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Mutation analysis of HSFY gene by DNA sequencing in Turkish men with idiopathic infertility

İdiyopatik infertilite tanılı Türk erkeklerinde HSFY geni mutasyonlarının araştırılması

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Abstract

Purpose: In this study we planned to investigate HSFY gene mutations in Turkish infertile men with azoospermia, oligospermia and/or poor motility/morphology.

Materials and methods: From three distinct medical centers, 41 Turkish infertile men contributed to this study. The patients included in the study had no endocrine or obstructive causes of spermatogenic failure and no Y microdeletion, and had normal karyotype. Mutation analysis of the HSFY gene was performed by DNA sequencing.

Results: No variant could be detected in coding regions and flanking introns of HSFY gene in the study population.

Conclusion: This study suggests no relation of HSFY sequence variant with spermatogenic failure and, the clinical molecular approach to diagnosis of individuals with spermatogenic failure is complicated due to extensive genetic heterogeneity and need more study to reveal causative genes and mutations of idiopathic infertility in men.

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Key words: Spermatogenesis, male infertility.

Özet

Amaç: Bu araştırmada azospermi, oligospermi ve/veya motilite/morfoloji bozukluğu olan Türk infertil hastalarda HSFY geninin mutasyon analizi planlanmıştır.

Gereç ve yöntem: Araştırmaya üç farklı merkezden toplam 41 infertil Türk hasta katılmıştır. Spermatogenez yetmezliğine neden olabilecek endokrin ve obstrüktif probleri olmayan, Y kromozomunda mikrodelesyonu olmayan ve normal karyotipe sahip olgular çalışmaya dahil edilmiştir. HSFY geninin mutasyon analizi DNA dizi analizi yöntemi ile gerçekleştirilmiştir.

Bulgular: Çalışma grubuna dahil olan olgularda HSFY geninin kodlanan bölgeleri ve bu bölgelere komşu intronik bölgelerinde herhangi bir değişim saptanmamıştır.

Sonuç: Bu çalışmada spermatogenez yetersizliği ile HSFY geni arasında bir ilişki saptanmamıştır. Genetik heterojenite nedeniyle spermatogenez yetersizliği olan bireylerde moleküler tanı uygulamaları oldukça güç olup erkeklerdeki idiyopatik infertileye neden olan gen ve mutasyonların aydınlatılması için daha çok çalışma yapılması gereklidir.

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Anahtar sözcükler: Spermatogenezis, erkek infertilitesi.

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Introduction

Primary spermatogenic failure is one of the major factors of male infertility and the most frequent genetic cause is microdeletion of Y chromosome with 5-10% [1]. After detectable deletions of the proximal Yq in azoospermic men was reported, the region with microdeletion caused azoospermia and severe oligozoospermia (A/O) was located Yq11 flanked by pseudoautosomal regions called azoospermia factor dividing in to three regions (AZFa, b, c) [2,3]. According to genotype-phenotype correlation while patients with microdeletion of AZFc region have residual spermatogenesis and good candidate for assisted reproduction, the patients with microdeletion of AZFa, AZFb and/or AZFb+c have no residual spermetogenesis for testicular sperm extraction, and not recommended for ICSI or TESE. So the genes on these regions are strong candidate genes for the patients with azoospermia/oligozoospermia. One of these genes is HSFY located on AZFb and belongs to the heat shock factor (HSF) family which has been mapped on the Y chromosome (Fig.1). In Sertoli and spermatogenic cells in the testis, it has a role in spermatogenesis [4-7].

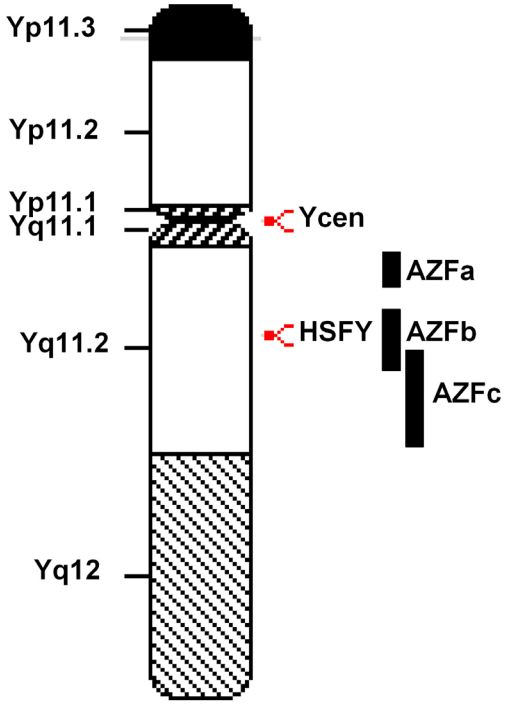

Figure 1. Ideogram of Y chromosome showing the locus of HSFY gene located proximal part of AZFb region.

Y microdeletion testing could not detect the other genetic alterations in the genes maybe because of maturation arrest of the spermatozoa. The aim of this study is to analyze the mutation of HSFY gene in infertile men having no microdeletion of Y chromosome for revealing spermatogenic failure.

