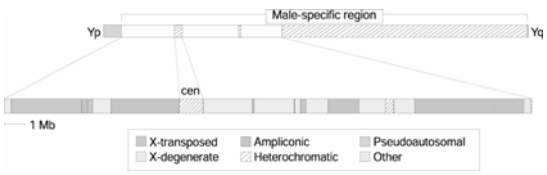
Ross, et al., Nature (2005)

Current contents – Y chromosome

The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes

Skaletsky, et al., Nature (2003)

Current contents – Y chromosome

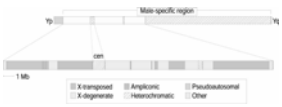


Skaletsky, et al., Nature (2003)

Current contents – Y chromosome

X-Y homologous sequences (PAR)
- Meiotic recombination with the X chromosome

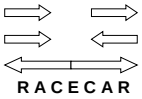
Male Specific Region of the Y (MSY)
- No meiotic recombination
- Four sequence classes:
  - Heterochromatic sequences
  - Euchromatic sequences: - X-transposed
    - X-degenerate
    - ampliconic



Skaletsky, et al., Nature (2003)

Current contents – Y chromosome

- Ampliconic sequences
  - >99% identical in sequence
- Organisation
  - Direct repeats
  - Inverted repeats
  - Palindromes
  - Complex structures



R A C E C A R

M A D A M I N E D E N , I ' M A D A M

Skaletsky, et al., Nature (2003)

Current contents – Y chromosome

- MSY genes
  - 156 transcription units
  - 78 likely to encode proteins
- Distribution
  - heterochromatic 0 genes
  - X-transposed 2 genes
  - X-degenerate 16 genes
    - widely expressed
  - ampliconic 60 genes
    - testis-specific expression

Skaletsky, et al., Nature (2003)

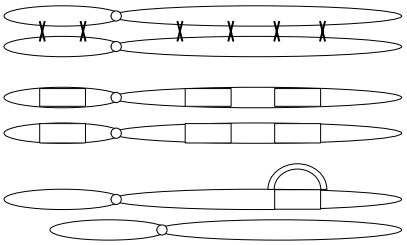
X Y comparison

- Size
  - X: 155 Mb
  - Y: 24 Mb (euchromatic MSY)
- Genes
  - X: 1,098 genes
  - Y: 78 genes
- Authors
  - X: 283 authors
    - 0.55 Mb sequence per author
    - 3.88 genes per author
  - Y: 40 authors
    - 0.60 Mb sequence per author
    - 1.95 genes per author

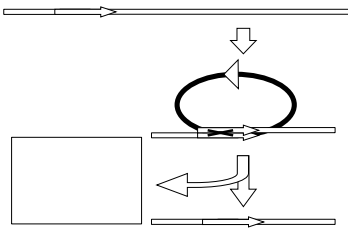
X Y comparison

- Repeats
  - X: …
  - Y: …
- Reproduction
  - X: 99 genes testis-specific (9%)
  - Y: 60 genes testis-specific (77%)

Meiotic recombination – genetic sex

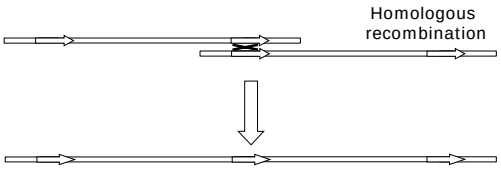


Structural variation - deletions

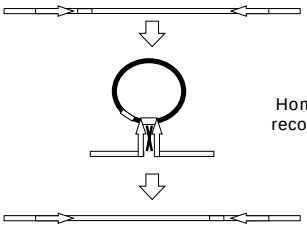


Sun, et al., Hum Mol Genet (2000), Kuroda-Kawaguchi, et al., Nat Genet (2001)
Repping, et al., AJHG (2002), Nat.Genet (2003), Genomics (2004)

Structural variation - duplications

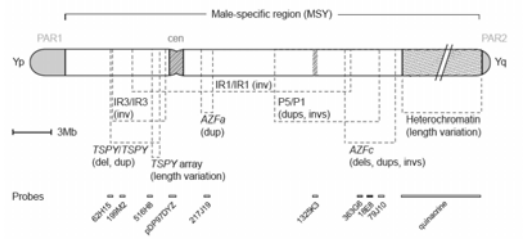


Structural variation - inversions



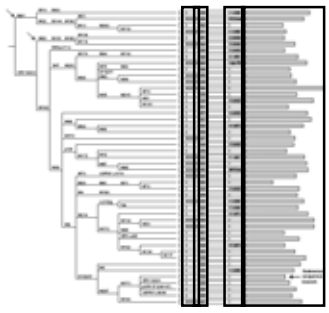
Structural variation - gene conversion

Structural variation – possibilities



Repping, et al., Nat Genet (2006)

Structural variation - results



Four classes
- *TSPY* array length (23-64 copies)
- Heterochromatin (29-54% chromosome)
- *IR3/IR3* inversion
- *AZFc* structure

Repping, et al., Nat Genet (2006)

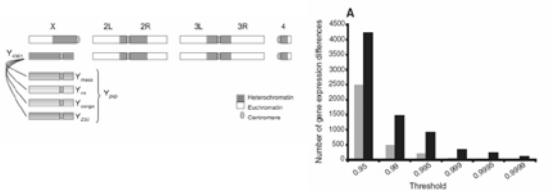
Structural variation - results

- 29 out of 47 chromosomes differ from reference sequence
  - Excluding *TSPY* array and heterochromatin length
- Degree of variation differs
  - Heterochromatin > *TSPY* > IR3/IR3 > *AZFc*
- "Only" 10 chromosomes carry a deletion or duplication
  - 79% identical copy number with reference genome
  - Evolutionary selection
    - against deletions
    - against other rearrangements?

⇨ via effects on spermatogenesis……

Repping, et al., Nat Genet (2006)

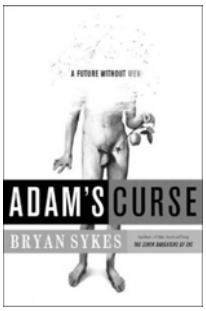
Structural variation – other effects



Lemos, et al., Science (2008)

The future

- Disappearing Y?
- Selection against
  - Deletions
  - Other rearrangements?
    - Effect on autosomes?
- Non-sexual reproduction…..



Conclusion

Acknowledgements

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X chromosome activity in gametogenesis and the strange phenomenon of sex chromosome silencing during male meiosis

Peter de Boer

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Learning objectives

- Biological understanding of sexual reproduction

- Emphasis on the role and regulation of the sex chromosomes in the soma versus the germline

- Appreciation of the functional stability of the sex chromosomes in especiallly spermatogenesis

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"Reproduction is at the center of Biology, everything else is at its service"

Development
Physiology
Behaviour
Genetics

"Sex is the queen of problems in evolutionary biology, Bell, 1982"

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Why is sexual reproduction so successfull despite of the two-fold cost of sex

- Favourable allele combinations are disrupted

- In sexual reproduction, one sex has few but fit gametes, the other many but "cheap".
A contribution of the latter sex to parenthood is no automatism . Yet this sex uses up food resources

Background: "Evolution an introduction", SC Stearns and RF Hoekstra, Oxford Univ press, 2000, 2006

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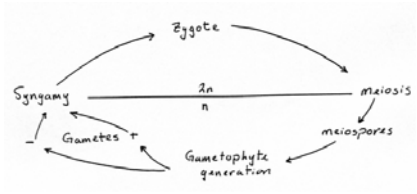
Population geneticists propose that genetic robustness, the resitance to genetic erosion and environmental change, as well as speciation, benefit from sexual reproduction



In sex, haploid and diploid phases alternate, the diploid phase becoming more and more elaborate

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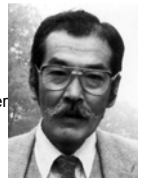
Robustness: the invariance of the phenotype or fitness in the face of genetic pertubations (say mutations) or environmental pertubations

"Robustness" includes resistance to environmental impact such as oxydative damage

Genetic robustness

"Evolution by gene duplication" S Ohno
- Genome doubling :
  for instance at first division of zygote
- Segmental chromosome doubling
- Gene doubling by unequal crossing-over (see later)
  causes duplications



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There is a trade-off between relaxation of the mechanism of
meiosis including DNA repair and the adaptive value of new
genotypes, opening up windows for the formation of new species

Yet the process of haploidisation through the meiotic divisions is tightly regulated, and firmly connected to fertility

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Sexual dimorphism started with the evolution of an allele that stimulated testis development SOX3 > SRY
(**S**ex determining **R**egion of the **Y** chromosome)

This autosomal chromosome pair specialized due to inhibition of crossing-over in the meiotic germ cells of the sex carrying the testis, leading to degeneration of the homologue with SRY

Inversions disrupt the gene order within the SRY carrying chromosome, reducing crossing-over: Muller's rachet at work

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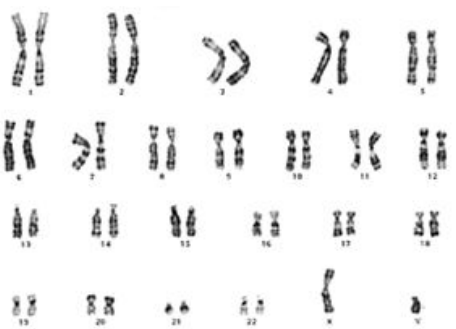

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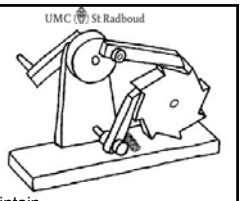

Muller proposed that any chromosomal segment that is devoid of crossing- over, as if it were left alone, will suffer genetic decay.
For survival it has to develope a strategy to maintain itself as an independent identity of its own.
Local evolution through gene duplication

Can the Y chromosome win this race?

