


Cytogenetic and Y chromosome microdeletion analysis in azoospermic patients: Insights into genetic causes of male infertility

 ¹Metin ESER

 ²Gulam HEKİMOĞLU

 ³Ferhat Yakup SUÇEKEN

¹Department of Medical Genetics, University of Health Sciences, Umraniye Training and Research Hospital, Istanbul, Turkey

²Department of Histology and Embryology, University of Health Sciences, International Faculty of Medicine, Istanbul, Turkey

³Department of Urology, University of Health Sciences, Umraniye Training and Research Hospital, Istanbul, Turkey

ORCID ID

ME : 0000-0001-7118-7958

GH : 0000-0002-5027-6756

FYS : 0000-0001-7605-4353



ABSTRACT

Objective: Azoospermia, the most severe form of male infertility, is characterized by the absence of sperm in the ejaculate as a result of spermatogenesis failure. The aim of this study was to identify genetic anomalies associated with Y chromosome microdeletions and sex chromosomal aneuploidy.

Material and Methods: A total of 134 azoospermic patients were included in the study. Following a general clinical evaluation and laboratory testing, karyotype analysis and Y chromosome microdeletion screening were performed.

Results: The study included 134 infertile males with azoospermia. The overall rate of cytogenetic anomalies was 9.7%. Chromosomal abnormalities were detected in 7 of 134 cases (5.2%). The most common genetic abnormality was 47,XXY (Klinefelter syndrome), with a prevalence of 3.7%. Y chromosome microdeletions were identified in 5 patients (3.7%).

Conclusion: This study highlights the significant role of genetic factors, particularly chromosomal abnormalities and Y chromosome microdeletions, in the etiology of azoospermia. In addition, Y chromosome microdeletions were identified in a notable subset of cases. These findings emphasize the importance of comprehensive genetic screening, including both karyotype analysis and Y chromosome microdeletion testing, in the diagnostic evaluation of azoospermic men to guide clinical management and genetic counseling.

Keywords: Azoospermia, infertility, microdeletion, Y chromosome.

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Correspondence: Gulam HEKİMOĞLU, MD. Sağlık Bilimleri Üniversitesi, Uluslararası Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, İstanbul, Türkiye.

Tel: +90 216 777 87 77 **e-mail:** gulam.hekimoglu@sbu.edu.tr

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INTRODUCTION

Azoospermia, defined as the complete absence of spermatozoa in the ejaculate, affects approximately 1% of men. Around 35–40% of azoospermia cases result from various acquired conditions.^[1] The congenital forms, on the other hand, are primarily attributed to genetic factors, many of which are routinely analyzed during the diagnostic evaluation of infertile males.^[2] Genetic screening plays a critical role in guiding clinical decisions, providing effective genetic counseling, and improving diagnostic accuracy.^[3] In clinical practice, patients with azoospermia due to primary testicular failure are typically assessed for chromosomal abnormalities and Y chromosome Azoospermia Factor (AZF) microdeletions.^[4] The advent of Next Generation Sequencing (NGS) technologies—particularly Whole Exome Sequencing (WES) and targeted gene panels—has led to the identification of numerous novel monogenic causes of azoospermia. Among the most frequent genetic causes is Klinefelter syndrome (KFS), characterized by the presence of an extra X chromosome. This condition occurs in approximately 1 in 600 male newborns (0.1–0.2%), but its prevalence rises to 3–4% in infertile men and up to 10–12% among those with azoospermia.^[5,6] Clinically, KFS is associated with features such as eunuchoid body habitus, hypergonadotropic hypogonadism, gynecomastia, small firm testes, azoospermia, and a range of neurocognitive impairments.^[7]

