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Review

Azoospermia factor (AZF) and male infertility

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Abstract

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/distalP1,

P4/distalP1, and AZFc deletions (Fig. 2b) [4,13,35]. 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 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].

Y chromosome haplogroups

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%

(14/518). 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 sever 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

364 counseling of infertile men. Furthermore, this information avoids unnecessary
365 treatments such as hormonal or surgical therapy.
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References

1 de Kretser DM: Male infertility. Lancet 1997;349:787-90.

2 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.

3 Vogt PH, Edelmann A, Hirschmann P, Kohler MR: The azoospermia factor (azf) of the human y chromosome in yq11: Function and analysis in spermatogenesis. Reprod Fertil Dev 1995;7:685-93.

4 Simoni M, Bakker E, Krausz C: Eaa/emqn best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. Int J Androl 2004;27:240-9.

5 de Kretser DM, Burger HG: The y chromosome and spermatogenesis. N Engl J Med 1997;336:576-8.

6 Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S, Page DC: The azfc region of the y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001;29:279-86.

7 Ali S, Hasnain SE: Molecular dissection of the human y-chromosome. Gene 2002;283:1-10.

8 Tilford CA, Kuroda-Kawaguchi T, Skaletsky H, Rozen S, Brown LG, Rosenberg M, McPherson JD, Wylie K, Sekhon M, Kucaba TA, Waterston RH, Page DC: A physical map of the human y chromosome. Nature 2001;409:943-5.

9 Lahn BT, Pearson NM, Jegalian K: The human y chromosome, in the light of evolution. Nat Rev Genet 2001;2:207-16.

10 Rozen S, Skaletsky H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Page DC: Abundant gene conversion between arms of palindromes in human and ape y chromosomes. Nature 2003;423:873-6.

11 UCSC Genome Bioinformatics. Genome Bioinformatics Group, Santa Cruz. 2009. http://www.Genome.Ucsc.Edu/. Accessed 10 May 2010.

12 MSY Breakpoint Mapper. MIT, Cambridge, 2007. http://breakpointmapper.Wi.Mit.Edu/. Accessed 18 May 2010.

13 Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, Page DC, Rozen S: Recombination between palindromes p5 and p1 on the human y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 2002;71:906-22.

14 Fukushima M, Koh E, Choi J, Maeda Y, Namiki M, Yoshida A: Reevaluation of azoospermic factor c microdeletions using sequence-tagged site markers with confirmed physical positions from the genbank database. Fertil Steril 2006;85:965-71.

15 Nakashima M, Koh E, Namiki M, Yoshida A: Multiplex sequence-tagged site pcr for efficient screening of microdeletions in y chromosome in infertile males with azoospermia or severe oligozoospermia. Arch Androl 2002;48:351-8.

16 Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, Lenzi A, Foresta C: Molecular and clinical characterization of y chromosome microdeletions in infertile men: A 10-year experience in italy. J Clin Endocrinol Metab 2007;92:762-70.

17 Simoni M, Tuttelmann F, Gromoll J, Nieschlag E: Clinical consequences of microdeletions of the y chromosome: The extended munster experience. Reprod Biomed Online 2008;16:289-303.

18 Camurri L, Novelli G, Gennarelli M, Cantarelli M, Dallapicolla B: Yq deletions and azf locus: Molecular analysis in two fetuses with non familial homogeneous yq rearrangements. Genet Couns 1993;4:223-6.

19 Simpson E, Chandler P, Goulmy E, Ma K, Hargreave TB, Chandley AC: Loss of the 'azoospermia factor' (azf) on yq in man is not associated with loss of hya. Hum Mol Genet 1993;2:469-71.

20 Vogt PH: Human chromosome deletions in yq11, azf candidate genes and male infertility: History and update. Mol Hum Reprod 1998;4:739-44.

21 Simoni M, Gromoll J, Dworniczak B, Rolf C, Abshagen K, Kamischke A, Carani C, Meschede D, Behre HM, Horst J, Nieschlag E: Screening for deletions of the y chromosome involving the daz (deleted in azoospermia) gene in azoospermia and severe oligozoospermia. Fertil Steril 1997;67:542-7.

