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GENETICS

# Clinical data for 185 infertile Iranian men with Y-chromosome microdeletion

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**Abstract**
*Abstract summary* Detection of Y-chromosome microdeletion is useful to obtain reliable genetic information for assisted reproductive techniques, thus avoiding unnecessary treatment and vertical transmission of genetic defects.
*Purposes* This research was conducted over a six-year period to analyze clinical data, somatic cytogenetic abnormalities, and types of microdeletions in men with fertility disorders in Iran.
*Methods and Patients* A total of 3654 infertile men were included in this study. Semen samples were analyzed according to standard methods. Conventional chromosomal karyotyping was used to analyze chromosome abnormalities. Polymerase chain reaction (PCR) amplification using nine specific sequence-tagged sites (STS) was used to detect AZF microdeletions.
*Results* Out of the 3654 patients who were analyzed, AZF region microdeletions were detected in 185 cases (5.06 %). Karyotype analysis was available for 157 men and among them abnormal karyotypes were found in 51 cases (32.48 %). One hundred and forty-seven cases with Yq microdeletions suffered from azoospermia and 38 from severe oligozoospermia. Our data show that the most frequent microdeletions were in the AZFc region, followed by the AZFb+c+d, AZFb+c, AZFb, AZFa, and AZF a+c regions.
*Conclusion* The study has confirmed that the detection of microdeletions in the **AZF** region is significant from a diagnostic viewpoint. It is also useful to obtain reliable genetic information from infertile men to determine the etiology of the deletions, and to avoid unnecessary treatments and vertical transmission of genetic defects.

**Keywords** Y-chromosome microdeletion · AZF · Male infertility · ART

*Capsule* It is essential to investigate the frequency and types of both major cytogenetic abnormalities and AZF microdeletions of infertile males with azoospermia and oligozoospermia to perform appropriate genetic counseling before assisted reproduction techniques.

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## Introduction

Infertility is a major reproductive health problem effecting more than 10 % of men and women of reproductive age around the world. [1]. Infertile couples should be evaluated to determine whether the problem resides in the male partner, the female partner, or both and if assisted reproductive techniques (ART) can help to treat the patient. All infertile men should undergo several semen analyses performed according to the World Health Organization (WHO) manual [2, 3], and infertile women, in addition to all other examinations, levels of

testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) should be measured.

It is estimated that approximately 50 % of infertility cases are due to an infertility factor in the male partner [4]. Evidence for direct involvement of the long arm of the Y-chromosome in male infertility was first established by cytogenetic analysis [5]. The azoospermia factor (AZF) locus, which contains one or more genes necessary for normal spermatogenesis and thus fertility have been mapped to deletions on the long arm of the Y chromosome (Yq-interval 6). Yq microdeletions are responsible for most cases of spermatogenic failure in men with azoospermia or severe oligozoospermia [6, 7]. In such cases a full karyotype test should be performed followed by a Yq microdeletions test. It is clear that men with large deletions of Yq are infertile but large deletions of the Yq-chromosome are rarely found. Several investigations have shown that 10-15 % of infertile men with azoospermia or severe oligozoospermia have small deletions of the Yq-chromosome, which are not detectable in a karyotype test [8]. A study performed on 3073 infertile men found that Yq microdeletions were detected in 5.5 % of patients who suffered from oligozoospermia and in 8.3 % of those suffering from non-obstructive azoospermia [9]. Yq microdeletions rarely occur in fertile men and men with sperm densities greater than $5\times10^6$/ml.

This study aimed to determine the incidence of Y chromosome AZF region microdeletions and their transmission to the sons of patients with azoospermia or severe oligozoospermia referred to our institute in Tehran, Iran. We analyzed Y-microdeletions in the AZFa, AZFb, and AZFc sub-regions using sequence-tagged sites (STS) using the multiplex polymerase chain reaction (PCR) technique.

## Patients and methods

The men included in this study underwent infertility treatments between the years 2005–2011 at the Royan Infertility Clinic (Tehran-Iran). Patients were found to carry the Yq microdeletion. Semen samples were analyzed and the sperm concentration, morphology and motility was measured according to the WHO guidelines [2]. The criteria for AZF deletion screening were as follows: inconspicuous family history of fertility, small testis with a soft consistency, raised levels of FSH together with normal levels of LH and testosterone, zero sperm count (azoospermia) or less than $2\times10^6$/ml ejaculate (severe oligozoospermia), without the consideration of any abnormalities in morphology and motility. Some patients with azoospermia or oligoa-astheno-teratozoospermia (OAT) went under testicular biopsy and we performed a comparative study between their spermatogenic activity and the result of the AZF deletion test.

