GENETIC CAUSES OF MALE INFERTILITY

Dijana Plaseska-Karanfilska
RCGEB "Georgi D Efremov", MASA

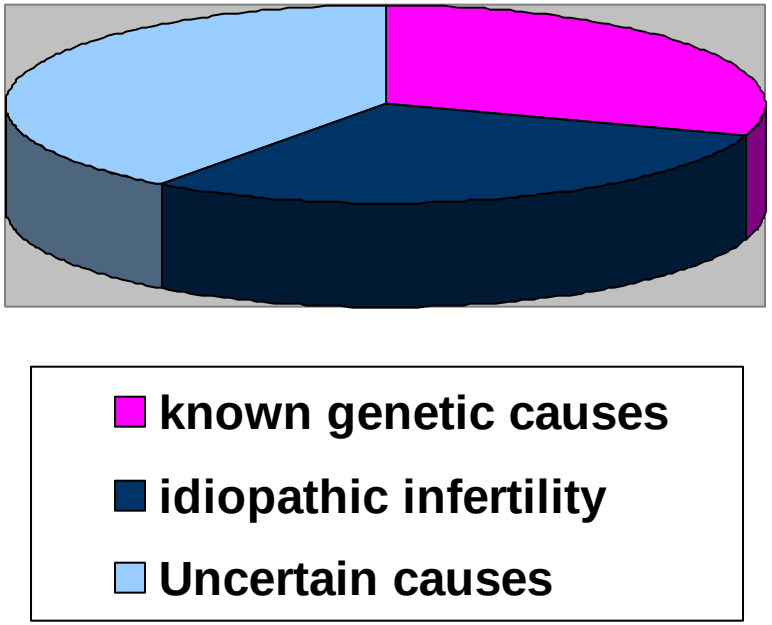
MACPROGEN Final Conference
Ohrid 29 March – 1 April 2012

Infertility

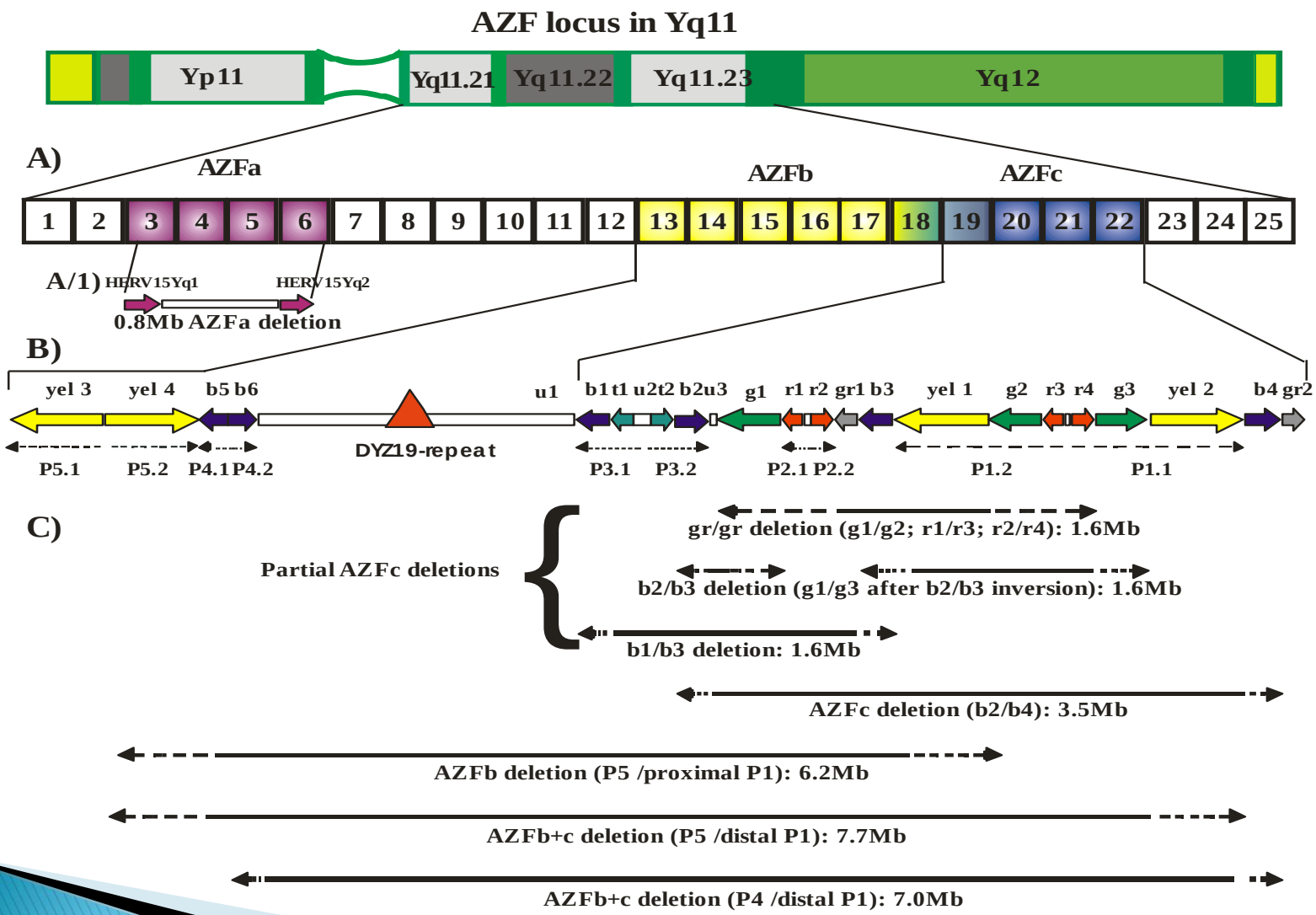
- Infertility affects 10-15% of couples world wide
- Male factor responsible for ~50% of these cases
- Spermatogenic failure (oligozoospermia, azoospermia)
- Genetic abnormalities account for ~15% of male infertility
- Natural selection prevents the transmission of mutations causing infertility
- Assisted reproduction techniques overcome this protective mechanism
- Identification of genetic causes – good practice for appropriate management of infertile couples

Genetic causes of male infertility

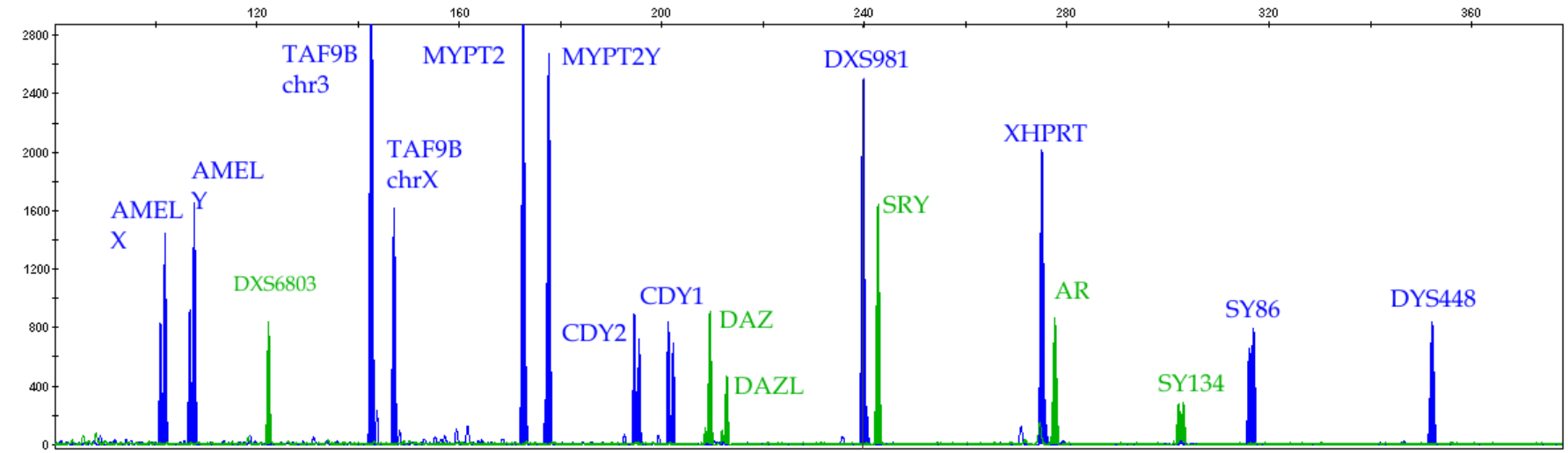
- Chromosomal abnormalities
- Y chromosome microdeletions
- CFTR mutations