22 Krausz C, Quintana-Murci L, Barbaux S, Siffroi JP, Rouba H, Delafontaine D, Souleyreau-Therville N, Arvis G, Antoine JM, Erdei E, Taar JP, Tar A, Jeandidier E, Plessis G, Bourgeron T, Dadoune JP, Fellous M, McElreavey K: A high frequency of y chromosome deletions in males with nonidiopathic infertility. J Clin Endocrinol Metab 1999;84:3606-12.

23 Seifer I, Amat S, Delgado-Viscogliosi P, Boucher D, Bignon YJ: Screening for microdeletions on the long arm of chromosome y in 53 infertile men. Int J Androl 1999;22:148-54.

24 Chiang HS, Wei HJ, Chen YT: Genetic screening for patients with azoospermia and severe oligo-asthenospermia. Int J Androl 2000;23 Suppl 2:20-5.

25 Kerr NJ, Zhang J, Sin FY, Benny P, Sin IL: Frequency of microdeletions in the azoospermia factor region of the y-chromosome of new zealand men. N Z Med J 2000;113:468-70.

445 26 Krausz C, Quintana-Murci L, McElreavey K: Prognostic value of y deletion
446 analysis: What is the clinical prognostic value of y chromosome microdeletion analysis?
447 Hum Reprod 2000;15:1431-4.
448 27 Lin YM, Chen CW, Sun HS, Hsu CC, Chen JM, Lin SJ, Lin JS, Kuo PL: Y-
449 chromosome microdeletion and its effect on reproductive decisions in taiwanese
450 patients presenting with nonobstructive azoospermia. Urology 2000;56:1041-6.
451 28 Martinez MC, Bernabe MJ, Gomez E, Ballesteros A, Landeras J, Glover G, Gil-
452 Salom M, Remohi J, Pellicer A: Screening for azf deletion in a large series of severely
453 impaired spermatogenesis patients. J Androl 2000;21:651-5.
454 29 Namiki M: Genetic aspects of male infertility. World J Surg 2000;24:1176-9.
455 30 Kleiman SE, Bar-Shira Maymon B, Yogev L, Paz G, Yavetz H: The prognostic
456 role of the extent of y microdeletion on spermatogenesis and maturity of sertoli cells.
457 Hum Reprod 2001;16:399-402.
458 31 Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L,
459 Roberts KP: Microdeletions in the y chromosome of infertile men. N Engl J Med
460 1997;336:534-9.
461 32 Saut N, Terriou P, Navarro A, Levy N, Mitchell MJ: The human y chromosome
462 genes bpy2, cdy1 and daz are not essential for sustained fertility. Mol Hum Reprod
463 2000;6:789-93.
464 33 Sun C, Skaletsky H, Birren B, Devon K, Tang Z, Silber S, Oates R, Page DC:
465 An azoospermic man with a de novo point mutation in the y-chromosomal gene usp9y.
466 Nat Genet 1999;23:429-32.
467 34 Foresta C, Moro E, Ferlin A: Prognostic value of y deletion analysis. The role of
468 current methods. Hum Reprod 2001;16:1543-7.

