Chromosomal disorders and male infertility

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INTRODUCTION

Infertility is defined as the inability to conceive after one or more years of unprotected intercourse[1] and occurs in approximately 15% of the population[2,3].
Approximately half of the cases due to male factors. In fact, infertility affects about 7% of all men[4]. Male factor infertility is considered a complex disorder with a largely unknown etiology[5,6]. In general, genetic abnormalities are thought to account for 15%-30% of this condition and chromosome abnormalities and Y chromosome microdeletions are frequently implied[6].
The study, based on our casuistic, aimed at contributing to a better understanding of the genetic causes of infertility, in order to improve genetic counseling of these conditions.

METHODS

We retrospectively evaluated the results of a group of 410 idiopathic infertile men with non-obstructive azoospermia (AZO), oligozoospermia (including oligo-asteno-teratozoospermia and cryptozoospermia) (OLIGO), as well as infertile men with no indicate or unknowed pathological semen quality (NIQ). Subjects were selected based on clinical evaluation and on sperm counts*.
We will focus on our current understanding of the chromosomal basis of male infertility specifically: structural or numerical karyotype abnormalities and Y chromosomal microdeletions.
Conventional karyotype was performed in all samples by *in situ* methods according to standard procedures.
Fluorescence *in situ* hybridization (FISH) analysis was performed according to standard procedures and included: whole chromosome painting probes (WCP) centromeric, pseudoautosomic region and unique sequence probes.
Molecular analysis was performed in 247 samples by three complementary and independent multiplex-PCR using specific sets of sequence-tagged sites markers for the three AZF regions in genomic DNA samples extracted from peripheral blood. The extension and breakpoints of microdeletions were confirmed using a subsequent multiplex-PCR with additional markers.
*Defined according to World Health Organization (WHO) recomendations and standards, 2010.

Table I – Chromosome abnormalities observed in 410 infertile men with azoospermia (AZO), oligozoospermia (OLIGO), oligo-asteno-teratozoospermia (OTA), cryptozoospermia (CRY) and infertile men with no indicated semen quality (NIQ).

Chromosomal abnormalities	AZO	OLIGO +OTA +CRY	NIQ	TOTAL
Nº Samples	86	144	180	410
Sex chromosomes				
47,XXY	9	2	7	18
mos 47,XXY[41]/46,XY[9]	1			1
mos 47,XXY[2]/46,XY[48]		1		1
47,XYY		1		1
mos 47,XXY[13]/46,XX[37]	1			1
46,X,der(X)t(X;Y)(p22.31;p11.2).ish der(X)t(X;Y)(p22.31;p11.2)(SRY+) (case A)	1			1
46,X,der(X)t(X;Y)(p22.33;p11.3).ish der (X)t(X;Y)(p22.33;p11.3)(wcpY+;SRY+)	1			1
46,X,+mar.ish der(Y)(wcpY+,DYZ3+)			1	1
46,X,inv(5)(p14.2p15.2),+mar[32]/45,X,inv(5)(p14.2p15.2)[18].ish i(Y)(p10)(SRY++,DYZ3+, inv(5)(wcp5+)(case B)	1			1
mos 45,X[34]/46,X,idic(Y)(q11.221)[16]	1			1
Total Sex chrossomes abnormalities (%)	15 (6.1)	4 (1.6)	8 (3.2)	27 (6.6)
Autosomes				
46,XY,t(1;3)(q42.3;q26.2)			1	1
46,XY,t(1;10)(p22.1;q25.2)			1	1
46,XY,t(4;8)(q27;q11.23)		1		1
46,XY,t(4;22)(p10;q10)			1	1
46,XY,t(7;22)(q21.23;q11.2)			1	1
46,XY,t(8;14)(p23.3;q11.2)			1	1
45,XY,rob(13;14)(q10;q10)			2	2
46,XY,rob(13;14)+mar.ish der(16)(wcp16+)		1		1
46,XY,t(4;14;15)(q22;q21;q21.39).ish der(4)t(14;15)(wcp15+),der(14)t(4;14)(wcp4+), der(15)t(14;15)(wcp14+)		1		1
Total translocations	0	3	7	10
46,XY,inv(2)(p11.2q13)			1	1
46,XY,inv(8)(q21.2q22.3)			1	1
46,XY,inv(14)(q13q22).ish inv(14)(p21.1)(RP11-388M7+)(q21.3)(RP11-,168D12+)			1	1
46,XY,dup(8)(p23.1p23.1).ish (wcp8+)	1			1
47,XY,+mar.ish der(14/22)(D14Z1/D22Z1+)			1	1
Other structural rearrangements	1	0	4	5
Total Autossomes abnormalities (%)	1 (0,4)	3 (1,2)	11 (4,4)	15 (6,1)
Total chromossomal abnomalities group (%)	16 (18.6)	7 (4.9)	19 (10.6)	42 (10.2)
% Total chromossomal abnomalities	3.9	1.7	4.6	10.2

