

40 years of AZF locus

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Summary

The most common genetic causes of male infertility are chromosomal conditions that affect sperm production. Klinefelter syndrome, discovered in 1959, is the first identified chromosomal alteration leading to infertility as the main clinical sign. Afterwards, several genes involved in the control of male reproduction have been disclosed and an alteration even just in one of these genes can be responsible for infertility. This paper provides an overview of the characterization of Yq chromosome regions, implicated in the failure of male gametogenesis, and of the molecular, diagnostic and phenotypic aspects linked to the Yq microdeletions. The discovery of the AZF locus in 1976, exactly 40 years ago, is the starting point of our history.

KEY WORDS: *Yq microdeletion, male infertility, AZF locus.*

Introduction

For all species living in our planet, reproduction represents a crucial step for the survival of their specific genome by the transmission of the genetic information to the offspring. From a genetic point of view, thus, infertility represents the most severe disease, affecting the survival of the species, although not the one of the single infertile subject.

In this view, it is not surprising that in human, several hundred genes are involved in the control of the reproduction, ensuring that the different steps of the male and female gametogenesis, as well as the fertilization and the embryo development, are correctly performed.

Nevertheless, infertility is a growing emergency in western countries. About one in ten couples suffer for problem of infertility, and in the majority of cases the pathogenesis of this condition is not detectable. As a consequence, infertility in many couples cannot be treated, and the only solution for generating a baby is the use of Assisted Reproduction Techniques (ART). Due to the large number of genes involved in the control of human reproduction, it is quite simple to hypothesize that an alteration even in just one of the genes involved in this process can be responsible for infertility. As a matter of fact, the history of the identification of genetics abnormalities in human starts with the discovery in 1959 of the chromosomal alteration responsible for a condition showing infertility as the main clinical sign, that is the Klinefelter syndrome (1). The 47, XXY karyotype associated to this syndrome, is responsible for a large part of the cases of azoospermia, being detected in up to the 25% of azoospermic patients, confirming that genetic alterations play a key role in the disruption of the spermatogenesis.

However, in the following 25 years no other relevant breakthrough in this field was recorded. The advances in the field of human gene mapping and cloning were still far to come, and cy-

togenetic investigation, the only available tool for the identification of genetic alterations related to human disease, was not able to identify alterations affecting single genes.

Nevertheless, it was just the cytogenetic approach that for the first time allowed to identify a genetic alteration specifically related to the failure of male gametogenesis, without any other clinical sign. The discovery of the AZF locus in 1976, 40 years ago, is the starting point of our history, which 40 years later appears far to be concluded (2).

The '70s: the discovery of the AZF locus

It was October the 28th 1976 when the journal Human Genetics published an article of Luciano Tiepolo and Orsetta Zuffardi entitled "Localization of factors controlling spermatogenesis in the non fluorescent portion of the human Y chromosome long arm". In this paper, the Authors reported the identification of acytogenetically detectable deletion of the Y chromosome long arm (Yq) at band q11 in 6 men with normal male habitus but with azoospermia. This finding was the result of a study carried out by cytogenetic investigation of 1170 subfertile males, and lead the Authors to conclude that "*on the distal portion of the non fluorescent segment of the long arm of the Y, factors are located controlling spermatogenesis*". This factor or these factors were defined by the Authors as *Azoospermia Factor (AZF)*, and this definition was conserved during the time. Starting from this moment, the search for the gene (or genes) mapped in Yq within AZF locus was launched.

Subsequently, several reports corroborated the findings of Tiepolo and Zuffardi, confirming that cytogenetically detectable Yq alterations, not only in form of terminal deletions, but also of more complex rearrangements, such as *idic(Y)* or *iso(Yp)* were associated to male infertility.

The '90s: molecular characterization of the AZF regions

The precise molecular characterization of AZF region took about 30 years to be carried out. Vogt et al. (1996) suggested for the first time