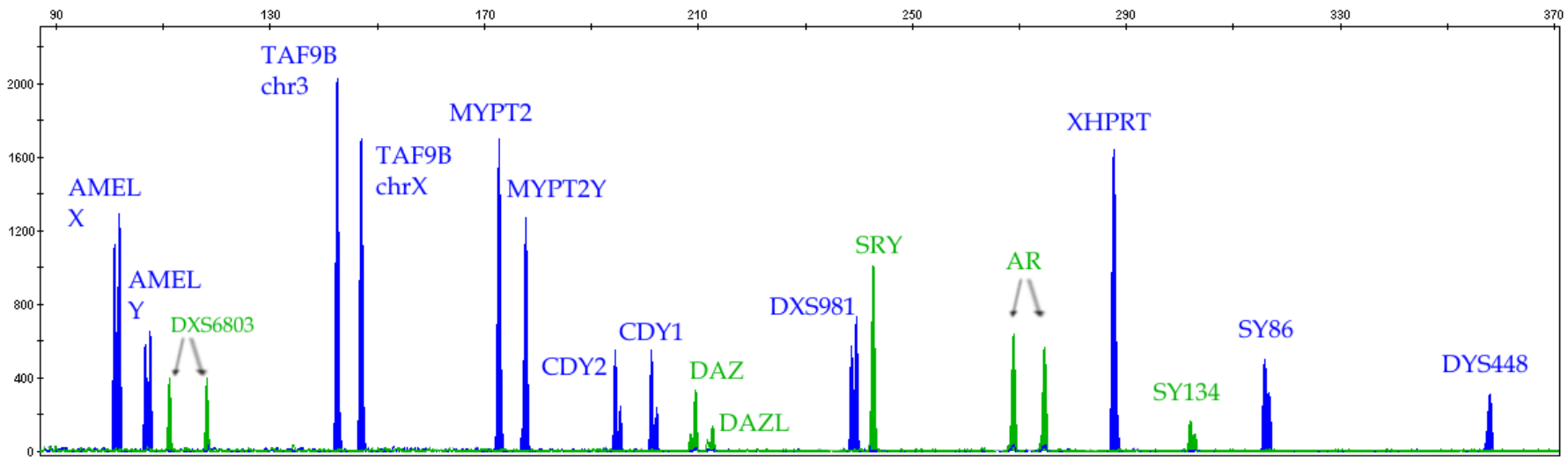


Y chromosome – AZF region

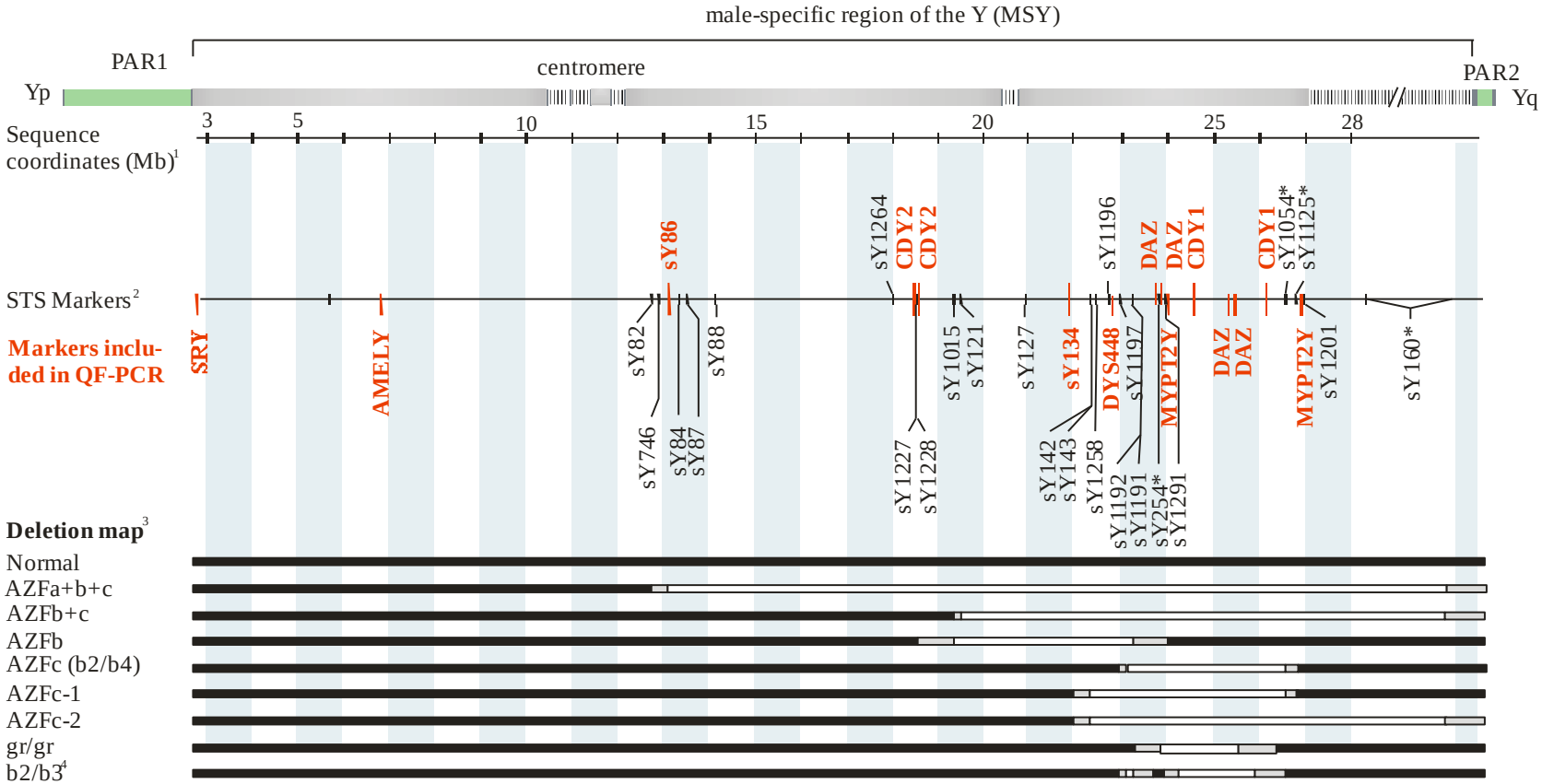


13-plex QF-PCR for the detection of the most common genetic causes of male infertility