469 35 Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T,
470 Kuroda-Kawaguchi T, de Vries JW, Oates RD, Silber S, van der Veen F, Page DC,
471 Rozen S: Polymorphism for a 1.6-mb deletion of the human y chromosome persists
472 through balance between recurrent mutation and haploid selection. Nat Genet
473 2003;35:247-51.
474 36 Lower R, Lower J, Kurth R: The viruses in all of us: Characteristics and
475 biological significance of human endogenous retrovirus sequences. Proc Natl Acad Sci
476 U S A 1996;93:5177-84.
477 37 Patience C, Takeuchi Y, Weiss RA: Infection of human cells by an endogenous
478 retrovirus of pigs. Nat Med 1997;3:282-6.
479 38 Sun C, Skaletsky H, Rozen S, Gromoll J, Nieschlag E, Oates R, Page DC:
480 Deletion of azoospermia factor a (azfa) region of human y chromosome caused by
481 recombination between herv15 proviruses. Hum Mol Genet 2000;9:2291-6.
482 39 Blanco P, Shlumukova M, Sargent CA, Jobling MA, Affara N, Hurles ME:
483 Divergent outcomes of intrachromosomal recombination on the human y chromosome:
484 Male infertility and recurrent polymorphism. J Med Genet 2000;37:752-8.
485 40 Kamp C, Huellen K, Fernandes S, Sousa M, Schlegel PN, Mielnik A, Kleiman S,
486 Yavetz H, Krause W, Kupker W, Johannisson R, Schulze W, Weidner W, Barros A,
487 Vogt PH: High deletion frequency of the complete azfa sequence in men with sertoli-
488 cell-only syndrome. Mol Hum Reprod 2001;7:987-94.
489 41 Kamp C, Hirschmann P, Voss H, Huellen K, Vogt PH: Two long homologous
490 retroviral sequence blocks in proximal yq11 cause azfa microdeletions as a result of
491 intrachromosomal recombination events. Hum Mol Genet 2000;9:2563-72.
492 42 Choi J, Koh E, Matsui F, Sugimoto K, Suzuki H, Maeda Y, Yoshida A, Namiki
493 M: Study of azoospermia factor-a deletion caused by homologous recombination

494 between the human endogenous retroviral elements and population-specific alleles in
495 japanese infertile males. Fertil Steril 2008;89:1177-82.
496 43 Ferlin A, Moro E, Rossi A, Dallapiccola B, Foresta C: The human y
497 chromosome's azoospermia factor b (azfb) region: Sequence, structure, and deletion
498 analysis in infertile men. J Med Genet 2003;40:18-24.
499 44 Kleiman SE, Yogev L, Hauser R, Botchan A, Bar-Shira Maymon B, Schreiber L,
500 Paz G, Yavetz H: Members of the cdy family have different expression patterns: Cdy1
501 transcripts have the best correlation with complete spermatogenesis. Hum Genet
502 2003;113:486-92.
503 45 Tse JY, Wong EY, Cheung AN, O WS, Tam PC, Yeung WS: Specific
504 expression of vcy2 in human male germ cells and its involvement in the pathogenesis of
505 male infertility. Biol Reprod 2003;69:746-51.
506 46 Stouffs K, Lissens W, Verheyen G, Van Landuyt L, Goossens A, Tournaye H,
507 Van Steirteghem A, Liebaers I: Expression pattern of the y-linked pry gene suggests a
508 function in apoptosis but not in spermatogenesis. Mol Hum Reprod 2004;10:15-21.
509 47 Kee K, Angeles VT, Flores M, Nguyen HN, Reijo Pera RA: Human dazl, daz
510 and boule genes modulate primordial germ-cell and haploid gamete formation. Nature
511 2009;462:222-5.
512 48 Lahn BT, Page DC: Retroposition of autosomal mrna yielded testis-specific
513 gene family on human y chromosome. Nat Genet 1999;21:429-33.
514 49 Huang WJ, Lin YW, Hsiao KN, Eilber KS, Salido EC, Yen PH: Restricted
515 expression of the human daz protein in premeiotic germ cells. Hum Reprod
516 2008;23:1280-9.

