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Review

Y CHROMOSOME MICRODELETIONS AS A CAUSE FOR MALE INFERTILITY

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Male infertility represents around half of all cases of infertility. The microdeletions of the azoospermia factor (AZF) region, located in the long arm of Y chromosome, are the second most common reason for reproductive problems among men. This genetic mutation results in low sperm count and fertility rate. The presence of Y microdeletions can lower the success rate of in vitro procedures and would be transmitted to the next generation. We have analyzed 30 articles about the connection between the deletions of the Y chromosome and the decreased sperm count. 25 of them confirm the role of this genetic mutation, while the rest do not, but they investigate only some AZF loci. The negative results could also be due to the different ethnic origin of the participants, difference between the research method and etc. Testing infertile men for Y chromosome deletions could lead to a major improvement in the options for treating infertility. Also, if such mutation is diagnosed, this is an indication for genetic counseling in order to avoid future fertility issues in the next generation. The review of the included articles proves the role of the Y chromosome microdeletions as a reason for male infertility and outlines the main principles when performing the genetic test for this mutation. ***Biomed Rev*** **2018; 29: 57-63**

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INTRODUCTION

Infertility is defined by the World Health Organization as a disease of the reproductive system and as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (1). Around 10-15% of the couples trying to conceive have reproductive problems and in around half of these cases this is due to male infertility (2). The reasons for these reproductive problems could be various, for example monogenic disorders such as cystic fibrosis or aberrations of the sex chromosomes such as Klinefelter syndrome.

After Klinefelter syndrome the microdeletions of the azoospermia factor (AZF) region of Y chromosome are the second most common reason for male infertility (3). The connection between male infertility and deletions of the long arm of Y chromosome was established when analyzing cells from idi-

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opathic infertile males (4). The AZF region was mapped to the long arm of Y chromosome, band 11.23, by testing 370 men with idiopathic azoospermia or severe oligozoospermia for deletions and proposed the presence of the 3 spermatogenesis loci in Yq11, which were named AZFa, AZFb and AZFc (5). Each of them could be deleted independently or all together. Depending on the type of the deletion, this could lead to a lower sperm count or to arrest of the spermatogenesis. However, these deletions are quite common even in the general population – it is estimated that they occur in about one in 4000 men (3). These phenomena could be explained by the complex structure of the Y chromosome.

In the period between 2001 and 2003 detailed structure of the Y chromosome was described and the possible mechanisms for the high frequency of microdeletions in AZFc region in its long arm (6, 7). They reported of a region, where there is no crossing over between the X and the Y chromosome and name it male-specific region. It represents 95% of the length of the Y chromosome and it consists of X-transposed sequences, X-degenerated sequences and ampliconic sequences. X-transposed sequences are the result of a transposition between X and Y chromosome, which took place 3-4 million years ago, and DNA, found here, is 99% identical to the DNA sequences of Xq21 band. The X-degenerated sequences contain sequences from autosomes, which were the basis for the formation of the sex chromosomes in the process of their evolution. The ampliconic sequences are organized in the so call palindromes. These are sequences, which could be read the same way from 5' to 3' on one strand and 5' to 3' on the other, complementary, strand (6, 7). When testing 48 infertile men with deletions of the AZFc region, the authors concluded that the distal and proximal breakpoints of all 48 AZFc deletions were very similar. The proximal and the distal breakpoint regions correspond to the amplicons, called b2 and b4 (6, 7). It is assumed that the palindromic organization rises the frequency of intrachromosomal aberrations during the meiotic recombination and this could lead to inversions/deletions/ duplications and etc. in the AZFc locus (6, 8). These findings suggest that homologous recombination between amplicons b2 and b4 is the reason for the high rate of deletions in this region (9).

STRUCTURE OF THE MALE-SPECIFIC REGION

The AZFa region (Fig. 1) covers 1,100 kb of Y chromosome and 2 genes are found there - *USP9Y* and *DDX3Y* (6, 10). *DDX3Y* gene codes RNA helicase and has a key role in the spermatogenesis (11). Mutations in this gene result in male infertility, a reduction in germ cell numbers, and can result in Sertoli-cell only syndrome, when in the seminiferous tubules there are only Sertoli cells, but no germ cells (12). The frequency of partial deletions of AZFa varies between 0.2% and 11% (13). Patients with such deletions have very poor fertility outcomes (14). However, if there is a partial deletion, involving only *USP9Y*, recent publication reports for patients with azoospermia and severe oligozoospermia, but with histological data for spermatogenesis (15). When testing for deletions in this locus, two markers are used – sY84 and sY86 (3).