the existence of more spermatogenesis loci in Yq11, namely AZFa, AZFb and AZFc (3). Several genes and families were detected in these three loci. RBM, encoding for proteins having RNA-binding properties, was the first gene family identified (4). Other genes were afterwards discovered: *DAZ* (Deleted in Azoospermia) (5) and *SPGY* (Spermatogenesis Gene locus on the Y) (3). Multiple gene copies of *RBM* and *DAZ* were then reported encoding for testis-specific RNA binding proteins of the RNA Recognition Motif (RRM) class (6). Lahn and Page (1997), described the presence of 12 genes in multiple copies mapped on the Y chromosome, showing homologous gene copies on the X chromosome (7). These genes were classified into two groups on the basis of their characteristics. The first class groups is formed of five genes with a testis-specific expression signature having X-Y homologous: *Drosophila* fat facets related Y (*DFFRY*), dead box Y (*DBY*), ubiquitous tetra tricopeptide repeat (TPR) motif Y (*UTY*), the eukaryotic translation-initiation-factor 1A Y isoform (*eIF-1AY*), selected mouse cDNA on the Y (*SMCY*) and the thymosin β 4 Y isoform (*Tb4Y*). The second group is constituted by Y-specific multi-copy genes: the RNA-binding-motif Y chromosome gene (*RBMY*) and its relatives, deleted in azoospermia (*DAZ*), chromodomain Y (*CDY*), XK-related Y (*XKRY*), protein-tyrosine phosphatase BAS-like (PTP-BL) -related Y (*PRY*) and the genes for basic proteins Y1 and Y2 (*BPY1* and *BPY2*). This group of genes are ubiquitously expressed (7). DNA sequencing and microdeletions characterization by PCR allowed the identification of a genetic map within the AZF loci and the differential genotype-phenotype correlations (8, 9). The AZFa region (chromosome location 12.9-13.7Mb) spans over 792 kb and was entirely sequenced in 1999 (10). AZFa, which maps to proximal Yq, is the only locus composed exclusively by single-copy genes (*DFFRY*, *DBY*, and *UTY*). The AZFa locus is flanked by two human endogenous retrovirus (HERV) elements, spanning about 10 kb each (11). AZFb locus spans from 6.2 to 7.7 Mb of MSY sequences, and it overlaps with the AZFc region by 1.5 Mb. The AZFb locus is composed by a very long, near identical direct and indirect repeats, named amplicons. The totality of the fourteen amplicons is fractionated in 6 families. The half of them is exclusive to AZFb, whereas the remain-

ing are shared with the AZFc locus (12). CDY and XKRY, SMCY, eIF-1AY and RBMY gene copies are mapped in the AZFb locus. Elliot et al. (1997) reported that the most active copies of the RBM genes are located within this critical region since deletions of distal AZFb are related to the RBMY epitopes defects in testicular sections (6).

The AZFc locus is a complex region having several transcripts mapped within (13, 14). It has been estimated that AZFc locus spans about 1.4 Mb and its complete sequence was described by Kuroda et al. (2001) using an iterative mapping-sequencing process. The AZFc region is formed by a sequence of three palindromes, each of them composed of six distinct families amplicons respectively (8). This peculiar structure might be originated by the tandem duplication and inversion during the primates evolution. Within this region 11 families of testis specific transcription units are mapped. In particular, six copies of DAZ gene (15, 16), and multiple copies of PRY, BPY2, CDY and XKRY are known.

The genes mapped in these three loci play a crucial role during the differentiation of male germ cells. They are currently deleted in infertile patients, leading to azoospermia (absence of sperm), severe oligozoospermia ($< 1 \times 10^6$ sperm/mL semen), moderate oligozoospermia ($1-5 \times 10^6$ sperm/mL semen) or mild oligozoospermia ($5-20 \times 10^6$ sperm/mL semen). Phenotype when Yq microdeletions occur.

Yq microdeletions

Yq microdeletions are the second most frequent genetic cause of male infertility after the Klinefelter syndrome (17, 18). They are generated by homologous recombinations between amplicons. Commonly, they produce spermatogenic failure leading to severe oligozoospermia or non-obstructive azoospermia (19). The deletions of the three AZF regions occur with different frequency. The AZFc deletions are the most common (79%), followed by AZFb (9%), AZFb,c (6%), AZFa (3%) and AZFa,b,c (XX male: 3%) (20-23). The clinical and molecular significance of this genetic alteration has widely been studied and nowadays Y chromosome microdeletions are routinely screened in male infertile patients worldwide.

The complete deletion of the AZFa, originated by the homologous recombination between the identical HERVYq1 and HERVYq2 sequence blocks flanking AZFa locus region, remove about 792 kb, including the genes USP9Y and DBY. The deletions encompassing only the USP9Y gene or the DBY gene have been reported by Ferlin et al. (1999); Foresta et al. (2000) (24, 25). The deletions involving *AZFa* locus seem to occur less frequently in infertile men but they are related with the SCO syndrome and azoospermia (3, 10, 11, 19).

The majority of Yq microdeletions induce the simultaneous loss of several genes mapped within the loci AZFb and AZFc (8, 26, 27). These rearrangements are caused by the large palindromes present in these regions (12). This peculiar structure makes the AZFc region prone to rearrangements, affecting almost the totality of amplicons contained in this locus (8).