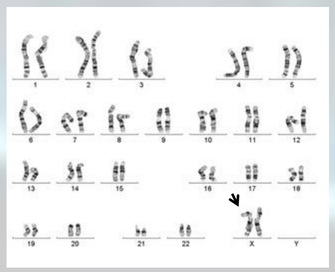

Figure 1a: Case A. GTL-banded karyotype with der(X)t(X;Y) in wich a segment of the Y chromosome with *SRY* is insert in X chromosome (→).

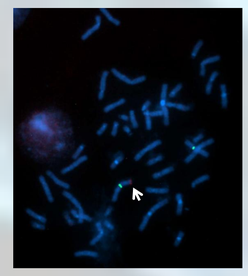

Figure 1b: FISH analysis with SRY probe (red) showing signals in the X chromosome (→).

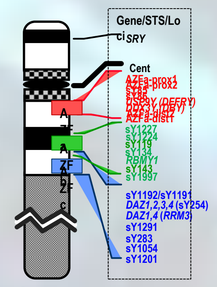

Figure 1c: Schematic representation of the Y chromosome, showing the three domains of spermatogenesis regulation (AZFa, AZFb and AZFc) and SRY localization.

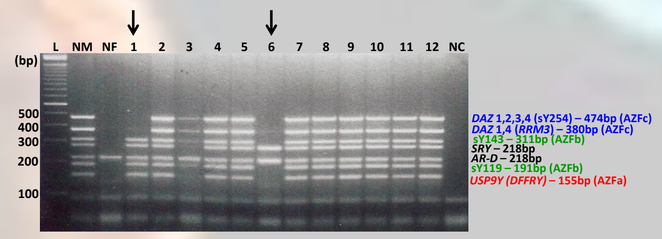

Figure 1d: Individuals 1 and 6 (our case A) show AZFc and AZFabc microdeletions characterized by the absence of the *DAZ* gene markers and absence of all AZF markers, respectively; bp- base pairs; L- 100 bp ladder; NM (normal male) and NF (normal female) used as PCR controls; *SRY* and *AR-D* genes used as internal positive PCR controls; NC- negative control.

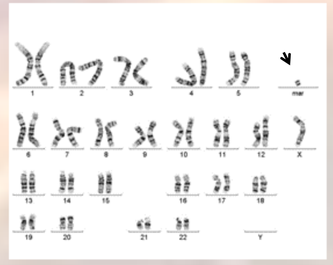

Figure 2a: Case B GTL-banded karyotype showing marker chromosome →

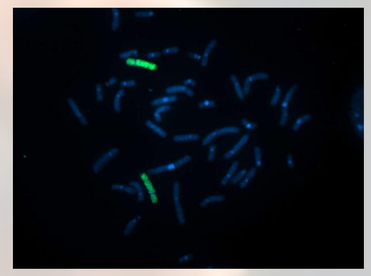

Figure 2b: FISH analysis with WCP 5 probe showing signal (green) for both 5 chromosomes and no involvement of other chromosomes

Figure 2c: FISH analysis with SRY probe showing two signals (red) → on the marker chromosome.

