

## Cytogenetic and AZF microdeletions on the Y chromosome of infertile men

K. Ben Makhoul<sup>1,2,3</sup>, I. Samri<sup>2</sup>, L. Bouguenouch<sup>2</sup>, N. Boudra<sup>3</sup>, N. Elghachtouli<sup>1</sup>, K. Ouldin<sup>2</sup>

<sup>1</sup>Laboratory of biotechnology, Faculty of Sciences and Techniques, PO Box : 2202, Road og Immouzer, Fez, Morocco

<sup>2</sup>Unit of Medical Genetics and Oncogenetics, Hospital University Center, Fez, Morocco

<sup>3</sup>Assisted reproductive technologies center, Fez, Morocco

\*Corresponding author : [kaoutar.benmakhoul@yahoo.com](mailto:kaoutar.benmakhoul@yahoo.com)

---

### Abstract

Intervals V and VI of Yq11.23 regions contain responsible genes for spermatogenesis, and are named as “azoospermia factor locus” (AZF). Deletions of these genes are thought to be pathogenetically involved in some cases of male infertility associated with azoospermia or oligozoospermia. The aim of this study was to develop a method for the detection of the AZF region of the human Y chromosome in the Unit of Medical Genetics and Oncogenetics UGMO at the Hospital University Center Hassan II-FEZ. We applied multiplex polymerase chain reaction (PCR) using several sequence-tagged site (STS) primer sets, in order to determine microdeletions in the Y chromosome. Our findings suggest that the technique is validated, so genetic screening should be advised to infertile men before starting assisted reproductive treatments.

**Key words:** Male infertility, Y chromosome microdeletion, AZF regions.

---

### Introduction

Infertility affects approximately 9% of couples attempting pregnancy, and it is estimated that a male factor is involved in approximately half of cases [1]. Evidence for direct involvement of the long arm of the Y chromosome in male infertility was first established by cytogenetic analysis [2]. The presence of the azoospermia factor (AZF) locus, containing one or more genes necessary for normal spermatogenesis, was then proposed as important for fertility and mapped to deletion Yq-interval VI. Finally, polymerase chain reaction (PCR) and recommended sequence-tagged sites (STS) allowed the definition of Y microdeletions as three close and nonoverlapping subregions: AZFa, AZFb, and AZFc.

Genotype and phenotype correlations have been described within the AZF region. Complete or incomplete deletions of the AZFa region were observed only in azoospermic patients, in association with Sertoli cell-only syndrome (SCO) [3].

Phenotype of men with a complete AZFb deletion corresponded to azoospermia with spermatogenic arrest at the pachytene stage [3]. In contrast, approximately half of men with incomplete AZFb deletion had spermatozoa in the ejaculate [4-6]. Patient phenotypes with AZFc deletions ranged from moderate oligozoospermia to azoospermia, with various histologic aspects. Finally, no testicular spermatozoa could be retrieved in cases of large deletions including and extending beyond the AZFc region (AZFbc or AZFac) [7-9].

Recently, infertility treatment has been performed by intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) techniques. However, deletions on the Y chromosome might be spread to the male offspring, causing the persistence of infertility problem over the next generations [10].

In this study, we develop a method to demonstrate the presence or absence of microdeletions on infertile cases with azoospermia and oligozoospermia before

ICSI/IVF, by using multiplex polymerase chain reaction (PCR) method.

## Material and methods

### Patients

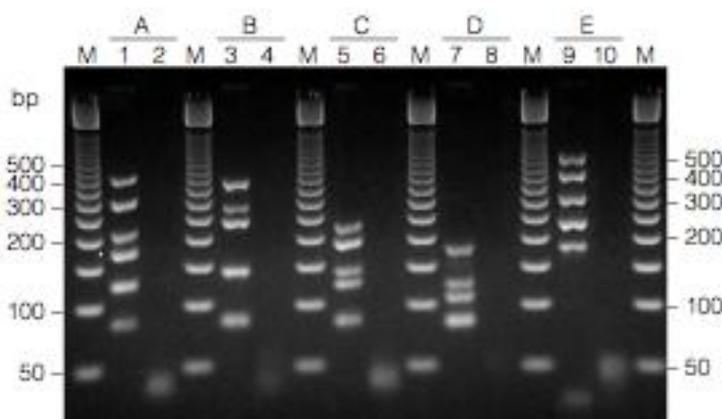
Deletion testing was performed in men with a sperm concentration  $<5.11/\text{mL}$  (azoospermia or severe oligozoospermia).

Extraction of genomic DNA from peripheral blood lymphocytes was performed using a standard technique.

