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Analysis of Y chromosome microdeletions and CFTR gene mutations as genetic markers of infertility in Serbian men

Analiza mikrodelecija Y hromozoma i mutacija CFTR gena kao genetskih markera infertiliteta kod muškaraca u Srbiji

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Abstract

Background/Aim. Impaired fertility of a male partner is the main cause of infertility in up to one half of all infertile couples. At the genetic level, male infertility can be caused by chromosome aberrations or gene mutations. The presence and types of Y chromosome microdeletions and cystic fybrosis transmembrane conductance regulator (CFTR) gene mutations as genetic cause of male infertility was tested in Serbian men. The aim of this study was to analyze CFTR gene mutations and Y chromosome microdelations as potential causes of male infertility in Serbian patients, as well as to test the hypothesis that CFTR mutations in infertile men are predominantly located in the several last exons of the gene. **Methods.** This study has encompassed 33 men with oligo- or azoospermia. The screening for Y chromosome microdeletions in the azoospermia factor (AZF) region was performed by multiplex PCR analysis. The screening of the CFTR gene was performed by denaturing gradient gel electrophoresis (DGGE) method. **Results.** Deletions on Y chromosome were detected in four patients, predominantly in AZFc region (four of total six deletions). Mutations in the CFTR gene were detected on eight out of 66 analyzed chromosomes of infertile men. The most common mutation was F508del (six of total eight mutations). **Conclusion.** This study confirmed that both Y chromosome microdeletions and CFTR gene mutations played important role in etiology of male infertility in Serbian infertile men. Genetic testing for Y chromosome microdeletions and CFTR gene mutations has been introduced in routine daignostics and offered to couples undergoing assisted reproduction techniques. Considering that both the type of Y chromosome microdeletion and the type of CFTR mutation have a prognostic value, it is recomended that AZF and CFTR genotyping should not only be performed in patients with reduced sperm quality before undergoing assisted reproduction, but also for the purpose of preimplantation and prenatal diagnostics in couples in which *in vitro* fertilization has been performed successfully.

Key words:
infertility; men; sex chromosome aberrations; genes; mutation; yugoslavia.

Apstrakt

Uvod/Cilj. Poremećena plodnost muškog partnera je glavni uzrok infertiliteta kod polovine neplodnih parova. Na genetskom nivou infertilitet kod muškaraca mogu uzrokovati hromozomske aberacije ili genske mutacije. U ovoj studiji analizirano je prisustvo i tip mikrodelecija Y hromozoma i mutacija u genu za regulator transmembranske provodljivosti u cističnoj fibrozi (CFTR) kao genetska osnova infertiliteta kod muškaraca u Srbiji. Cilj studije je bio da se analiziraju mutacije u CFTR genu i mikrodelecije Y hromozoma, kao potencijalni uzroci infertiliteta kod muškaraca u Srbiji, kao i da se testira hipoteza da su CFTR mutacije kod infertilnih muškaraca predominantno locirane u nekoliko poslednjih egzona. **Metode.** Studija je obuhvatila 33 muškarca sa oligo ili azospermijom. Detekcija mikrodelecija Y hromozoma u regionu faktora azospermije (AZF) vršena je pomoću multipleks PCR metode. Pretraživanje CFTR gena vršeno je metodom elektroforeze u gelu sa gradijentom denaturišućeg agensa (DGGE). **Rezultati.** Delecije Y hromozoma su detektovane kod četiri bolesnika, predominantno u AZFc regionu (četiri od ukupno šest). Mutacije u CFTR genu su detektovane na osam od 66 analizovanih hromozoma infertilnih muškaraca. Najčešće detektovana CFTR mutacija je F508del (šest od osam). **Zaključak.** Ova studija je potvrdila da mikrodelecije Y hromozoma i mutacije u CFTR genu igraju važnu ulogu u etiologiji infertiliteta kod muškaraca u Srbiji. Genetsko testiranje koje obuhvata detekciju mikrodelecija Y hromozoma i mutacija u CFTR genu uvedeno je u rutinsku dijagnostičku praksu i ponuđeno je parovima koji pristupaju asistiranoj reprodukciji. S obzirom da tip mikrodelecija Y hromozoma i tip mutacija u CFTR genu imaju prognostički značaj, preporuka je da se genotipizacija AZF regiona i CFTR gena ne vrši samo kod bolesnika sa umanjenim kvalitetom sperme pre pristupanja asistiranoj reprodukciji, već i u svrhe preimplantacione i prenatalne dijagnostike kod parova kod kojih je uspešno izvršena *in vitro* fertilizacija.

Ključne reči:
neplodnost; muškarci; hromosomi, polni, aberacije; geni; mutacija; srbija.