517 50 Kim B, Lee Y, Kim Y, Lee KH, Chun S, Rhee K, Seo JT, Kim SW, Paick JS:
518 Polymorphic expression of daz proteins in the human testis. Hum Reprod
519 2009;24:1507-15.
520 51 Reijo RA, Dorfman DM, Slee R, Renshaw AA, Loughlin KR, Cooke H, Page
521 DC: Daz family proteins exist throughout male germ cell development and transit from
522 nucleus to cytoplasm at meiosis in humans and mice. Biol Reprod 2000;63:1490-6.
523 52 Stuppia L, Gatta V, Fogh I, Gaspari AR, Morizio E, Mingarelli R, Di Santo M,
524 Pizzuti A, Calabrese G, Palka G: Genomic organization, physical mapping, and
525 involvement in yq microdeletions of the vcy2 (bpy 2) gene. Genomics 2001;72:153-7.
526 53 Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S,
527 Jaffe T, Straus D, Hovatta O, et al.: Diverse spermatogenic defects in humans caused by
528 y chromosome deletions encompassing a novel rna-binding protein gene. Nat Genet
529 1995;10:383-93.
530 54 Saxena R, de Vries JW, Repping S, Alagappan RK, Skaletsky H, Brown LG, Ma
531 P, Chen E, Hoovers JM, Page DC: Four daz genes in two clusters found in the azfc
532 region of the human y chromosome. Genomics 2000;67:256-67.
533 55 Premi S, Srivastava J, Chandy SP, Ali S: Azfc somatic microdeletions and copy
534 number polymorphism of the daz genes in human males exposed to natural background
535 radiation. Hum Genet 2007;121:337-46.
536 56 Fernandes S, Huellen K, Goncalves J, Dukal H, Zeisler J, Rajpert De Meyts E,
537 Skakkebaek NE, Habermann B, Krause W, Sousa M, Barros A, Vogt PH: High
538 frequency of daz1/daz2 gene deletions in patients with severe oligozoospermia. Mol
539 Hum Reprod 2002;8:286-98.

57 Fernandes S, Paracchini S, Meyer LH, Floridia G, Tyler-Smith C, Vogt PH: A large azfc deletion removes daz3/daz4 and nearby genes from men in y haplogroup n. Am J Hum Genet 2004;74:180-7.

58 Lin YW, Thi DA, Kuo PL, Hsu CC, Huang BD, Yu YH, Vogt PH, Krause W, Ferlin A, Foresta C, Bienvenu T, Schempp W, Yen PH: Polymorphisms associated with the daz genes on the human y chromosome. Genomics 2005;86:431-8.

59 Zhang F, Li Z, Wen B, Jiang J, Shao M, Zhao Y, He Y, Song X, Qian J, Lu D, Jin L: A frequent partial azfc deletion does not render an increased risk of spermatogenic impairment in east asians. Ann Hum Genet 2006;70:304-13.

60 Visser L, Westerveld GH, Korver CM, van Daalen SK, Hovingh SE, Rozen S, van der Veen F, Repping S: Y chromosome gr/gr deletions are a risk factor for low semen quality. Hum Reprod 2009;24:2667-73.

61 Machev N, Saut N, Longepied G, Terriou P, Navarro A, Levy N, Guichaoua M, Metzler-Guillemain C, Collignon P, Frances AM, Belougne J, Clemente E, Chiaroni J, Chevillard C, Durand C, Ducourneau A, Pech N, McElreavey K, Mattei MG, Mitchell MJ: 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.

62 Lynch M, Cram DS, Reilly A, O'Bryan MK, Baker HW, de Kretser DM, McLachlan RI: The y chromosome gr/gr subdeletion is associated with male infertility. Mol Hum Reprod 2005;11:507-12.

63 de Carvalho CM, Zuccherato LW, Fujisawa M, Shirakawa T, Ribeiro-dos-Santos AK, Santos SE, Pena SD, Santos FR: Study of azfc partial deletion gr/gr in fertile and infertile japanese males. J Hum Genet 2006;51:794-9.

64 Wu B, Lu NX, Xia YK, Gu AH, Lu CC, Wang W, Song L, Wang SL, Shen HB, Wang XR: A frequent y chromosome b2/b3 subdeletion shows strong association with male infertility in han-chinese population. Hum Reprod 2007;22:1107-13.

65 Yang Y, Ma M, Li L, Zhang W, Chen P, Ma Y, Liu Y, Tao D, Lin L, Zhang S: Y chromosome haplogroups may confer susceptibility to partial azfc deletions and deletion effect on spermatogenesis impairment. Hum Reprod 2008;23:2167-72.

66 Giachini C, Laface I, Guarducci E, Balercia G, Forti G, Krausz C: Partial azfc deletions and duplications: Clinical correlates in the italian population. Hum Genet 2008;124:399-410.