Y chromosome markers used in the QF-PCR analysis



Notes

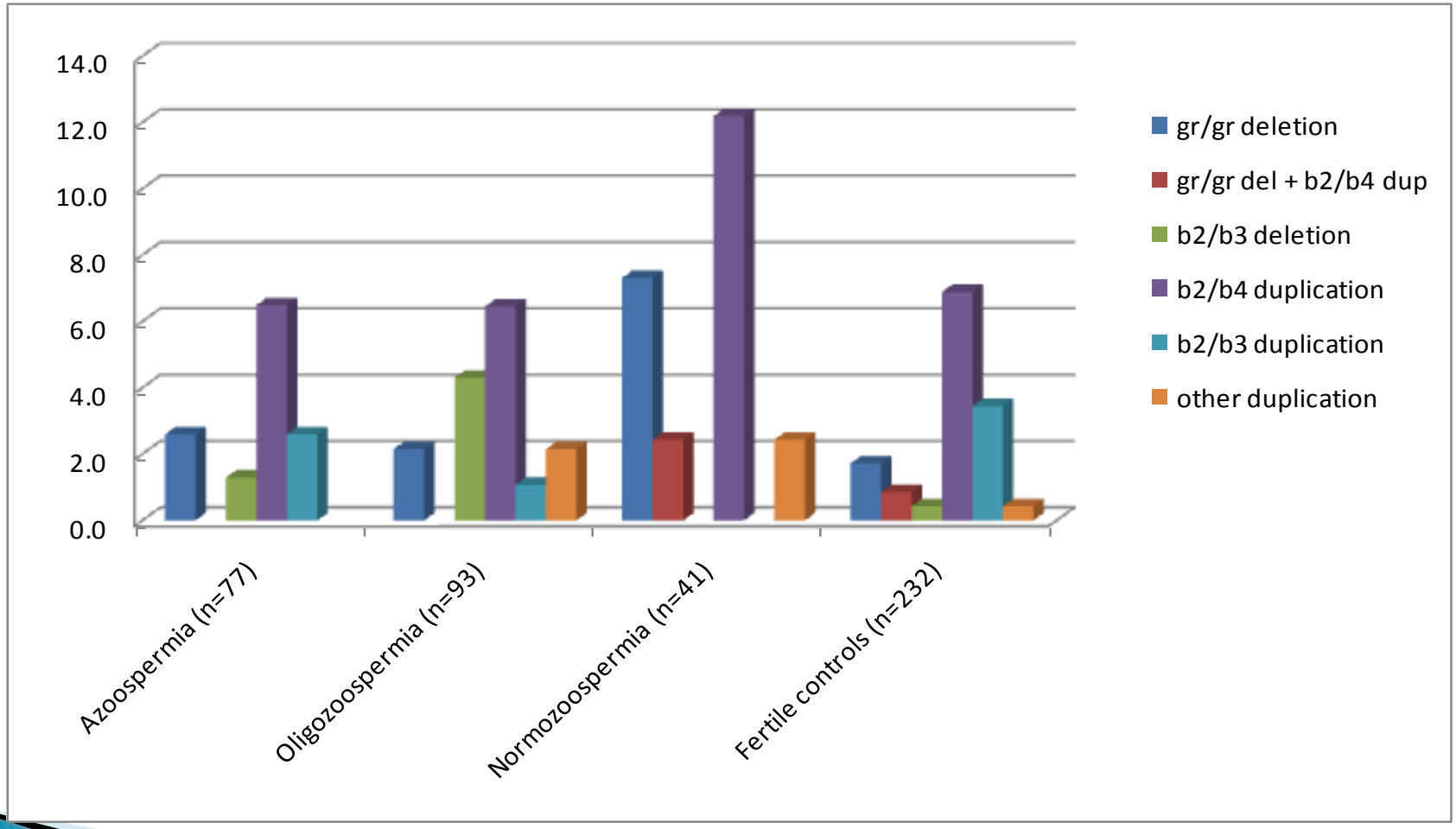
[1]NCBI MSY assembly based on Scaletsky et al, 2003

[2]Multicopy markers are designated with *

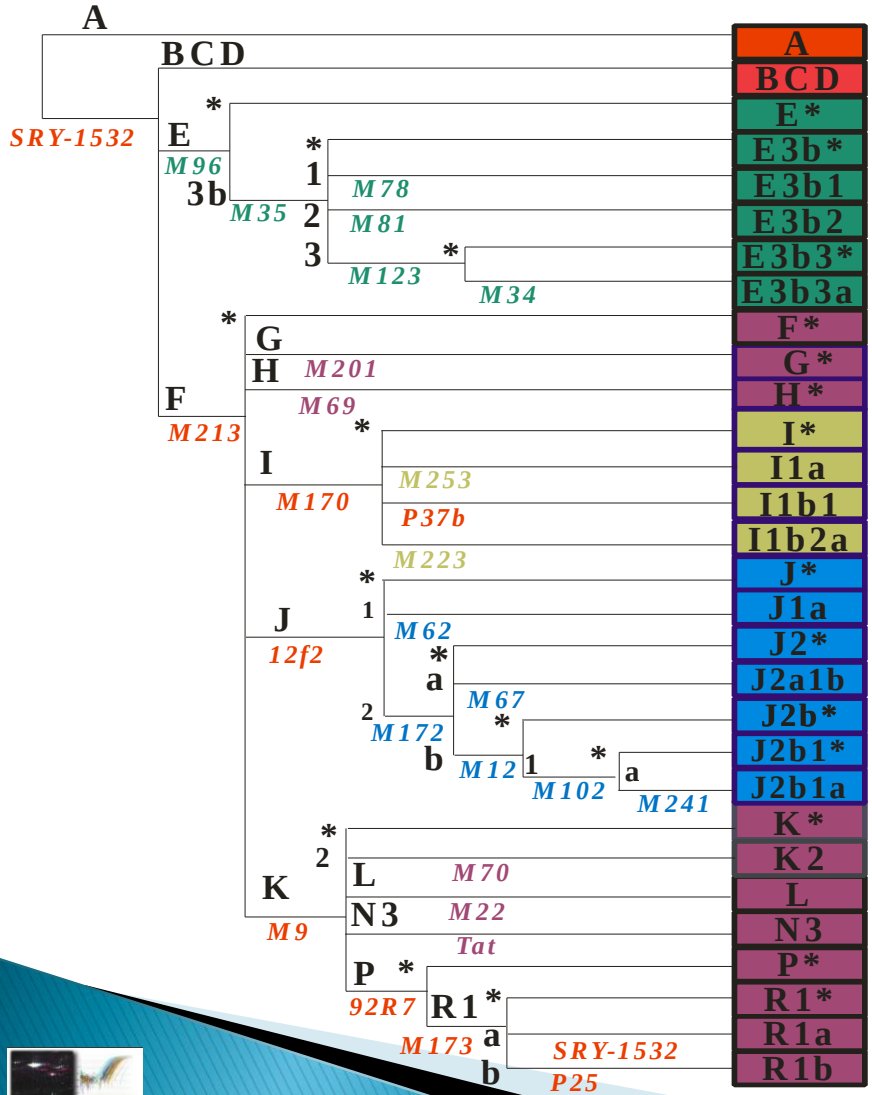
[3]For each deletion, black boxes indicate presence of STS, gray boxes indicate breakpoint intervals, and white boxes indicate deletions

[4]The b2/b3 deletion is contiguous; it appears as shown because the deletion arises on Y chromosome with an inversion in this region

Partial AZFc deletions and duplications and male infertility

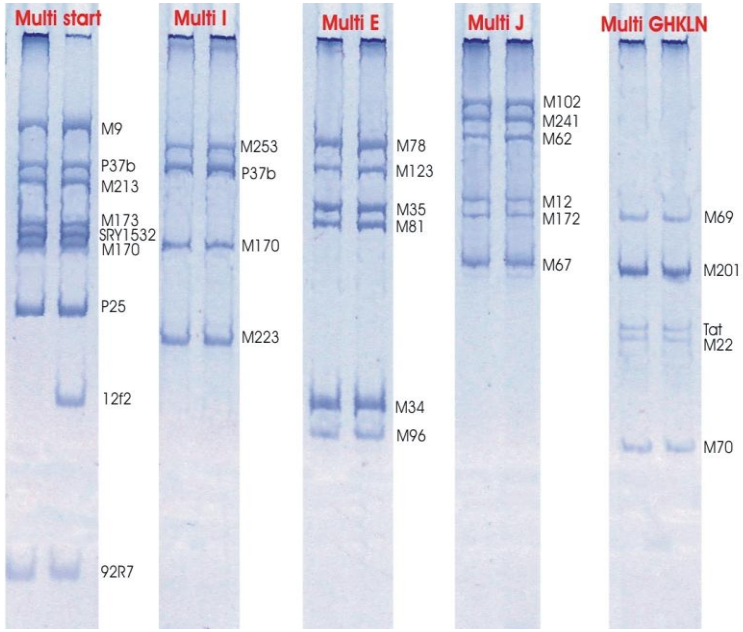


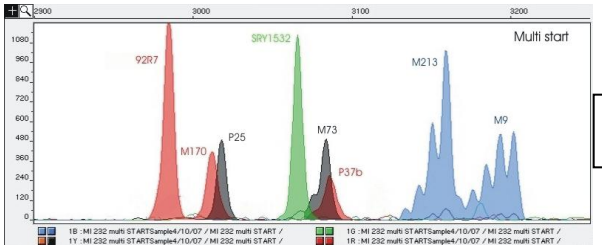
Y haplogroups and male infertility



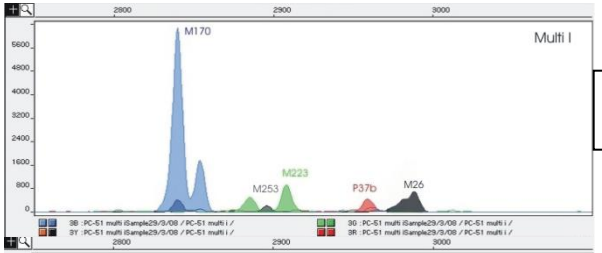
- Slowly mutating binary markers on the MSR of the Y chromosome
- Studying the origin of human populations and measuring the variability among them
- Association studies of male specific and male associated diseases
- Association of Y Hgr with infertility and Y deletions – contradictory results