Hermann Muller 1890 - 1967

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Recombination by crossing-over produces for all chromosomes endless versions (by allele content) one of which by chance ends up in one gamete

After fertilization, minor detrimental alleles (for a number of genes) collect in one conceptus.
This will lead to embryonic death or sterility (genetic death) or reduced fitness before the end of reproduction

Selection for fertility is a force to shape the Y (and X) chromosome

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Two consequences of sex chromosome specialisation

- Genetics differences for sex chromosomes between the sexes, how big can this difference be before population fitness is reduced?

-The balance between autosome and sex chromosome gene product

Why is this balance important?

The bulk of proteins does not work alone but in multi-protein complexes with a flexible composition to allow for adaptation of function. So autosomal and sex
chromosomal gene products often function in the same pathways

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Equalisation of sex chromosome activity between sexes is regulated by sex chromosome inactivation

- Every sex chromosome in excess of 1 is inactivated

- Inactivation is random and happens early in embryonic life

- Inactivation is an example of an epigentic process. Not allele content decides but chromosome wide downregulation of gene activity is transmitted to daughter cells within the soma

- This type of transmission is less stable than genetic transmission, so local reactivation can occur later in life

- Germ cells do not obey this rule

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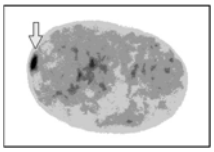
Inactivatition is triggered by one gene XIST of which the RNA is not translated into protein. The RNA product induces the spread of inactivation from the site of transcription. XIST works in cis. The inactivated chromosome has a deviant chromatin make-up, is late replicating. Occupies its own territory within the nucleus (Barr body)

Staining now is also with antibodies that recognize histone modifications that are typical for facultative and/or constitutive heterochromatin

Inactivation is incomplete, for some 5% of X linked genes the sexes differ in gene activity



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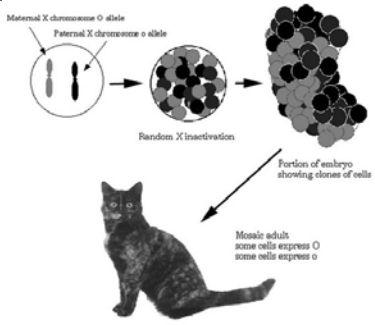

In 1961, Mary Lyon proposed random X chromosome inactivation to explain the female phenotypes of mice heterozygous for X linked coat markers

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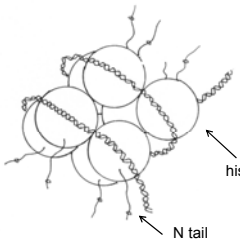
The use of histone N tail modifications to describe chromatin* status,
for instance the facultative heterochromatin status of the inactive X chromosome

8 histone molecules, 2 of each of 4 types, (H2A, H2B, H3 and H4) assemble as a nucleosome around which 147 basepairs of DNA are wrapped



The N tails stick out and are enzymatically modified in many positions. Locally the functionality of chromatin can be read

For instance, Histone 3 trimethylated at Lysine position 27 is characteristic for
facultative heterochromatin. Antibodies have been generated to detect this modification, hence the inactive Xp. chromatin is the substance of chromosomes

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Would in the next generation, the expression of the paternal X equal the expression of the maternal X?

In females, this comparison exists, but a parent of origin effect has not been described for the human, also not for the embryonic membranes

Turner syndrome as an alternative: Xmaternal O versus Xpaternal O

XmO: more kidney problems, lower cholesterol

XpO: more eye problems, better educated

Earlier work indicated more social behaviour in XpO

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A comparison in the male may be affected by another hormonal regime

(hormone - gene expression interactions)

XmXmY versus XmXpY

Speech and motor development problems, both stronger with XmXpY

Body size parameters: XpXmY > XmXmY. Xp favours growth in a male environ

Both XO and XXY provide some evidence for a genetic imprinting effect of the X
(selected genes of this chromosome have a parent-of-origin specific mode of functioning in the offspring)

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In the female germline, when the primordial germ cells enter the ovary, the inactive X chromosome is reactivated in order to participate in meiosis

Spermatogonia do not function properly with two X chromosomes (the Klinefelter case)

In male meiotic prophase, the X and Y chromosomes are inactivated, a situation called meiotic sex chromosome inactivation (MSCI)

X and Y are set apart in a nuclear domain called the sex body (or XY body)

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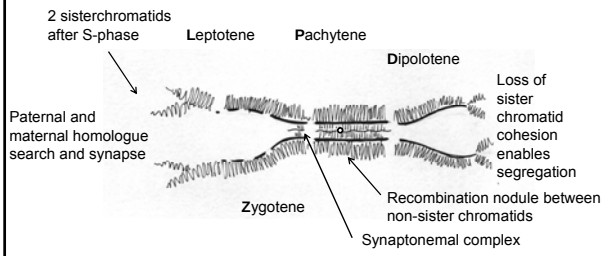
A closer look at meiosis



Human male: **L**, 3.75 days, **Z**, 3 days, **P**, 15.5 days **D**, short

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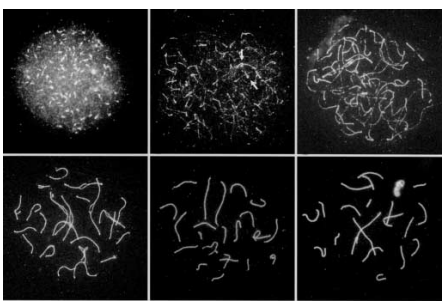
Anti-SCP3* fluorescence in male meiosis



* SCP3 = synaptonemal complex protein 3

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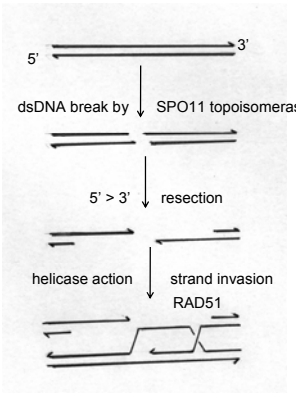

The homology search starts by SPO11 induced dsDNA breaks

- SPO11 action in leptotene-zygotene is controlled

- Only subset of breaks leads to non-randomly placed crossing-over, ma only to gene conversion (leads ao to minisatellite variation)

- Absence of crossing-over leads to aneuploidy, hence embryonic death

- Crossing-over frequency is heritable

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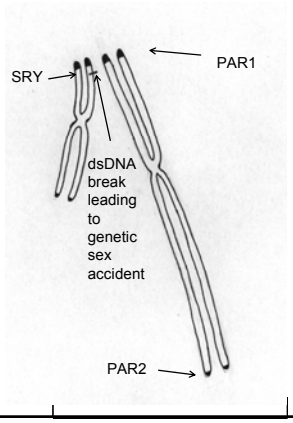

X and Y are later to find each other

Limited homology?

The evolutionary decision to position SRY close to PAR1 gives rise to genetics accidents:

XXSRY Klinefelters and XY females

PAR = pseudo autosomal region

Note: sysnapsis at both PAR1 and PAR2 produces a sex chromosomes meiotic ring

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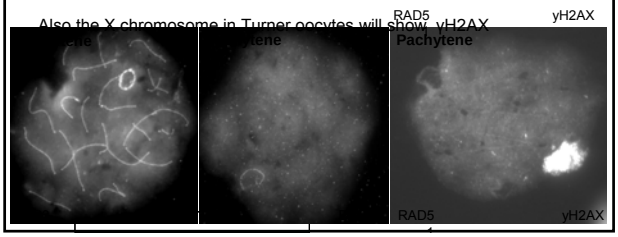
As in mouse, both BRCA1 and the DNA damage triggered kinase ATR are involved in the detection of unsynapsed chromosome segments, BRCA1 providing a platform for ATR to phosphorylate on a grand scale, among which H2AX (a H2A variant). S139 phosphorylated H2AX = γH2AX

These reactions are not sex chromosome confined, nor sex specific but asynapsis specific

Also the X chromosome in Turner oocytes will show γH2AX



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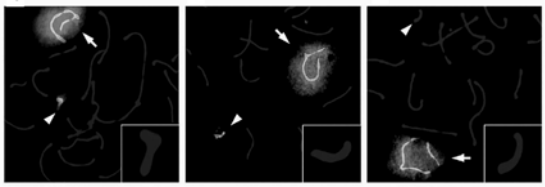
In the mouse, it was discovered that any deviation from a regular synaptonemal complex translates in some phosphorylation of H2AX

Hence, any synaptic irregularity is detected by BRCA1/ATR. Recently this principle was also confirmed in the human



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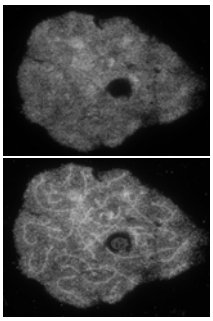
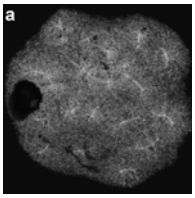
Recently it was also found that the presence of γH2AX announces a nucleosome change. This change likely happens parallel to MSCI and the inactivation of unsynapsed autosomal chromatin, collectively called MSUC (meiotic silencing of unpaired chromatin)

In the mouse, Xist is not involved

human



mouse



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X,Y linked genes have a role before and after meiosis, but not so much during first meiotic prophase

Of 25 genes found to be expressed in spermatogonia, 3 are Y linked and 10 X linked

The X chromosome is enriched in genes related to sexual reproduction and brain function and almost devoid of genes that function in spermatocytes

The Y chromosome is largely spermatogenesis specialised

The presence of spermatogonial genes on the X likely is a case of hemizygous selection

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Hemizygous selection is a force in shaping the X (and Y) chromosomes

Genetic changes that are beneficial to males are directly selected for, and negative changes are selected against

Example: males have a wider variation in IQ scores and more males are hospitalized in institutions for the mentally ill