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Introduction

Impaired fertility of a male partner is the main cause of infertility in up to one half of all infertile couples. At the genetic level, male infertility can be caused by chromosome aberrations or gene mutations [1]. So far, molecular genetics has discovered a large number of genes involved in reproduction which, when mutated or deleted, can cause pathological changes in male reproductive system [2]. The most frequent genetic causes of male infertility are considered to be Y chromosome microdeletions and mutations in the cystic fybrosis transmembrane conductance regulator (CFTR) gene [3].

The genes located on the long arm of the Y chromosome play an essential role in spermatogenesis, and deletions of these regions are suggested to be involved in male infertility [4]. These spermatogenesis loci have been characterized as the three non-overlapping regions on Y chromosome and named the azoospermia factors (AZF) a, b and c [5].

The main role of the CFTR protein is to transport chloride ions through the apical membrane of epithelial cells. Mutations in this gene result in the protein whose transporting function has been impaired or missing, which leads to fluid imbalances in epithelium-lined organs, including the male reproductive system. Genetic analyses have indicated that CFTR gene plays an important role in spermatogenesis and, when mutated, leads to obstructive azoospermic conditions including congenital bilateral absence of vas deferens (CBAVD) [6]. Although CFTR gene mutations are scattered within essentially the entire gene, the most frequent mutations and polymorphisms have been found in certain exons and flanking introns (especially exons 4, 10, 11, 20 and 21) [7]. The CFTR variations most frequently detected in infertile men, are mutation F508del in exon 10 and 5T allele of Tn polymorphism on the boundary of intron 8 and exon 9 [8]. The data from literature, along with the search of the CFTR gene database, indicate that a large number of other detected mutations in the infertile men are located in last several exons (exon 17b to exon 24) of the CFTR gene [7, 8].

The aim of this study was to analyze CFTR gene mutations and Y chromosome microdeletions as potential causes of male infertility in the Serbian patients, as well as to test the hypothesis that CFTR mutations in infertile men are predominantly located in the last several exons of the gene.

Methods

This study included 33 men with oligo- or azoospermia who had been diagnosed at the Urological Clinic in Belgrade. The patients were divided in three groups: 10 patients with obstructive azoospermia, 11 patients whose infertility had been caused by impaired spermatogenesis or sperm maturation and 12 patients with an unknown cause of infertility. The patients were grouped according to the results of their spermatogram, including sperm volume, pH, level of fructosis or alpha-glucosidase and spermatozoid count [9]. For the purpose of the analysis, DNA was isolated from the peripheral blood lymphocytes [10].

An Y chromosome microdeletion screening was performed by multiplex PCR analysis. The primers for the analysis of six markers were previously described [9]. The analysis was performed for two markers in each of the AZF region: sY84 and sY86 in AZFa, sY127 and sY134 in AZFb and sY254 and sY255 in AZFc. All products of amplification were analyzed by electrophoresis on 10% (29:1) polyacrylamide gel, followed by silver staining. Whenever failure of amplification indicated deletion, PCR analysis was repeated twice in single reactions to confirm the absence of each marker.

The screening of the CFTR gene was performed by previously described denaturing gradient gel electrophoresis (DGGE) method [11]. The exons in which band pattern variations were detected were further analyzed by direct DNA sequencing. The polymorphism IVS8-5T was analyzed by the PCR-mediated site-directed mutagenesis (PSM) method [12].

Results

The detected AZF region microdeletions and CFTR gene mutations are shown in table 1. Deletions on Y chromosome were detected in four patients, two with azoospermia and two with unknown cause of infertility. Mutations in the CFTR gene were detected on eight out of 66 analyzed chromosomes of infertile men, while the presence of 5T

Table 1

Y chromosome microdeletions and CFTR gene mutations detected in infertile men

Group of patients	Number of patients	Y chromosome microdeletion	CFTR gene mutation / polymorphism
Obstructive azoospermia (n = 10)	1	AZFc	F508del / 711+3A/G
	1	AZFb	-
	3	-	F508del / 5T
	1	-	F508del
Impaired spermatogenesis (n = 11)	1	-	F508del / D1152H
	2	-	5T
Unknown cause of infertility (n = 12)	1	AZFa + AZFc	-
	1	AZFc	-
	1	-	5T

variant of Tn polymorphism was detected on six chromosomes. Mutations were found in the groups of the patients with obstructive azoospermia and the patients with impaired spermatogenesis, while in the group of the patients with an unknown cause of infertility no CFTR mutations were found. Common polymorphisms F508C, 2694T/G, 4002A/G and 4029A/G were detected in several patients in each of the three groups.

