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Y microdeletion in moroccan population
DOI: 10.1111/j.1745-7262.2007.00290.x

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·*Original Article*·

AZF microdeletions and partial deletions of AZFc region on the Y chromosome in Moroccan men

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Abstract

Aim: To evaluate for the first time the frequency of Y chromosome microdeletions and the occurrence of the partial deletions of AZFc region in Moroccan men, and to discuss the clinical significance of AZF deletions. **Methods**: We screened Y chromosome microdeletions and partial deletions of the AZFc region of a consecutive group of infertile men (n = 149) and controls (100 fertile men, 76 normospermic men). AZFa, AZFb, AZFc and partial deletions of the AZFc region were analyzed by polymerase chain reaction (PCR) according to established protocols. **Results**: Among the 127 infertile men screened for microdeletion, four subjects were found to have microdeletions: two AZFc deletions and two AZFb+AZFc deletions. All the deletions were found only in azoospermic subjects (4/48, 8.33%). The overall AZFc deletion frequency was low (4/127, 3.15%). AZF microdeletions were not observed in either oligoasthenoteratozoospermia (OATS) or the control. Partial deletions of AZFc (gr/gr) were observed in a total of 7 of the 149 infertile men (4.70%) and 7 partial AZFc deletions (gr/gr) were found in the control group (7/176, 3.98%). In addition, two b2/b3 deletions were identified in two azoospermic subjects (2/149, 1.34%) but not in the control group. **Conclusion:** Our results suggest that the frequency of Y chromosome AZF microdeletions is elevated in individuals with severe spermatogenic failure and that gr/gr deletions are not associated with spermatogenic failure. *(Asian J Androl 2007 Sep; 9: 674–678)*

Keywords: Y microdeletions; haplogroups; gr/gr; infertility; bi-allelic markers

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Received 2006-10-19 Accepted 2007-04-04

1 Introduction

Infertility is a major health problem affecting approximately 10%–20% of couples. Male factor infertility is assumed responsible in approximately 50% of infertile

couples [1, 2]. There are several known causes of male infertility, such as varicocele, endocrine disorders, cryptorchidism and infection [3]; however, 30%–40% of male cases of infertility are of unknown origin [4]. Genetic abnormalities are considered to make an important contribution to these cases of unexplained spermatogenesis failure. Early cytogenetic studies show that microscopic deletions in the long arm of Y chromosomes are responsible for azoospermia [5]. Microdeletions in the long arm (Yq) are known to represent the pathogenic mechanism for infertile males. Three non-overlapping regions named azoospermia factor AZFa, AZFb and AZFc have been defined [6]. These regions contain several genes involved in spermatogenesis [1].

AZFc is the most commonly deleted interval in men with azoospermia or severe oligozoospermia [7, 8]. AZFc deletions are generated by intrachromosomal homologuous recombination between repeated sequence blocks called "amplicons" organized in palindromic structures with nearly identical sequences in each palindrome arm. Within the AZFc region there are several candidate fertility genes, including three copies of *BPY2* (basic protein on Y chromosome 2), two copies of *CDY1* (*CDY1a* and *CDY1b*; chromodomain protein, Y chromosome 1), and four copies of the *DAZ* (deleted in azoospermia) gene family [7, 9]. A partial deletion termed gr/gr was recently described in the AZFc region, and has been described as a risk factor for spermatogenic failure in some studies [10–12]. Other studies suggest that it is a polymorphic deletion with no clinical relevance [13]. This deletion removes half the AZFc gene content, including two copies of the major AZFc candidate gene, *DAZ* [13].

Another deletion termed b2/b3 (1.8 Mb), which also results in the absence of half the AZFc gene compliment, seems to have no effect on fertility status and it is found on a certain chromosome background commonly present in northern Eurasian populations [13, 14].

The present study aimed for the first time to assess the prevalence of Y microdeletions and to investigate the association of partial deletion of the AZFc region in Moroccan men with fertility status and with clinical parameters.

