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Y Chromosome Microdeletions in men with Azoospermia and Oligoasthenoteratozoospermia

Spas D. Dzhoglov[1], Evgeniya N. Ivanova[2*], Doychin T. Boyadzhiev[3]

1 - Central Clinical Laboratory of Medical University, Hospital St. George, 66, Pestersko shose bul. Plovdiv 4000 , BULGARIA
2 - Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of Developmental Biology, 24, Tsar Asen Str. Plovdiv 4000 , BULGARIA
3 - Plovdiv University "Paisii Hilendarski", Faculty of Mathematics and Informatics, Department of Applied Mathematics and Modeling, 236, Bulgaria bul., Plovdiv 4000, BULGARIA
[*]Corresponding author: geneiv@uni-plovdiv.bg

Abstract. The causes of male infertility are different and significantly related to both genetic components and a variety of environmental factors. Approximately 5 to 10% of men with azoospermia or severe oligoasthenoteratozoospermia have microdeletions in the area of the azoospermic factor AZF - Yq11, which contains genes that control the processes of spermatogenesis. In the present study totally 48 men with azoospermia and oligoastenoteratozoospermia were included in the Y-microdeletion analysis by real time PCR. The set of primers used to detect microdeletions on the Y chromosome included: SRY (sY14) and ZFX / Y (short arm), sY84 and sY86 (for AZFa); sY127 and sY134 (for AZFb); sY254 and sY255 (for AZFc). No microdeletions in the Y chromosome were found in 29.2% of the men studied. Among all others, single microdeletion was found in the AZFa subregion of Yq (2.1%) and in the SRY zone of Yp (22.9%). For all other men included in the study, different combinations of microdeletions were found in two (AZFb + AZFc, AZFa + SRY, AZFb + SRY, AZFc + SRY and SRY + ZFX / Y), three (AZFa + AZFc + SRY, AZFb + AZFc + SRY and AZFb + SRY + ZFX / Y), four (AZFa + AZFb + AZFc + SRY, AZFa + AZFb + SRY + ZFX / Y, AZFa + AZFc + SRY + ZFX / Y and AZFb + AZFc + SRY + ZFX / Y) or five (AZFa + AZFb + AZFc + SRY + ZFX / Y) sections / subsections simultaneously. The obtained results support the opinion that interactions between different deletions in the AZF region of the Y-chromosome with other genes or gene groups are possible, which is a significant factor influencing spermatogenesis.

Key words: male infertility, Y chromosome, microdeletions, semen quality, reproductive health.

Introduction

Infertility is a complex medical problem affecting about 15% of couples in reproductive age worldwide. Approximately half of these cases are related to the male factor. Despite many studies in this field, the origin of infertility remains unknown in about 30% of individuals suffering from it (Poongothai et al., 2009). The causes of male infertility are different and significantly related to both genetic and environmental factors (Matzuk & Lamb, 2008). Changes in chromosome structure (e.g. Robertsonian translocation, originating from chromosomes 13 and 14; reciprocal translocations, etc.), in the number of sex chromosomes (such as Klinefelter syndrome - 47, XXY and 46, XX male syndrome) and autosomes, as well as gene mutations have been discussed as reasons for diminished reproductive potential in men (Poongothai et al., 2009). Such mutations typically affect genes responsible for the spermatogenesis course or associated with the differentiation of the male reproductive system (Matzuk & Lamb, 2008). Microdeletions in the Y chromosome associated with manifestation of azoospermia have been studied by a number of authors (Sadeghi-Nejad & Farrokhip, 2007; O'Flynn O'Brien et al., 2010). According to Ferlin et al. (2003; 2007) and Fernandes et al. (2006), they currently are one of the most common identified causes for severe spermatogenic damage. The Y chromosome contains genes associated with sex determination and differentiation. It has been established that the SRY region of the short arm of the Y chromosome is responsible for the maturation of Sertol cells and the normal course of spermatogenesis. The genetic changes in the SRY region are due to molecular genetic mechanisms associated with abnormalities in the testicular formation (Schumacher et al., 2008; Bradbury, 2017. This region is related