### Yq microdeletion screening

Genomic DNA was extracted from peripheral blood samples using the Genomic DNA Extraction Kit (Bioneer, Korea). Detection of microdeletions on the Y-chromosome was based on three multiplex PCRs [10, 11]. As summarized in Table 1, there are nine STS amplified in three multiplex PCRs. PCR products were run by electrophoresis on a 3.3 %

**Table 1** Primer sequences of multiplex PCR

| STS name | Sequence 5′—3′ | Tm (°C) | Size (bp) | Region | Multiplx PCR Mix |
|---|---|---|---|---|---|
| SY157 | CTTAGGAAAAAGTGAAGCCG<br>CCTGCTGTCAGCAAGATACA | 52 | 285 | AZF c | A |
| SY154 | TTTGCACCAGGATTAAGTGA<br>TTTTTTCAGATAAACTTTCAGTGG | 52 | 245 | AZF c | A |
| SY142 | AGCTTCTATTCGAGGGCTTC<br>CTCTCTGCAATCCCTGACAT | 52 | 196 | AZF b | A |
| SY84 | AGAAGGGTCTGAAAGCAGGT<br>GCCTACTACCTGGAGGCTTC | 61 | 326 | AZF a | B |
| SY83 | CTTGAATCAAAGAAGGCCCT<br>CAATTTGGTTTGGCTGACAT | 61 | 275 | AZF a | B |
| SY158 | CTCAGAAGTCCTCCTAATAGTTCC<br>ACAGTGGTTTGTAGCGGGTA | 61 | 231 | AZF c | B |
| SY14 | GAATATTCCCGCTCTCCGGA<br>GCTGGTGCTCCATTCTTGAG | 61 | 472 | SRY | C |
| SY254 | GGGTGTTACCAGAAGGCAAA<br>GAACCGTATCTACCAAAGCAGC | 61 | 380 | AZF c | C |
| SY134 | GTCTGCCTCACCATAAAACG<br>ACCACTGCCAAAACTTTCAA | 61 | 301 | AZF b | C |

agarose gel, stained with ethidium bromide and visualized under UV light. We studied two to four STS markers for each AZF region to cover the sparse region of the Yq-chromosome. Each analyzed deletion was verified by repeating the PCR experiments.

### Couple information and genetic counseling

Genetic counseling is proposed when an Yq-chromosome is detected. Patients are informed of their genetic problem and its probable effect on both spermatogenesis and the predicted success rate of sperm retrieval during testis biopsy in the case of azoospermia patients. Genetic counselors would also explain the risk of transmitting infertility due to Yq-microdeletions to male progeny conceived using an intra-cytoplasmic sperm injection (ICSI). Some patients prefer to select the sex using pre-implantation genetic diagnosis (PGD) to prevent having an infertile male child [12], but we don't encourage them.

### Testicular sperm extraction and the ART procedure

After semen analysis, patients suffering from azoospermia were offered the possibility of undergoing a testis biopsy to recover any spermatozoa suitable for ICSI. Testis biopsy samples were sent to the laboratory for sperm extraction. The methodology for ovulation induction, trans-vaginal retrieval of oocytes and spermatozoal microinjection has been described previously [13].

## Results

### Patients

Of the 3654 infertile men referred to the Genetics laboratory at the Royan institute (Tehran-Iran), a total of 185 (5.06 %) men with AZF deletion were selected to take part in the study. The patients mean age was 34.65±6.98 years (range: 21–66 years) at the time of inclusion and all had primary infertility. The different STS deletion patterns and the number of patients showing these patterns are shown in Table 2.

### Semen analysis

This study revealed that 147 (79.46 %) of the cases with Yq microdeletions had azoospermia and 38 (20.54 %) had severe oligozoospermia. All men with AZFa, AZFb, AZFa+c, AZFb+c and Yq deletions were azoospermic, thus they were confirmed to have a non-obstructive etiology based on standard clinical evaluations.

The only patients with evidence of sperm production were those with isolated AZFc and partial AZF deletions. However, 64.2 % (61/95) of men with isolated AZFc deletions were azoospermic and only 35.8 % (34/95) of the cases sustained a level of spermatogenesis sufficient to produce sperm within their ejaculate.