Y chromosome SNP typing by SNaPshot analysis in Macedonian infertile men

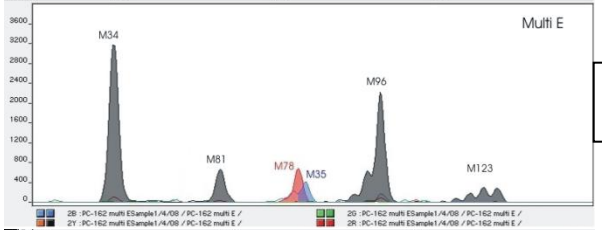




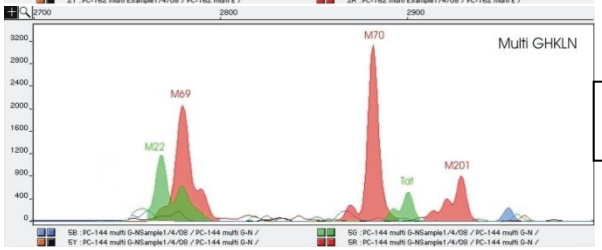
R1a-SRY1532



I1b2a-M223

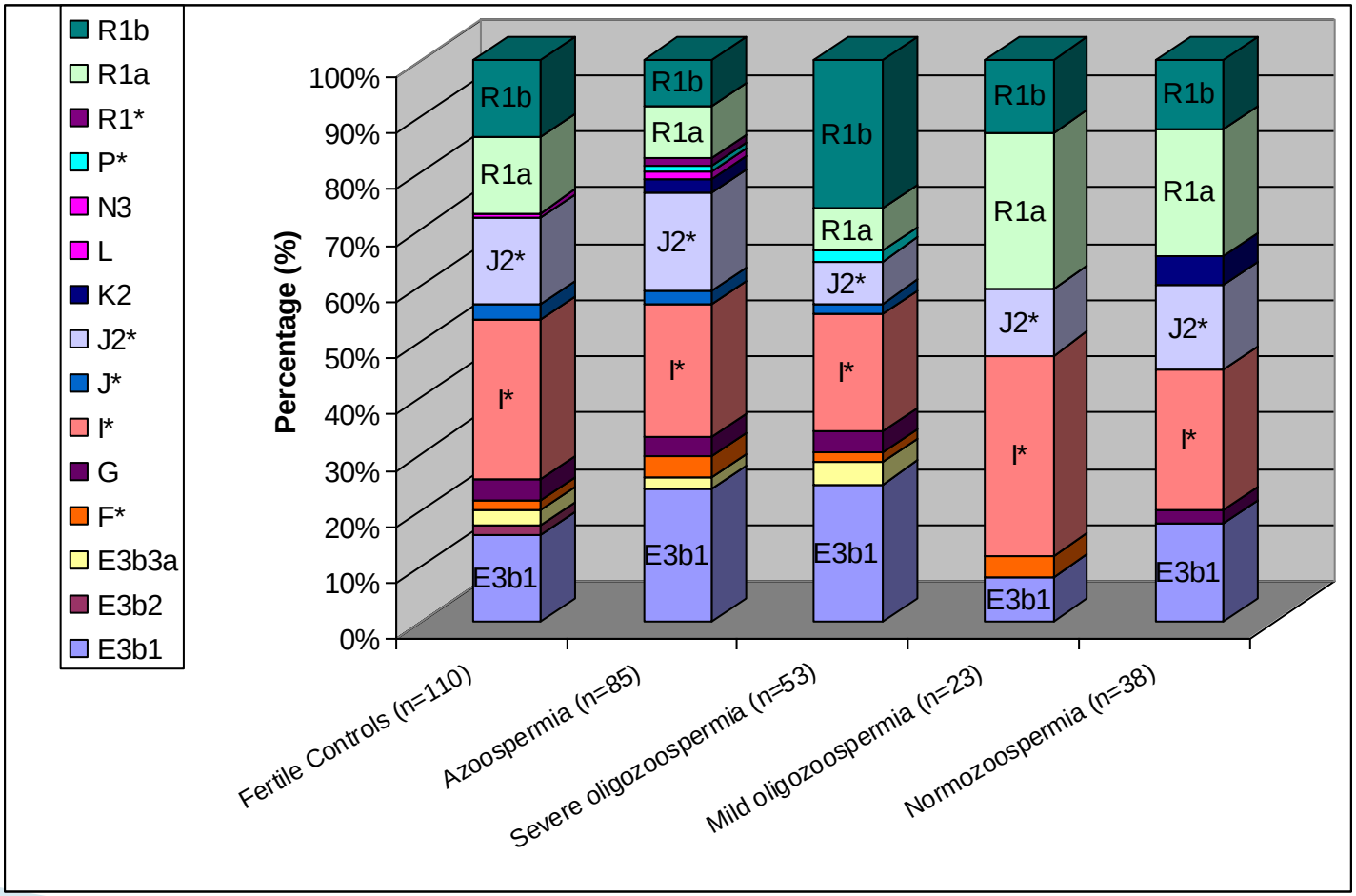


E3b1a-M78

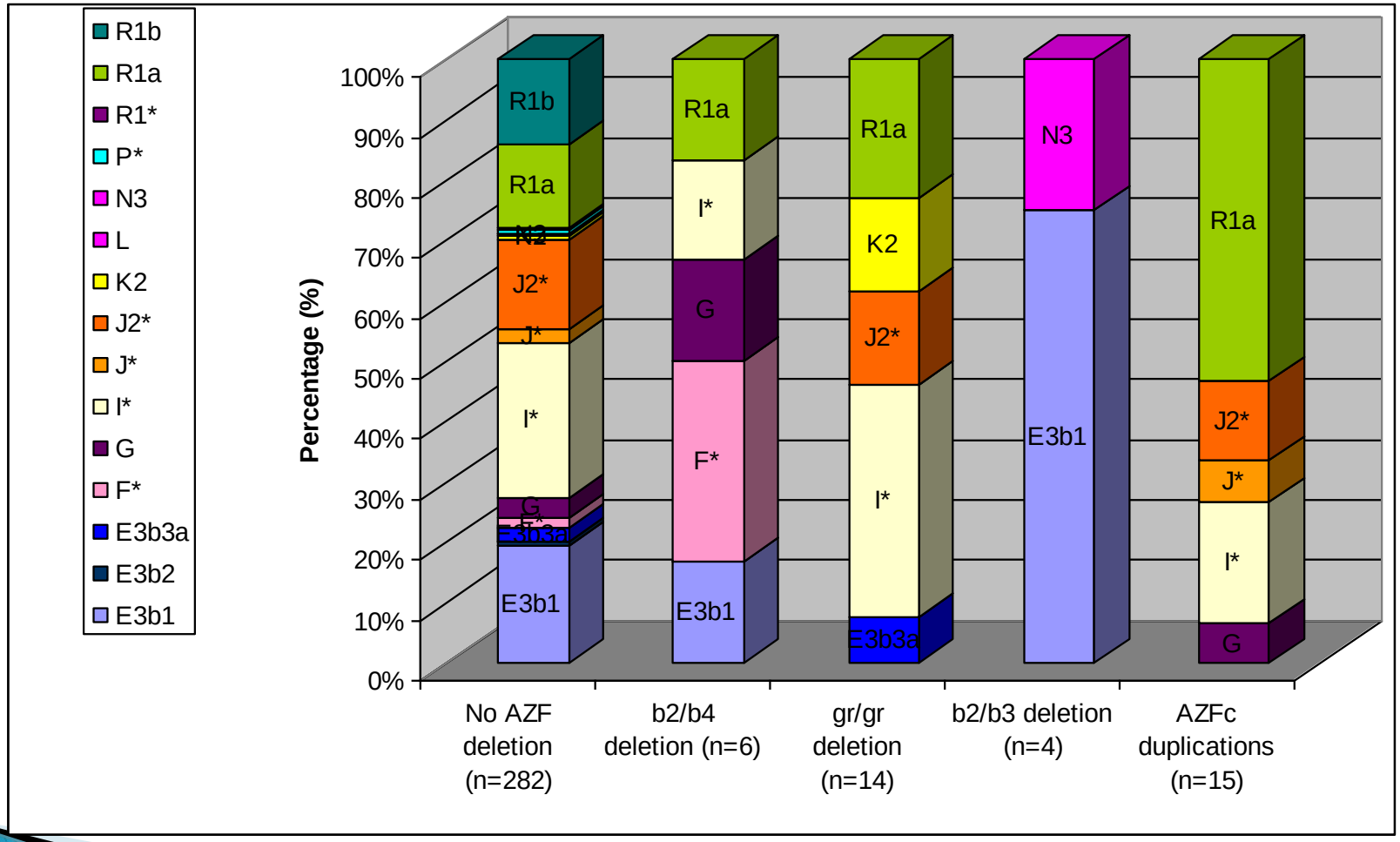


G-M201

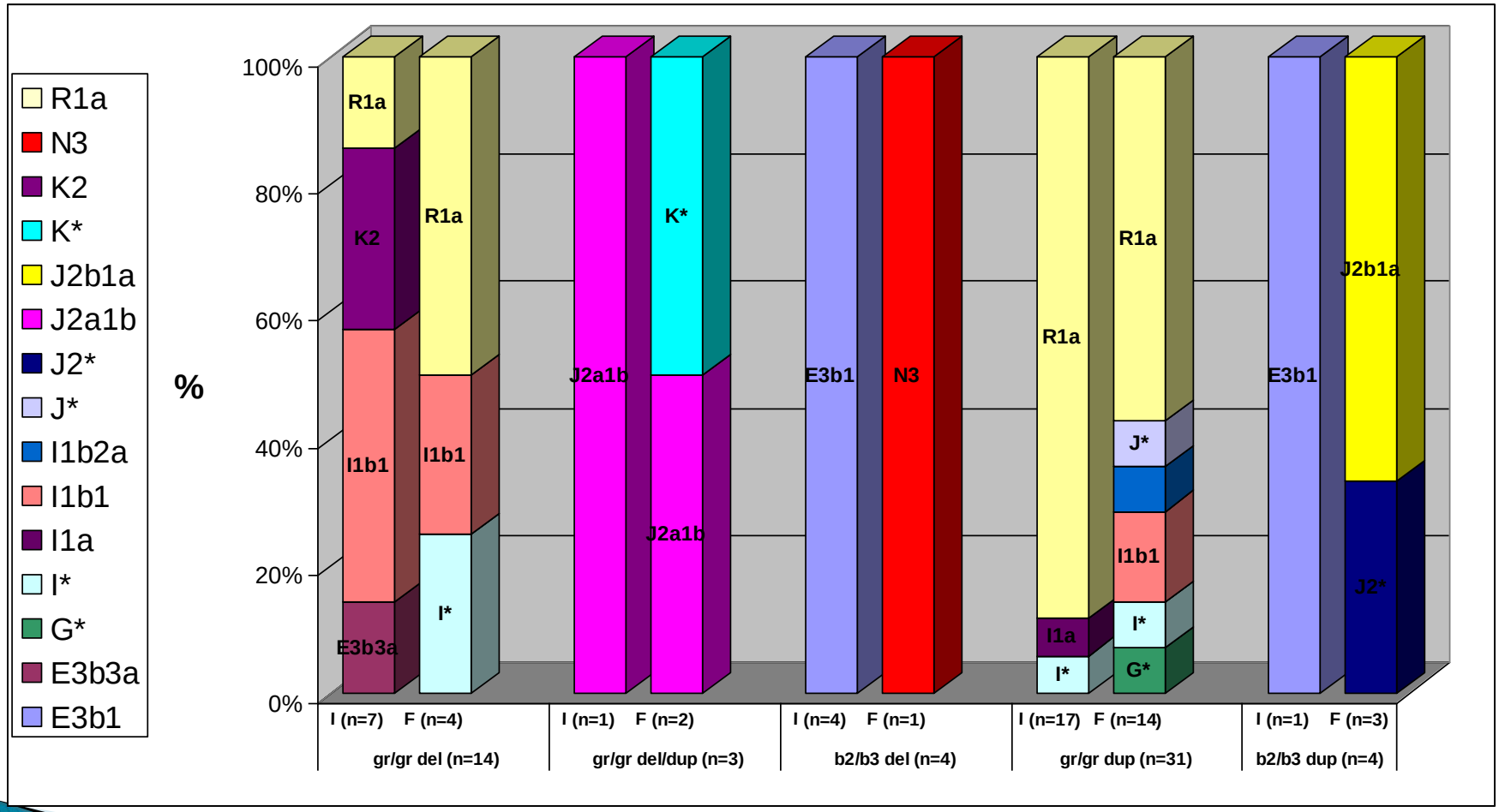
Y haplogroups in Macedonian infertile men



Y haplogroups in Macedonian men with AZF rearrangements



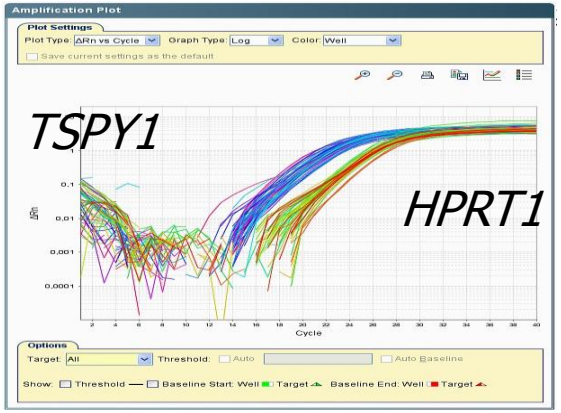
Y haplogroups in Macedonian infertile and fertile men with AZF rearrangements