2 Materials and methods

2.1 Subjects

The study population consisted of an unselected group of 149 infertile men and 176 fertile men. Informed consent was obtained from each subject. All subjects and controls were of Moroccan ethnic origin. The subjects were divided into four groups according to seminal profiles: azoospermia (n = 48), severe oligoasthenoteratozoospermia (OATS) (n = 79) and asthenozoospermia (n = 22). The control population consisted of 176 men with either known fertility (at least one child, n = 100) and normospermic men (> 20 million sperm/mL, n = 76). The seminal analysis was done according to the World Health Organization criteria.

2.2 Yq microdeletion analysis by sequence tagged site (STS) polymerase chain reaction (PCR)-based strategy

Genomic DNA was extracted from peripheral blood samples using standard DNA extraction methods, and amplified in multiplex polymerase chain reaction (PCR). Each of these subjects was tested for six AZF loci: the STS primers used for AZFa (sY84, sY86), AZFb (sY127, sY134) and AZFc (sY254, sY255). The internal control used was SRY14, samples from normal fertile men, without Y chromosome microdeletions and from healthy women, were used as normal controls, blank served as negative control. PCR was carried out in 25 μL reaction volume containing 150 ng of DNA, 1.5 mmol/L $MgCl_2$, dNTPs mix (0.2 mmol/L each), oligonucleotide primers (0.2 μmol/L each) and Taq DNA polymerase (1 unit). Amplification was carried out in the following thermal profile: initial denaturation at 94ºC for 5 min followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s, extension at 72ºC for 30 s, followed by a final extension at 72ºC for 7 min.

2.3 Screening for partial AZFc deletions

Individuals who did not carry Y AZF microdeletions were screened for partial AZFc deletions. Each individual was screened with two STSs specific for the gr/gr region, sY1291 and sY1191. A gr/gr deletion was identified by the absence of amplification of marker sY1291 and presence of marker sY1191. The b2/b3 deletions were characterized by the absence of the STS sY1191 and the presence of sY1291. All negative PCR reactions were repeated for at least three times.

2.4 Y chromosome haplogroup analysis

The Y chromosome haplogroup of individuals carrying either partial or complete AZF deletions was performed using the binary markers YAP and M81 that are highly informative for North African populations [15].

2.5 Statistical analysis

Differences among frequencies were calculated using the chi square (χ^2)-test and Fisher's exact test (two sides). Probability values of $P < 0.05$ were regarded as statistically significant.

3 Results

3.1 Y chromosome microdeletions

One hundred and twenty-seven subjects were screened for the presence of Yq microdeletions. Among them, 48 were azoospermic and 79 were OATS. We did not screen the subjects with asthenozoospermia for microdeletions (Table 1). Four subjects were found to have microdeletions. Microdeletions were not observed in control samples. The deletions were present in 4 azoospermic men (4/48, 8.33%): three of them were idiopathic and one was cryptorchid. No microdeletions were found in men with OATS (Table 1). The overall frequency of microdeletions in infertile men was 3.15% (4/127).

In two cases, two large deletions involving the AZFb + AZFc regions were seen: one of them had a positive chlamydia test and both had Sertoli-cell-only syndrome (SCOS) in the testicular biopsy. In two azoospermic men, two deletions of the AZFc were detected: one of them had cryptorchidism and a *Trichomonas vaginalis*. The deleted STS loci and clinical characteristics of the subjects carrying microdeletions are shown in Table 2.

3.2 Partial AZFc deletions

Using AZFc specific STS markers we identified seven cases of AZFc *gr/gr* deletions (absence of sY1291 and the presence of sY1191) among the infertile group (7/149, 4.70%) and seven cases (7/176, 3.98%) among the control groups (Table 3). This difference is not statistically significant. STS analysis identified b2/b3 deletions (absence of sY1191 and the presence of the marker sY1291) in two infertile men and none in the control group.

In the infertile population, the gr/gr deletion was associated with azoospermia (3/48, 6.25%), OATS (3/79, 3.80%) and asthenozoospermia (1/22, 4.54%). The b2/b3 deletion was found in two azoospermic men (2/48, 4.16%). In the control population, we found two normospermic men carrying the gr/gr deletion (2/76, 2.63%) and five men of known fertility carrying the gr/gr deletion (5/100, 5.00%).