The complete AZFc deletion, takes eight gene families out, including the members of the DAZ family, that act as the strongest candidate accountable for the AZFc phenotype (5, 28-31). Complete deletions of AZFb or AZFb and c lead to azoospermia associated with the SCO phenotype or the pre-meiotic spermatogenic arrest (31). The most frequent AZFc deletion induces azoospermia or severe oligozoospermia, which associates with different spermatogenic phenotypes in the testis. About 60% of men showing AZFc deletion, have mature spermatozoa in the ejaculate or in the testis (32). Two types of partial AZFc deletions have also been identified. In particular, a 1.6 Mb DNA segment is excised from the AZFc region, and it is considered a significant risk factor for spermatogenic failure in Dutch, Spanish, Italian and Australian studies (31, 33-36). However, the association of this deletion with spermatogenic failure was not confirmed in French, German, Brazilian, Japanese, Sri Lankan and Chinese men (37-44). The other partial deletion removes a 1.8 Mb DNA segment and its association with male infertility was recently reported in Chinese men, whereas in other populations no relation with male infertility was detected (37, 45, 46). Nowadays, the relation between the partial deletions of AZFc and the spermatogenic impairment is still controversial. Recently, it was reported that the partial deletions frequencies were not significantly different between the azoospermic/severe oligozoospermic men and

normozoospermic controls. These data suggest that the partial deletion is not associated with azoospermia/oligozoospermia in an Iranian population (47).

Best Practices for Yq microdeletions analysis

The analysis of the Y chromosome microdeletions is recommended for men showing a severe impairment of spermatogenesis, in particular before intracytoplasmic sperm injection (ICSI) (48). The last European Academy of Andrology (EAA) guidelines for laboratory Practices (48) recommends the screening for Yq deletions as a routine diagnostic test that highlights the etiology of spermatogenic disturbances so that the prognosis for testicular sperm retrieval is defined by the type of deletion found. Men carrying Y microdeletions could refer to assisted reproductive techniques as the most useful symptomatic therapy, however, the genetic defect on the Y chromosome are transmitted to the male offspring, thus impairing their fertility. The diagnosis of the complete deletion of the AZFa locus is the only that could be associated with the impracticability to retrieve spermatozoa in the testis for ICSI. Several studies have reported the successful use of ICSI for infertile couples with male partners carrying AZFc deletions (18).

Although an impaired fertilization rate with lower embryo quality (49), a reduced blastocyst rate (50) and lower overall success of ICSI (51) have been detected, no differences in fertilization and pregnancy rates were reported between men carrying Y microdeletions and normal controls. Existing the risk of conceiving offspring with spermatogenesis impairment, the genetic counselling remains mandatory. In addition, the genetic counselling is suitable for the male members of families having a case of partial AZFa or AZFb and AZFc deletion (52-54). Female fetuses from a father with a Y chromosome deletion have no increased risk of congenital abnormalities or infertility. Even though deletions carried by the father are obligatory transmitted to male offspring, who will show sperm production defects, the testicular phenotype is not predictable due to the genetic variability. Despite their infertility phenotype, some males with AZF deletion have occasional-

ly spontaneously fathered infertile. It was reported in about 4% of couples with severe oligospermia mainly if the female partner is young and very fertile (55-57).

The sex of the fetus and the presence of the Y deletion may be determined with specific prenatal or pre-implantation testing in pregnancies known to be at risk of resulting in a male with Y chromosome deletion (57).

Inside the Testis transcriptome of infertile patients

To better understand the molecular mechanism affecting spermatogenesis in patients having Y microdeletions, the testis transcriptome in infertile and fertile men have been studied by microarray (58). It was described a particular testicular gene expression signature in patients carrying AZFc microdeletions or who were affected by idiopathic infertility. This study highlighted that distinct infertility forms share common pathogenic mechanisms. In particular, alterations in the mRNA storage were identified. Intriguingly, four idiopathic infertile patients presented no testicular expression of DAZ despite the absence of AZFc deletion in the peripheral blood. These data suggest that the lack of DAZ expression in the testis could trigger the infertile phenotype. The same mechanism could explain the infertile status for those patients carrying AZFc deletions in mosaic or showing the loss of function of AZFc genes in absence of Yq deletions. Thus the testicular functions of DAZ should be investigated in order to unravel the molecular basis of infertility in patients negative for the peripheral detection of Yq deletions.

Conclusion

Since the discovery of the AZF locus in 1976, many efforts have been made to disclose the molecular mechanisms related to the idiopathic male infertility which still represents 30% of all male infertility cases (59). The use of advanced reproductive techniques will overcome some of these problems, however it is also important to underline that there is a need, for routine fertility care, of paying greater attention to couple's emotional needs. It has been consistently

demonstrated that a diagnosis of infertility as well as ART treatment have a negative impact on both the individuals' well-being, with manifestations of depression, anxiety, frustration, isolation and low self-esteem (60). Future researches in this field will shed in light useful clinically tools for counseling, diagnosis, and treatment of infertile couples.

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