

Prevalence of Y chromosome microdeletions in infertile Tunisian men

Prévalence des microdélétions du chromosome Y chez les hommes tunisiens infertiles

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Abstract. Yq microdeletions are the leading genetic cause of male infertility and its detection is clinically relevant for appropriate genetic counseling. The objective of this study was to determine the frequency of Y microdeletion in a group of Tunisian infertile men and to compare the prevalence of these abnormalities with other countries and other Tunisian reported series. Totally, 105 Tunisian idiopathic infertile men (74 azoospermic and 31 severe oligozoospermic) were screened for the presence of Y chromosome microdeletions. The screening of Yq microdeletions was performed by two multiplex PCRs using six STS markers recommended by the EAA/EMQN. No microdeletions were detected in the men with severe oligozoospermia. In the azoospermic group, 2/74 (2.7%) patients showed Y chromosome microdeletions. Both had complete deletion of the AZFc region. No microdeletion was identified in the AZFa region or in the AZFb region. The estimated frequency of Y chromosome microdeletions in the present survey was similar to some other reports but lower than that of previous reports in Tunisian populations.

Key words: male infertility, non-obstructive azoospermia, severe oligozoospermia, Y chromosome deletions

Résumé. Les microdélétions du chromosome Y représentent, après les causes chromosomiques, l'une des causes majeures d'infertilité masculine d'origine génétique. Nous rapportons dans ce travail les résultats de la recherche de microdélétions dans la région AZF du chromosome Y chez des patients tunisiens dont le spermogramme est altéré. La recherche des microdélétions dans la région AZF a été réalisée chez les patients ayant un caryotype normal par PCR multiplexe dont les STS ont été choisis selon la recommandation de l'EAA/EMQN (the European academy of andrology/the European molecular genetics quality network). L'étude moléculaire du locus AZF, effectué chez 105 patients ayant un caryotype normal (74 azoospermiques et 31 oligozoospermiques sévères) a révélé l'existence de microdélétions dans 2 cas (1,9 %), tous deux azoospermiques. Par ailleurs, aucune microdélétion dans les loci AZFa ou AZFb n'a été décelée. La prévalence de microdélétions retrouvée dans notre série est similaire à certaines autres études mais reste inférieure à la plupart des séries tunisiennes.

Mots clés : infertilité masculine, azoospermie non obstructive, oligozoospermie sévère, chromosome Y, microdélétion

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The Y chromosome plays a fundamental role in the control of spermatogenesis. Microdeletions of the long arm of the Y chromosome removing the azoospermia factor (AZF) region or parts are found in men suffering from azoospermia or oligozoospermia and are the second most frequent genetic cause of spermatogenic failure after Klinefelter's syndrome [1].

The AZF region mapped to Yq11, is subdivided into three subregions called from proximal to distal as "AZFa" "AZFb" and "AZFc" respectively [2]. In the two last decades, many investigators have described the occurrence of microdeletions in infertile patients around the world and the molecular diagnosis of deletions have become a routine diagnostic step in the work-up of male infertility [3] and has prognostic value in couples who want to undergo assisted reproductive techniques (ART) [3, 4]. Nevertheless, data from molecular studies have demonstrated that the prevalence of Y chromosome microdeletions among men with non-obstructive azoospermia or severe oligozoospermia ranges from 1 to 58% [5]. According to the previous published studies, there are wide variations in the estimated frequency of Yq microdeletions among Tunisian infertile men, which vary from 1.31% [6] to as high as 48% [7].

The objective of this study was to determine the frequency of Yq microdeletions in a Tunisian population of infertile men with non-obstructive azoospermia and severe oligozoospermia and then, to evaluate the reasons of discordance between the different studies performed in Tunisia.

Materials and methods

Patients

In the present study, we studied 105 karyotypically normal infertile men (age range from 28 to 58 years) who were referred from various Tunisian infertility clinics to the department of histology and cytogenetics at the Institut Pasteur de Tunis for evaluation of male factor infertility, during the period from September 2010 to December 2012. All participants were informed about the purpose of the study and signed a written, informed consent. Blood samples were taken on a single occasion, and all the steps of the tests were explained to the patient along with their possible complications. All subjects were of Tunisian ethnic origin. Physical examination was performed in order to detect known causes of male infertility. Patients were excluded if there is consecutive clinical evidence of obstructive azoospermia or spermatogenic failure resulting from endocrine abnormality.

The patients were classified into two groups according to alterations detected in semen analysis based on the World

health organization guidelines [8]: the first one includes 74 non-obstructive azoospermia (no sperm in ejaculate even after centrifugation) and the second one contains 31 severe oligozoospermia (sperm count $< 5 \times 10^6/\text{mL}$).

