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Azoospermia factor (AZF) and male infertility

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Recently, work has shown that azoospermia factor (AZF) microdeletions result from homologous recombination between almost identical blocks in this gene region. These microdeletions in the Y chromosome are a common molecular genetic cause of spermatogenetic failure leading to male infertility. After completion of the sequencing of the Y chromosome, the classical definition of AZFa, AZFb, and AZFc was modified to five regions, namely AZFa, P5/proximal-P1, P5/distal-P1, P4/distal-P1, and AZFc, as a result of the determination of Y chromosomal structure. Moreover, partial AZFc deletions have also been reported, resulting from recombination in their sub-ampliconic identical pair sequences. These deletions are also implicated in a possible association with Y chromosome haplogroups. In this review, we address Y chromosomal complexity and the modified categories of the AZF deletions. Recognition of the association of Y deletions with male infertility has implications for the diagnosis, treatment, and genetic counseling of infertile men, in particular candidates for intracytoplasmic sperm injection.

Keywords: AZF, intrachromosomal recombination, male infertility, palindrome, Y chromosome
Introduction

Infertility affects about 10% of couples, and a genetic basis of infertility may exist in many men currently classified as having idiopathic infertility. In fact, in about 15% of cases, an unknown cause of male infertility could be present, including chromosome aberrations and alterations at the gene level. Approximately 7% of infertile men harbor microdeletions of the Y chromosome that are not detectable on routine karyotype analyses [1]. Cytogenetic studies in infertile men have revealed a gene that controls spermatogenesis, designated as azoospermia factor (AZF), localized on the long arm of the Y chromosome [2]. The presence of three spermatogenesis loci in Yq11 was initially proposed, namely AZFa, AZFb, and AZFc [3]. These microdeletions of AZF are now recognized as the second most frequent genetic cause of spermatogenetic failure in infertile men after Klinefelter syndrome [4], and deletions in the AZF regions are the most common known molecular genetic cause of human male infertility involving spermatogenic failure [5]. Thus, the molecular diagnosis of Y chromosomal microdeletions is routinely performed worldwide in the workup of male infertility in men with azoospermia or severe oligozoospermia.

The complete sequencing of the Y chromosome revealed its structure and organization. In particular, it was shown that most AZF microdeletions result from intrachromosomal homologous recombination between repeated sequence blocks organized into palindromic structures in the long arm of the Y chromosome. The greater understanding of Y chromosome structure led to some reclassification of AZF microdeletions into five categories, and further work has identified a further set of partial deletions in AZFc.

In the clinical field, great progress has been made in the last 15 years or so with respect to assisted reproductive techniques. Among these, intracytoplasmic sperm
injection (ICSI) is a leading method of treatment for male factor infertility. However, one risk consists in a potential increase in the genetic causes of infertility in the future; thus, identification of genetic factors has become good practice for appropriate management of infertile couples, and genetic testing for infertile men has increased in importance in the reproductive clinic.

In this review, we discuss the complexity of the human Y chromosome and the change in how AZF deletions are categorized. Finally, we analyze Y chromosome microdeletions possibly associated with male infertility in a Japanese population.

**The Y chromosome**

The completion of the sequencing of the Y chromosome as part of the Human Genome Project revealed a relatively low number of functional genes but a high frequency of repeat elements (Fig. 1)[6,7]. There are two pseudoautosomal regions (PAR) on the short (Yp) and long (Yq) arms of the Y chromosome, respectively, where crossing over occurs in meiosis. However, no other part of the Y chromosome crosses over with the X chromosome in meiotic recombination, thus leaving about 95% of the human Y as non-recombining [8]. The euchromatic and heterochromatic regions lie between the PARs. The euchromatic region contains nucleotides of about 24 Mb, consisting of 8 Mb in the Yp and 15 Mb in the Yq. The heterochromatic region consists of about 1 Mb in the centromere and approximately 40 Mb in the distal portion of the long arm. The euchromatic and heterochromatic regions are independent from the X chromosome and designated as male-specific regions of the Y chromosome (MSY). Therefore, MSY does not recombine with the X chromosome and is transmitted from father to son, and the
lack of recombination between X and Y chromosomes was thought to be responsible for
the decay of Y-linked genes [9].