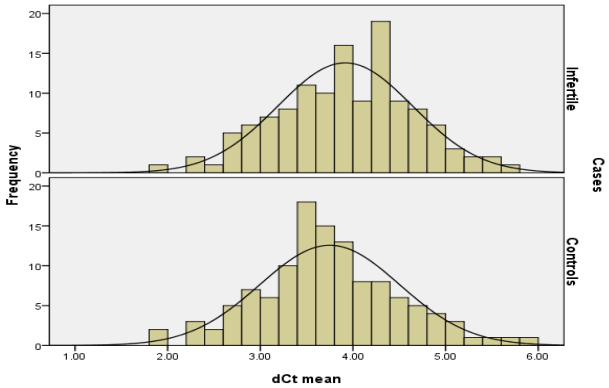


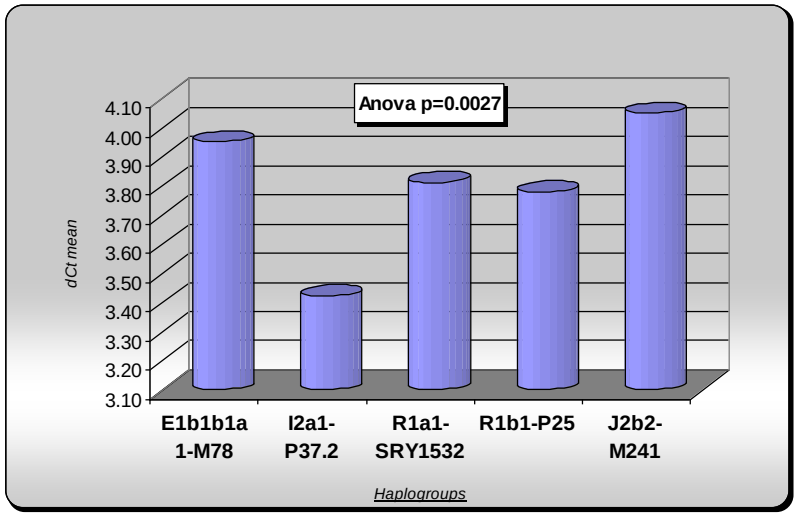
TSPY1 gene copies and male infertility

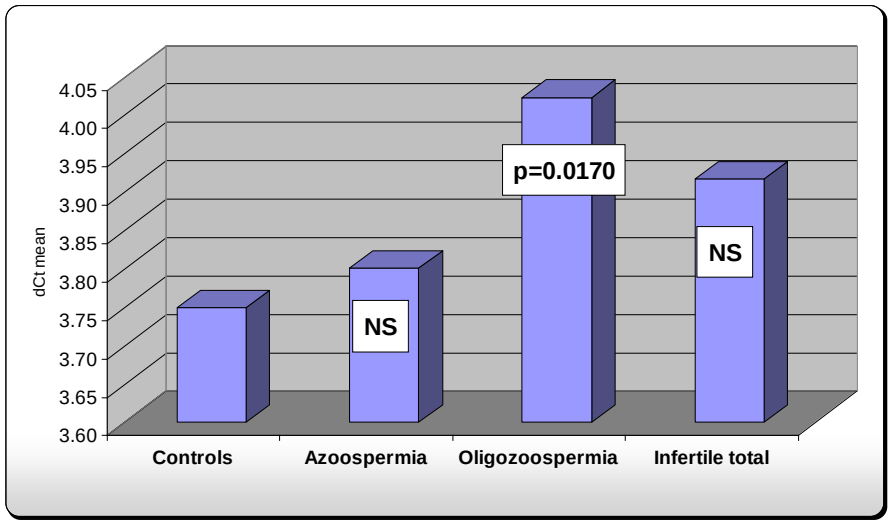
- Human testis-specific protein Y-encoded 1
- 18 to 76 copies
- in the MSY (male-specific) region of the Y chr.
- Gene with a testis-specific expression pattern (in spermatogonia and primary spermatocytes)
- Critical region that predisposes dysgenetic gonads of intersex patients to gonadoblastoma development
- *TSPY1* copy number influences spermatogenesis?
  - Contradictory results