Detection of AZF microdeletions by multiplex PCR

Genomic DNA was extracted from blood lymphocytes using a commercially available kit (FlexiGene Kit; Qiagen), according to the manufacturer's instructions. Each patient was examined for six AZF loci. The STS primers used were: for AZFa sY84 and sY86, for AZFb sY127 and sY134 and for AZFc sY254 and sY255. This primer set was suggested by Simoni *et al.* [9] and is prescribed by the European academy of andrology (EAA) and European molecular genetics quality network (EMQN) [1, 3]. In addition, sY14 (STS within the SRY gene located in Yp) was tested as an internal positive control.

Two multiplex PCRs were carried out in 50 μL reaction volumes containing: 200 ng of each DNA sample, 1.5 mM MgCl_2 , 0.4 mM of each dNTP, 1.6 μM of each oligonucleotide primer: sY86, sY127, sY254 (Mix I) for the first multiplex PCR, sY84, sY134, sY255 (Mix II) for the second one, 5% dimethyl sulfoxide (DMSO) were added to 1 μL of Taq DNA polymerase.

A positive control (sample from a normal fertile male), two negative controls [(i) normal female sample, (ii) every constituent except DNA], and one infertile male with 46,del(Yq) karyotype, were included in every PCR assay. The reaction mixture included thermocycling consisted of an initial denaturation of 5 minutes to 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds annealing at 59°C, 30 seconds extension at 72°C, finally, 7 minutes extension step at 72°C. PCR products were analyzed by electrophoresis on 3% agarose gels containing ethidium bromide and visualized by exposure to ultraviolet light. In the event of detecting deletion with a primer, the PCR assay was repeated thrice for confirmation. A STS was considered absent only after 3 amplification failures in the presence of successful amplification of internal control (SRY).

Results

Using the EAA/EMQN criteria, microdeletions of the Y chromosome were found in two out of the 105 subjects (1.9%). The failed amplification of sY254 and sY255 markers indicates a complete deletion in the AZFc sub-region. All two microdeletions were present in non-obstructive azoospermic men (2.7%; 2/74) and none were observed in the 31 severe oligozoospermic cases.

Table 1. Frequency of Y-chromosome microdeletions in infertile men detected with the EAA/EMQN STSs markers in different countries.

Authors	Regions	Patients		Group study		Karyotype
		Total	AZF deletion (%)	Frequency of AZF deletion in each group	Type of AZF deletion in each group	
Sargin <i>et al.</i> 2004 [13]	Turkey (Antalya)	60	2 (3.33%)	AS: 4.25% (2/47) OS: 0% (0/13)	AS: 1 AZFb, 1 (AZFb, AZFc) -	Normal karyotype
Imken <i>et al.</i> 2007 [25]	Morocco	127	4 (3.15%)	AS: 8.33% (4/48) OATS: 0% (0/79)	AS: 2 AZFc, 2 AZFb+c -	NA
Song <i>et al.</i> 2007 [16]	China (Nanjing)	62	12 (19.35%)	AS: 27.27% (9/33) sOS: 10.34% (3/29)	AS: 8 AZFc, 1 AZFb sOS: 1 AZFc, 1 AZFb+c, 1 AZFb	Normal karyotype
Ristanovic <i>et al.</i> 2007 [17]	Serbia	90	14 (15.55%)	AS: 15.52% (9/58) sOS: 15.62% (5/32)	AS: 1 AZFa, 5 AZFc, 2 (AZFa+partial AZFb), 1 (partialAZFb+AZFc) sOS: 5 AZFc	Normal karyotype
Elhawary <i>et al.</i> 2010 [18]	Egypt	49	18 (36.73%)	AS: 39.29% (11/28) sOS: 33.33% (7/21)	AS: 2 partial AZFa, 1 AZFb, 4 partial AZFb, 2 (AZF partial a+partial b), 1 (AZF partial a+partial c), 1 (AZF partial b+partial c) sOS: 6 partial AZFb, 1 (AZF partial b+partial c)	Normal karyotype
Alkhalaf <i>et al.</i> 2010 [14]	Kuwait	116	9 (7.75%)	AS: NA sOS: NA	AS: 1AZFa, 3 AZFc, 3 AZFa-c sOS: 2 AZFc	Normal karyotype
Puzuka <i>et al.</i> 2011 [26]	Latvia	105	5 (4.76%)	AS: NA sOS: NA	AS: 2 AZF a+b+c, 1 AZFc sOS: 2 AZFc	Normal karyotype except two cases (46, X, del (Y) (q))
Behulova <i>et al.</i> 2011 [27]	Slovenia	226	8 (3.54%)	AS: 3.54% (8/226)	AS: 4 AZFc, 1 AZFb+c, 1 AZFa+b+c, 2 partial AZFa	Normal karyotype
Sun <i>et al.</i> 2012 [15]	China (Shanghai)	507	45 (8.87%)	AS: 9.32% (33/354) sOS: 7.84% (12/153)	AS: 23 AZFc, 3 AZFb, 3 AZFb+c, 2AZFa, 2 AZFa+b+c sOS: 12 AZFc	Normal karyotype
Wettasinghe <i>et al.</i> 2012 [11]	Sri Lanka	207	3 (1.45%)	AS: 1.96% (3/153) sOS: 0% (0/54)	AS: 1 AZFc, 2 partialAZFc. -	Normal karyotype
Saliminejad <i>et al.</i> 2012 [12]	Iran	115	2 (1.74%)	AS: 2.13% (2/94) sOS: 0% (0/21)	AS: 1 AZFc, 1 AZFb+c.	Normal karyotype
Cavkaytar <i>et al.</i> 2012 [28]	Turkey (Ankara)	332	24 (7.23%)	AS: 10.71% (21/196) sOS: 2.20% (3/136)	AS: 7 AZFc, 6 AZFb, 5 AZFa, 2 AZFb,c, 1 AZFa,b,sOS: 3 AZFc	Normal and abnormal karyotypes
Chellat <i>et al.</i> 2013 [10]	Algeria	80	1 (1.25%)	AS: 2.04% (1/49) OATS: 0% (0/31)	AS: 1 AZFc -	NA
Our results	Tunisia	105	2 (1.90%)	AS: 2.70% (2/74) sOS: 0% (0/31)	AS: 2 AZFc -	Normal karyotype