to the sex determination in the fetus, to the androgens' secretion and the activation of their receptors (Toncheva, 2010). In the short arm of the Y chromosome, there is also a ZFY sex-determining region homologous to that on the X chromosome (ZFX / Y), which encodes a zinc-finger protein (Page, 1988; Bradbury, 2017). In the AZF (azoospermia factor) region of the long arm of the Y chromosome, more than 20 genes involved in spermatogenesis have been identified (Navarro-Costa et al., 2010a, b). The small chromosomal deletions, spanning several neighboring genes, due to their size, cannot be detected by conventional chromosome analysis. Their frequency in men with azoospermia or severe oligozoospermia is high. Microdeletions in the long arm of the Y chromosome are specifically associated with abnormalities in spermatogenesis. Approximately 5 to 10% of men with azoospermia or severe oligozoospermia have deletions in the Yq11 AZF area, which contains genes that control the processes of spermatogenesis. Three different AZF areas, designated as "a", "b" and "c", are located in this region. Disorders of spermatogenesis in patients with deletions in the a, b, or c regions of AZF have been found to have different specificity and expression, suggesting that interactions between various deletions in AZF region and other genes (or gene groups) are possible, which also affects spermatogenesis (Navarro-Costa, 2010a, b). From a biomedical point of view, in Bulgaria this problem is insufficiently studied. Linev et al. (2017), Mitkovska et al. (2019) and Ivanova et al. (2021) emphasize the fact that different types of aberrations in gonosomes and autosomes are often in the root of reproductive problems in men, which determines the necessity for complex cytogenetic and molecular genetic approaches in the study of male reproductive health. One of the most commonly identified molecular genetic causes for male infertility are submicroscopic deletions in the long arm of the Y chromosome, and establishing the type and specificity of their manifestation are important to clarify the prospects for patients with azoospermia and oligozoospermia (Linev et al., 2017).

The aim of the present study was to analyze the specificity and frequency of occurrence of Y chromosome microdeletions in men with azoospermia and oligoasthenoteratozoospermia and to characterize the possible relations between the abnormalities found.

Material and Methods

This study was approved by the Institutional Ethical Committee with Certificate N 2 / 16.01.2019. Totally 48 men with azoospermia and oligoastenoteratozoospermia were included in the Y-microdeletion analysis. All study participants signed an informed consent form and voluntarily answered questions included in the survey, providing data on their medical history. Genomic DNA isolated from frozen (-20 ° C) semen was used for PCR analysis to identify deletions in the Y chromosome. Semen material from fertile men was used as a positive control. A complex of all components necessary for the reaction with the exception of the template DNA replaced with ddH2O, as well as a DNA sample from a female individual were used as negative controls. For detection of microdeletions in certain regions of the Y chromosome, Real time PCR was applied. The set of primers used to diagnose microdeletions on the Y chromosome includes: SRY (sY14) and ZFX / Y (short arm), sY84 and sY86 (for AZFa); sY127 and sY134 (for AZFb); sY254 and sY255 (for AZFc) – Table 1.

The lack of hybridization between the primers and their complementary regions of the template single-stranded DNA was graphically reported on the monitor and accepted as an evidence of a microdeletion in the relevant region of the Y chromosome.

Statistical analyzes were performed with the software package SPSS, version 22. Descriptive statistics were used to characterize the frequency of the compared groups. The differences (dependencies) between the compared groups were analyzed by the Pearson Chi-Square test ($\chi 2$) and the t-test. Statistical significance was defined as $P < 0.05$ for the different analysis schemes.

Table 1. Sequences of the primers used: F – forward; R – reverse.

Primers	Sequences
ZFX/Y – F	5′ – ACC R* CT GTA CTA CTG ACT GTG ATT ACA C – 3′
ZFX/Y – R	5′ – GCA C Y* T CTT TGG TAT C Y* G AGA AAG T – 3′
SRY – F	5′ – GAA TAT TCC CGC TCT CCG GA – 3′
SRY – R	5′ – GCT GGT GCT TTC TTG AG – 3′
sY86 – F	5′ – GTG ACA CAC AGA CTA TGC TTC – 3′
sY86 – R	5 ′ – ACA CAC AGA GGG ACA ACC CT – 3′
sY 127 – F	5′ – GGC TCA CAA ACG AAA AGA AA – 3′
sY 127 – R	5′ – CTG CAG GCA GTA ATA AGG GA – 3′
sY 254 – F	5′ – GGG TGT TAC CAG AAG GCA AA – 3′
sY 84 – F	5′ – AGA AGG GTC TGA AAG CAG GT – 3′
sY84 – R	5′ – GCC TAC TAC CTG GAG GCT TC – 3
sY134 – F	5′ – GTC TGC CTC ACC ATA AAA CG – 3′
sY134 – R	5′ – ACC ACT GCC AAA ACT TTC AA – 3′
sY255 – F	5′ – GTT ACA GGA TTC GGC GTG AT – 3′
sY255 – R	5′ – CTC GTC ATG TGC AGC CAC – 3′

Results

The results of the current study show the presence of microdeletions both – on the long and on the short arm of the Y chromosome. The data concerning microdeletions' frequencies calculated for the different subareas of the AZF region as well as concerning the SRY and ZFX / Y regions are presented in Table 2. Statistical information was presented in Table 3.

Table 2. Frequency of established microdeletions in the Y chromosome by regions in its short and long arm and combinations of them in the analyzed men with azoospermia and oligoasthenoteratozoospermia.