Discussion

In the group of ten men with obstructive azoospermia, the two patients had partial Y chromosome microdeletions, one in AZFb and the other in AZFc region. The most common CFTR mutation in this group was F508del, which led to the absence of the CFTR protein on the epithelial cell membrane, and it was detected in five patients. In one patient who was a carrier of F508del mutation on one chromosome, mutation 711+3A→G was detected on the other. This mutation has not yet been examined at a functional level [7]. The location of mutation (intron five of the CFTR gene) indicates that it might be involved in splicing of CFTR pre-mRNA and, therefore, lead to the reduced quantity of the CFTR protein in the cell. It is worth mentioning that the patient with F508del/711+3A→G genotype was exactly the same one found to have partial AZFc microdeletion. In three patients who were heterozygous for F508del mutation, 5T variant of Tn polymorphism was detected on the other chromosome. Polymorphic Tn locus (located in intron eight of the CFTR gene) has a role in pre-mRNA splicing and in 90% of all the cases in which 5T allele is present, exon nine is deleted from mRNA, resulting in non-functional CFTR protein [13].

Twenty two chromosomes were analyzed in the group of infertile men with impaired spermatogenesis or sperm maturation, but no Y chromosome microdeletions were detected, while two CFTR mutations were found, both in the same patient. This patient was a compound heterozygote for F508del and D1152H mutations. Mutation D1152H is considered to be mild, exerting with borderline or even normal levels of chloride in sweat, with relatively preserved permeability of the chloride channel, but severely reduced cAMP-activated flow of the chloride ions in the cell [14]. This indicates that this mutation interferes with correct gating of the chloride channel. The patient carrying this mutation had severe oligoastenospermia. Two patients with azoospermia were heterozygous carriers of 5T allele.

In the group consisted of patients with an unknown cause of infertility, Y chromosome microdeletions were detected in two patients. While one patient had a combination of partial microdeletions of AZFa and AZFc regions, the other had complete AZFc microdeletion. No CFTR mutations were found in this group. One patient was found to be a carrier of 5T allele.

In four men, Y chromosome microdeletions were found to be the genetic cause of infertility and they were detected predominantly in AZFc region. The frequency of Y chromosome microdeletion depends mostly on the selection criteria of infertile patients varying from 1% to 55%, and, therefore, the frequency increases with the severity of spermatogenic defect [15]. In general, deletions of AZFa region are mainly associated with Sertoli cell-only syndrome (SCOS), deletion of AZFb region with spermatogenic arrest, while the absence of AZFc region is associated with a range of phenotypes, from azoospermia to oligozoospermia [5].

In two patients who were found to be compound heterozygotes for CFTR mutations (F508del/711+3A→G and F508del/D1152H) azoospermia and impaired spermatogenesis could be explained by the presence of mutations on both alleles of the CFTR gene. Three men with obstructive azoospermia had F508del mutation in combination with 5T allele. Since F508del mutation results in non-functional protein and 5T allele variant results in the reduced production of the CFTR protein, infertile men with obstructive azoospermia with F508del mutation on one chromosome and 5T allele on the other have very low quantity of functional CFTR protein in the cell. Low quantity of functional CFTR protein probably leads to changes only in those organs that are most sensitive to dysfunction of the CFTR protein, such as the male reproductive system [16].

The detection of mutations and 5T alleles in the patients with impaired spermatogenesis or sperm maturation indicates a possible role of the CFTR protein in spermatogenesis. Expression of the CFTR gene in the testicles is confirmed in infertile men both with and without CBAVD and also in CBAVD patients with the presence of morphologically impaired spermatozoides with the reduced quantity of normal CFTR gene transcripts [17]. This indicates that this protein could be involved in the later phases of the development and maturation of the germ cells.

This was the first study in the Serbian population addressing the issue of involvement of Y chromosome microdeletions and CFTR gene variations in the etiology of male infertility. The results of this study correspond to the data published so far for Y chromosome microdeletions and confirm that they are found more often in men with more severe spermatogenic defect [15]. When it comes to CFTR gene, mutation F508del and 5T allele of Tn polymorphism, which have already been implicated in etiology of male infertility, are found to be associated with this condition. Their frequency was the highest in the group of men with obstructive azoospermia. Although some studies showed the increased number of mutations in the last several exons of the CFTR gene in infertile men, in this study however, we did not obtain such results. It is confirmed that infertile men have an elevated risk of being the carriers of mutation within the CFTR gene, in comparison with the general population [6].

Conclusion

This study confirmed that both Y chromosome microdeletions and CFTR gene mutations play an important role in etiology of male infertility. Genetic testing for Y chromosome microdeletions and CFTR gene mutations has been introduced in routine daignostics and offered to couples undergoing assisted reproduction techniques. Since infertile men are the candidates for assisted reproduction, detection of Y

chormosome deletions and CFTR gene mutations in these patients represent an important element of a genetic counseling. Considering that both the type of Y chromosome microdeletion and the type of CFTR mutation have a prognostic value, it is recommended that AZF and CFTR genotyping should not only be performed in patients with the reduced sperm quality before undergoing assisted reproduction, but also for the purpose of preimplantation and prenatal diagnostics in the couples in which *in vitro* fertilization has been performed successfully.

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