So inactivation of unsynapsed sex chromosome segments is at a higher level of importance than the activity of spermatogenesis beneficial genes on X and Y during meiosis

Also, inactivation of autosomal segments is negative for spermatogenesis

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It is now understandable why the X chromosome has no interest in genes that function during male meiosis

In evolution, backup copies have been made on autosomes for those genes
who's activity could not be missed during meiosis. Because of Ohno's law, this likely is the case for the human too : "Genes that in one mammal are on the X chromosome are also on this chromosome for the other mammals"

But there is no dogma without exception, the first X linked mouse gene for which gene ablation results into a male meiotic phenotype has recently been reported

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Conclusions

The different roles for the sex chromosomes in the sexes are due to evolutionary pressures that are different for males and females and differ from the autosomes

Epigenetic regulation is at the basis of somatic X chromosome inactivation

Large scale chromatin remodeling is at the basis of sex chromosome inactivation in spermatocytes

Spermatocytes suffer from remodeling and inactivation extending into autosomal domains

Oocytes also are sensitive to inactivation of unsynapsed chromatin, irrespective
of chromosomal content

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Elucidating the functions of the mammalian Y chromosome in spermatogenesis

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NOTE: There are no conflicts of interest with respect to this presentation

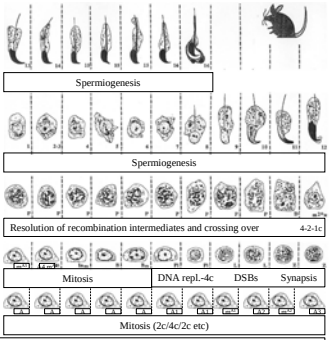
LEARNING OBJECTIVES

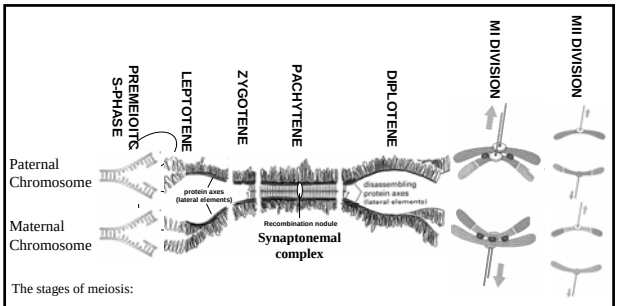
To help those providing male ART services and counselling to infertile men to better understand the link between perturbed Y gene activity during spermatogenesis and infertility.
The presentation will describe:

1. The basics of spermatogenesis including an overview of the salient features of meiosis.
2. The advantages of the mouse for learning about Y gene functions in spermatogenesis.
3. The puzzling phenomenon of Y (and X) gene silencing during male meiosis (MSCI).
4. How a failure to silence the Y leads to pachytene spermatocyte death in XYY males.
5. That there is substantial post-meiotic repression of the Y (and X) during sperm development; nevertheless, there are some Y (and X genes) that are expressed post-meiotically.
6. The phenomenon of gene product sharing between X- and Y-bearing round spermatids.
7. Describe our progress in ascribing functions to Y genes during the period of sperm development

THE SPERMATOGENIC CELL CYCLE (mouse)

1. Testis tubule cross-sections are classified into stages based on their spermatogenic cell complement.
2. Spermatogenesis begins (bottom left of diagram) with the mitotic proliferation of spermatogonia; DNA content oscillates between 2c and 4c
3. The meiotic phase begins with the replication of the DNA (2c to 4c) without an ensuing cell division (DNA content remains at 4c). During this 4c prophase stage there is a re-shuffling of maternal and paternal genomes (recombination). There are then two cell divisions without further DNA replication thus reducing the DNA content from 4c to 1c. The cells throughout this meiotic phase are termed spermatocytes.
4. The resulting post-meiotic cells are termed spermatids. The dramatic transformation of the initially round spermatids into spermatozoa is termed spermiogenesis.



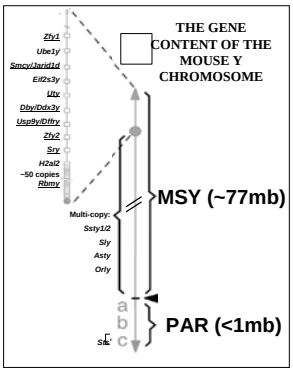


The stages of meiosis:

1. Premeiotic S when the DNA replicates (2c to 4c).
2. Leptotene when meiotic chromosome axes first appear.
3. Zygotene when maternal and paternal chromosomes intimately pair (synapse to form the synaptonemal complex).
4. Pachytene when there is exchange (recombination) between the maternal and paternal chromosomes.
5. Diplotene when the paired chromosome axes separate (while retaining associated at the centromeres – not shown).
6. MI - the first meiotic division (4c to 2c)
7. MII the second meiotic division (2c to 1c)

WHY STUDY MOUSE SPERMATOGENESIS?

1. Man and mouse share a number of Y genes that are expressed during spermatogenesis



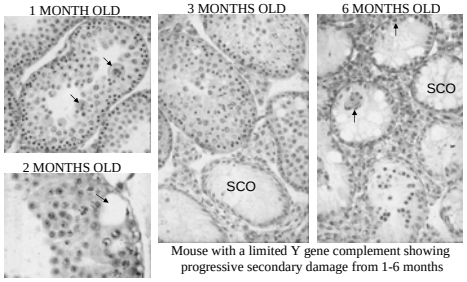
MSY is the male-specific part of the Y comprising:

- Short arm: ~1/10th of the MSY 7 single copy genes, 2 duplicated genes (*Zfy*, *H2al2*) and 1 multi-copy gene (*Rbmy*). [Genes with human Y homologues underlined]
- MSYq: ~9/10th of the MSY *Ssty*/*Sly*/*Asty*/*Orly* multi-copy genes [*Sly* is essential for later stages of mouse sperm development; no homologues on human Y]

Y chromosome PAR pairs (synapses) and recombines with X PAR. This is essential for subsequent progression beyond the first meiotic division in mouse and human.

WHY STUDY MOUSE SPERMATOGENESIS?

2. In the mouse you can study testicular changes from puberty to adulthood. This allows the separation of the primary consequences of a genetic deficiency from the secondary damage which is a consequence of ongoing germ cell death



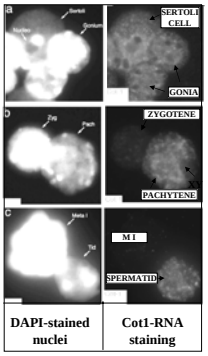
Mouse with a limited Y gene complement showing progressive secondary damage from 1-6 months

MEIOTIC SEX CHROMOSOME INACTIVATION (MSCI)

1. The silencing of the X and Y chromosomes throughout the pachytene stage of meiotic prophase in males; this occurs immediately after the completion of the phase of intimate chromosome pairing (synapsis) that takes place during the zygotene stage.
2. This silencing is a response to the fact that the X and Y chromosomes remain unsynapsed along most of their length and is a manifestation of a meiotic silencing mechanism that targets unsynapsed chromosome segments in males and females.
3. The silencing is due to chromatin restructuring which prevents the cellular machinery from transcribing messenger RNA from the DNA.

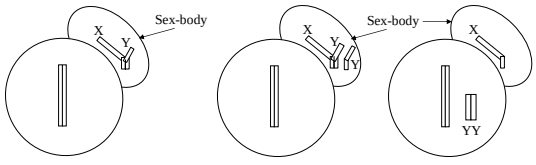
Reviewed by Turner and Burgoyne, 2007

MSCI AS REVEALED BY Cot1 RNA FISH – A MEASURE OF GLOBAL mRNA TRANSCRIPTION IN THE NUCLEUS (Turner et al., 2005)



1. Spermatogonia actively transcribe messenger RNAs at levels similar to those in Sertoli cells.
2. Global transcription is repressed on entry to meiotic prophase and is barely detectable at zygotene. It resumes and increases during pachytene, but the X and Y chromatin domain (sex body) is silenced.
3. Transcription is again repressed during meiotic divisions and resumes in spermatids

FAILURE OF Y CHROMOSOME SILENCING IN XYY MALES LEADS TO PACHYTENE SPERMATOCYTE DEATH (Turner et al, 2006)



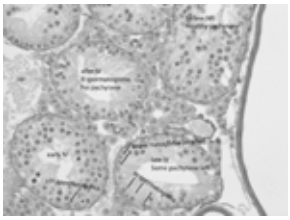
In XY males the X and Y of pachytene spermatocytes are silenced and form a peripheral structure termed the sex-body

In 88% of XYY pachytene spermatocytes the two Ys did not synapse and were silenced in the sex body. These cells completed meiotic prophase.

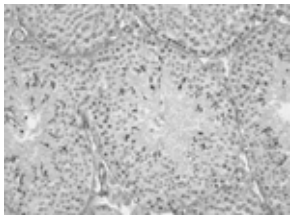
In 12% of XYY pachytene spermatocytes the two Ys fully synapsed, were not silenced, and were not in the sex body. These cells were eliminated during mid pachytene.

Note: In XYY men, XYY pachytene spermatocytes have predominantly YY synapsis with associated extensive pachytene cell loss (Solari and Valzacchi, 1997)

ELIMINATION OF XYY CELLS WITH YY SYNAPSIS IS DUE TO 'INAPPROPRIATE' EXPRESSION OF *ZFY* (Burgoyne & Turner labs, unpublished)

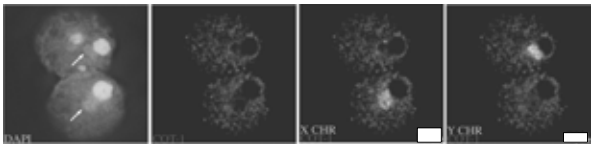


Testis section for an XY male with an extra copy of *Zfy* inserted on an autosome (non-sex chromosome). Because the autosome synapses normally with its partner the extra copy of *Zfy* is expressed during pachytene. All spermatocytes are eliminated at mid pachytene (in the course of epithelial stage IV).



