First Infertile Case with *CSTF2T* Gene Mutation

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Established Facts

- Male infertility is a genetically heterogeneous group of diseases.
- Biallelic mutations of the *CSTF2T* gene cause male infertility in animals.

Novel Insights

- *CSTF2T* is a candidate gene that can contribute to this heterogeneity.
- Biallelic mutations of the *CSTF2T* gene can also cause male infertility in humans.

Keywords

Clinical exome sequencing · *CSTF2T* · Infertility · Novel mutation

Abstract

Male infertility is multifactorial and presents with heterogeneous phenotypic features. Genetic factors are responsible for up to 15% of the male infertility cases. Loss of the *Cstf2t* gene in male mice results in infertility. No disease-associated mutations have been described for this gene in infertile men. Here, we report a patient diagnosed with infertility in whom a homozygous nonsense mutation in the *CSTF2T* gene was detected by clinical exome sequencing. This case is the first description of an infertile patient who has a homozygous *CSTF2T* mutation.

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Introduction

Approximately 7% of all men are affected by infertility and commonly present with spermatogenic failure. The primary cause of spermatogenic failure may be genetic factors, however the etiologies are undetermined in the majority of cases. In humans, approximately 1,000 genes are expressed in a testis-enriched manner, although until now only a scant selection of these have been found to contribute to male infertility [Yin et al., 2019]. Consequently, it is of considerable importance to understand the genetic basis of spermatogenic failure for the clinical diagnosis and treatment of male infertility. All eutherian mammals, including humans and mice, maintain the *CSTF2T* gene but the τ CstF-64 protein is only expressed in a subgroup of mammalian tissues, mostly testis and brain. Studies in mice have shown that males lacking *Cstf2t* (*Cstf2t*^{-/-} mice) experience spermatogenesis irregularity and infertility, however female fertility is unaffected.

ed [Harris et al., 2016]. Here, we report a patient diagnosed with infertility in whom a homozygous mutation (c.979C>T, p.Arg327*) in the *CSTF2T* (NM_015235) gene was detected using clinical exome sequencing. This is the first description of a patient who has a homozygous mutation in the *CSTF2T* gene associated with severe infertility.

Patient and Methods

Case Presentation

A 31-year-old male patient, born to consanguineous parents, was referred to the Genetics Department with infertility. No other symptoms had been observed besides infertility. He has 3 sisters and 8 brothers. His 3 elder brothers are also infertile. Physical examination of the patient was normal. The semen samples were obtained by masturbation after 3–7 days of sexual abstinence. Semen analyses were performed according to the World Health Organization recommendations. The patient presented with severe oligoasthenospermia with 100% immotile spermatozoa and a low sperm count (0.96×10^6 sperm/mL). There was no analysis for sperm morphology. LH, FSH, prolactin, and total testosterone were within the normal range (FSH 5.1 ng/dL, LH 7.52 ng/dL, prolactin 10.08 ng/dL, total testosterone 533 ng/dL).

Genetic Analysis

In the present study, microdeletions of the Y chromosome and karyotype anomalies were excluded first. Conventional cytogenetic analysis was performed to detect karyotype anomalies, and the Devyser AZF Extension kit (Devyser, Stockholm, Sweden) was applied for Y chromosome microdeletion analysis according to the manufacturer's recommendations. No pathology was detected.

Next-generation sequencing and Sanger sequencing were performed, and genomic DNA was extracted from peripheral venous blood using the QIAamp® DNA Mini Kit (Qiagen, Ankara, Turkey). The Clinical Exome Solution (Sophia Genetics SA, Saint-Sulpice, Switzerland) was used for exome enrichment, with all procedures carried out according to the manufacturer's protocols. This capture-based target enrichment kit covers 4,493 genes related to inherited diseases. Paired-end sequencing was performed on NextSeq 500 system (Illumina, San Diego, CA, USA) with a read length of 150×2 , while the base calling and image analysis were conducted using Real-Time Analysis (integrated to the NextSeq 500 system; Illumina) software. The BCL (base calls) binary was converted into FASTQ utilizing the Illumina package bcl2fastq.

All bioinformatic analyses were performed on Sophia DDM™ platform (Sophia Genetics SA), which includes algorithms for alignment, calling single nucleotide polymorphisms and small insertions/deletions (Pepper™, Sophia Genetics SA patented algorithm), calling copy number variations (Muskat™, Sophia Genetics SA patented algorithm), and functional annotations (Moka™, Sophia Genetics SA patented algorithm). The raw reads were aligned to the human reference genome (GRCh37/hg19). Variant filtering and interpretations were performed on the Sophia DDM™ platform (Sophia Genetics SA), and an Integrative Genomics Viewer was used to visualize the BAM (binary alignment map) files.

Next-generation sequencing showed a homozygous nonsense variation, c.979C>T, p.Arg327* in the *CSTF2T* (NM_015235) gene which was confirmed by Sanger sequencing (Fig. 1). The variant has not been previously reported in the Human Gene Mutation Database (HGMD; <http://www.hgmd.cf.ac.uk/ac/index.php>) and in 1000 G (1000 Genomes Project). In silico analysis programs showed that the change may have pathogenic effects (Table 1).

Discussion

In this report, we present a case with a homozygous nonsense mutation (c.979C>T, p.Arg327*) in the *CSTF2T* gene, which has never been reported in an infertile patient. A definitive molecular diagnosis was made in the patient through the application of clinical exome and Sanger sequencing.

