Y chromosome microdeletions in infertile Moroccan males: 10 years laboratory experience in AZF deletions

Brahim EL HOUATE, Zouhair ELKARHAT, Hicham CHAROUTE, Houda HARMAK, Salaheddine REDOUANE, Abdelhamid BARAKAT, and Hassan ROUBA

1 Higher Institute of Nursing and Health Techniques, Ouarzazate, Morocco
2 Laboratory of Human Genetics, Department of Scientific Research, Pasteur Institute, Casablanca, Morocco.

*Corresponding Author E-mail: hassan.rouba@pasteur.ma

SUMMARY

Genetic causes of male infertility are abnormalities in chromosome numbers and/or structures, Y-chromosome deletions and gene mutations. Genetic screening of male infertility is rarely done in our country.

The purpose of the study was to investigate the frequencies and types of Y chromosome microdeletions in infertile men, based on studies done in the Human Genetics Laboratory of the Pasteur Institute in Morocco.

A total of 543 infertile men were screened for Y chromosome microdeletions. The prevalence of AZF Y-chromosome microdeletions among infertile men range from 3% to 10% depending on patients selected. The most frequent microdeletions were detected in the AZFc region, followed by AZFbc, AZFb, AZFa, AZFab. These results indicate the need for Y chromosome microdeletion screening for better management of infertile patients.

We hope to encourage use of genetic diagnosis and also research in this field to initiate collaboration for clinical management and appropriate genetic diagnosis and counselling for male infertility.

Introduction

Male infertility is a complex multifactorial pathology with a very heterogeneous phenotype. Male infertility has generally been defined as men reporting the experience of infertility (usually >12 months in duration). A simplistic and frequently used approach to assessing male infertility has been to examine sperm parameters and determine the frequency of sperm abnormalities (Cooper et al., 2010; Virtanen et al., 2017).

Alterations in spermatogenesis are evaluated for abnormalities ranging from complete absence of sperm in the testes to distinct sperm alterations (Zegers-
The causes of Male infertility are many and varied, including accidental causes, congenital malformations, functional deficiencies, pollutants, or genetic factors. Alongside these main mechanisms, there remain so-called "idiopathic" male infertilities where the etiology is difficult to identify with current diagnostic tools.

The most common genetic abnormalities in male infertility are abnormalities in chromosome numbers and/or structures, Y-chromosome deletions and ultimately gene mutations. There are several genes on the Y chromosome that are necessary for spermatogenesis. Tiepolo and Zuffardi in 1976 identified deletions of the long arm of the Y chromosome associated with abnormalities in spermatogenesis (Tiepolo and Zuffardi, 1976). Subsequently, these deletions were characterized in the long arm of the Y chromosome and designated as AZoospermia factors (AZFa, AZFb and AZFc) Figure. These regions contain several genes or families of genes that are expressed in the testes and involved in spermatogenesis (Pryor et al., 1997).

Between 15 and 20 genes essential for spermatogenesis are located on the non-recombinant portion of the long arm of the Y chromosome. They are divided into 2 categories, on the one hand the ubiquitous genes that have a homologue on the X chromosome (DFFRY, DBY, SCMY) and on the other hand the so-called "testicular" genes that are specific (DAZ, RBMY, CDY, BPY, PRY) (Skaletsky et al., 2003).

After Klinefelter's syndrome, microdeletions of the Y chromosome are the second most frequent genetic cause of male infertility (Hawksworth et al., 2018). The frequencies of Y-chromosome microdeletions differ within populations and shows heterogeneity and this may be due to several factors such as the choice of inclusion criteria, the STS markers tested or the ethnicity of the population (Flannigan and Schlegel, 2017a; Krausz et al., 2014a).

Men with azoospermia are most likely to be carriers Yq microdeletions and this risk decreases progressively with the increase in the quantitative and qualitative quality of the semen (Krausz and Riera-Escamilla, 2018). Currently, with the development of assisted reproduction technologies, the diagnosis of a genetic cause of infertility is more important than ever. In fact, karyotype analysis, screening for Y chromosome microdeletions in the AZF region, analysis and screening for mutations in candidate genes are part of the management and diagnostic follow-up of male infertility.