Testis section form an XY male with an extra copy of *Zfy* inserted on the X chromosome. Because the X is silenced (MSCI) the extra copy of *Zfy* is silenced during pachytene. Spermatogenesis now goes through to completion and the mice are fully fertile.

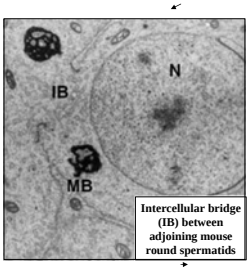
THE X AND Y CHROMOSOMES REMAIN SUBSTANTIALLY REPRESSED IN SPERMATIDS



These images (that will be in colour in the live presentation) show the nuclei of a pair of spermatids. The upper cell is Y-bearing and the lower cell X-bearing. The brightly DAPI-fluorescent structures are composed of centromeric heterochromatin, but adjacent to these are structures of intermediate fluorescent intensity (arrowed). Cot1 RNA fluorescent in situ hybridisation, which detects messenger RNAs as they are generated within the nucleus, shows that the regions of centromeric heterochromatin are transcriptionally silent (as expected). The adjacent DAPI fluorescent structures are also markedly depleted of transcripts. The last two panels with combined Cot1 RNA FISH and chromosome painting show that the transcript poor regions are the X and Y chromosome domains.

The fact that the majority of mouse Y genes are reactivated in spermatids (following their complete silencing in pachytene spermatocytes) or are exclusively expressed in spermatids, point to their having important functions in spermiogenesis.

X- AND Y-BEARING ROUND SPERMATIDS SHARE TRANSCRIPTS VIA INTERCELLULAR BRIDGES



Intercellular bridge (IB) between adjoining mouse round spermatids

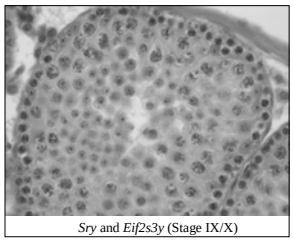
The two meiotic divisions generate four spermatids (2 X-bearing and 2 Y-bearing) from each primary spermatocyte. The cell divisions are incomplete such that substantial cytoplasmic 'bridges' remain between the four cells, through which messenger RNAs can move freely. Thus X and Y bearing spermatids are each expected to have X and Y gene products.

In the mouse all the Y genes so far identified have closely similar X homologues and it is often assumed that the proteins encoded by the X and Y homologues fulfil similar functions. Nevertheless, in mouse and man Y gene deficiencies can seriously impact on spermiogenesis.

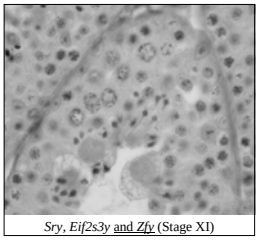
MOUSE Y GENES EXPRESSED IN SPERMATIDS AND THEIR PUTATIVE FUNCTIONS

Mouse Y gene	Human Y location	Putative function	X v Y gene amino acid identity/similarity
Zfy1/2	Distal short arm	Transcription factor	67 / 79%
Smcy/Jarid1d	*AZFb*	Chromatin remodelling in sperm head?	83 / 89%
Uty	*AZFa*		76 / 84%
Usp9y/Dffry	*AZFa*	Histone replacement with protamines in sperm head?	81 / 89%
Ube1y	X copy only		82 / 89%
H2al2	No Y (or X) copy	Spermatid nucleosomal histone	70 / 73%
Dby/Ddx3y	*AZFa*	RNA helicase	90 / 94%
Sly (multi-copy)	Possible X copies	Acrosome development	58 / 59%

ZFY IS ESSENTIAL FOR MOUSE SPERM HEAD ELONGATION



Sry and *Eif2s3y* (Stage IX/X)



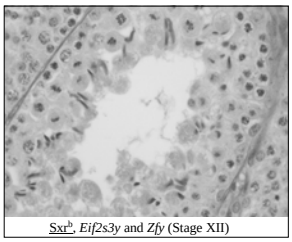
Sry, *Eif2s3y* and *Zfy* (Stage XI)

In mice with *Sry* and *Eif2s3y* as the only Y genes, spermatid development arrests at step 7 just prior to the elongation of the sperm head.

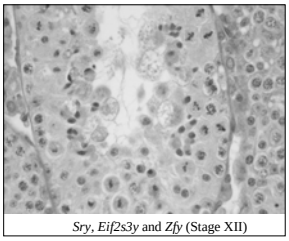
In mice with *Sry*, *Eif2s3y* and *Zfy* there is now some elongation of the sperm head and sperm tails are forming. However, head elongation is incomplete and the cells soon die.

Zfy is a transcription factor that would be expected to control the expression of one or more spermatid expressed genes – this may include some other Y genes.

H2AL2 IS ALSO IMPLICATED IN SPERM HEAD ELONGATION



Sxr^b, *Eif2s3y* and *Zfy* (Stage XII)



Sry, *Eif2s3y* and *Zfy* (Stage XII)

Mice carrying the mouse Y short arm-derived chromosomal fragment Sxr^b together with *Eif2s3y* and *Zfy*, have much more extensive sperm head elongation than mice with *Sry*, *Eif2s3y* and *Zfy*. Thus Sxr^b must include a Y gene or genes that is involved in the chromatin restructuring associated with sperm head elongation.

The most likely candidate is *H2al2* of which two (or 4?) copies are present in Sxr^b. This gene, together with related X copies, encodes a spermatid-specific histone that can substitute for the nucleosomal histone H2A. It first appears during step 9 of spermatid development and has already been implicated in chromatin restructuring during the elongation of the sperm head (Govin et al., 2007), although these authors did not know that genes encoding histone H2AL2 were on the Y chromosome.

PRELIMINARY DATA ALSO SUGGEST A ROLE FOR SMCY DURING SPERM HEAD ELONGATION

It is hoped that definitive data will be available in time for the ESHRE July meeting

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Klinefelter syndrome: genetic variability and consequences for spermatogenesis

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Learning objectives

- To get a brief overview of the commonest clinical and cytogenetic features of KS
- To review data on sperm chromosome abnormalities in KS
- To gain insight into meiotic events and spermatogenesis in KS
- To discuss genetic reproductive risk in KS
- To identify issues that require further investigation

Klinefelter syndrome

- Brief History

  Description by Klinefelter et al. (1942)

  Individuals affected were "X-chromosome positive" (Ferguson-Smith et al. 1957)

  47,XXY karyotype reported (Jacobs and Strong 1959)

Klinefelter syndrome

- General clinical features

  Small firm testes, gynaecomastia, azoospermia, hipergonadotrophic hypogonadism.

  Slight increased rate of major and minor congenital anomalies, abnormal body proportions.

  Associated disorders.

Heterogeneous syndrome. Variable clinical presentation

Lanfranco et al. 2004; Aksglaede et al. 2006

Klinefelter syndrome

- General prevalence

  1/600 in newborns (~0.15%)
  1/300 in spontaneous abortions (~0.3%)

  ~5% in infertile males ~33 fold increase vs. newborns

  ~2% in oligozoospermic males
  ~9% in azoospermic males

de Braekeleer & Dao 1991; Pandiyan & Jequier, 1996; Mau-Holzmann 2005; Aksglaede et al. 2006; Ferlin et al. 2005

Klinefelter syndrome

- Cytogenetics

  ~ 80% karyotype 47,XXY
  ~ 20% karyotype 46,XY/47,XXY

  Variants (48,XXXY; 48,XXYY; 49,XXXXY; ....)

Mosaicism grade, assessment, cell lines, ...

Lanfranco et al. 2004; Mau-Holzmann 2005

Klinefelter syndrome

- Fertility

  Traditionally described as infertile. Germ cell depletion, absence spermatogenesis, hyalinization of seminiferous tubules.

  Residual foci with spermatogenesis (mosaics?, cell line?).

  Rare natural conceptions

Advent of ICSI and TESE changed the picture

Lanfranco et al. 2004; Mau-Holzmann 2005 Laron et al. 1982; Terzoli et al. 1992

Klinefelter syndrome

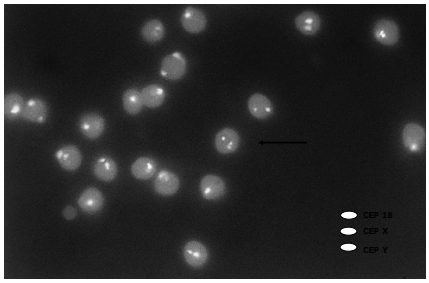
Sperm chromosome abnormalities

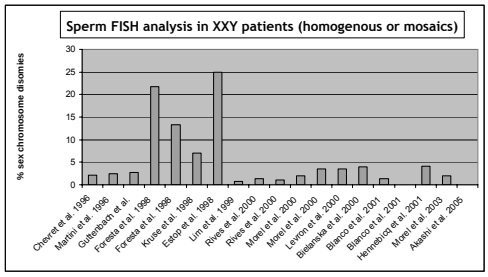
Meiotic competence 47,XXY cell line

Genetic risk

Sperm chromosome abnormalities

FISH in sperm nuclei





Number of cases = 23
- ✓ Statistical analysis = 15
- ✓ Increase incidence of sex chromosome disomies = 13/15 (87%)
- ✓ Range = 0 - 25%
- ✓ Mean and standard deviation = 5.2% ± 7.1

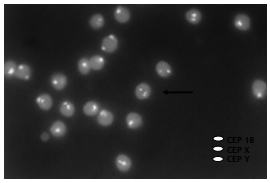
Sperm chromosome abnormalities

Table 1. Percentage of chromosomal abnormalities in spermatozoa of Klinefelter's syndrome patients. [a]No statistical analysis has been performed; [b]statistically significant versus internal controls.