Microdeletions by regions and combinations of them	Number of individuals	Valid %
Absence	14	29.2
AZFa	1	2.1
AZFb+AZFc	1	2.1
SRY	11	22.9
AZFa+SRY	1	2.1
AZFb+SRY	1	2.1
AZFc + SRY	1	2.1
AZFa+AZFc+SRY	1	2.1
AZFb+AZFc+SRY	4	8.3
AZFa+AZFb+AZFc+SRY	2	4.2
SRY+ZFX/Y	2	4.2
AZFb+SRY+ZFX/Y	1	2.1
AZFa+AZFb+SRY+ZFX/Y	1	2.1
AZFa+AZFc+SRY+ZFX/Y	1	2.1
AZFb+AZFc+SRY+ZFX/Y	4	8.3
AZFa+AZFb+AZFc+SRY+ZFX/Y	3	6.3
Total	48	100.0

Table 3. Data concerning the relations in the groups compared.

Crosstab		AZFa		Total	Sig.
		none	yes		
SRY	none	15	1	16	0.07
	yes	23	9	32	
Total		38	10	48	
Crosstab		AZFb		Total	Sig.
		none	yes		
SRY	none	15	1	16	0.002
	yes	16	16	32	
Total		31	17	48	
Crosstab		AZFc		Total	Sig.
		none	yes		
SRY	none	15	1	16	
	yes	17	15	32	0.004
Total		32	16	48	
Crosstab		ZFX/Y		Total	Sig.
		none	yes		
SRY	none	16	0	16	
	yes	20	12	32	0.003
Total		36	12	48	

The data presented in Table 2 show that microdeletions in the Y chromosome were not found only in 29.2% of the studied men in this group. Single microdeletions were established in the AZFa subregion of Yq (2.1%) and in the SRY region of Yp (22.9%). For all other men included in this study, different combinations of microdeletions were detected in two (AZFb + AZFc, AZFa + SRY, AZFb + SRY, AZFc + SRY and SRY + ZFX / Y), three AZFc + SRY, AZFb + AZFc + SRY and AZFb + SRY + ZFX / Y), four (AZFa + AZFb + AZFc + SRY, AZFa + AZFb + SRY + ZFX / Y, AZFa + AZFc + SRY + ZFX / Y and AZF + AZFc + SRY + ZFX / Y) or five (AZFa + AZFb + AZFc + SRY + ZFX / Y) regions / subregions simultaneously (Table 2). The anomalies in the SRY region (alone and in combination) were a total of 66.7% in the examined men with azoospermia and oligoasthenoteratozoospermia.

Discussion

Submicroscopic deletions in the AZF zone of the long arm of the Y chromosome are one of the most common molecular genetic causes for male infertility (Harton & Tempest, 2012). It has been found that most of these microaberrations occur over again, as a result of interchromosomal homologous recombination within the region (Navarro-Costa, 2010a; b). They damage one or more of the genes and this results in different defects in spermatogenesis.

Genes in the AZF region have been suggested to have a regulatory function in the germ cell cycle and during meiosis. The diverse clinical manifestations in gametogenesis disorders are results of this complex regulation (Harton & Tempest, 2012).

Differences in the registered frequencies have been found when analyzing the literature data on microdeletions in the AZF subregions. Microdeletions in the Y chromosome were found in 12% of 100 men with azoospermia according to the study of Mirfakhraie et al. (2010). The authors found that 66.7% of these microdeletions were in the AZFb, 41.7% - in AZFc, 33.3% - in AZFd - and 8.3% - in AZFa region. Linev et al. (2017) reported the presence of microdeletions on the Y chromosome in approximately 22% of the 73 men studied with abnormalities in the quantitative and qualitative sperm parameters. The main percentage of microdeletions they found was in the AZFc region.

In the course of the present study, a high total percentage of microdeletions in the Y chromosome was found (Table 2), which is due to the fact that the analysis included patients with established azoospermia and oligoastenoteratozoospermia.

The results found in our study show that the highest percentage of microdeletions is in the AZFb subregion, but it is significantly lower than indicated by Mirfakhraie et al. (2003).

According to Navarro-Costa (2010a, b): deletions in the AZFa region are most often associated with azoospermia represented by Sertoli cell only syndrome (SCOS) and less frequently – with oligospermia; deletions in the AZFb region are associated with blocking spermatogenesis on spermatocyte level (Spc), and deletions in the AZFc region – with SCOS and fewer number of spermatogonia (Spg).

In the present study, the statistically significant dependences between the presence of microdeletions in the SRY region from Yp and the microdeletions in the other studied regions of the Y chromosome were analyzed (Table 3). The statistic data show that microstructural anomalies in the SRY region are statistically significantly associated with microdeletions in the ZFX / Y region of Yp and in the AZFa, AZFb and AZFc subregions of Yq (significance by $\chi 2$ test: 0.005; 0.079; 0.003; 0.005, respectively by Fisher's exact test: 0.003; 0.078; 0.002 and 0.004, respectively).

Conclusions

The obtained results support the opinion that interactions between different deletions in the AZF region of the Y-chromosome with other genes or gene groups are possible, which is a significant factor influencing spermatogenesis.

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