TSPY1 gene copies and male infertility









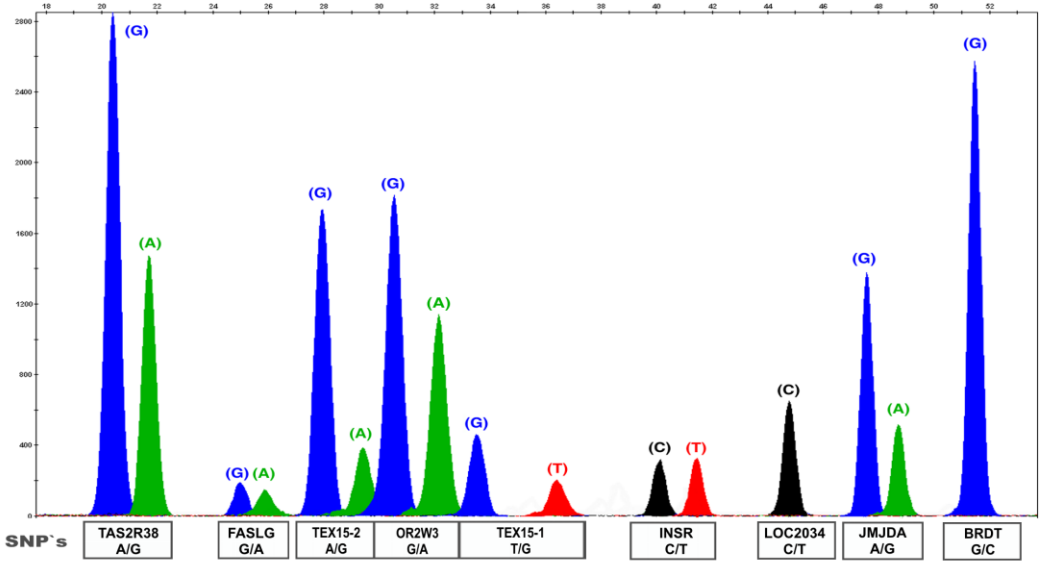
SNPs and male infertility

- SNPs in association with male infertility
  - Contradictory results
  - SNPs on specific genetic background or environmental factors can lead to spermatogenic impairment?
- SNPs studied in RCGEB
  - CAG repeat in POLG gene *(BJMG, 2007)*
  - AR CAG repeats *(BJMG, 2007)*
  - Folate-related genes: MTHFR, MTR, MTRR *(ECHG, 2009)*
  - FASLG, JMJDIA, LOC203413, TEX15, BRDT, OR2W3, INSR and TAS2R38 genes *(J Androl, 2011)*
  - SLC6A14 gene *(ECHG, 2012)*

Association study of nine SNPs in FASLG, JMJDIA, LOC203413, TEX15, BRDT, OR2W3, INSR and TAS2R38 genes with male infertility

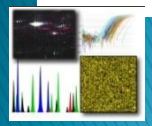
Subjects

- Infertile men (n=136)
  - Azoospermia (n=60)
  - Oligozoospermia (n=76)
- Fertile men (n=161)