The AZFb region (Fig. 1) contains 7 genes, which were proven experimentally to have a role in the spermatogenesis. These are the genes *EIF1AY, RPS4Y2* and *SMCY*, localized in the X-degenerative sequences, and *HSFY, XKRY, PRY, RBMY* found in the ampliconic sequences (6, 16). The size of the deletions in the AZFb region is around 4.96 -6.92 Mbs (16). Their frequency is 10-16% of the total Y microdeletions (17). The complete deletions of this region result in azoospermia and Sertoli-cell only syndrome and early stop of maturation of the spermatozoa cells (18). When testing for deletions in this locus, two markers are used – sY127 and sY134 (3).

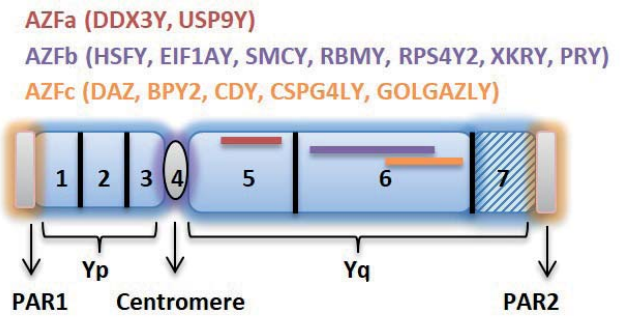

Figure 1. *Schematic diagram of Y chromosome showing AZF loci and their candidate gene associated with male infertility.*

The AZFc region covers 4.5 Mb and contains 5 genes, coding proteins, involved in the spermatogenesis - *BPY2, CDY, DAZ, CSPG4LY* and *GOLGAZLY* (19). This gene family is organized in a palindromic sequence *Deleted in azoospermia* (*DAZ1/2 and DAZ3/4*) (6, 7). Most often the deletions involve regions b2/b4, b1/b3, b2/b3 and gr/gr, which leads to the lack of *DAZ* gene (6). The AZFc deletions are the most common and make up to 60% of the Y microdeletions of clinical significance (19). Around 70% of the men with AZFc deletion have sperm cells in the ejaculate, but their count is usually below 1 million per milliliter (20-22). Men, diagnosed with

this type of deletion, could undergo microdissection testicular sperm extraction (TESE) in order to obtain sperm cell and use them later for intracytoplasmic sperm injection (ICSI) (21). This procedure increases the fertility chances of men with such deletions, who otherwise have poor outcomes for normal conception. When testing for deletions in this locus, two markers are used – sY254 and sY255 (3).

However, the Y microdeletions would be inherited by the next generation and children of such men usually also have impaired spermatogenesis due to Y microdeletion (23). Also, partial AZFc deletions can increase the risk of complete AZFc deletion in the following generation (24) and to combined deletions, involving for example AZFb and AZFc regions (20). The patients have Sertoli cell only syndrome or arrest of the spermatogenesis and have low successful rate of obtaining sperm cells, when applying TESE (20, 21). The frequency of Y microdeletions depends on the study population. For example, when investigating microdeletions in 1,226 infertile patients from Korea, the reported frequency of AZFc deletions was 10.93% (134/1,226) (25). In another study from China the incidence of AZF deletions was 10.80% from 1,333 tested men (26). In a similar study in Slovakia the reported frequency was 3.35% from 226 patients with azoospermia (27). This could be due to different genetic background, different haplogroups of the Y chromosome.

Y MICRODELETIONS AND INFERTILITY

We have analyzed 30 articles about the connection between the deletions of the Y chromosome and the decreased sperm count. The biggest patient cohort consisted of 3,170 men with total sperm count below 5×10^6/ml and in 12 of the articles a control group of men with normal sperm count was also analyzed for the frequency of Y microdeletions. Twenty five of the articles (26, 28-51) confirm the role of this genetic mutation, while the rest do not, but they investigate only some AZF locuses, not all 3 of them. The authors conclude that the Y microdeletions, which involve AZF region, are possible etiological factor for the fertility problems among the tested men. Also, the authors establish a correlation between the low sperm count and the presence of such microdeletions. For example, Liu *et al* investigated the frequency of Y microdeletions among 3731 infertile men and 341 (9.14%) were diagnosed with microdeletion of AZFa, AZFb or AZFc. 13 of them had AZFa deletion and the authors sequenced the fathers of the subjects to check if the deletion was inherited (28). In one case the deletion was passed from the father's subject, but the others were *de novo* mutations, which could be due to the presence of hot spots for new deletion in the palindromic sequences (52).