Reference	Authors	Karyotype	XY	XX	YY	Diploid
15	Chevret *et al.* (1996)	46,XY/47,XXY	2.09[b]	0.11	0.003	0.33
16	Martini *et al.* (1996)[a]	46,XY/47,XXY	1.30	0.5	0.7	–
17	Guttenbach *et al.* (1997)	47,XXY	1.36[b]	1.22[b]	0.09	0.23[b]
18	Foresta *et al.* (1998)	47,XXY	14.58[b]	6.92	0.21	0.05
		47,XXY	10.03[b]	3.34	0.09	0.03
19	Kruse *et al.* (1998)[a]	46,XY/47,XXY/48XXXY	5	2	–	–
20	Estop *et al.* (1998)[a]	47,XXY	25[b]	–	–	4.2[b]
21	Lim *et al.* (1999)	46,XY/47,XXY	0.41[b]	0.29[b]	0.06	1.70[b]
22	Rives *et al.* (2000)	47,XXY	0.54[b]	0.45[b]	0.37[b]	0.23[b]
		46,XY/47,XXY	0.62[b]	0.24[b]	0.20	0.36[b]
23	Morel *et al.* (2000)	46,XY/47,XXY	1.3[b]	0.71[b]	–	0.24[b]
		46,XY/47,XXY	1.73[b]	0.86[b]	0.86[b]	0.25[b]
24	Levron *et al.* (2000)[a]	47,XXY (5)	0.89	1.79	0.89	–
25	Bielanska *et al.* (2000)[a]	46,XY/47,XXY	2.23	1.12	0.56	0.84
26	Blanco *et al.* (2001)	47,XXY	1.37[b]	–	–	1.37[b]
		46,XY/47,XXY	–	–	–	–

Sarrate et al. 2005

Sperm chromosome abnormalities



- ↑ **sex chromosome disomies** (range 0%-25%)
- ↑ **diploidy** (range 0.03%-4.2%)

Origin?

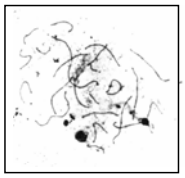
- XXY germ cells are able to go though meiosis and produce both normal and aneuploid spermatozoa (?)
- XY germ cells are the only ones meiotically competent and responsible for sperm production. Sperm production is related to germ cell mosaicism (?)

Meiotic competence 47,XXY line

Early meiotic studies

Skakkeabaek et al. 1969; Luciani et al. 1970; Hultén and Pearson, 1971; Laurent et al. 1973; Chandley et al. 1976; Vidal et al. 1984

- Contradictory results

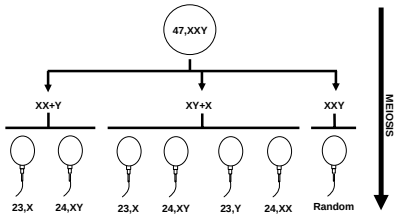


- Disruption meiotic process

Meiotic competence 47,XXY line

Sperm FISH studies

- Indirect results → X:Y ratio, sex chromosome disomies



Theoretical expected percentage of sperm chromosome anomalies
- 50% of sex chromosome aneuploidies in sperm (3 out of 6)
- non equivalent 1:1 X/Y ratio

Meiotic competence 47,XXY line

Sperm FISH studies

- Indirect results → X:Y ratio, sex chromosome disomies

	Karyotype	X:Y	XY	XX	YY	Diploid
Chevret *et al* (1996)	46,XY/47,XXY	53:44	2.09	0.11	0.003	0.33
Martini *et al* (1996)*	46,XY/47,XXY	47:43	1.3	0.5	0.7	-
Guttenbach *et al* (1997)	47,XXY	43:49	1.36	1.22	0.09	0.23
Foresta *et al* (1998)	47,XXY	52:25	15.58	6.92	0.21	0.05
	47,XXY	56:29	10.03	3.34	0.09	0.03
Kruse *et al* (1998)	46,XY/47,XXY/48XXXY	50:42	5	2	-	-
Estop *et al* (1998)*	47,XXY	21:29	25	-	-	4.2
Lin *et al* (1999)	46,XY/47,XXY	47:50	0.41	0.29	0.06	1.70
Rives *et al* (2000)	47,XXY	50:48	0.54	0.45	0.37	0.23
	46,XY/47,XXY	50:49	0.62	0.24	0.20	0.36
Morel *et al* (2000)	46,XY/47,XXY	50:43	1.3	0.71	-	0.24
	46,XY/47,XXY	47:45	1.73	0.86	0.86	0.25
Levron *et al.* (2000)	47,XXY (5)	48:52	0.89	1.78	0.89	-
Bielanska *et al* (2000)*	46,XY/47,XXY	50:44	2.23	1.12	0.56	0.84
Blanco *et al* (2001)	47,XXY	52:44	1.37	-	-	1.37
	46,XY/47,XXY	43:50	-	-	-	0

PCC-ESHRE'08

Meiotic competence 47,XXY line

Sperm FISH studies

- Indirect results → X:Y ratio, sex chromosome disomies

XXY meiotically competent:

- **X:Y ≠ 1:1** Lower percentage of Y, slightly higher percentage of X
- XX- and XY-disomies increased with respect to controls; normal percentage of YY-disomies.

⇩

No coincident results between reported studies

PCC-ESHRE'08

Meiotic competence 47,XXY line

Sperm FISH studies

- Indirect results → X:Y ratio, sex chromosome disomies

XY responsibles for the residual spermatogenesis:

- **X:Y = 1:1**
- Sex chromosome disomies increased with respect to controls (but far away from the 50% theoretically expected)
- Increase in diploidy
- Autosomal aneuploidy

⇩

Most reported studies

PCC-ESHRE'08

Meiotic competence 47,XXY line

Sperm FISH studies

- Indirect results → X:Y ratio, sex chromosome disomies

XY responsibles for the residual spermatogenesis:

- **X:Y = 1:1**
- Sex chromosome disomies increased with respect to controls (but far away from the 50% theoretically expected)
- Increase in diploidy
- Autosomal aneuploidy

⇩

Germ cell mosaicism

Gonadal mosaicism

Mosaicism grade, assessment, somatic karyotype, cell lines, ...

Mosaic: when at least 5% of cells are 46,XY

•FISH analyis on interphase nuclei

Cases	Lymphocytes	Testicular tissue
1	47,XXY (100)	46,XY/47,XXY (11:89)
2	46,XY/47,XXY (35:65)	46,XY/47,XXY (72:28)

Evidence of germ cell mosaicism

Meiotic competence 47,XXY line

Meiotic analysis

- Testicular tissue. Direct study

  - Classical meiotic analysis
  - FISH analysis of meiotic figures
  - FISH analysis of interphase nuclei

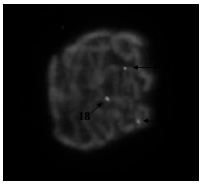
Evaluation of meiotic stages, presence of XY/XXY lines, pre-meiotic and post-meiotic cells, ...

Meiotic competence 47,XXY line

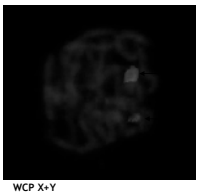
Meiotic analysis

- Testicular tissue. Direct study

Meiotic behaviour of the sex chromosomes

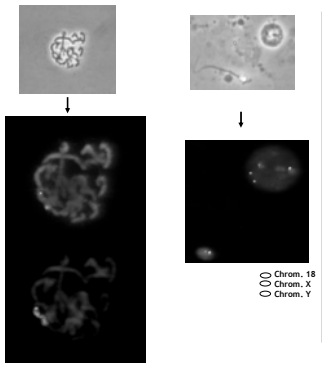


CEP X+Y+18



WCP X+Y

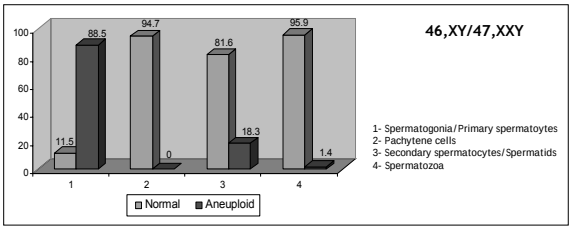
Prophase I



Meiotic figures: XY

No evidence for 47,XXY cell line proceeding through meiosis

Blanco et al. (2001) Hum Reprod 16:887-892



46,XY/47,XXY

1- Spermatogonia/Primary spermatoytes
2- Pachytene cells
3- Secondary spermatocytes/Spermatids
4- Spermatozoa

Spermatogenic foci: 46,XY cell line. General disruption meiotic process

Chromosomally abnormal spermatozoa: Development of meiosis in a compromised testicular environment (Mroz et al. 1999)

Good agreement with animal model studies

Genetic risk in KS

- ↑ **sex chromosome disomies** (range 0%-25%)
- ↑ **diploidy** (range 0.03%-4.2%)

ICSI in XXY males

Mosaic and non-mosaic (?)
Ejaculated and Testicular spermatozoa

Staessen C et al (2003). PGD in 47,XXY Klinefelter's syndrome patients. Hum Reprod Update, 9: 319-330

Denschlag M et al (2004). Assisted reproductive techniques in patients with Klinefelter syndrome: a critical review. Fertil Steril, 82: 775-779

Harper JC et al (2008). ESHRE PGD consortium data collection VII: cycles from January to December 2004 with pregnancy follow-up to October 2005.Hum Reprod, 23:741-755

Discussion of data

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Turner syndrome and deletions in the X chromosome: genetic variability