Depending on the origins of its sequences, the MSY can be classified into three
regions, X-transposed, X-degenerate, and ampliconic sequences. X-transposed and X-
degenerate regions are characterized by sequences with 99% identity to the X
chromosome and with single-copy genes or pseudogene homologues of X-linked genes,
respectively. Furthermore, the ampliconic sequences, which are Y-specific sequences
and represent 45% of the euchromatic MSY, are arranged in direct and inverted repeats,
including eight major palindromes in which sequences having higher than 99.9%
homology are present in pairs. These eight palindromes comprise 5.7 Mb, or one-
quarter of the MSY euchromatin, and harbor several distinct gene families unique to the
Yq. In addition, frequent gene conversion has been thought to prevent the progressive
decay of the Y chromosome over time [10].

Genes on the Y chromosome

The Y chromosome contains over genes and many testis-specific transcripts, and several
deletions have been described that remove some of these transcripts, causing
spermatogenic failure. The identified genes have been made available online with
symbol, aliases, accession ID, and cytogenetic map position [11]. Recent work on the Y
chromosome has added even more information, available in another online database
[12]. From the MSY, 18 distinct protein or 9 gene families have been identified.
Interestingly, the majority of testis-specific genes are present in multiple copies ranging
from one (TGIF2LY) to two (VCK, XKRY, HSFY, PRY) to three (BPY2) to four (CDY,
DAZ) to six (RBMY) to approximately 35 (TSPY) on the Y chromosome. These genes
are present in the proximal and distal palindromic complexes encompassing the AZF region [13]. A total of 23 testis-specific transcripts (TTY1–23) have been described; of these, TTY3, 4, 5, 6, 9, 10, 13, and 14 of the palindromic complex have shown deletions in patients with spermatogenic failure [13]. Screening for such deletions in infertile men is now a standard part of the clinical evaluation. Many other Y-chromosome structural variants, some of which affect gene copy number, have also been investigated recently.

**STS (sequence-tagged sites)-based analysis**

Studies on the structural organization of the chromosome have advanced our understanding of Y chromosomal microdeletions. Large sets of primers encompassing palindromic complexes can also be used for sequence-tagged sites (STS)-based analysis of the genetic integrity of the Y chromosome [10,13].

STS-based markers can be used to screen patient DNA samples to assess the loss or gain of the critical region(s) involved in Y chromosomal microdeletion. Many of these sites have proved to be either repetitive sequences or polymorphic between individuals or races. In general, genomic DNA has a linear and contiguous sequence, and STS is defined as the determination of their unique position within the whole genome. However, after the genomic sequence was fully verified, some of the original STSs were found to have either repetitive or polymorphic sequences. Screening of such a large number of patient DNA samples with a varying spectrum of Y chromosome anomalies is a laborious task [14], but today, reliable STS markers on Y are available online [12].

**Classical AZF**
In clinical terms, particular regions of the MSY are consistently deleted, which is attributed to causes of spermatogenic failure. Indeed, the most well-characterized association of the AZF region seems to be its link to male infertility [15-17].

From an initial observation in 1976 [2], cytogenetic studies in infertile men revealed genes controlling spermatogenesis, localized on the Yq, and the identified region was designated AZF. A number of studies ascertained that microdeletions in the Yq represent the most frequent molecular genetic cause of severe infertility, observed with a prevalence of 5–15% in non-obstructive azoospermia and severe oligozoospermia. Therefore, the AZF region is thought to be essential for spermatogenesis in some part [18,19].

In 1998, a large collaborative screening project involved 370 men with idiopathic azoospermia or severe oligozoospermia who were analyzed for deletions of 76 loci in Yq11, including testis biopsies in patients with deletions in different regions of Yq11. The presence of three spermatogenesis loci in Yq11, which the authors designated as AZFa, AZFb, and AZFc, was proposed (Fig. 2a). Histopathologically, the AZFa defect causes Sertoli-cell–only (SCO) syndrome, AZFb deficiency leads to maturation arrest as observed on the testicular biopsies, and AZFc is responsible for various histopathologic changes [20].

Each region is thought to be rich in various functional genes and transcript units. Individuals with microdeletions on the Yq seem to exhibit spermatogenic failure and infertility [15,21-30]. Interestingly, microdeletions occur in 3–15% of not only azoospermic or oligozoospermic men but also in 2% of fertile men [31]. Some studies have indicated no association between spermatogenesis and candidate genes in the AZFc region [32].
The most common microdeletions occur in the AZFc region, which carries active copies of the DAZ (deleted in azoospermia) gene. Much less common are microdeletions of the AZFa carrying the DFFRY and DBY (dead box on the Y) genes and of the AZFb area carrying the RBM gene [8]. These latter two deletions are more likely to be associated with azoospermia than is deletion of the AZFc region. However, deletion of any or all of the three azoospermia factors—AZFa, AZFb, or AZFc—disrupts spermatogenesis [33,34].