3.3 Y chromosome haplogroups

All samples with either AZF or partial AZFc deletion were analyzed for their haplogroup. All samples were Y chromosome Alu polymorphism (YAP) positive and harbored the M81 T allele, indicating that each belonged to the Y chromosome haplogroup E3b2 [15].

4 Discussion

A number of genes on the Y chromosome and auto-

Table 1. Frequency of Y chromosome microdeletion. OATS, severe oligoasthenoteratozoospermia.

Phenotype	*n*	Deletions (%)	AZFc regions
Azoospermia	48	4 (8.33)	AZFc, AZFb + AZFc
OATS	79	0 (0)	
Total	127	4 (3.15)	

Table 3. Partial AZFc micodeletion analysis in infertile men and controls.

	gr/gr deletion (%)	b2/b3 deletion (%)	Total partial AZFc deletions (%)
Infertile	7/149 (4.70)	2/149 (1.34)	9/149 (6.04)
Controls	7/176 (3.98)	0	7/176 (3.98)

Table 2. Clinical characteristics of patients with Y microdeletions. SCOS, sertoli-cell-only syndrome.

Number of patients	Phenotype	Clinic	Testicular histology	Infections	STS deletion	AZF deletion
1	Azoospermia	Cryptorchidism	Not done	*Trichomonas vaginalis*	sY254, sY255	AZFc
3	Azoospermia	Idiopathic	SCOS	*Chlamydia*	sY127, sY134 sY254, sY255	AZFb + AZFc
2	Azoospermia	Idiopathic	SCOS	–	sY254, sY255	AZFc
4	Azoospermia	Idiopathic	SCOS	–	sY127, sY134, sY254, sY255	AZFb + AZFc

Table 4. Phenotype in patients and controls with partial AZFc deletions. OATS, severe oligoasthenoteratozoospermia.

	gr/gr deletions (%)	b2/b3 deletions (%)	Total partial AZFc deletions (%)
Azoospermia	3/48 (6.25)	2/48 (4.16)	5/48 (10.41)
OATS	3/79 (3.80)	0	3/79 (3.80)
Asthenzoospermia	1/22 (4.54)	0	1/22 (4.54)
Fertile	5/100 (5.00)	0	5/100 (5.00)
Normospermic	2/76 (2.63)	0	2/76 (2.63)

somes regulate spermatogenesis and Y chromosome deletions are emerging as a prevalent cause of male factor infertility [4]. The frequency of Y chromosome deletions increases with the severity of spermatogenic defect [1, 16]. The reported incidence of Y chromosome deletion varies among the studies: approximately 15% of azoospermic and 5%–10% of oligozoospermics men. The frequency of deletions was reported to be in a range of 0.7%–34.5% in various studies [17].

In the present study, we report the first analysis of Y microdeletions in the Moroccan population. We found that 4 of the 127 infertile Moroccan subjects tested harbored microdeletion in the AZF region (3.15%). In three of the subjects, testicular histology was available and each had SCOS. All the subjects had the entire AZF region deletion and showed an azoospermic phenotype, which is in accordance with the suggestion that deletions in these regions have an adverse prognosis for finding sperm in the testicular biopsies [18]. Our results are similar to the published data; the entire deletions of such AZF region are associated with SCOS and spermatogenic arrest [4, 19]. The deletions found in the present study concern the AZFc and AZFb + AZFc regions. No deletions were found in the AZFa region.

The frequency of AZF deletions in severe oligozoospermia was found to be lower than those in azoospermia. In OATS subjects, we did not find any deletion; this could be due to our sample size of subjects with sperm concentration < 1 million sperm/mL. In the literature, the vast majority of deletions were found in azoospermic men with deletion frequencies up to 15% [4]. In men with sperm concentration < 5 million, microdeletions were found sporadically [20], and in moderate oligospermia the deletions were rare.

Several partial AZFc deletions have been described in earlier studies [11]. These deletions result in the absence of several AZFc genes and in the case of the gr/gr deletion, it has been suggested to be an important genetic risk factor for spermatogenic failure [10, 11]. Using a PCR approach, we found the types of partial deletions, the gr/gr deletion and the *b2/b3* deletion, in our study population. The gr/gr deletions were present in both controls (4%) and infertile (4%) men at similar frequencies. This result is similar to several studies that have not detected an association between the *gr/gr* deletions and reduced sperm counts [13].