CSTF2T is an autosomal retrotransposed gene that encodes the human variant CstF-64 polyadenylation protein τ CstF-64. τ CstF-64 is a vital protein linked with mRNA polyadenylation – a primary controller of gene expression in specific tissues [Licatalosi et al., 2008; Idler and Yan, 2011]. The shortening or lengthening of mRNA 3' ends is the typical manner in which polyadenylation influences gene expression, which hides or reveals RNA control elements in the 3' untranslated areas [Ivshina et al., 2014; Wang and Yi, 2014], though it can additionally change protein isoforms [Takagaki et al., 1996; Martincic et al., 1998].

τ CstF-64 has high levels of expression in the testes and brain, and lower levels in other tissues and cell types. τ CstF-64 expression in the testes occurs solely in germ cells, due to male sex chromosome inactivation which leads to transcriptional inactivation of the somatic CstF-64 [Li et al., 2012].

In mice, *Cstf2t*^{-/-} subjects were found by Dass et al. [2007] to present no obvious abnormalities. Nonetheless, *Cstf2t*^{-/-} males presented with infertility and aberrant spermatogenesis, resulting in a condition that resembled oligoasthenoteratozoospermia [Dass et al., 2007; Tardif et al., 2010; Hockert et al., 2011; Li et al., 2012; Grozdanov et al., 2018]. Male infertility is caused by defects in post-meiotic germ cell development as a consequence of targeted deletion of *Cstf2t*. The symptomatic defects, which include a lower primary spermatocyte count as well as round and elongating spermatids, cause the resulting epididymal contents to comprise few motile sperm cells and to be unable to fertilize eggs in vitro [Dass et al., 2007; Tardif et al., 2010; Hockert et al., 2011]. Our patient presented with severe oligoasthenospermia with 100% immotile spermatozoa and a low sperm count (0.96×10^6 sperm/mL). There was no analysis of sperm morphology.

Table 1. Features of the variant identified in this study

Gene	Nucleotide change	Amino acid change	Type	Zygosity	ACMG scoring	ACMG pathogenicity	DANN score	Frequency (gnomAD)	R/N
<i>CSTF2T</i> (NM_015235)	c.979C>T	p.Arg327*	Nonsense	Homozygous	PSV1+PM2+PP3	LP	0.9981	1:250158	N

ACMG, American College of Medical Genetics; LP, likely pathogenic; R/N; reported/novel; gnomAD, Genome Aggregation Database. DANN is a pathogenicity scoring methodology developed by Daniel Quang, Yifei Chen, and Xiaohui Xie at the University of California, Irvine. It is based on deep neural networks. The value range is 0–1, with 1 given to the variants predicted to be the most damaging [Quang et al., 2015].

Fig. 1. Molecular genetic analysis of the patient. **A** Excerpt of next-generation sequencing data visualized using Integrative Genomics Viewer. The mutation (chr10:53458331 G>A) is indicated by a red arrow. **B** Result of DNA sequencing. The nonsense germline mutation c.979C>T, p.Arg327* in the *CSTF2T* (NM_015235) gene is indicated by the red frame.

However, both *Cstf2t*^{+/-} male and *Cstf2t*^{-/-} female mice were fertile. It has been observed that loss of τ CstF-64 in mice results in infertility for many reasons including extensive changes in gene expression, altered splicing, genomic misregulation, and altered polyadenylation [Dass et al., 2007; Tardif et al., 2010; Hockert et al., 2011; Li et al., 2012; Grozdanov et al., 2018].

τ CstF-64 is critical for brain function and has elevated levels of expression there. A study performed by Harris et al. [2016] on 185-day-old wild-type and *Cstf2t*^{-/-} mice of

both sexes examined their motor function, learning, general activity, and memory. The tests were conducted by open field activity, rotarod, an 8-arm radial arm maze, and Morris water maze tasks. No learning or memory abnormalities were observed in male wild-type and *Cstf2t*^{-/-} mice. However, female *Cstf2t*^{-/-} mice were markedly better than female wild type in the retention of learned maze tasks. It can be concluded from these results that τ CstF-64 plays an important role in memory function in female mice. Male *Cstf2t*^{-/-} mice showed less thigmotactic be-

havior than wild-type mice did, implying that *Cstf2t* may be integral to anxiety in males [Harris et al., 2016]. Our patient does not have any behavior problems.

The variants in the *CSTF2T* gene have been previously reported to be associated with autism in the literature [Iossifov et al., 2012, 2014; Luo et al., 2012]. Our patient has normal intelligence.

To our knowledge, an infertile patient with a mutation in the *CSTF2T* gene has not been reported in the literature yet. There are some studies performed on knockout *Cstf2t*^{-/-} mice to show the effect on spermatogenesis. This case is the first description of a patient who has a homozygous *CSTF2T* pathogenic mutation associated with severe infertility.

In summary, our patient, having a novel mutation (c.979C>T, p.Arg327*) in the *CSTF2T* gene, is the first case with infertility reported in the literature.

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Statement of Ethics

Molecular genetic studies of the patient were performed in the Medical Genetics Department of Bursa Yuksek Ihtisas Training and Research Hospital with the written consent of the patient. Informed consent was also obtained from the patient for the publication. Approval for the study was granted by the local ethics committee.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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Author Contributions

Dr. Ozlem Gorukmez examined the patient, made molecular genetic analyzes and wrote the article. Table and figure of the article were prepared by Dr. Orhan Gorukmez.