67 Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonne-Tamir B, Bertranpetit J, Francalacci P, Ibrahim M, Jenkins T, Kidd JR, Mehdi SQ, Seielstad MT, Wells RS, Piazza A, Davis RW, Feldman MW, Cavalli-Sforza LL, Oefner PJ: Y chromosome sequence variation and the history of human populations. Nat Genet 2000;26:358-61.

68 Ravel C, Chantot-Bastaraud S, El Houate B, Mandelbaum J, Siffroi JP, McElreavey K: Gr/gr deletions within the azoospermia factor c region on the y chromosome might not be associated with spermatogenic failure. Fertil Steril 2006;85:229-31.

69 Li Z, Haines CJ, Han Y: "Micro-deletions" Of the human y chromosome and their relationship with male infertility. J Genet Genomics 2008;35:193-9.

70 Krausz C, Giachini C, Xue Y, O'Bryan MK, Gromoll J, Rajpert-de Meyts E, Oliva R, Aknin-Seifer I, Erdei E, Jorgensen N, Simoni M, Ballesca JL, Levy R, Balercia G, Piomboni P, Nieschlag E, Forti G, McLachlan R, Tyler-Smith C: Phenotypic variation within european carriers of the y-chromosomal gr/gr deletion is independent of y-chromosomal background. J Med Genet 2009;46:21-31.

589 71 Habermann B, Mi HF, Edelmann A, Bohring C, Backert IT, Kiesewetter F,
590 Aumuller G, Vogt PH: Daz (deleted in azoospermia) genes encode proteins located in
591 human late spermatids and in sperm tails. Hum Reprod 1998;13:363-9.
592 72 Y Chromosome Consortium. The Y Chromosome Consortium , Tucson,. 2002.
593 http://ycc.Biosci.Arizona.Edu/. Accessed 20 May 2010
594 73 Lu C, Zhang J, Li Y, Xia Y, Zhang F, Wu B, Wu W, Ji G, Gu A, Wang S, Jin L,
595 Wang X: The b2/b3 subdeletion shows higher risk of spermatogenic failure and higher
596 frequency of complete azfc deletion than the gr/gr subdeletion in a chinese population.
597 Hum Mol Genet 2009;18:1122-30.
598 74 Kuroki Y, Iwamoto T, Lee J, Yoshiike M, Nozawa S, Nishida T, Ewis AA,
599 Nakamura H, Toda T, Tokunaga K, Kotliarova SE, Kondoh N, Koh E, Namiki M,
600 Shinka T, Nakahori Y: Spermatogenic ability is different among males in different y
601 chromosome lineage. J Hum Genet 1999;44:289-92.
602 75 Paracchini S, Stuppia L, Gatta V, Palka G, Moro E, Foresta C, Mengua L, Oliva
603 R, Ballesca JL, Kremer JA, van Golde RJ, Tuerlings JH, Hargreave T, Ross A, Cooke H,
604 Huellen K, Vogt PH, Tyler-Smith C: Y-chromosomal DNA haplotypes in infertile
605 european males carrying y-microdeletions. J Endocrinol Invest 2000;23:671-6.
606 76 Quintana-Murci L, Krausz C, Heyer E, Gromoll J, Seifer I, Barton DE, Barrett T,
607 Skakkebaek NE, Rajpert-De Meyts E, Mitchell M, Lee AC, Jobling MA, McElreavey
608 K: The relationship between y chromosome DNA haplotypes and y chromosome
609 deletions leading to male infertility. Hum Genet 2001;108:55-8.
610 77 Hammer MF, Horai S: Y chromosomal DNA variation and the peopling of japan.
611 Am J Hum Genet 1995;56:951-62.
612 78 Shinka T, Tomita K, Toda T, Kotliarova SE, Lee J, Kuroki Y, Jin DK, Tokunaga
613 K, Nakamura H, Nakahori Y: Genetic variations on the y chromosome in the japanese