Paul N Scriven PhD

Cytogenetics Department

Guy's Hospital, London

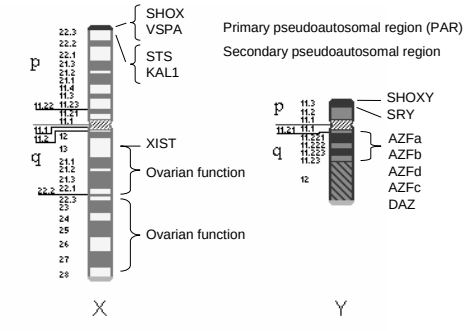
Conflict of interest

No commercial relationships or other activities

Learning objectives

- The clinical features associated with 45,X Turner syndrome and their aetiology
- The genetics of significant regions and genes on the sex chromosomes associated with the Turner syndrome phenotype and its variations
- The clinical significance of mosaic Turner karyotypes
- The clinical significance of X,abn(X) Turner variant karyotypes

Sex chromosome regions



Sex Chromosome Genetics

- The normal male (46,XY) has only one X chromosome in every cell

- The normal female (46,XX) has two X chromosomes in every cell

- Approximately two weeks after conception one X chromosome in every cell of female conceptuses is randomly genetically inactivated (lyonization); normal females can have skewing of the maternal or paternal X up to 9:1 and the proportion may be different in different tissues
- Descendant cells have the same active or inactive X chromosome

X-Inactivation Centre (XIC)

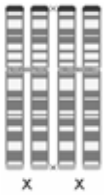
Xq13.2

- XIC contains the XIST gene (X inactive specific transcript, MIM *314670)
- XIST mRNA molecule is the primary effector, which is transcribed and acts only on the inactive X chromosome (cis-acting)
- The mechanism remains speculative

Sex Chromosome Genetics

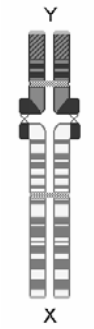
- Many genes on the inactivated X chromosome are not transcribed ("silenced") resulting in functional monosomy and dosage compensation between normal males and females
- Not all genes are inactivated; the pseudoautosomal regions (PAR1 and PAR2) and approximately 15% of genes outside these regions escape inactivation (predominantly in the short arm)
- PAR1 and PAR2 and some genes outside these regions have homologous regions or genes on the Y chromosome and functional disomy in normal females and males

Primary Pseudoautosomal Region (PAR)



Xpter – p22.3

- Genes in the PAR1 region (terminal 2.6 megabases) escape inactivation and have functional homologues on the Y chromosome short arm
- Synapse and recombination between Xp and Yp PARs in male meiosis forms the sex vesicle
- Absence of pairing along most of the X-Y bivalent may make it vulnerable during disjunction – the paternal X is absent in 75% of cases of Turner syndrome



Xp22.3

Haploinsufficiency (only one copy where two are usual) of genes with functional disomy is thought to be the basis of the abnormal clinical features of Turner syndrome

- *SHOX*– Short stature homeo box (MIM *312865), pseudoautosomal gene causing short stature in Turner syndrome and likely to at least in part associated with the other skeletal defects
- *VSPA* - Visuospatial/perceptual abilities (MIM %313000), Turner syndrome-associated neurocognitive phenotype, which is associated with paternal or maternal inherited deletions within PAR1 and with complete or incomplete skewing of X inactivation

Premature Ovarian Failure

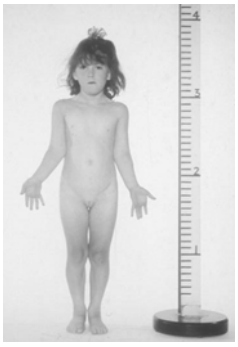
Secondary amenorrhea with elevated gonadotrophins occurring before age 40 years.

Usually idiopathic and due to depletion of ova. Turner variant genetic disorders are associated with rapid atresia of follicles resulting in premature ovarian failure

- Xq21 - *FLJ22792* (OMIM #300604), premature ovarian failure 2B
- Xq22 - *POF2* (OMIM #300511), premature ovarian failure
- Xq26-q28 – *POF1* (OMIM #311360), Premature ovarian failure

Turner Syndrome Historical Context

- "Sexagen dwarfism" (Rossle, 1922), an association between short stature and defective ovarian development
- Turner syndrome (Turner, 1938), short stature, sexual infantilism, webbed neck and cubitus valgus (deviation of the extended forearm to the outer side of the axis of the limb)
- Association of Turner syndrome with monosomy for the X chromosome (45,X) (Ford et al. 1959)



Turner Syndrome Karyotypes

• Monosomy X	45,X	46%
• Isochromosome Xq	45,X/46,X,i(Xq), 46,X,i(Xq)	18%
• Ring X	45,X/46,X,r(X)	16%
• X mosaicism	X/XX, X/XXX, X/XX/XXX	7%
• Y abnormality	X/XY	6%
• Xp deletion	45,X/46,X,del(Xp), 46,X,del(Xp)	5%
• Other		2%

Monosomy X (45,X)

High *in utero* lethality: ~0.5% of conceptions reach term; 75% of cases detected at amniocentesis (16 weeks gestation) spontaneously abort. Live born 45,X (1 in 2000 phenotypic females) is associated with Turner syndrome:

- Short stature (147cm ± 7cm; 4 feet 10 inches ± 2¾ inches)
- Gonadal failure with infertility
- IQ in the normal range but reduced compared to siblings
- Physical defects – micrognathia, cubitus valgus, short neck, high arched palate, neck webbing, lymphoedema, coarctation (narrowing) of the aorta
- Increased adult morbidity and risk of common diseases - obesity, hypothyroidism, diabetes mellitus, heart disease, hypertension, stroke, liver cirrhosis, osteoporosis; cardiovascular disease is the most common cause of death in adults (50%)

X Mosaicism

1. Female phenotype with clinical features of Turner syndrome: 45,X/46,XX; 45,X,46,XX,47,XXX; 45,X/47,XXX
- Ovarian function is typical but premature failure is common
- Increased risk (up to 15%) of 45,X offspring
- An increased risk of aneuploidy for other chromosome or structural malformations has not been established
2. Low-level 45,X/46,XX mosaicism (less than 10%) in females with a normal phenotype is likely to have no reproductive risk. Loss of an X chromosome is a normal finding and age-related

45,X/46,XY Mosaicism

Female phenotype with clinical features of Turner syndrome. The presence of Y chromosome material is associated with gonadoblastoma in dysgenetic gonads

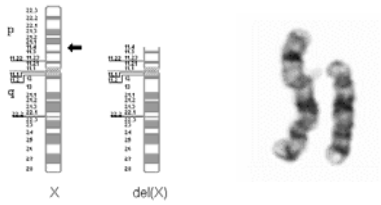
- The risk increases with age; 2% at 10 years and 28% at 30 years
- Malignant transformation occurs in 60%
- Early prophylactic removal of the dysgentic gonads is recommended

46,X,abn(X) Turner Variants

46,X,abn(X) karyotypes: partial terminal deltion of Xp, isochromosome Xq, ring X (partial deletion of Xp and Xq), X;Y translocations

- Female phenotype with some clinical features of Turner syndrome
- Normal secondary sexual development and fertility is typical
- Premature ovarian failure is likely
- Partial X chromosome deletion is typical and associated with phenotypes that range from minor menstrual perturbation through to Turner syndrome

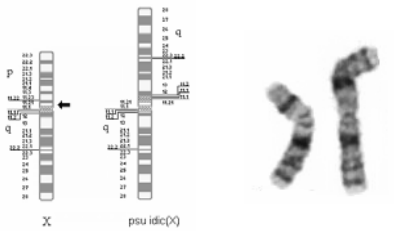
Xp Deletion



46,X,del(X)(p11.4)

Referred with short stature and difficulty conceiving, age 33 years

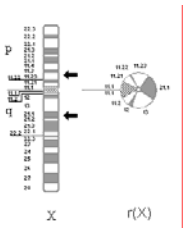
Isochromosome Xq



45,X[30]/46,X,psu idic(X)(p11.21)dn[5]

Referred with short stature, age 15 years

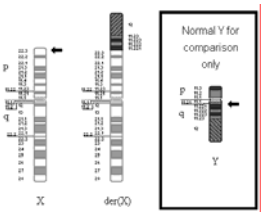
Ring X Chromosome



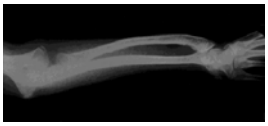
45,X[48]/46,X,r(X)(p11.23q21.1)[12]

Referred with infertility, age 26 years

Classical X-Y Translocation



46,X,der(X)t(X;Y)(p22.3;q11 or q12)

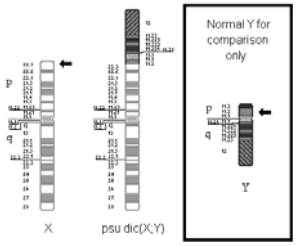


Unbalanced translocations that result in a terminal deletion of the short arm of the X chromosome (including PAR1)

- Females are typically fertile with normal intelligence
- Phenotype depends on which genes are haploinsufficient

SHOX – short stature, wrist abnormality

X-Y Translocation Variant



46,X,psu dic(X;Y)(p22.3;p11.2)

.ish psu dic(X;Y)(SHOX-,STS+,DXZI+,SRY-,DYZ3+,DYZI+)

Referred with short stature, age 7 years

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Y gene mutations interfering with gametogenesis

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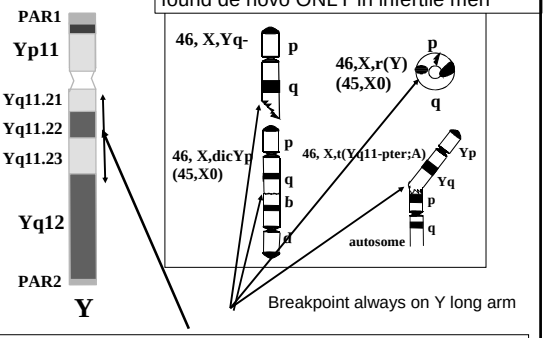
Learning objectives

1. AZoospermia Factor (AZF) dysfunction in Yq11 causes male infertility
2. AZF chromatin regions are involved in pre-meiotic X-Y pairing
3. AZF can be disrupted by 3 distinct microdeletions, AZFa, AZFb, AZFc
4. Y-genes with a role in spermatogenesis are concentrated in AZF
5. AZF-Y genes interfering with male fertility: how to analyse ?