**Recent categories of AZF regions and deletions**

The ampliconic sequences of Y consist of eight major palindromes (P1–P8) in which sequences have higher than 99.9% homology (Fig. 1). These eight palindromes can serve as substrates for structural rearrangements. AZF deletions can result from intrachromosomal recombination events between non-reciprocal homologous sequences, such as palindrome, direct, or inverted sequences in the Yq. Consistent patterns of these rearrangements have led to a reclassification of the AZF microdeletions.

Recently, the mechanism of the AZFb deletions was identified as resulting from homologous recombination between the palindromes P5/proximal P1 [13]. The classical complete deletion of AZFc, the most frequent pattern among men with deletions of the Y chromosome, removes 3.5 Mb and originates from a homologous recombination between blue-amplicons b2 and b4 (see below) in palindromes P3 and P1, respectively (Fig. 3). Deletions of both AZFb and AZFc together occur via two major mechanisms involving homologous recombination between P5 and distal P1. Therefore, five main interstitial deletions have been defined, namely the AZFa, P5/proximal P1, P5/distal P1,
these five deletions share the same deletion mechanism of non-allelic homologous recombination between palindrome pairs.

**Mechanism and type of deletions**

**AZFa deletion**

The proximal and distal regions of the Y chromosome have been found to harbor 10 kb each of the proviral sequences of the HERV15 of endogenous retroviruses that are 94% identical [36,37]. Recombinations between these proviruses have been implicated in most of the AZFa deletions. As noted, these deletions usually lead to SCO syndrome histologically [38-42].

**AZFb deletion**

The P5/proximal-P1 deletion is the result of homologous recombination between the P5 palindrome and the proximal part of the P1 palindrome, which is called a complete AZFb deletion. This recombination removes 6.2 Mb, including 32 genes and transcripts. P5/distal-P1 deletions have breaks in the P5 and P1 palindromes spanning 7.7 Mb, namely the AZFb+c deletion, as classically defined. The P4/distalP1 deletion is also caused by homologous recombination between these palindrome pairs.

Complete deletions of AZFb or AZFb+c lead to azoospermia associated with SCO syndrome or pre-meiotic spermatogenic arrest. Genes in the AZFb region reside in this interval, and most are testis-specific transcripts [43]. In the classical definition of AZFb and AZFc, the proximal end of the AZFc region overlaps with the distal end of AZFb [13].
AZFc deletion

The most frequent AZFc deletion leads to azoospermia or severe oligozoospermia, associated with different spermatogenic phenotypes in the testis. The full AZFc sequence represents 3.5 Mb of the Yq and consists of palindromic repeats (sub-amplicons) that are organized into sequence families (Fig. 3). These sub-ampliconic sequences have levels that are more than 99.9%, making them substrates for structural rearrangements. Five different sub-amplicons (color-coded as blue, green, red, grey, and yellow) map to the reference AZFc sequence, harboring a total of 13 different ampliconic units. Conventional AZFc regions in fact result from recombination between two direct repeats, blue sub-amplicon b2 and b4 (b2/b4) [6].

Genes in the AZFc region

Active copies of four protein-coding gene families map to the AZFc interval: PRY2, BPY2, DAZ, and CDY1 [44-47]. These genes localize to the blue, green, red, and yellow-coded amplicons, respectively, with one transcription unit per amplicon copy. AZFc genes are reported to exhibit germline-specific expression [45,46,48-50]. The complete AZFc deletion, the b2/b4 deletion, removes eight gene families including all members of the DAZ gene family, that represent the foremost candidates for determining the AZFc phenotype [3,6,29,43,51,52]. The complete deletion of AZFc mainly influences azoospermia because of removal of genes and transcripts within the whole AZFc region.

DAZ genes
DAZ belongs to a family of germ-cell–specific RNA-binding proteins that are essential for gametogenesis [51,53]. A two-gene cluster was duplicated, generating a two-cluster/four-gene arrangement (DAZ1/2, DAZ3/4) 1.6 Mb apart within the AZFc region [54,55]. The gr/gr (gr = green-red) deletion may result in the elimination of DAZ1/2 or DAZ3/4 depending on the location of the recombination site within the gr sub-amplicon repeats, with the DAZ1/2 deletion being the most likely if there are no recombination hot spots [56-58].