Materials and Methods

Patients

From three distinct medical centers, 41 Turkish infertile men were contributed to this study. All patients underwent an evaluation including physical examination, hormonal studies, and karyotype. The patients included in the study had no endocrine or obstructive causes of spermatogenic failure and no Y microdeletion, and had normal karyotype. Semen analysis were performed according to the World Health Organization guidelines (WHO 1999) and study group were divided into subgroups; the patients had no spermatozoa in ejaculate accepted azoospermia, sperm concentrations $<3x10^6$/ml were accepted severe oligozoospermia, and $3\text{-}19x10^6$/ml were accepted oligozoospermia. Sperm morphology evaluation was performed using Kruger criteria.

Written informed consents were obtained from all the patients. The study protocol was approved by the Pamukkale University Ethics Committee (approval number: 2011/16).

Molecular Analysis

Peripheral blood samples were obtained from all patients following written informed consent. Genomic DNA was isolated by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the “blood and body fluid protocol” using peripheral blood samples. According to ENSEMBL and UCSC Human Genome databases (GRCh37/hg18) coding exons of HSFY gene were amplified by polymerase chain reaction (PCR). Primers were designed using Primer3 web software. Following primers were used: For HSFY exon1F1 GGCAAGAGATTTTTGCAGCTTAC, exon1R1 TCTGGCTCAGAGACACAAACTG, exon1F2 GGACTCAGACTTACGGTCAATG, exon1R2 TAAAAGGTTAACGCTCAAGCTG, exon2F ATGAGGTTTTCTGGATCTGAGG, exon2R TTCCAATCTAGTCTTTCCCAGAG. PCR reactions were done in a total volume

of 50 µl, including, extracted DNA, 10 pmol of each forward and reverse primers, and 25 µl of HotStarTaq Master Mix [containing 2.5 units of HotStarTaq DNA polymerase, 1x PCR buffer with 1.5 mM $MgCl_2$, and 200 µM of each dNTP (Qiagen, Hilden, Germany)]. The thermal cycling was performed as follows: initial activation of HotStarTaq DNA polymerase at 95 ˚C for 15 min, followed by 35-45 cycles of denaturation at 94˚C for 1 min, annealing at 54˚C-62˚C for 1 min and, extension at 72˚C for 1 min, with final extension at 72˚C for 10 min. The PCR amplification products were separated by 2% agarose gel. PCR products were directly sequenced using ABI PRISM 3130 DNA analyzer (Applied Biosystems).

Results

Ages of the study group ranged from 29 to 42. All patients had normal physical examination findings, no dysmorphic signs. FSH, LH and testosterone levels were in normal range (1.5-8.0 IU/l; 1.5-6.0 IU/l; 9.0-34.0 nmol/l respectively). All had normal 46, XY karyotype. Among 41 patients 28 had azoospermia, 7 had poor motility or poor morphology, 3 had oligozoospermia, 1 had severe oligozoospermia, 1 had oligoasthenospermia, and 1 had severe oligozoospermia and poor motility. Mutation analysis of HSFY gene was performed by direct sequencing. However, no variant could be detected in coding regions and flanking introns (Table 1).

Table 1. Clinical and molecular data of study group.

Study Group	n	Age (years)	HSFY variation
Azoospermia	28	29-42	No variation
Severe oligozoospermia	1	35	No variation
Oligozoospermia	3	30-36	No variation
Oligoasthenospermia	1	31	No variation
Severe oligozoospermia with poor motility	1	31	No variation
Poor morphology and/or poor motility	7	29-40	No variation
Toplam	41	29-42	No variation

Discussion

In this study we performed mutation analysis of HSFY gene isoform 1 in 41 idiopathic infertile Turkish men and could not find any mutation in HSFY gene in the study population. HSFY gene is a member of heat shock protein transcriptional factor (HSF) located on chromosome Yq11.221 (AZFb region) in multiple copies and related with sperm maturation. There are two copies and three mRNA transcripts located in AZFb region [8]. HSFY isoform 1 is strongly candidate form for sperm maturation because of having heat shock factor-like DNA-binding domain. Sato et al. [9]. showed that the HSFY expression decreased in men with maturation arrest. In another study, Kinoshita et al. [10] suggested that HSFY is candidate for azoospermic factor on the human Y chromosome due to predominant expression in round spermatids. Stahl et al. [11] did expression analysis of ten candidate genes located microdeletion region by quantitative RT-PCR in testicular tissue. They suggested CDYZ and HSFY expression could be implicated in the pathogenesis of maturation arrest. On the other hand, Kichine et al [12] found that four patients have Y microdeletion, one of them has azoospermia, and the others have oligospermia. They had deletion of proximal and of the AZFb region including HSFY gene. They did not found this deletion in controle group of 1179 fertile men. Although these studies report strong evidence related with HSFY and normal spermatogenesis, we could not find sequence variant to explain spermatogenesis defect of our patients.

The genes on the sex chromosomes have been added from the autosomes like DAZ (deleted in azoospermia) and CDY (chromodomain protein, Y chromosome) via retroposition [13,14]. On the other hand Y chromosome which is different from X chromosome has palindromes which contain eight massive inverted repeat segments. These repeats prevent mutation. This heterochromatic structure of Y chromosome and the effect of other genes on autosomal chromosomes on

spermatogenesis make difficult to search the causative genes of A/O [15].

In conclusion, this study suggests no relation of HSFY sequence variant with spermatogenic failure and the clinical molecular approach to diagnosis of individuals with spermatogenic failure is complicated due to extensive genetic heterogeneity and need more study to reveal causative genes and mutations of idiopathic infertility in men.

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