614 population and implications for modern human y chromosome lineage. J Hum Genet
615 1999;44:240-5.
616 79 Tajima A, Hayami M, Tokunaga K, Juji T, Matsuo M, Marzuki S, Omoto K,
617 Horai S: Genetic origins of the ainu inferred from combined DNA analyses of maternal
618 and paternal lineages. J Hum Genet 2004;49:187-93.
619 80 Hammer MF, Karafet TM, Park H, Omoto K, Harihara S, Stoneking M, Horai S:
620 Dual origins of the japanese: Common ground for hunter-gatherer and farmer y
621 chromosomes. J Hum Genet 2006;51:47-58.
622 81 Wen B, Xie X, Gao S, Li H, Shi H, Song X, Qian T, Xiao C, Jin J, Su B, Lu D,
623 Chakraborty R, Jin L: Analyses of genetic structure of tibeto-burman populations
624 reveals sex-biased admixture in southern tibeto-burmans. Am J Hum Genet
625 2004;74:856-65.
626 82 Krausz C, Degl'Innocenti S: Y chromosome and male infertility: Update, 2006.
627 Front Biosci 2006;11:3049-61.
628 83 Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Paz G, Lessing JB,
629 Yaron Y, Yavetz H: Three-generation evaluation of y-chromosome microdeletion. J
630 Androl 1999;20:394-8.
631 84 Cram DS, Ma K, Bhasin S, Arias J, Pandjaitan M, Chu B, Audrins MS,
632 Saunders D, Quinn F, deKretser D, McLachlan R: Y chromosome analysis of infertile
633 men and their sons conceived through intracytoplasmic sperm injection: Vertical
634 transmission of deletions and rarity of de novo deletions. Fertil Steril 2000;74:909-15.
635 85 Rolf C, Gromoll J, Simoni M, Nieschlag E: Natural transmission of a partial
636 azfb deletion of the y chromosome over three generations: Case report. Hum Reprod
637 2002;17:2267-71.

638 86 Katagiri Y, Neri QV, Takeuchi T, Schlegel PN, Megid WA, Kent-First M,
639 Rosenwaks Z, Palermo GD: Y chromosome assessment and its implications for the
640 development of icsi children. Reprod Biomed Online 2004;8:307-18.
641 87 Kihaile PE, Kisanga RE, Aoki K, Kumasako Y, Misumi J, Utsunomiya T:
642 Embryo outcome in y-chromosome microdeleted infertile males after icsi. Mol Reprod
643 Dev 2004;68:176-81.
644
645

646 Figure legends

647

648 Fig.1 Whole Y chromosome structure

649

650 Fig.2 Recent model of AZF deletions

651 a. Classical categorization. b. Recent categorization of AZF deletions based on

652 palindrome structure.

653

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

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

656

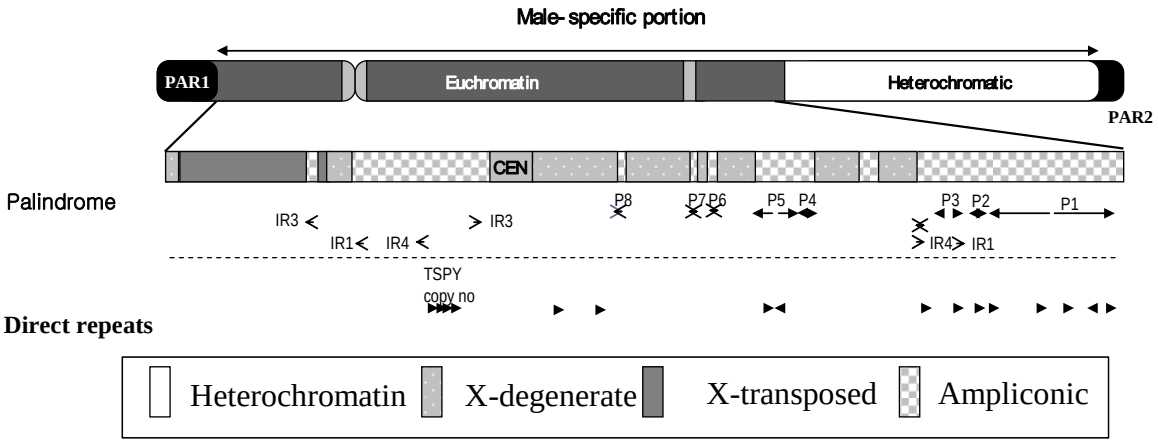
Male-specific portion
PAR1
Euchromatin
Heterochromatic
PAR2
CEN
Palindrome
IR3
IR3
IR1
IR4
IR4
IR1
P8
P7
P6
P5
P4
P3
P2
P1
TSPY
copy no
Direct repeats
Heterochromatin
X-degenerate
X-transposed
Ampliconic