We have carried studies on the genetic causes of male infertility in the Moroccan population. We have analyzed chromosomal abnormalities, deletions of the AZF (Azoospermia factor) region, partial deletions of the AZFc region (gr/gr, b2/b3...) and mutations of genes involved in spermatogenesis (Protamine, MTHFR, Aurokinase, USP26) (Eloualid et al., 2014; Imken et al., 2009; Ravel et al., 2006). In this study we present the cumulative analyze of AZF Y-chromosome microdeletions among infertile Moroccan men and discuss the relevance of this deletions to phenotype in our population.

**Patients and methods**

**Patients**

We retrospectively analyzed 543 infertile men, screened for Y-chromosome microdeletions between 2006 and 2015. All patients included were Moroccan. More detail about patients is included in previous studies (Eloualid et al., 2012; Imken et al., 2007; Naasse et al., 2015). All studies were approved by local ethical committee, Pasteur institute, in accordance with the declaration of Helsinki standard and all subjects gave written informed consent.
Methods

Yq microdeletions screening

In all the studies performed in our laboratory we followed the EAA “European Academy of Andrology” guidelines recommendation by testing the set of six sequence-tagged sites (STS) markers covering the AZFa (sY84 and sY86), a multiplex PCR protocol described in all published results (Eloualid et al., 2012; Imken et al., 2007; Naasse et al., 2015). This set of markers can detect almost all (over 95%) clinically important deletions in the AZF region. AZFb (sY127 and sY134), and AZFc (sY254 and sY255) regions. Yq microdeletions analysis were performed using...

Results and discussion

The frequency of AZF deletions in the Moroccan population

We found that the prevalence of Yq microdeletions was in the range of frequencies already described in other studies, namely 18.83%, 3.15% and 5.63 % (Table 1). The difference observed in these studies can be explained by the number of patients analyzed and also the phenotype included in studies. We observed the highest Yq microdeletions frequency in patients with a sperm count below $5 \times 10^6$ sperm/ml (Naasse et al., 2015). In fact, the count of the sperm is inversely proportional to the presence of genetic abnormalities (Krausz et al., 2014b).

Considering the accumulated patients included in the studies conducted in our laboratory, 198 infertile patients with azoospermia and 345 patients with oligospermia were analyzed.

The frequency of Y chromosome deletions in the azoospermic patients in the Moroccan population was 10%. This frequency is consistent with the previous studies in many Mediterranean and North African geographically related studies (Table2). For oligospermic patients we have analyzed 345 individuals. The prevalence of Y microdeletion was 3.47 % and most of them had a sperm count below $5 \times 10^6$ sperm/ml. This frequency indicates the value of systematic screening for these deletions in patients with azoospermia and severe oligospermia.

Table 1: AZF deletions in Moroccan patients

<table>
<thead>
<tr>
<th>Studies</th>
<th>AZF deletions</th>
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<tbody>
<tr>
<td></td>
<td>Azoospermia % (n/ N)</td>
</tr>
<tr>
<td>(Naasse et al., 2015)</td>
<td>14.5 % (9/62)</td>
</tr>
<tr>
<td>(Eloualid et al., 2012)</td>
<td>9.09 % (8/88)</td>
</tr>
<tr>
<td>(Imken et al., 2007)</td>
<td>6.25 % (4/48)</td>
</tr>
<tr>
<td>Total</td>
<td>10.6% (21/198)</td>
</tr>
</tbody>
</table>

Phenotype associated with AZF deletions

The nature of the deletions appears to have phenotypic impacts depending on the genes affected and also on the management of patients during assisted human reproduction (Flannigan and Schlegel, 2017b; Hotaling and Carrell, 2014).