NA= not available; AS= azoospermia (0 spermatozoa [spz]/mL); OS= oligozoospermia (< 20 × 10⁶ spz/mL); sOS = severe OS (≤ 5 × 10⁶ spz/mL); OATS = severe oligoastheno-teratozoospermia.

Discussion

Y chromosome deletions are emerging as a prevalent genetic cause of male factor infertility and the frequency of Y chromosome deletions increases with the severity of spermatogenic defect [5]. As different numbers of Y-chromosome deletions have been reported from different

parts of the world and in Tunisia but with wide variation frequencies, we have investigated 105 patients consecutively referred to our department in order to compare our results with earlier published reports.

In the present study, six STSs strongly recommended by European academy of andrology (EAA) and European molecular genetics quality network (EMQN) [1, 3],

Table 2. Frequency of Yq microdeletions reported by different Tunisian studies.

Authors	Number STSs markers	STSs markers used	Patients		Group study		Karyotype
			Total	AZF deletion (%)	Frequency of AZF deletion in each group	Type of AZF deletion in each group	
Hadj-Kacem <i>et al.</i> 2006 [19]	6	AZFa: sY84, sY86. AZFb: sY127, sY134. AZFc: sY254, sY255 +SRY	163	26 (16%)	NS: 1.53% (1/65) AS: 28.89% (13/45) OS: 22.64% (12/53)	NA	Normal karyotype
HadjKacem-Loukil <i>et al.</i> 2007 [7]	14	AZFa: sY81, DFFRY, sY86, DBY1, sY84, sY87, sY182. AZFb: sY127, sY130, sY134, RBMY. AZFc: sY254, sY255, sY157.	210	102 (48%)	NS: 18.82%(16/85) AS: 54.72% (29/53) OS: 79.17% (57/72)	NA	Normal karyotype
Rejeb <i>et al.</i> 2008 [21]	20	AZFa: sY82, sY83, sY84, sY86, sY87, sY88. AZFb: sY135, sY114, sY127, sY134, sY142. AZFc: sY152, sY154, sY254, sY255, sY277, sY283, sY157, sY158, sY159.	146	10 (6.85%)	AS: 11.84% (9/76) sOS: 1.42% (1/70)	AS: 1 AZFb, 3 AZFb+c, 2 AZFc, 3 AZFa+b+c sOS: 1 AZFc	Normal karyotype
HadjKacem-Loukil <i>et al.</i> 2009 [29]	14	AZFa: sY81, sY86, sY87, sY84, DBY1, DFFRY. AZFb: sY182, sY134, sY130, sY127, SHGC-102574. AZFc: sY254, sY255, sY157.	27	7 (25.92%)	AS-KFS: 67% (6/9) AS-WCA: 5.55% (1/18)	AS-KFS: 3 AZFa+b+c, 1 AZFa, 1 AZFb, 1 AZFc. AS-WCA: 1 AZFc	Normal and abnormal karyotypes
Ghorbel <i>et al.</i> 2012a [6]	6	AZFa: sY84, sY86. AZFb: sY127, sY134. AZFc: sY254, sY255. +SRY	76	1 (1.31%)	AS: 0% (0/54) sOS: 4.54% (1/22)	- sOS: 1 AZFa+b+c	Normal and abnormal karyotypes
Ghorbel <i>et al.</i> 2012b [20]	6	AZFa: sY84, sY86. AZFb: sY127, sY134. AZFc: sY254, sY255. +SRY	261	7 (2.68%)	NS: 3.06% (3/101) AS: 1.29% (1/79) OS: 3.70% (3/81)	NS: 2 AZFa, 1 AZFa+c AS: 1 AZFa OS: 2 AZFa, 1 AZFa+b	NA
Our results	6	AZFa: sY84, sY86. AZFb: sY127, sY134. AZFc: sY254, sY255. +SRY	105	2 (1.9%)	AS: 2.70% (2/74) sOS: 0 % (0/31)	AS: 2 AZFc -	Normal karyotype