Fig. 1

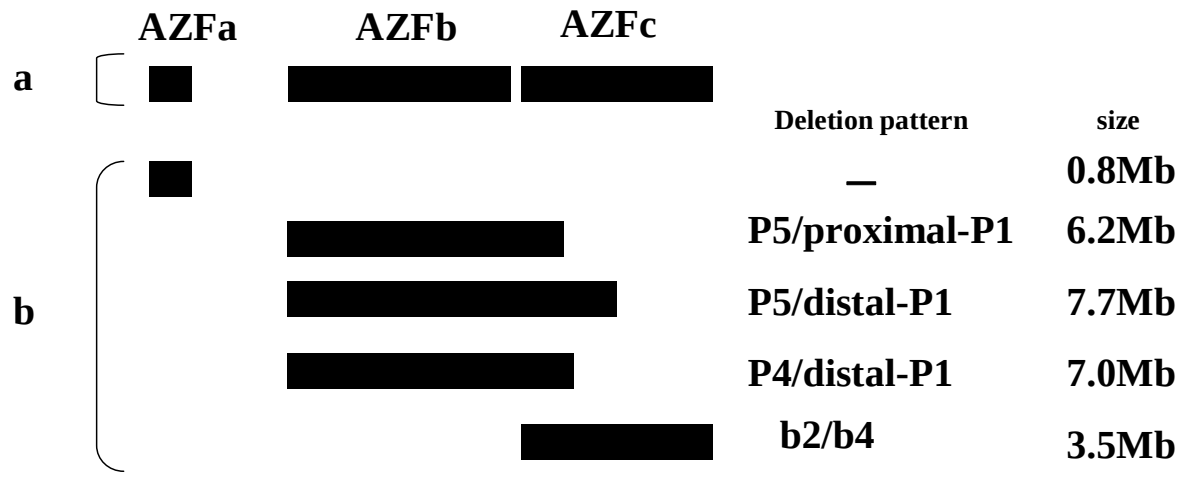
AZFa
AZFb
AZFc
a
b
Deletion pattern
size
—
0.8Mb
P5/proximal-P1
6.2Mb
P5/distal-P1
7.7Mb
P4/distal-P1
7.0Mb
b2/b4
3.5Mb

Fig.2

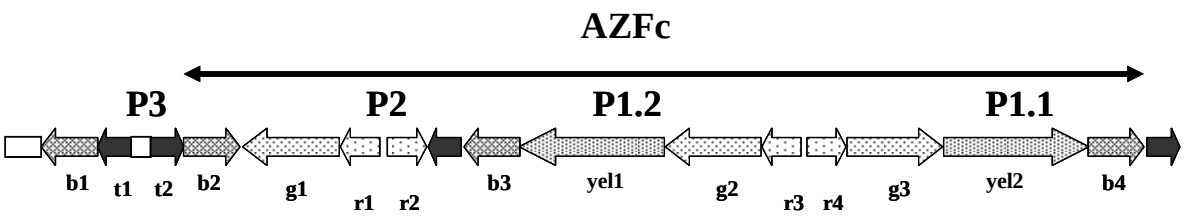

Fig. 3

Table Summmary of deletions in Japanese infertility

	Frequncy % (n)	
Deletion	Azoospermia	Oligozoospermia
AZFa	1.2% (6)	
P5/proximalP1	1.2% (6)	
P5/distalP1	0.4% (2)	
AZFc (b2/b4)	0.6% (3)	2.3% (10)
gr/gr	33.2% (172)	40.8% (177)
b1/b3	0.6% (3)	
b2/b3	2.3% (12)	2.1% (9)
Total	518	434