The *b2/b3* deletion was found only in two infertile men with azoospermia (2/149). This observation is not statistically significant: the Fisher's test was performed and the *P*-value was 0.2125. This data is supported by several studies that have reported *b2/b3* deletion in normospermic individuals [12, 14]. Repping *et al.* [14] reported that the b2/b3 deletion seems to have no or little effect on fertility, probably representing common polymorphism almost exclusively associated with some Y chromosome haplogroup. Although a strong correlation exists between classical AZFc deletions and spermatogenic failure, there is no evidence of a correlation between phenotype and genotype in the case of the gr/gr and b2/b3 deletions in our population. All individuals that carried an AZF deletion or a partial AZFc deletion belonged to haplogroup E3b2. This observation is not surprising because the E3b2 haplogroup is found at high frequencies (approximately 60%) in Moroccan men [15].

Y chromosome AZF microdeletions were found at a frequency of 3.15% in our population and consist of AZFc or AZFb + AZFc deletions. In the partial AZFc deletions, we found gr/gr deletions in infertile men and control groups, which suggests that this deletion may not be sufficient for spermatogenic failure in Moroccan men. The b2/b3 was only observed in azoospermic men and it is possible to have an effect on spermatogenesis.

Acknowledgment

Authors appreciate the kind participation of the

patients. We are grateful to all members of the IVF Centre and private laboratories of Morocco for their contributions to this work.

References

1 Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, *et al*. Microdeletions in the Y chromosome of infertile men. N Engl J Med 1997; 336: 534–9.

2 Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Lessing JB, *et al*. Genetic evaluation of infertile men. Hum Reprod 1999; 14: 33–8.

3 World Health Organization. Environmental Health Criteria 225. Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. Geneva: World Health Organization, 2001.

4 Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod 2000; 15:1431–4.

5 Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976; 34: 119–24.

6 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.

7 Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, *et al*. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001; 29: 279–86.

8 Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, *et al*. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003; 423: 825–37.

9 McElreavey K, Krausz C, Bishop CE. The human Y chromosome and male infertility. Results Probl Cell Differ 2000; 28: 211–32.

10 Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, *et al*. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 2003; 35: 247–51.

11 Ferlin A, Tessari A, Ganz F, Marchina E, Barlati S, Garolla A, *et al*. Association of partial AZFc region deletions with spermatogenic impairment and male infertility. J Med Genet 2005; 42: 209–13.

12 Giachini C, Guarducci E, Longepied G, Degl'Innocenti S, Becherini L, Forti G, *et al*. The gr/gr deletion(s): a new genetic test in male infertility? J Med Genet 2005; 42: 497–502.

13 Machev N, Saut N, Longepied G, Terriou P, Navarro A, Levy N, *et al*. Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. J Med Genet 2004; 41: 814–25.

14 Repping S, van Daalen SK, Korver CM, Brown LG, Marszalek JD, Gianotten J, *et al*. A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. Genomics 2004; 83: 1046–52.

15 Arredi B, Poloni ES, Paracchini S, Zerjal T, Fathallah DM, Makrelouf M, *et al*. A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa. Am J Hum Genet 2004; 75: 338–45.

16 Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996; 347: 1290–3.

17 Foresta C, Bettella A, Moro E, Roverato A, Merico M, Ferlin A. Sertoli cell function in infertile patients with and without microdeletions of the azoospermia factors on the Y chromosome long arm. J Clin Endocrinol Metab 2001; 86: 2414–9.

18 Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 2003; 18: 1660–5.

19 Kamp C, Huellen K, Fernandes S, Sousa M, Schlegel PN, Mielnik A, *et al*. High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. Mol Hum Reprod 2001; 7: 987–94.

20 Foresta C, Ferlin A, Moro E, Marin P, Rossi A, Scandellari C. Microdeletion of chromosome Y in male infertility: role of the DAZ gene. Ann Ital Med Int 2001; 16: 82–92.

Edited by Aleksander Giwercman