AZFc are the most frequent deletions found in infertile men. They are associated to variable phenotype, from azoospermia to mild oligozoospermia (Table3). 22/543 patients presented with AZFc deletion. The fact that this
deletion can be transmitted, show the diagnostic and preventive values of screening of AZFc deletions. AZFbc was found in 11 subjects analyzed in our studies. 10 deletions resulted in impaired spermatogenesis (azoospermia) and 1 patient with oligo-terato-asthenozoospermia. AZFbc deletion is associated with severe phenotype with no chance to retrieve mature sperm upon testicular sperm extraction.

The complete deletion of AZFa region blocks the production and maturation of germ cells in the seminiferous tubule. 2 patients with AZFa were found in all studies. Patient carrying this deletion has no chance to retrieve mature testicular sperm for the use in assisted techniques (IVF/ICSI).

Table 2: Frequency of AZF deletions in some populations compared to the Moroccan population

<table>
<thead>
<tr>
<th>Authors</th>
<th>Regions</th>
<th>Prevalenceof AZFdeletions %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Azoospermia</td>
<td>SevereOligospermia</td>
</tr>
<tr>
<td>National studies</td>
<td>Morocco</td>
<td>10.6 % (21/198)</td>
<td>3.47 % (12/345)</td>
</tr>
<tr>
<td>(Gumus et al., 2021)</td>
<td>Turkey</td>
<td>4.9% (16/327)</td>
<td></td>
</tr>
<tr>
<td>(Amouri et al., 2014)</td>
<td>Tunisia</td>
<td>14.10 % (46/328)</td>
<td>4.05 % (6/148)</td>
</tr>
<tr>
<td>(Chellat et al., 2013)</td>
<td>Algeria</td>
<td>2 % (1/49)</td>
<td>-</td>
</tr>
<tr>
<td>(Ferlin et al., 2007)</td>
<td>Italy</td>
<td>8.3% (52/625)</td>
<td>5.5 % (45/811)</td>
</tr>
</tbody>
</table>

Without consensus guidelines, authors recommend screening for Yqmicodeletion with a variable threshold of sperm counts. (Flannigan and Schlegel, 2017b; Krausz et al., 2014b; McLachlan and O’Bryan, 2010). We believe that screening AZF deletions in azoospermia and severe oligospermia (< 2.10^6 spz/ml) in the Moroccan populations should be indicated for routine Y-chromosome microdeletion screening. The genetic diagnosis integrated into the fertility check-up, will reduce medical wandering and accompany the care of couples in desire for children. The identification of genetic abnormalities could have a prognostic value of testicular sperm to be extracted for couples undergoing assisted reproduction attempts (ICSI and IVF). In fact, identifying the genetic cause of male infertility in a subject allows:

1- to better choose the appropriate assisted reproduction technique,
2- to offer appropriate genetic counseling and the risk of transmission of these genetic defects to future generations.
3- to get a better patient management to quickly guide the clinician on the right course of action and avoid unprofitable treatments and follow-ups.

Conclusion

The work carried out on the genetic component of infertility in Morocco at the Human Genetics Laboratory at the Pasteur Institute of Morocco has allowed us to clarify a number of facts concerning the genetic causes of male infertility.

Indeed, the identification of genetic causes and their
frequency in patients with male infertility is of importance for diagnostic management. Karyotype analysis and Y-chromosome microdeletions are tests recommended for all patients with azoospermia or severe oligospermia (< $2.10^6$ spz/ml). In the near future, the identification of genes associated with infertility is likely to help open future paths in the search for better management of men infertility.

Table 3: Phenotype associated with variable AZF deletions in the Moroccan patients analyzed

<table>
<thead>
<tr>
<th>AZF Deletions</th>
<th>N(%)</th>
<th>Phenotype associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZFc</td>
<td>12</td>
<td>Azoospermia</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Oligo–terato-asthénospermia</td>
</tr>
<tr>
<td>AZFbc</td>
<td>10</td>
<td>Azoospermia</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Oligo–terato-asthénospermia</td>
</tr>
<tr>
<td>AZFa</td>
<td>1</td>
<td>Azoospermia</td>
</tr>
<tr>
<td>AZF a+b</td>
<td>2</td>
<td>Azoospermia</td>
</tr>
</tbody>
</table>

Conflicts of Interest:

There is no conflict of interests

References