### Sequence-Tagged Sites and Polymerase Chain Reaction

The screening method for microdeletions was consisted of 20 primer pairs that are homologous to previously identified and mapped sequence-tagged sites STS [11-17]. These primers will amplify nonpolymorphic short DNA segments from the Y chromosome when

used in PCR [16, 18]. Y chromosome deletions in the regions that are amplified by these primer sets have been associated with male infertility [12, 16, 19-27]. The primers have been combined into five Multiplex Master Mix sets for use in multiplex PCR. This makes it possible to determine the presence or absence of all 20 STS by performing five concurrent PCR amplifications (Figure 1). Adjacent regions of the Y chromosome do not generally appear as sequential amplification products within a single multiplex amplification reaction. Exceptions are SY242 and SY208 of the DAZ locus, which are represented as sequential amplification products using Multiplex B Master Mix reactions, and SY84 and SY86 of the DYS273 and DYS148 loci, which are represented as sequential amplification products in Multiplex E Master Mix reactions.



**Figure 1.** Example of amplification using male genomic DNA as template. Amplification of male genomic DNA (MD115A) (lanes 1, 3, 5, 7, 9), as well as a negative DNA control (lanes 2, 4, 6, 8, 10), for each of the five Multiplex Master Mixes.

Multiplex Master Mixes A–D contain a control primer pair that amplifies fragments of the X-linked SMCX locus. The fifth Multiplex Master Mix, Multiplex E, contains a control primer pair that amplifies a unique region in both male and female DNA (ZFX/ZFY). These control primer pairs are internal controls for the multiplex amplification reactions and test the integrity of the genomic DNA sample. Finally, Multiplex E Master Mix also includes a primer pair that amplifies a region of the SRY gene, acting as control amplification for the testis-determining

factor on the short arm of the human Y chromosome.

## Results

To detect microdeletions in the Y chromosome following multiplex PCR, the worksheets presented in Table 1 are used to analyze the PCR reactions (presence or absence of the expected PCR products). Therefore, if there are any PCR products absent from the reactions, they can be mapped using the Y Chromosome Map Worksheet (Table 2). All deletions should be contiguous. If multiple amplification

bands are missing for a sample, and those bands do not map to adjacent regions of the Y chromosome (Table 2), they represent dropout bands and are not deletions. In this case, the test is repeated. A single missing amplification band in a sample may also represent a dropout band and thus the test is repeated to confirm that the locus is not amplified. A locus that is present in a sample may not be amplified if there is a mutation in one of the primer binding sites for the locus. Diagnostic results obtained with this method should

**Table 1.** PCR Amplification Product Profile for Test Sample. Worksheets: We record the presence (+) or absence (-) of the PCR amplification products for each sample.

STS	Locus	Product Size (bp)	Map Position	Sample (+/-)
Multiplex A Master Mix				
SY254	DAZ	380	18	+
SY157	DYS240	290	20	+
SY81	DYS271	209	2	+
SY130	DYS221	173	11	+
SY182	KAL_Y	125	5	+
	SMCX	83	Control	+
Multiplex B Master Mix				
SYPR3	SMCY	362	7	+
SY127	DYS218	274	9	+
SY242	DAZ	233	16	+
SY208	DAZ	140	17	+
	SMCX	83	Control	+
Multiplex C Master Mix				
SY128	DYS219	228	10	+
SY121	DYS212	190	6	+
SY145	DYF51S1	143	14	+
SY255	DAZ	124	19	+
	SMCX	83	Control	+
Multiplex D Master Mix				
SY133	DYS223	12	SY133	+
	177			
SY152	DYS236	15	SY152	+
	125			
SY124	DYS215	8	SY124	+
	109			
	SMCX	83	Control	+
Multiplex E Master Mix				
	ZFX/ZFY	496	Control	+
SY14	SRY	400	1	+
SY134	DYS224	303	13	+
SY86	DYS148	232	3	+
SY84	DYS273	177	4	+

only be interpreted in conjunction with other clinical or laboratory data. Figure 2 shows an example of gel analysis of sample DNA.

We have observed some nonspecific bands that appear above or below the expected amplification product on the agarose gel. These nonspecific bands represent different conformations of the same amplification product. This phenomenon is sometimes seen in gel electrophoresis because of the pH. In addition, no PCR amplification products were detected with the negative controls (no DNA template). Accordingly, these nonspecific bands have no effect on the analysis.

## Discussion

Recent advances in molecular biology suggested that microdeletions of the Y chromosome represent an important cause of male infertility and the most frequent cause of severe testiculopathy [28]. Y chromosome was thought to be poor in terms of gene content as its q arm constitutes mostly of heterochromatic region. But recently, it came into consideration due to the discovery of genetic complexity of AZF region which is divided into three nonoverlapping loci: AZFa, AZFb, AZFc spanning through intervals V and VI. Molecular mapping analysis has shown that these loci harbor not all but at least some of the genes responsible for spermatogenesis [29, 30].