Partial AZFc deletions

AZFc deletions, including all members of the DAZ gene family, represent the most frequently identified molecular cause of spermatogenic impairment. Based on the mechanism of deletion, a recombination of the AZFa homologous sequence, it was predicted that the AZFc region was prone to two additional deletions, one resulting from recombination between sub-amplicons b1 and b3 (b1/b3), and one resulting from recombination between the sub-amplicon gr complex. Indeed, both deletions, the b1/b3 deletion and the gr/gr deletion, were subsequently identified on the basis of this prediction [13]. These deletions are performed by AZFc-specific STSs, DAZ-specific Sequence family variants (SFV), or gene dosage analysis. The gr/gr removes 1.6 Mb, b1/b3 and b2/b3 remove 1.8 Mb, and others are more infrequent. In spite of abundant gene losses from these deletions, partial deletions of the AZFc region (i.e., b1/b3, b2/b3, and gr/gr deletions) are still controversial issues in terms of whether these events are associated with infertility or not [59].

The gr/gr deletion
Three candidate sub-amplicon recombinations involving g1/g2, r1/r3, or r2/r4 cause the gr/gr deletions (Fig. 3). Analyses thus far have been unable to distinguish which deletions occur. Moreover, following gr/gr deletions, there have been subsequent duplications that again are mediated through homologous recombination between amplicons and that seem to restore gene copy number [35,60].

Identification of a phenotypic association between the gr/gr deletion and spermatogenic impairment has been variously reported depending on populations and countries [56,59-66]. According to Y chromosome haplogroup analysis, the Db2 type occurs primarily in Japan [67] and consists of only gr/gr-deleted chromosomes. The gr/gr deletion removes 1.6 Mb of the AZFc region but does not remove an entire AZFc gene family; instead, it reduces the copy number of five families. These microdeletions could cause reduced sperm production [68,69].

The gr/gr region harbors CDY1, the DAZ family, and several pairs of genes that are divided into combinations of sub-amplicons that occur as four different gene loss types: CDY1a + DAZ1/2, CDY1a + DAZ3/4, CDY1b + DAZ1/2, and CDY1b + DAZ3/4 [66,70]. The DAZ family is expressed in testis. CDY1 encodes the chromodomain histone acetylase transferase, which occurs exclusively in mature spermatids and spermatozoa and may be required in a later stage of spermatogenesis [44,51,71].

The biological function of the CDY and DAZ families is not yet confirmed, but the expression ranges and patterns seem to be highly involved in spermatogenesis. Much research has focused on the deletion frequency and types of CDY and DAZ and the relationship with infertility. Phenotypic abnormalities associated with each deletion subtype are currently being investigated in a European population [70].
Phenotypic diagnosis of the gr/gr deletion has been inconsistent across study populations of different geographic origins, which have shown a great deal of variation compared with phenotypes associated with complete deletion of AZF a, b, and c. In studies using binary markers on the MSY, the Y polymorphism in diverse populations has provided clues to biogeographical ancestry [72]. A few groups have studied the possible association of Y chromosome haplogroups with Yq microdeletions or with particular phenotypes of infertility.

In fact, the correlation of infertility with the frequency and gene loss of the gr/gr deletion differs among Y haplogroups. For instance, haplogroup Q1 has been uniformly revealed to have a gr/gr deletion, and DAZ3/4 copies were deleted in haplogroup N, but without any apparent relevance regarding sperm concentration [57,73]. In contrast, the gr/gr deletion has been associated with infertile males in an Italian population [66]. In the case of haplogroup D, an almost-fixed gr/gr deletion has been identified, for which there is not significant evidence of an association with infertility [63,74]. An absence of a significant association between Y haplogroups and Y microdeletions has been found in a European sample [75] and in a northwestern European sample [76]. Therefore, no conclusions have yet been reached about the role of Y haplogroups in infertility or in association with Y microdeletions.

**Haplogroup D and a Japanese population**

One insertion that is particularly useful in population studies is the Y Alu polymorphism (YAP). The Alu sequence exists as half a million copies in a particular region in human
males in some populations [77]. Therefore, a comprehensive study of the YAP marker can be useful in the context of population dynamics and delineation of major human populations. A YAP-positive result is classified into haplogroup D, in which the almost-fixed presence of gr/gr has been identified. Haplogroup D was present in Japan ~12,000 years ago and today occurs in 34.7% of the Japanese population [78,79]. In contrast, the O lineage started immigrating to Japan only ~2,300 years ago but has spread to include 51.8% of the Japanese Y haplogroup [80]. Following their appearance, these two major haplogroups expanded over the past several centuries. Interestingly, as noted, haplogroup D appears to have been highly susceptible to gr/gr deletion. Generally, haplogroup D was distributed sparsely in northeast Asia; however, it was dispersed among the African, Tibetan, and Japanese populations [77,81].