Rare spermatozoa could only be observed after centrifugation of the total ejaculate for 8 (4.32 %) patients who were classified as cryptoazoospermic and 23 (12.43 %) other

**Table 2** Different sequence-tagged sites (STS), AZF regions and the different deletion pattern of all patients in this study. '-' denotes deletion of specific STS. The column on the left shows the number of patients in this study with the deletion pattern indicated

| % of Patients | SY14 (SRY) | AZFa | | AZFb | | AZFc | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SY83 | SY84 | SY134 | SY142 | SY154 | SY157 | SY158 | SY254 (DAZ) |
| 10.54 | | | - | | | | | | |
| 31.62 | | | | | - | | | | |
| 10.54 | | | | | | | | | - |
| 42.16 | | - | - | | | | | | |
| 84.32 | | | | - | - | | | | |
| 10.54 | | | | | | | - | - | |
| 10.54 | | | | | | | - | - | - |
| 42.16 | | | | - | - | | | | - |
| 31.62 | | | | - | - | - | | | - |
| 51.35 | | | | | | - | - | - | - |
| 31.62 | | | | | - | - | - | - | - |
| 21.05 | | | | - | | - | - | - | - |
| 15.13 | | | | - | - | - | - | - | - |
| 10.54 | - | | | - | - | - | - | - | - |
| 10.54 | | - | - | | | - | - | - | |
| 15.13 | | - | - | - | - | - | - | - | - |
| 10.54 | - | - | - | - | - | - | - | - | - |

cases were diagnosed with severe oligozoospermia (sperm concentration $\leq 10^6$/mL). Sperm concentrations $>1\times10^6$/mL were found in only 7 (3.78 %) men (Table 3).

### Y microdeletion definition

Most Y microdeletions (95 of 185, 51.35 %) were found in the AZFc regions while AZFa regions were deleted in only 4 (2.16 %) and AZFb regions were deleted in only 8 cases (4.32 %). Combined deletions that included AZFac and AZFbc were also detected in 1 (0.54 %), and 29 (15.67 %) of the patients respectively. Further distribution of these patients by specific deletion is shown in Table 3. In the case of an isolated AZFc deletion ($n$=95), all AZFc markers (sY154, sY157, sY158 and sY254) were removed. There were 26 patients (27.36 %) with AZFc deletions who had oligospermia in their ejaculate, whereas 8 cases were cryptoazoospermic and 61 azoospermic. In patients with an AZFc deletion, the average age of azoospermic patients was 35.10±7.7 years compared with 32.55±4.88 for oligospermic patients.

AZFb deletion was also detected in 65 patients. All 8 patients with isolated AZFb deletions were azoospermic (Table 3). A deletion of AZFa alone was found in 4 cases; and an AZFa partial deletion was detected in 1 patient where only the sY84 STS were deleted.

### Karyotype analysis

Karyotype analysis was available for 157 (84.9 %) of the 185 male patients. Abnormal karyotypes were found in 51 cases (32.48 %) of which 20 cases (41.17 %) demonstrated the disorder 46,XX SRY-positive male syndrome. All abnormal karyotypes, with one exception, were azoospermic. Except in two patients, the karyotype abnormality involved sex chromosomes either numerically or structurally. The results and details of patients' karyotypes are summarized in Table 4. Abnormal karyotypes may also have existed in the 28 men without karyotype results.

### ART enrollment of couples

ICSI was offered to 17 couples (9.18 %) and at the time of publishing this article, two patients (11.76 %) successfully had a male child. However, genetic testing has not yet been performed on these newborns.

Of the 17 patients who went through ICSI, 15 male patients had AZFc deletions, one had an AZFc partial deletion, and the other was diagnosed with an AZFa partial deletion. From the spermogram aspect, three patients were azoospermic, one cryptoazoospermic and 13 oligozoospermic. All 17 men had a normal karyotype except for one patient who showed abnormalities in chromosomes 13 and 15 (Table 4). The average age of those 17 men who underwent ICSI was 37.06±6.30 years (range: 27–48 years), and the average age of their female partners were 32.4± 5.79 years (range: 24–41 years). None of the female partners were infertile. A total of 27 ICSI cycles with either testicular ($n$=3) or ejaculated spermatozoa ($n$=24) were carried out. Preimplantation genetic diagnosis (PGD) was performed on six cycles (22.22 %) for sex selection.