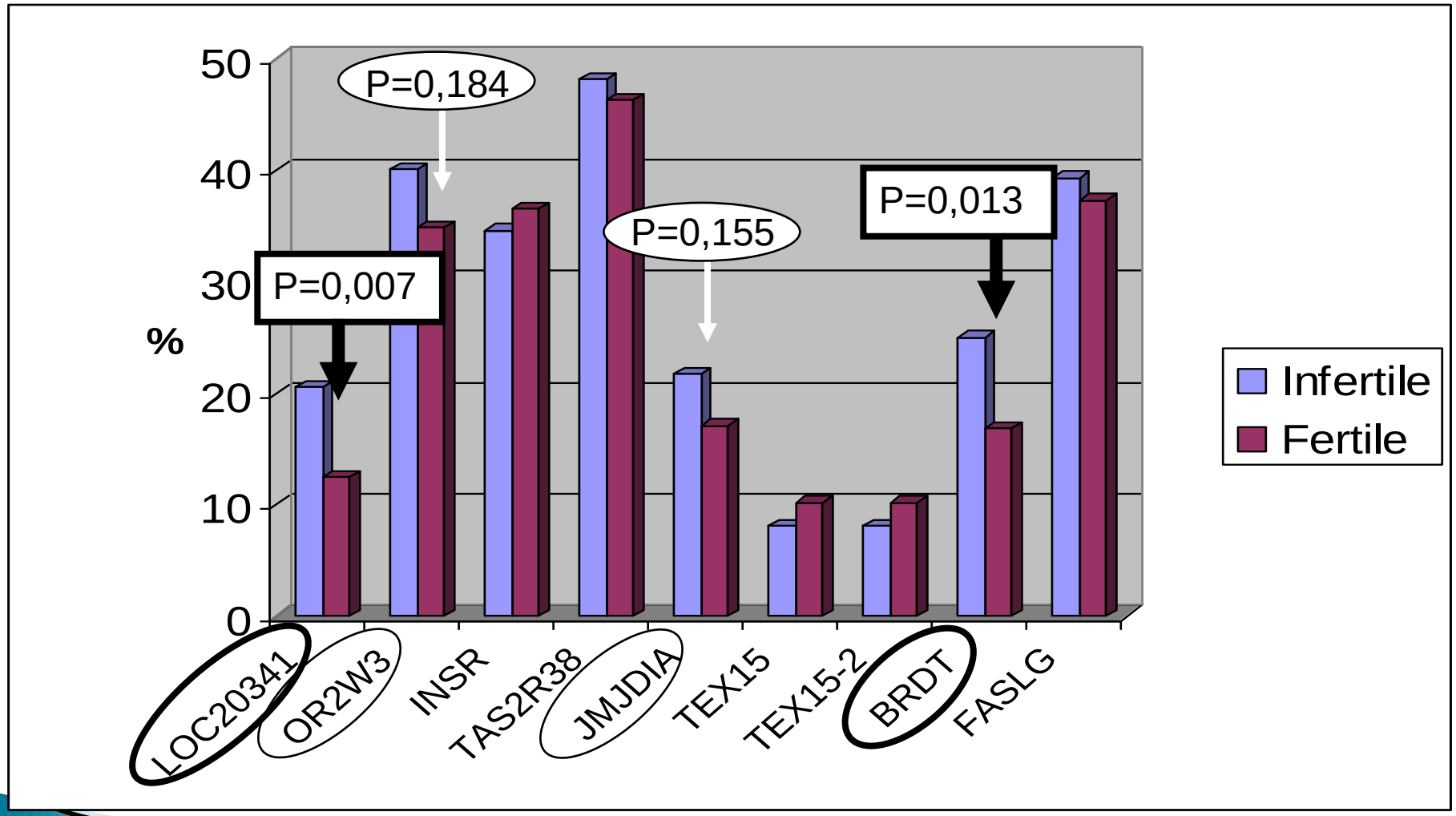


Methods

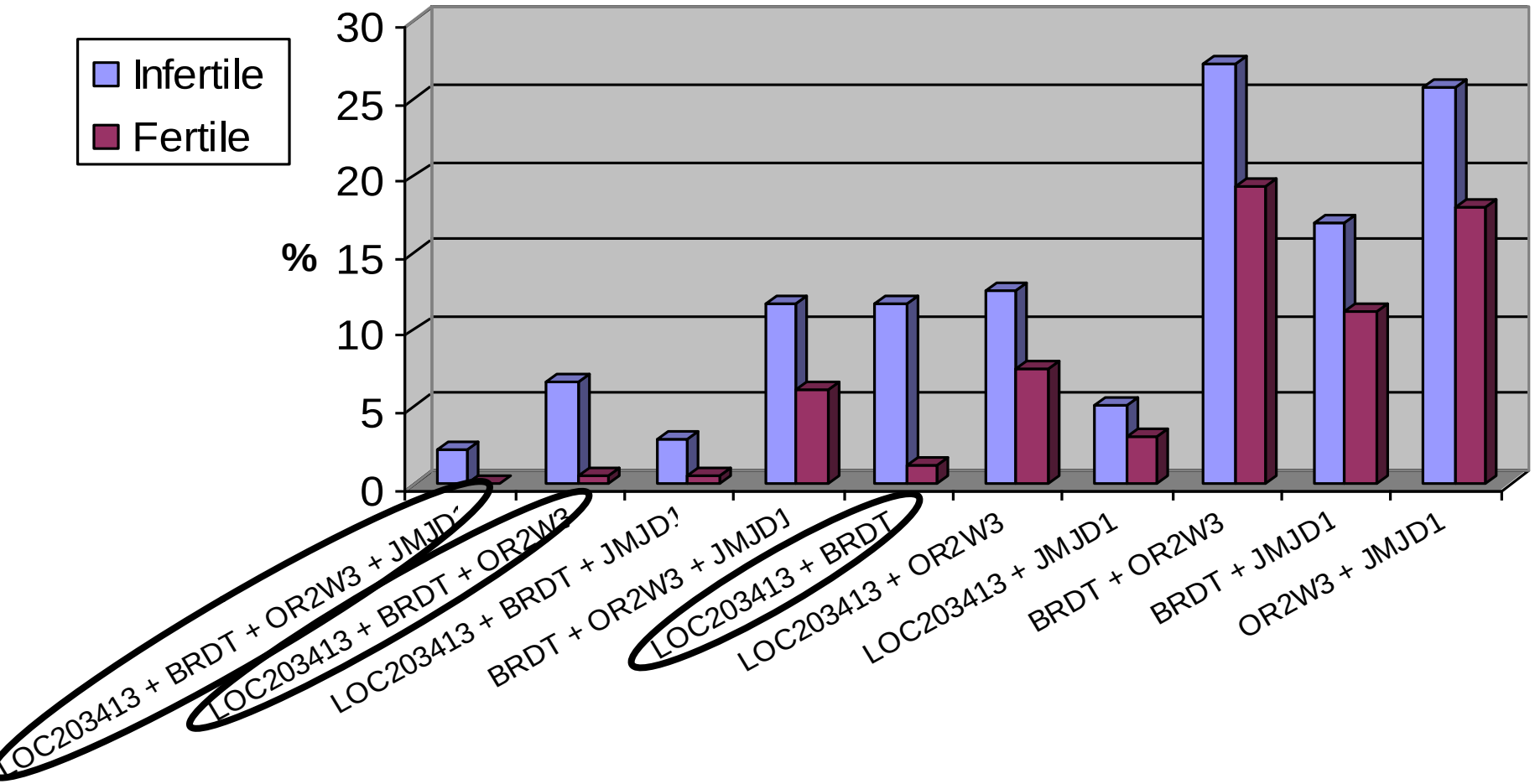
- Multiplex PCR of eight fragments
- Multiplex SNaPshot analysis od nine SNPs
- Capillary electrophoresis on ABI 3130 Genetic Analyzer



Allele frequencies of the nine SNPs among 136 infertile and 161 fertile men



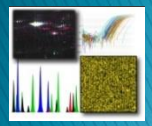
Risk for infertility is increased in individuals that carry ≥ 2 of the LOC203413, BRDT, OR2W3 and JMJD1A SNPs



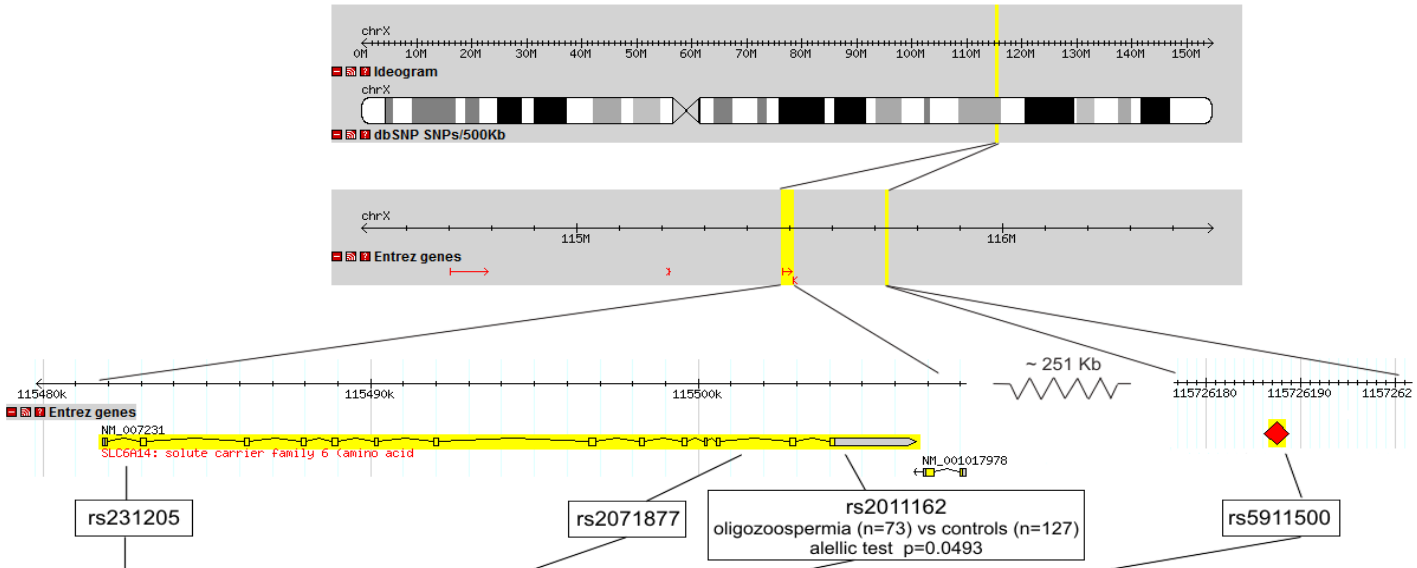
SNPs associated with male infertility

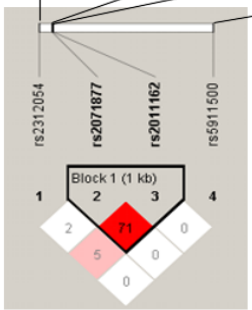
- **rs3088232 in the BRDT gene (chr 1)**
  - spermatogenesis gene involved in chromatin remodelling
  - testis-specific protein that interacts with acetylated lysine residues of histones and other proteins
  - candidate gene for disruption of spermatogenesis

- **rs5911500 in LOC203413 (X chr.)**
  - ~200kb downstream of *SLC6A14* gene that was found in association with obesity
  - obesity contributes to infertility by reducing semen quality, changing sperm proteome, affecting erectile dysfunction
  - association of *SLC6A14* SNPs with male infertility ?