The frequency of AZF deletion in the Japanese population

We analyzed the frequency of AZF deletions in a Japanese population. Our study involved 952 infertile men visiting the Department of Urology, Kanazawa University Hospital and Center for Reproductive Medicine, Kiba Park clinic. The participants represented 518 cases of azoospermia and 434 of oligozoospermia (sperm count <20 million/mL).

In that study, we described AZF deletions in 952 infertile men. Of these, 32 massive deletions excluding the gr/gr deletion were observed in 518 azoospermic patients. The respective frequencies of the AZFa, P5/proximal-P1, and AZFc (b2/b4) deletions were only 1.2%, 1.2%, and 0.6%. Only 10 AZFc (b2/b4) and nine b2/b3 deletions excluding the gr/gr deletion were observed in 434 oligozoospermic patients (Table 1). The frequency of the classical AZF deletion in azoospermia was only 2.7%
This result indicated that AZF deletions make up a very small proportion in Japan, as expected. According to a previous report, the complete AZFc deletion with a prevalence of 1/4000 in men is responsible for about 10% of azoospermia and 5–7% of severe oligozoospermia [4,82], although complete AZFa and AZFb deletions are less frequent than AZFc deletions.

**ICSI**

Most men with azoospermia or severe oligozoospermia require ICSI (with ejaculated or testicular spermatozoa) to overcome their infertility. Because all spermatozoa from men with Y microdeletions harbor the same microdeletions, ICSI allows the transmission of these genetic changes [83-87]. Male offspring of men with Yq microdeletions will therefore also carry the deletion and will have spermatogenic impairment in adulthood. Transmission of AZF deletions appears not to affect the psychological and physical development of children derived from ICSI [86]. Screening for Y chromosome microdeletions provides crucial information in the counseling of couples seeking infertility treatment.

**Conclusion**

Contrary to expectations, the frequency of AZF deletion in Japanese populations is appear to be relatively small compared with Cocacians. Almost all cause of non-obstructive azoospermia still remains unexplained. However, the importance of examining molecular genetics approach including AZF deletions must be emphasized for these who are considered intracytoplasmic sperm injection, because this genetic defect is transmitted to their sons affecting fertility. Recognition of the association of Y deletions with male infertility has implications for the diagnosis, treatment, and genetic
counseling of infertile men. Furthermore, this information avoids unnecessary
treatments such as hormonal or surgical therapy.

Acknowledgments

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References


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Hammer MF, Horai S: Y chromosomal DNA variation and the peopling of japan.


Shinka T, Tomita K, Toda T, Kotliarova SE, Lee J, Kuroki Y, Jin DK, Tokunaga K, Nakamura H, Nakahori Y: Genetic variations on the y chromosome in the japanese


Fig. 1  Whole Y chromosome structure

Fig. 2  Recent model of AZF deletions

a. Classical categorization.  b. Recent categorization of AZF deletions based on palindrome structure.

Fig. 3 Five sub-amplicons mapped in the AZFc region

Sub-amplicons color-coded as blue(b), green(g), red (r), grey (g), and yellow (yel).
Fig. 1

Deletion pattern size

<table>
<thead>
<tr>
<th></th>
<th>AZFa</th>
<th>AZFb</th>
<th>AZFc</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
<tr>
<td>b</td>
<td>[ ]</td>
<td>[ ]</td>
<td>[ ]</td>
</tr>
</tbody>
</table>

- a
- b

- P5/proximal-P1: 6.2Mb
- P5/distal-P1: 7.7Mb
- P4/distal-P1: 7.0Mb
- b2/b4: 3.5Mb

Fig. 2
Table Summary of deletions in Japanese infertility

<table>
<thead>
<tr>
<th>Deletion</th>
<th>Azoospermia</th>
<th>Oligozoospermia</th>
</tr>
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<tbody>
<tr>
<td>AZFa</td>
<td>1.2% (6)</td>
<td></td>
</tr>
<tr>
<td>P5/proximalP1</td>
<td>1.2% (6)</td>
<td></td>
</tr>
<tr>
<td>P5/distalP1</td>
<td>0.4% (2)</td>
<td></td>
</tr>
<tr>
<td>AZFc (b2/b4)</td>
<td>0.6% (3)</td>
<td>2.3% (10)</td>
</tr>
<tr>
<td>gr/gr</td>
<td>33.2% (172)</td>
<td>40.8% (177)</td>
</tr>
<tr>
<td>b1/b3</td>
<td>0.6% (3)</td>
<td></td>
</tr>
<tr>
<td>b2/b3</td>
<td>2.3% (12)</td>
<td>2.1% (9)</td>
</tr>
<tr>
<td>Total</td>
<td>518</td>
<td>434</td>
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