## Discussion

Lifestyle and interactions between somatic and sex chromosome genes, contribute to spermatogenesis [14]. Most cases of severe spermatogenic failure occur as a result of Yq-

**Table 3** Total number of patients in each deletion group, previous history of Varicocele and sperm concentration according to the nature of the Y deletion in 185 Y-deleted men

| Deletion site | | Total No. of patients | History of Varicocele | | | Sperm concentration ($\times10^6$/mL) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Yes | No | NA[a] | 0 | >0 and ≤1 | >1 |
| AZFa | | 4 (2.16 %) | 1 | 2 | 1 | 4 | 0 | 0 |
| AZFb | | 8 (4.32 %) | 1 | 6 | 1 | 8 | 0 | 0 |
| AZFc | | 95 (51.35 %) | 6 | 85 | 4 | 61 | 27 | 7 |
| AZFa+b | | 0 (0 %) | 0 | 0 | 0 | 0 | 0 | 0 |
| AZFa+c | | 1 (0.54 %) | 0 | 1 | 0 | 1 | 0 | 0 |
| AZFb+c | | 29 (15.67 %) | 2 | 25 | 2 | 29 | 0 | 0 |
| AZF partials | Partial a | 1 (0.54 %) | 0 | 1 | 0 | 0 | 1 | 0 |
| | Partial b | 3 (1.62 %) | 0 | 3 | 0 | 2 | 1 | 0 |
| | Partial c | 3 (1.62 %) | 1 | 2 | 0 | 1 | 2 | 0 |
| | b+partial c | 7 (3.78 %) | 1 | 6 | 0 | 7 | 0 | 0 |
| | c+partial b | 5 (2.70 %) | 0 | 5 | 0 | 5 | 0 | 0 |
| AZFa+b+c(Yq) | | 29 (15.67 %) | 1 | 27 | 1 | 29 | 0 | 0 |
| Total | | 185 | 13 | 163 | 9 | 147 | 31 | 7 |

[a]Not Available

**Table 4** Overview of all patients karyotype details according to the nature of the Y deletion in 185 Y-deleted men

| Deletion Site | NA | Kind of Karyotype | |
|---|---|---|---|
| | | Normal | Abnormal |
| AZFa (*n*=4) | 0 | 4 | 0 |
| AZFb (*n*=8) | 1 | 7 | 0 |
| AZFc (*n*=95) | 13 | 79 | 3 45;X[13]/46;X;del(Y)(q11.2)[13]/46;XY[26] (*n*=1)<br>45;X[6]/46:XY[9] (*n*=1)<br>46; XY; 9? (*n*=1) |
| AZF a+c (*n*=1) | 0 | 0 | 46;X;del(Y)(q11.2) (*n*=1) |
| AZF b+c (*n*=29) | 6 | 6 | 17 46;X;del(Y)(q11.2) (*n*=5)<br>45;X[]/46:XY[] (*n*=6)<br>45;X[12]/47;XXY[4]/46:XY[5] (*n*=1)<br>46;X; inv(Y)(p11.2;q12)[]/45;X[] (*n*=2)<br>46;X; inv(Y)(p11.2;q12) (*n*=3) |
| Partial a (*n*=1) | 0 | 0 | 46;XY;dic(13;15)(p11.2 q13) (*n*=1) |
| Partial b (*n*=3) | 1 | 2 | 0 |
| Partial c (*n*=3) | 0 | 2 | 45;X[12]/46:XY[3] (*n*=1) |
| b+Partial c (*n*=7) | 2 | 4 | 46;X;del(Y)(q11.222 q11.23) (*n*=1) |
| c+Partial b (*n*=5) | 3 | 2 | 0 |
| AZF a+b+c (Yq) (*n*=29) | 2 | 0 | 27 46;XX (Sex Reverse) (*n*=20)<br>46;X;del(Y)(q11.2) (*n*=6)<br>45;X (*n*=1) |

*NA* Not Available

chromosome deletions [10]. Intra-chromosomal recombination events between large homologous repetitive sequence blocks, lead to AZF microdeletions [15]. Since 1976, when Tiepolo et al. [5] recognized the relationship between deletions in the long arm of the Y-chromosome and spermatogenic failure, there have been studies of the association between AZF microdeletions and male infertility [16–23]. AZFc microdeletions are now believed to be the most prevalent genetic lesions that cause male infertility [24].

In Western populations the frequency of Y-chromosome microdeletions in men with azoospermia and oligozoospermia varies between 1–35 %, depending on the population studied [25]. Stringent selection of patients according to histologic, endocrinologic, and clinical criteria are believed to be related to the detection of high deletion frequencies [26–28].