Association study of SNPs in obesity gene SLC6A14 with male infertility

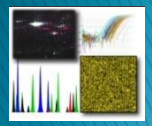




Haplotype of two SNP`s in SLC6A14 gene associated with male infertility				
rs rs2071877 (C/T)	rs rs2011162 (C/G)	Controls (n=127)	Infertile (n=123)	p value
		n (%)	n (%)	
C	G	66 (53.7)	84 (66.7)	p=0.0439
T	C	45 (36.6)	36 (28.3)	NS
C	C	12 (9.8)	7 (5.5)	NS

Copy Number Variants and male infertility

- Important source of genetic diversity with remarkable differences between individuals
- Play a role in complex diseases CNVs and male infetrility
  - CNVs can cause spermatogenic failure by:
    - Increased number or specific distribution of CNVs could result in deffective recombination, meiotic failure and loss of germ cells
    - CNVs might affect the activity of genes important for spermatogenesis
  - *Tuttelmann et al, PLOS One 2011* – first study investigating CNVs in patients with severe oligozoospermia and SCOS
    - Provided a number of candidate genes possibly causing or being risk factors for spermatogenic failure
    - Larger case-control and re-sequencing studies are needed

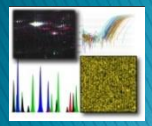


CNVs study design in RCGEB

- Patients:

Patient No.	Ethnicity	Spermogram	Histo-pathology	AZFc deletion
MI-88	Macedonian	azospermia	maturity arrest	no
MI-91	Macedonian	azospermia	maturity arrest	no
MI-100	Albanian	azospermia	maturity arrest	no
MI-114	Albanian	azospermia	maturity arrest	no
Mi-38	Macedonian	azospermia	n.d.	gr/gr
MI-7	Macedonian	oligozoospermia	n.d.	gr/gr
MI-215	Macedonian	oligozoospermia	n.d	gr/gr
MI-137	Macedonian	normozoospermia	n.d	gr/gr

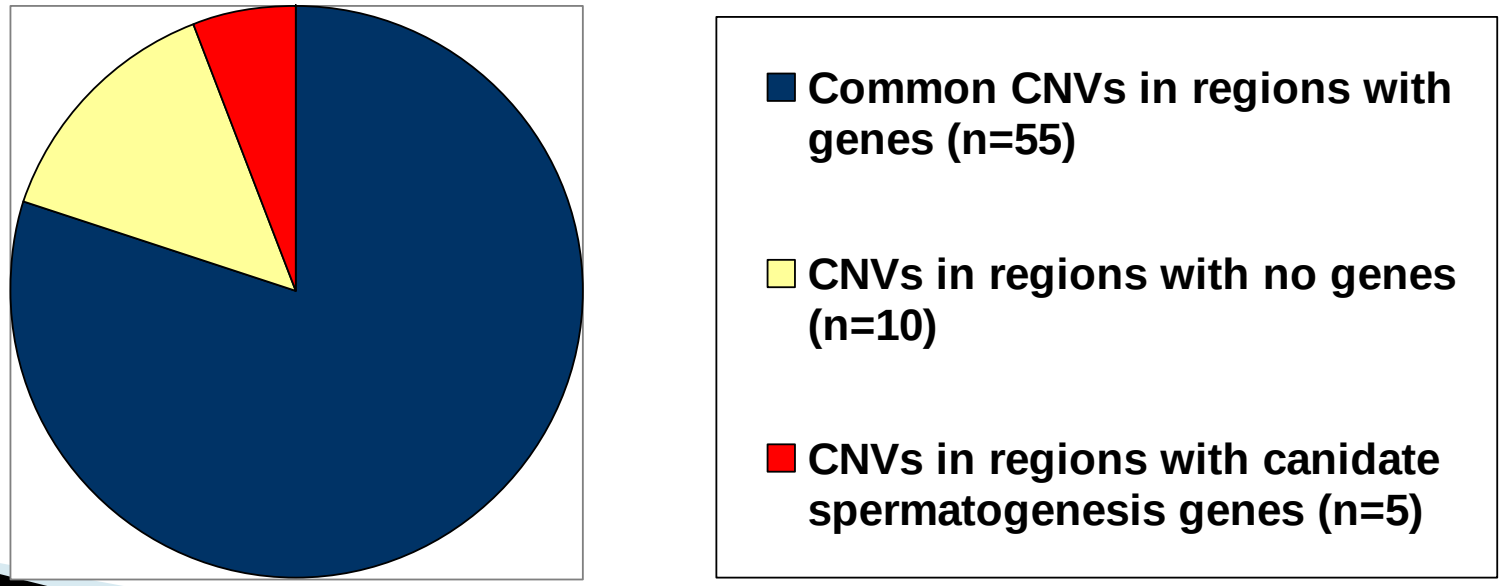
- Methods: array-CGH
  - 180K Agilent Human Genome CGH Microarrays
  - Genomic Workbench software
  - Database of Genomic Variants (DGV)



CNVs detected

- 70 CNVs
- 6 - 700 kb in lenght
- 4 -15 CNVs in each patient

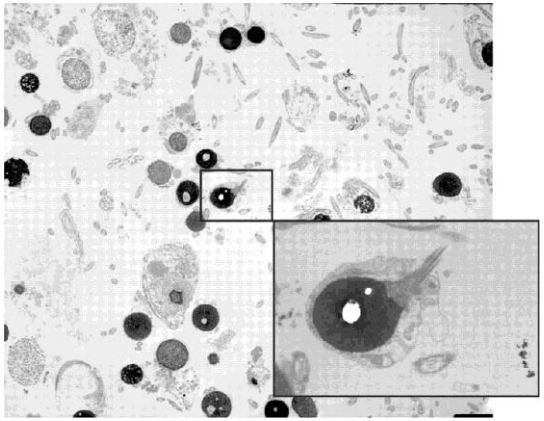
- Duplications (n= 40)
- Deletions (n=30)



CNVs in candidate spermatogenesis genes

Patient details	Genomic region	Size (kb)	CNV type	Genes	*Tuttelmann et al, 2011*
Azoospermia (maturity arrest)	chr4 q13.2	70	del	UGT2B17	Increased frequency in SCOS
Azoospermia (maturity arrest)	chr4 q13.2	70	dup	UGT2B17	Decreased frequency in SCOS
Azoosprermia (gr/gr deletion)	chr7 q21.13	69	dup	STEAP2	
Oligozoospermia (gr/gr deletion)	chr21 p11.2-p11.1	279	dup	TPTE	
Normozoospermia (gr/gr deletion)	chrX q22.2	117	dup	H2BFWT	Found in oligozoospermia

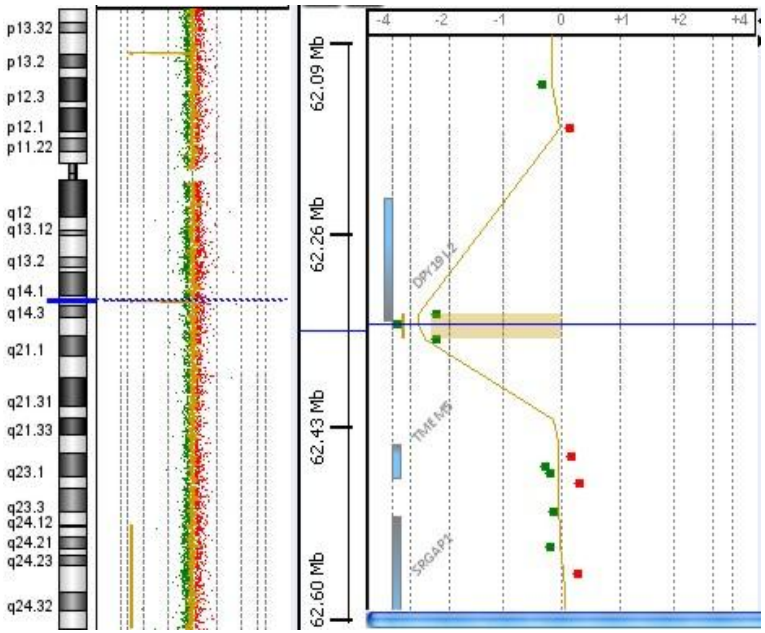
Globoozoospermia



Dam A et al. Hum. Reprod. Update 2007;13:63-75

- rare (0.1% of male infertility)
- severe disorder
- low success rates with ICSI
- described in 1971
- round-headed spermatozoa that lack an acrosome
- familial cases suggest a genetic component
- Research in mice identifies several implicated genes
  - *Gopc*, *Hrb*, *Csnk2a2, Pick1*
- In humans
  - 2 patients with homoz. mutations in SPATA and PICK1 genes
  - homozygous deletion of DPY19L2 – the major cause (Am J Hum Gen 2011)

Globoozoospermia – DPY19L2 gene



- highly expressed in the testis
- its ortholog in C elegans is involved in cell polarity
- In men described as a CNV
  - duplications (3x more freq)
  - deletions
- Do heterozygous deletions affects fertility?
- Could this explain selection against the deletion

Acknowledgements

- **Participants:**
  - ***MASA, RCGEB "Georgi D. Efremov"***

    Noveski P *Trivodalieva S*
    Moneva Z *Maleva I*

  - ***Fac. of Medicine, Clinic Endocrinol & Metabol. Dis.***

    Plaseski T *Dimitrovski C*
    Kocevska B

- **Grants:**
  - ***ICGIB, Trieste***
  - ***Ministry of Education and Science, R. Macedonia***
  - ***Macedonian Academy of Sci. & Arts, Science Funds***
  - ***European Commission, FP7-REGPOT 2008-1***