NA= not available; NS= normozoospermia (more than 20×10^6 spermatozoa [spz]/mL); AS= Azoospermia (0 spz/mL); OS= oligozoospermia (less than 20×10^6 spz/mL); sOS =severe OS ($\leq 5 \times 10^6$ spz/mL); mOS = mild OS (5×10^6 spz/mL- 20×10^6 spz/mL); AS-KSF= AS with klinefelter syndrome; AS-WCA= AS without chromosomal abnormalities.

were used in the detection of classical microdeletions and revealed 2 azoospermic men (1.9%) with microdeletion of the AZFc region. The low frequency of the AZF microdeletions in our cohort is in accordance with some previous studies reported from Algeria (1.3%) [10], Sri Lanka (1.45%) [11] and Iran (1.74%) [12]. However, this rate is lower than the frequencies reported from Morocco (3.1%) [25], Turkey (3.3 and 6%) [13, 28], Latvia (4.8%) [26], Kuwait (7.75%) [14], China (8.9 and 19,4%)

[15, 16], Serbia (15.5%) [17], and Egypt (36.7%) [18] (table 1).

All these studies were performed by the EAA/EMQN STS markers. A number of factors have been implicated in the wide variation of Y deletion frequencies reported, such as patient selection criteria, experimental design, environmental influences and ethnic variation.

Moreover, there are wide variations in the reported frequency of microdeletion in Tunisian population. Using the

6 STSs markers, Hadj-Kacem *et al.* [19] have reported an overall of 16% of Y chromosome microdeletions in 163 infertile Tunisian men with normal karyotype subdivided into 3 groups: azoospermic, oligozoospermic (less than 20×10^6 spz/mL), and normozoospermic men (more than 20×10^6 spz/mL). With the same STSs markers, two studies performed by Ghorbel *et al.* have detected a lower frequency in azoospermic and oligozoospermic groups: 1.3% [6] and 2.7% [20], but chromosomal abnormalities in these reports were included in the first study and unspecified in the latter one (table 2).

However, estimated frequency of Yq microdeletions among Tunisian infertile men with normal karyotypes in the study by Hadj-Kacem *et al.* using 14 STSs markers [7] revealed a high frequency of Yq microdeletions (48%), while Rejeb *et al.* have found 6.84% Yq microdeletions using 20 STSs markers [21]. Differences in the methods applied for the Yq microdeletion screening in Tunisian studies might account for varying deletion frequencies. The choice and number of STS markers differed widely, ranging from 6 [6, 19, 20] to 20 STS [21] despite the fact that Simoni *et al.* showed that the detection rate did not depend on the number and choice of markers used [22].

In the present study, 2/74 azoospermic men (2.7%) showed Y chromosome microdeletions. Both had complete microdeletion of the AZFc region. None of the patients in the present study, showed deletion neither in AZFa nor in AZFb regions. Microdeletions in the AZFa and AZFb regions are extremely rare [23] while AZFc microdeletion is the most frequently detected in the most published reviews [1].

Histologically, AZFc microdeletion is associated with various spermatogenetic alterations, is compatible with residual spermatogenesis and, in rare cases, can even be transmitted naturally to the male offspring [24].

Conclusion

Our data which are the result of the study of a Tunisian population, add the growing body of evidence supporting that there is a cause and effect relation between Yq11 microdeletions in the AZF locus and azoospermia. However, despite a relatively low frequency of microdeletion in our study, molecular genetic examination of the Y-chromosome should be offered to men with severely impaired spermatogenesis. This is in part because identification of a genetic reason for infertility improves the patients' ability to cope psychologically with the defect. Genetic counseling with Yq microdeletion candidates for ICSI (Intra-Cytoplasmic Spermatozoa Injection) should consider the obligate transmission of the deletion to male offspring [5].

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