In the present study, microdeletions in the AZFc region were the most prevalent (51.35 %), followed by the combination of AZFb/AZFc (15.67 %), AZFb (4.32 %) and AZFa (2.16 %). As mentioned before, in the present study, the AZFc deletion was the most common AZF microdeletion in patients with azoospermia and oligozoospermia; this finding was in agreement with other previous investigations [18, 29–31]. Approximately 1:4000 males (~13 % of azoospermic men and ~6 % of men with severe oligospermia) were found to have microdeletions in the AZFc region [18, 26, 27, 32]. Several candidate fertility genes have been discovered within the AZFc region. It is still not clear why the AZFc deletion is so frequent but it could be caused by repetitive sequences of the genes in this region. It has been suggested that men with AZFc deletions are capable of producing sperm, but some patients do not have any sperm production inside their seminiferous tubules [33]. In several studies patients with AZFc deletions have shown a good prognosis for successful retrieval of sperm during testicular sperm extraction (TESE), whereas patients with deletions in the AZFa and AZFb regions did not [19, 26, 34]. These findings are in agreement with those from this study where AZFa, AZFb, and all AZFb+c deletions were found only in azoospermic males. Some studies suggested a link between a partial deletion in the AZFc region and spermatogenic failure [35, 36]. However other studies have disagreed, proposing it to be simply a polymorphic deletion with no clinical ramification [37].

Some patients with Y-chromosomal microdeletions have also shown a decrease in spermatogenesis with age [38]. However, a study of four AZFc-deleted oligozoospermic men conducted over a period of seven years by Oates et al., reported a fluctuation, but no decrease in the sperm concentration of such patients [29]. These data prove that spermatogenesis does not decline abruptly in males with AZFc-deleted regions and that, like all males, they show a moderate decline over decades [39]. The azoospermic men with positive testis biopsies are usually younger than those with negative results [25, 34]. In this study, no statistical difference was detected between the mean age of oligospermic

and azoospermic patients with AZFc deletions. Even though establishing precise genotype/phenotype correlations in patients with Y-chromosomal microdeletions is difficult deletion of AZFa+b and deletions of more than one region (AZFb +c or AZFa+b+c) result in more severe deleterious consequences for spermatogenesis than deletions limited to AZFc. To our knowledge, completion of spermatogenesis in men with full deletions of the AZFa or AZFb regions has not been demonstrated. The microdeletion of the AZFa region is relatively rare, whereas deletion of the AZFb region is more prevalent, either alone or accompanied by deletion of the AZFc region (Table 2). Although a complete AZFa deletion is reported to be associated with the absence of spermatozoa, there have been cases of spermatozoa retrieval in males with partial AZFa deletions [40]. sY84 has been previously considered as a polymorphic locus of the Y-chromosome not associated with sterility phenotypes in men [41]. This study supports this assumption.

Several deletion patterns involving the AZFb and AZFc regions are evident in Table 2. Although spermatogenesis varies with the deletion of the AZFc region alone, none of the patients with AZFb region deletions, either isolated or accompanied by deletion of the AZFc region, or as part of the Yq deletion, have demonstrated evidence of mature spermatid production. Just one patient with a partial AZFb deletion (sY142) was diagnosed with oligospermia.

Partial deletions within an AZF region are not sufficient for accurate prognosis because in this study some patients with partial deletions within the AZF region demonstrated different levels of sperm production (Table 2).

We observed a high frequency of chromosomal abnormalities (22.6 %) to coexist with Y-chromosome microdeletions in infertile men. In addition, large microdeletions of the Yq tip can cause chromosomal instability and be responsible for chromosomal rearrangements or Y-chromosome loss. A higher frequency of Y-microdeletions has been well demonstrated in patients with sex chromosome mosaicism (45,X/46,XY or 47,XXY/46,XX) [42–44]. However, it is still unknown whether it is karyotypic abnormality or Y-microdeletions that primarily cause spermatogenesis failure in such patients. The 20 men diagnosed with Yq deletion by microdeletion tests have been found to lack the entire Y-chromosome on karyotype analysis. We did not observe translocations of Yq regions to other chromosomes in this study (Table 3).

In conclusion, deletions of the AZFa, AZFb and AZFc regions are associated with abnormal spermatogenesis and although certain deletions have a detrimental effect on sperm retrieval, the majority of men with AZFc deletions have no problems during sperm retrieval for use in ART. However, men with these deletion(s) may optionally be subjected to further evaluations with more STS markers to determine the extent of the effects of such deletions. This will help decrease the cost and technical difficulty of the procedure and allow for more widespread use of Y-chromosome microdeletion screening in infertility clinics. The high prevalence of Y-chromosome deletions in infertile men and its potential transmission to future offspring make screening prior to ICSI an important part of the work-up for assisting the reproduction of infertile men.

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