1. AZoospermia Factor (AZF) dysfunction in Yq11 causes male infertility:

cytogenetic analyses of Y abnormalities found de novo ONLY in infertile men

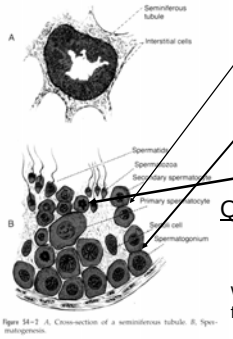


Breakpoint always on Y long arm

AZoospermia Factor in Yq11: AZF (Tiepolo & Zuffardi, 1976)

1. AZoospermia Factor (AZF) dysfunction in Yq11 causes male infertility:

based on the testicular pathologies oberved in infertile patients with Yq11 aberrations it was concluded that AZF is functional at *different phases* of the male germ cell development (Vogt et al. 1993).



1. X-Y chromatin function during pairing process at meiosis
2. proliferation and differentiation of spermatogonia
3. maturation of spermatids and spermatozoa

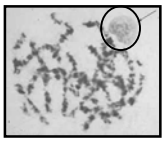
Questions ?

Is there an AZF *chromatin function* active during the premeiotic X-Y pairing process ?

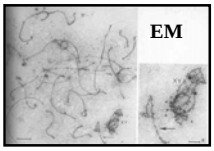
Which are the AZF-Y genes that encode proteins functional during the indicated germ line phases?

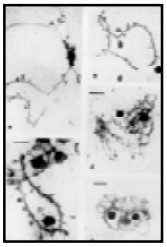
2. AZF chromatin regions are involved in pre-meiotic X-Y pairing:

by a germ line extending chromatin structure

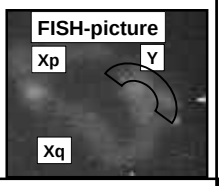


At meiotic pachytene homologous X and Y domains are forming a pairing complex called „ *sex vesicle*“ !





This pairing process starts at leptotene and is dynamic !

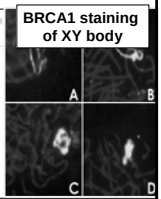


FISH painting of X and Y chromosome shows bending of X to Y at pachytene

2. AZF chromatin regions are involved in pre- meiotic X-Y pairing:

Significant impairment of X-Y centromere and telomere pairing in two cases of infertile men with AZFb-c deletions
(Yogev et al. 2004; J. Androl. 25:110-116)

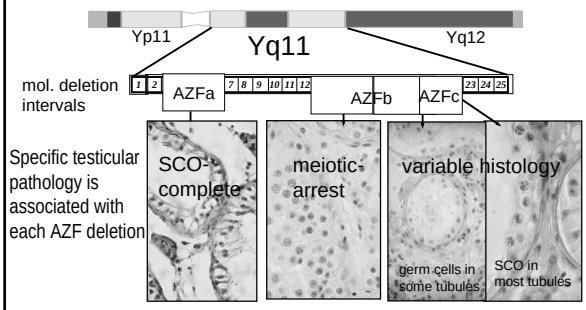
Specimen no.	Y chromosome	Bivalent 16 (centromeres)	Bivalent X-Y (centromeres)	Paired p telomeres	Paired q telomeres	All telomeres in proximity
1	AZFc del	99	86	98	87	94
2	AZFc del	81	57	84	64	70
3	AZFc del	94	56	81	62	74
7	AZFb-c del	87	29	51	24	29
8	AZFb-c del	81	28	—	—	—
9	Normal	99	90	97	92	95
10	Normal	99	88	96	86	96
11	Normal	99	90	98	90	96



AZFc deletions seems to be *not critical* for meiotic X-Y pairing although the leptotene & zygotene stage is extended and chromosome condensation is reduced (see on the right: BRCA1 staining)
(Geoffroy-Siraudin et al. 2007; Hum. Reprod. 6: 1567-1572)

X-Y chromatin pairing sites are concentrated in AZFb.

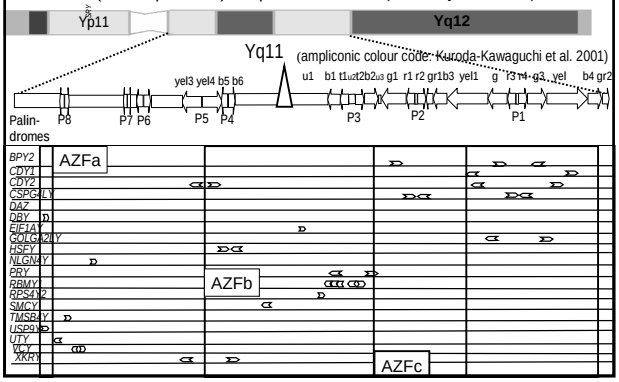
3. AZF can be disrupted by 3 distinct microdeletions: AZFa, AZFb, AZFc



Major conclusion:
at least 3 Y genes functional in human spermatogenesis must be located in AZF locus (called „AZF-Y genes“)

4. Y-genes with role in spermatogenesis are concentrated in AZF:

14 AZF-Y genes based on knowledge of Y chromosomal MSY (Male-Specific-Y) sequence structure (Skaletsky et al. 2003)



4. Y-genes with role in spermatogenesis are concentrated in AZF

What is known about their function in germ cells ?

- expression studies of AZF Y-gene transcripts (RNA) display *restriction to testis tissue* (Skaletsky et al. 2003)

  but not for: *USP9Y, DDX3Y, SMCY, EIF1AY*

- expression profiles of transcripts in testicular tissue studied for 7 AZF Y-genes display *germ cell dependence* for 5 AZF genes (Kleiman et al. 2006)

  DDX3Y-t, *PRY*, *RBMY*, *DAZ*, *CDY1*

- IHC-localisation studies of AZF proteins in male germ cells were performed for 8 AZF genes:

  USP9Y, DBY, HSFY, PRY, RBMY, BPY2, CDY1/2, DAZ

5. AZF-Y genes interfering with male fertility: how to analyse ?

For a functional understanding of each of the encoded AZF proteins in human spermatogenesis four major research lines are helpful:

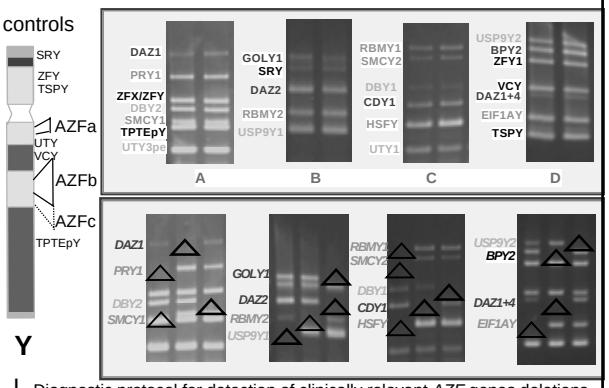
I. Diagnostic detection of *AZF* gene deletions in men with non-obstructive azoospermia or severe oligozoospermia

II. Localisation of AZF proteins in male germ cells of men with *normal* spermatogenesis

III. Localisation of AZF proteins in male germ cells of men with distinct disruption of spermatogenesis

IV. Functional studies of Y genes in model animals which encode proteins homologous to the AZF proteins

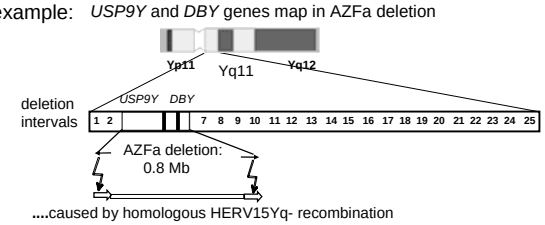
5. AZF-Y genes interfering with male fertility: how to analyse ?



I. Diagnostic protocol for detection of clinically relevant *AZF* genes deletions

5. AZFa-Y genes interfering with male fertility: how to analyse?

example: *USP9Y* and *DBY* genes map in AZFa deletion



....caused by homologous HERV15Yq- recombination

High deletion frequency of the AZFa sequence in men with complete Sertoli-cell-only (SCO) syndrome !

Kamp et al. in Mol. Hum. Reprod. vol. 7, 987-994 (2001)

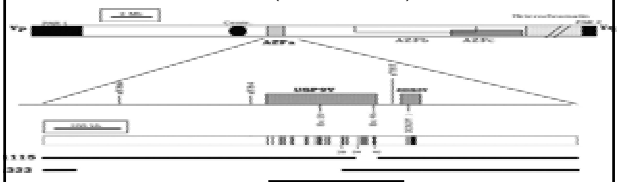
Which Y gene in AZFa causes SCO ?

5. AZFa-Y genes interfering with male fertility: how to analyse?

pathological phenotypes of AZFa Y-gene deletions

USP9Y deletions and point mutations cause:

- postmeiotic disruption of spermatogenesis (Qureshi et al. 1996;Sun et al.1999)
- and can be *natural* transmitted (Krausz et al., 2006)

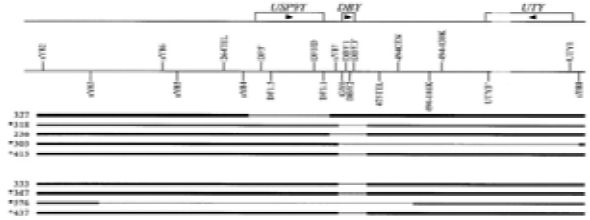


II. Immunohistochemical localisation of USP9Y proteins in human testicular tissue sections with normal spermatogenesis points to functional expression in spermatids

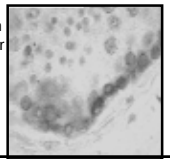
USP9Y dysfunction no significant influence on postmeiotic sperm maturation ?