Table II – Karyotypes and AZF microdeletions: results in 247 cases with Y microdeletions studies.

Pathological status	Karyotype	AZF microdeletions (A)			Regions deleted	SRY in chrom.
		AZFa	AZFb	AZFc		
Azo	46,X,der(X)t(X;Y)(p22.33;p11.3)	A	A	A	3	X
Azo	46,X,der(X)t(X;Y)(p22.31;p11.2)	A	A	A	3	X
Azo	46,X,inv(5),+mar/45,X,inv(5).ish i(Y)(p10)	A	A	A	3	YY
Azo	46,XY		A	A	2	Y
Azo	46,XY	A			1	Y
Azo	46,XY			A	1	Y
Azo	46,XY			A	1	Y
Azo	46,XY			A	1	Y
Cry	46,XY			A	1	Y
OTA	46,XY			A	1	Y
OTA	46,XY			A	1	Y
Infertility	46,XY		A		1	Y
Infertility	46,XY			A	1	Y
Infertility	46,XY			A	1	Y
Infertility	46,XY			A	1	Y
Infertlity	46,XY			A	1	Y
Total deletions		4 (1.6%)	5 (2.0%)	14 (5.7%)	23(9.3%)	

RESULTS AND DISCUSSION

In the 410 samples, 42 abnormal karyotypes (10.2%) were found, indicating an elevated frequency of chromosome abnormalities among the selected infertile men (Table I), when compared to that of newborn populations (~0.4%)[4]. This frequency is higher than that reported in most similar studies that pointed to frequencies ranging from 2.2%-14.3%[7].
-There are 27 sex chromosomes abnormalities and 15 autosome (structural) rearrangements.
-Sex chromosomes anomalies are present in 6.6% of total cases and represents 64.3% of the chromosomal abnormalities.
-As expected, Klinefelter´s syndrome was the most common chromosome disorder (4.4%).
-2 cases with 46,X,der(X)t(X;Y) occurs. In this cases SRY is present in the X chromosome and regions AZFa,b,c were deleted (Figure 1). The XY bivalent is particularly susceptible to errors in meiosis because of homology between the X and the Y chromosome[8]. A case with a mosaic marker chromosome i(Y)(p10) presents two signals for SRY (Figure 2).
-Reciprocal translocations were identified in 10 cases (2.4%), particularly in men with OLIGO, OTA and NIQ (Table I) and may have a strong impact on the spermatogenesis process lead to oligozoospermia or even azoospermia[2,3]**.** In fact, chromosomal translocations may cause reductions in testicular volume and testosterone level, which may impact spermatogenesis, resulting in male infertility[9] . A review study reveals that have been found in approximately 1% of the infertile men and are more common in azoospermic than in oligozoospermic males [4], a value and a relation not found in the present study.

- Y microdeletions cases were identified in 16 of the 247 Y microdeletions cases studied (6.5%) with a total of 23 deletions (Table II).
-There are more frequent in azoospermics (13.3% of this group), corresponding to 8/60 azoospermics. Among these 8 cases, 7 presented deletions at the AZFc region and 3 presented deletion at the entire AZF region.
-Oligozoospermics present exclusively deletions on AZFc region. Men with complete AZFc deletions have variable seminal and testicular phenotypes, with sperm products levels ranging from azoospermia to oligozoospermia[10].

-Sperm counts and other clinical data are essential for results interpretation and a genotype/phenotype correlation. The marked presence of chromosomal abnormalities and Y microdeletions enphasizes the relevance of studying both factors in infertile men to improve genetic counseling, to allow the development of appropriate therapies, and to expand the knowledge about the ethiology of male infertility.

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