The negative effects of abnormal semen characteristics and sperm quality on fertility can be overcome with in vitro fertilization (IVF) using conventional methods of fertilization, or with intracytoplasmic sperm injection (ICSI). The use of ICSI provides an effective treatment for severe male factor infertility. Indeed, ICSI is recommended following failure of fertilization using conventional methods of IVF because the technique bypasses the zona pellucida and oolemma



## Reference

- [1] Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 2007; **22**:1506–12.
- [2] Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the Y chromosome. *Hum Genet* 1976; **34**:119–24.
- [3] Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996; **5**:933–43.
- [4] Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD, et al. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. *Hum Reprod* 1998; **13**:2812–5.
- [5] Page DC, Silber S, Brown LG. Men with infertility caused by AZFb deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod* 1999; **14**: 1722–6.
- [6] Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis. *Hum Reprod* 2000; **15**:1431–4.
- [7] Krausz C, McElreavey K. Y chromosome and male infertility: update, 2006. *Front Biosci* 1999; **15**:E1–8.
- [8] Krausz C, Degl'Innocenti S. Y chromosome and male infertility. *Front Biosci* 2006; **11**:3049–61.
- [9] Catherine Patrat, Thierry Bienvenu (2010) Clinical data and parenthood of 63 infertile and Y-microdeleted men. *American Society for Reproductive Medicine. Fertil Steril* **93**:822–32.
- [10] Canan Figen Sargin, Sibel Berker-Karaüzüm (2003) AZF microdeletions on the Y chromosome of infertile men from Turkey. *Annales de génétique* 2004 ; **47**; 61–68
- [11] Vollrath, D. et al. (1992) The human Y chromosome: A 43-interval map based on naturally occurring deletions. *Science* **258**, 52–9.
- [12] Reijo, R. et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet* 1995. **10**, 383–93.
- [13] Foote, S. et al. The human Y chromosome: Overlapping DNA clones spanning the euchromatic region. *Science* 1992 ; **258**, 60–6.
- [14] Affara, N. et al. Report of the second international workshop on Y chromosome mapping 1995. *Cytogenet. Cell. Genet.* 1996 **73**, 33–76.
- [15] Kent-First, M.G. et al. Gene sequence and evolutionary conservation of human SMCY. *Nat. Genet.* 1996 ; **14**, 128–9.
- [16] Kent-First, M.G. et al. Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. *Mol. Reprod. and Dev.* 1999 ; **53**, 27–41.
- [17] Vogt, P.H. et al. (1997) Report of the third international workshop on Y-chromosome mapping 1997. *Cytogenet. Cell Genet.* **79**, 1–20.
- [18] Skaletsky, H. et al. (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **423**, 825–37.
- [19] Pryor, J.L. et al. (1997) Microdeletions in the Y chromosome of infertile men. *New Eng. J. Med.* **336**, 534–39.
- [20] Kent-First, M.G. et al. (1996) The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injections and their fathers. *Mol. Hum. Reprod.* **2**, 943–50.
- [21] Kostiner, D.R., Turek, P.K. and Reijo, R.A. (1998) Male infertility: Analysis of

the markers and genes on the human Y chromosome. *Hum. Reprod.* **13**, 3032–8.

[22] Kuroda-Kawaguchi, T. et al. (2001) The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat. Genet.* **29**, 279–86.

[23] Lahn, B.T. and Page, D.C. (1997) Functional coherence of the human Y chromosome. *Science* **278**, 675–80.

[24] Reijo, R. et al. (1996) Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet* **347**, 1290–3.

[25] Repping, S. et al. (2002) Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am. J. Hum. Genet.* **71**, 906–22.

[26] Saxena, R. et al. (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was

transposed, repeatedly amplified and pruned. *Nat. Genet.* **14**, 292–9.

[27] Simoni, M. et al. (1999) Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *Int. J. Androl.* **22**, 292–9.

[28] J.L. Pryor, M. Kent-First, A. Muallem, A. Van Bergen, W. Nolten, L. Meisner, K.P. Roberts, Microdeletions in the Y chromosome of infertile men, *New England Journal Medicine* **8** (1997) 534–539.

[29] C. Foresta, E. Moro, A.Y. Ferlin, Chromosome microdeletions and alterations of spermatogenesis, *Endocrine Reviews.* 2011 ;**22**; 226–239.

[30] A. Friel, J.A. Houghton, M. Maher, T. Smith, S. Noel, A. Nolan, D. Egan, M. Glennon, Molecular detection of Y chromosome microdeletions: an Irish study, *International Journal of Andrology.* 2011 ; **24** ; 31–36.