5. AZFa-Y genes interfering with male fertility: how to analyse?

DDX3Y gene deletions cause a variable testicular pathology including the SCO-syndrome (Foresta et al. 1998, 2000)



II. Immunohistochemical localisation of DDX3Y protein in human testicular tissue with normal spermatogenesis points to function in spermatogonia

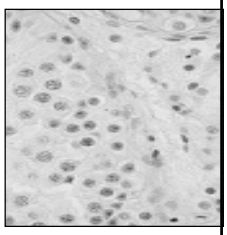


If DDX3Y deletion results in loss of spermatogonia, it would the major AZFa gene !

5. AZFb-Y genes interfering with male fertility: how to analyse ?

Major question:

Is meiotic arrest associated with dysfunction of any AZFb Ygene or is disruption of X-Y chromatin pairing *sufficient* to produce this pathology?



AZFb genes expressed in male germ cells are multicopy genes !

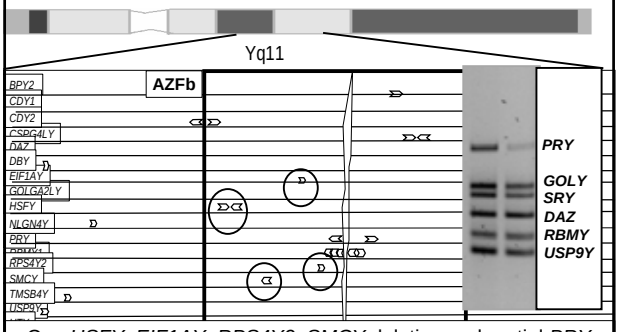
- 1 *CDY2* copy
- 2 *HSFY* copies
- 2 *PRY* copies
- 15-20 *RBMY* copies

- 1 *CDY1* copy
- 1 *BPY2* copy
- 2 *DAZ* copies

these are *also* AZFc genes

5. AZFb-Y genes interfering with male fertility: how to analyse?

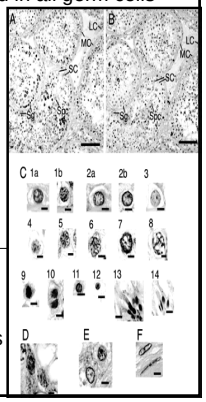
I. Diagnostic protocol for AZFb gene deletion identifies deletion of *HSFY,EIF1AY RPS4Y2, SMCY* and partial *PRY in* patient with complete teratozoospermia



Can *HSFY, EIF1AY, RPS4Y2, SMCY* deletion and partial *PRY deletion* cause teratozoospermia ?

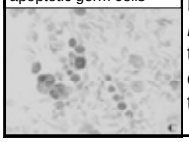
5. AZFb-Y genes interfering with male fertility: how to analyse?

HSFY protein is expressed in Sertoli cells and in all germ cells (Shinka et al. 2004; Biol. Reprod. 71, 297-306)



PRY protein is expressed in late spermatids and sperms (Stouffs et al. 2004; Mol. Hum. Reprod. 10, 15-21)

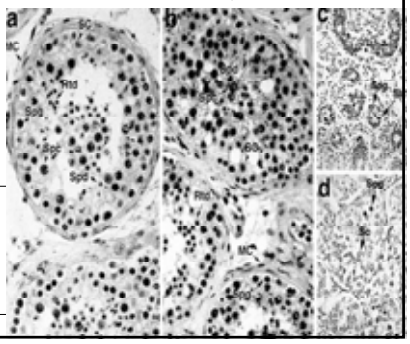
increased PRY expression in apoptotic germ cells



Loss of one functional *PRY* and /or loss of the two *HSFY* genes can contribute to the patient´s teratozoospermia

5. AZFb-Y genes interfering with male fertility: how to analyse?

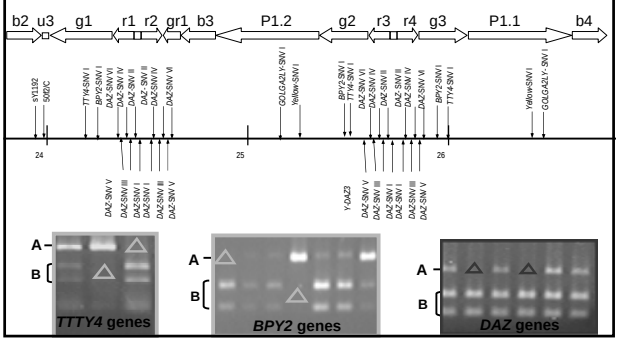
RBMY protein is involved in the nuclear metabolism of many newly synthesized transcripts in fetal, pepuberal and adult spermatogonia (Elliott et al. 1997; PNAS 94: 3848-3853)



RBMY gene family when causing meiotic arrest after deletion would be the major AZFb genes

5. AZFc-Y genes interfering with male fertility: how to analyse?

AZFc-Y genes are multicopy genes: deletion pattern of associated STS/SNV markers can identify *Which* of the gene copies is deleted



5. AZFc-Y genes interfering with male fertility: how to analyse?

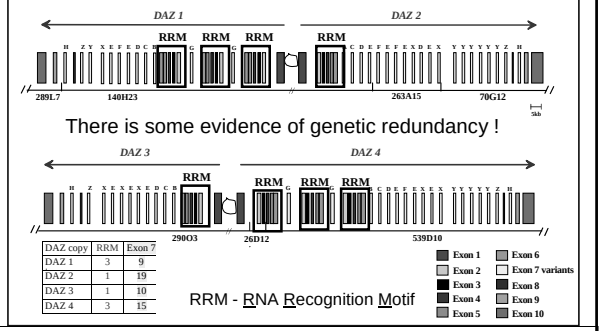
Putative *DAZ* gene deletions characterised by 13 *DAZ* haplotypes !

DAZ deletions	SNV I	SN V II	SNV III	SNV IV	SNV V	SNV VI	Y-RRM3	Y-DAZ3	DAZ haplotyp
DAZ 1	A+B	B	A+B	A+B	A+B	A+B	+	+	1
DAZ 2	A+B	A+B	B	B	A+B	A+B	+	+	2
DAZ 3	A+B	A+B	A+B	A+B	A+B	A+B	+	-	3
DAZ 4	A	A+B	A+B	A+B	A+B	A	+	+	4
DAZ 1+2	A+B	B	B	B	A	A+B	+	+	5
DAZ 1+3	A+B	B	A+B	A+B	A+B	A+B	+	-	6
DAZ 1+4	A	B	A+B	A+B	A+B	A	-	+	7
DAZ 2+3	A+B	A+B	B	B	A+B	A+B	+	-	8
DAZ 2+4	A	A+B	B	B	A+B	A	+	+	9
DAZ 3+4	A	A+B	A+B	A+B	B	A	+	-	10
DAZ 1+2+3	B	B	B	B	A	B	+	-	11
DAZ 1+2+4	A	B	B	B	A	A	-	+	12
DAZ 2+3+4	A	A	B	B	B	A	+	-	13

However, today we know that there are multiple *DAZ* gene copy variations also in fertile men probably depending on Y-lineage !

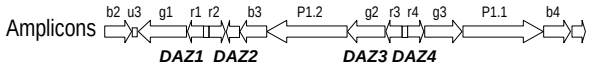
5. AZFc-Y genes interfering with male fertility: how to analyse?

DAZ has usually four gene copies in AZFc



How many *DAZ* gene copies are needed for male fertility ?

5. AZFc-Y genes interfering with male fertility: how to analyse?

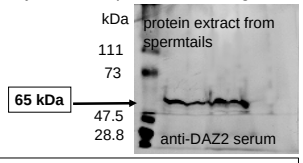


DAZ* genes structure in men from Y lineage R

Molecular weight of DAZ proteins in Y lineage R* :

DAZ1	83 kDa
DAZ2	64 kDa
DAZ3	49 kDa
DAZ4	99 kDa

⇒ IHC analysis of DAZ proteins in male germ line



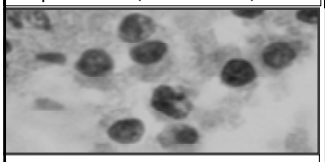
DAZ2 antiserum displays cross reaction with sperm tail protein of 65 kDa

DAZ proteins also present in testicular germ cells.

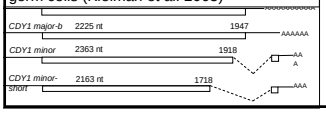
5. AZFc-Y genes interfering with male fertility: how to analyse?

However, not only DAZ proteins are expressed in male germ cells

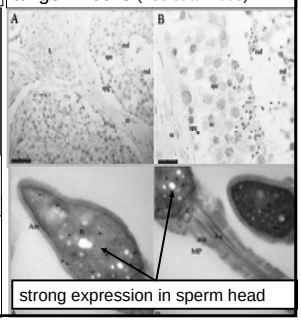
CDY protein is expressed in spermatids (Lahn et al. 2002)



Alternative spliced *CDY1 RNA* with different poly A sites probably expressed in specific germ cells (Kleiman et al. 2003)



BPY2 protein is expressed in all germ cells (Tse et al. 2003)



strong expression in sperm head

Summary

- Human Y chromosome contains X-Y genes and repetitive gene families functional in male fertility.
- Y-genes with a role in spermatogenesis are concentrated in AZF
- Major candidate for AZFa function is *DDX3Y* gene.
- Major candidate for AZFb function is *RBMY* gene
- Major candidates for AZFc function are *BPY2; DAZ; CDY genes*
- Germ line expression of the repetitive Y genes in AZFb/c might be functionally redundant.
- Mainly AZFb chromatin region is involved in X-Y pairing.

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