Another example is the research of Kim *et al* who tested 1,306 infertile men for Y microdeletion and 101 (7.7%) turned out to be carriers. The most common type of deletion was in the AZFc region (87.1%). They confirmed the role of Y microdeletions as a risk factor for infertility and recommended testing patients with oligo- and azoospermia before the ICSI procedure for this deletion in order to avoid future fertility problems in the next generation (39). Patrat and colleagues did a research on Y microdeletions as a factor for male infertility and confirmed that the incidence of AZFc deletions was highest (73,1%) than the other types of deletions (35). The authors investigated what was the success rate of the ICSI procedure among 23 couples, the men being diagnosed with AZFc deletion. From 42 ICSI there were 18 clinically diagnosed pregnancies, which led to the birth of 14 children. The role of Y microdeletions as a factor lowering the fertility rate was confirmed (35).

Johnson *et al* published an article about Y microdeletions among 1473 men from London, UK. Among 58 (4%) of them there was a deletion, involving one of the regions. All patients positive for this deletion had a sperm concentration below 0.5×10^6/ml (50). The authors concluded that the sperm concentration was the predictive factor, when offering test for Y microdeletions. In spite of that, five of the included articles (53-57) reject the role of Y microdeletions as an etiological factor for male infertility. This is mainly due to the low frequency of the found deletions among the tested men. Further, the partial deletions gr/gr, b1/b3 and b2/b3 in AZFc region among 348 men with non-obstructive oligo- or azoospermia and 170 men with normal sperm count were analyzed (53). The authors found that the frequency of these partial deletions was not significantly higher among the men with impaired spermatogenesis and that this finding could not explain the infertility issues. However, a disadvantage of the study is that the authors chose only specific deletions without investigating the frequency of the other common ones, which could also explain the observed fertility problems.

The frequency of Y microdeletions among 67 Romanian men with oligo- or azoospermia was published (54). These latter authors found only two cases of Y microdeletions (3%). Both men had a deletion of the AZFc region. The authors concluded that Klinefelter is the most common reason for male infertility, but did not reject the idea of also testing men with azoospermia for Y microdeletions because of the high

percentage of chromosomal rearrangements among this patient group. Godoy and colleagues investigated the frequency of Y microdeletions among 94 men with oligo- or azoospermia, all of whom are from Mato Grosso, Brazil (55). The authors found very low frequency of Y microdeletions among this population – 1,1%, but the sample is not representative, since all of the patients are form one Brazilian state. Another study tested 154 infertile Iranian men with oligo- and azoospermia for two types of deletions - gr/gr and b2/b3 in the AZFc region (56). 111 fertile men were also included in the study. The authors concluded that there is a slight difference of the frequency of these mutations – for example the frequency of the gr/gr deletion was 4.4% (5/113), 7.3% (3/41) and 1.8% (2/111) for the men with azoospermia, oligozoospermia and the control fertile men. That is why the authors suggested that these deletions did not have a major impact on the fertility outcomes (56). Despite that the study model has two disadvantages – the participants are only from Iranian origin and only two types of deletions were analyzed, but there are also other possible types of mutations. Miraghazadeh *et al* published data about the success outcomes of TESE in patients with AZFc deletions (57). The authors tested 200 infertile men for the presence of partial AZFc deletions and found 11 carriers. Then they investigated the TESE success rate if it depended on the mutation status and concluded that partial AZFc microdeletions was not a predictor of microTESE outcome.

CONCLUSION

The Y chromosome has a high frequency of microdeletions, which could be due to the presence of palindromic structures and the high rate of homologous recombination between the ampliconic sequences. This deletion results in fertility problems in the affected men, which is confirmed by the cited articles. The studies, which reject the role of this mutation, tested the patients only for certain types of deletions and some of them included only men of specific ethnic background, which could explain the different results. However, such mutations have an impact on the fertility potential of the men and result in oligo- and azoospermia. These deletions would be inherited by the next generation, which illustrates the importance of genetic counseling of affected couples. That is why testing infertile men for Y chromosome deletions could lead to a major improvement in the options for treating infertility.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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