Another common genetic cause of azoospermia involves deletions in the Y chromosome's long arm (Yq), specifically within the Azoospermia Factor (AZF) regions essential for normal spermatogenesis. Three major deletion patterns have been identified—AZFa, AZFb, and AZFc—located in the proximal, middle, and distal Yq11 regions, respectively.^[8] Among these, AZFc deletions are the most frequent (approximately 80%), followed by AZFa (0.5–4%), AZFb (1–5%), and combined AZFbc deletions (1–3%). Because the AZF loci harbor multiple genes vital for spermatogenesis, microdeletions in these areas are well-established genetic causes of male infertility.^[9] Determining the specific gene responsible for the clinical phenotype is challenging, as these regions contain multiple gene families. Within the 792 kb AZFa region, two single-copy genes—USP9Y and DDX3Y—are broadly expressed. The USP9Y gene encodes a ubiquitin C-terminal hydrolase that likely plays a regulatory role in protein turnover,^[10] whereas DDX3Y encodes an ATP-dependent RNA helicase belonging to the conserved DEAD-box family. The loss of DDX3Y is thought to underlie the Sertoli Cell-Only Syndrome phenotype associated with complete AZFa deletions, characterized by small testicular volume and a total absence of germ cells in the seminiferous tubules.^[11] Complete AZFb deletions, in contrast, remove approximately 6.2 Mb of DNA containing 32 gene copies and transcriptional units. Since their removal results in spermatogenic arrest, these genes are probably involved in germ cell maturation. There are 12 genes and transcription units in the AZFc area, each of which is present in a different number of copies, for a total of 32 copies. Complete AZFc deletion carriers have a wide range of clinical manifestations. Although sperm concentrations are usually <2 million/mL, spermatozoa can be found in the ejaculate.^[12] In this study, we assessed the genetic reasons for azoospermia in male infertility patients.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of Umraniye Training and Research Hospital (Ethics No: B.10.1.TKH.4.34.H.GP.01/329, 30/09/2025), School of Medicine, University of Health Sciences, Istanbul, Türkiye. Following ethical approval, this study included 134 patients who were admitted to the Department of Medical Genetics at the University of Health Sciences Umraniye Training and Research Hospital between January 2021 and September 2025 for infertility. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was not required due to the retrospective design of the study and ethics committee regulations, and all records were kept confidential.

Y Microdeletion

Peripheral blood samples were collected, and genomic DNA was extracted using standard methods. DNA extraction from blood samples was performed using a semi-automated robot as recommended by the manufacturer (Qiagen). DNA concentration and quality control (260/280 nm and 260/230 nm values) were determined by fluorometry (Qubit v3.0) and UV spectrophotometry. Disease-associated gene regions were amplified by polymerase chain reaction (PCR), and the samples were loaded onto the ABI SeqStudio 8 Flex instrument. Fragment analysis was performed using SeqStudio 8 Flex Software v6.0.

Cytogenetics

For karyotype analysis, peripheral blood lymphocytes were cultured in special culture media. During the last 2 hours of the 72-hour culture period, colcemid was added to arrest the cells in mitosis. After colcemid treatment, trypsin-EDTA was used to detach the cells from the bottom of the flask. The cells were collected and transferred to tubes. For harvesting, the cells were treated with a hypotonic solution (potassium chloride or sodium citrate) for approximately 10–15 minutes after centrifugation. Immediately following the hypotonic treatment, the cells were fixed two to three times with a cold methanol:glacial acetic acid solution prepared at a 3:1 ratio. The prepared cell suspensions were dropped onto clean slides, and the aging process was carried out by air-drying or brief exposure to high temperatures. The aged preparations were banded and stained with trypsin and Giemsa for routine analysis. Karyotype analysis was performed using the Leica Biosystems Cytovision analysis system.

Statistical Analysis

GraphPad Prism software version 8.4.2 was used for data analysis. Continuous variables are presented as mean±standard deviation. Statistical significance was considered when $p < 0.05$.

RESULTS

The mean age of the 134 infertile men evaluated in this study was 30.9±7.0 years. Cytogenetic abnormalities were detected in 9.7% of cases overall. Chromosomal abnormalities were identified in 7 of 134 cases (5.2%). Structural and numerical chromosomal abnormalities are summarized in Table 1. Klinefelter syndrome

Table 1: Karyotype and Y chromosome deletion information of the patients

Patient no.	Age	Karyotype (peripheral blood)	Y Chromosome microdeletion
1	26	47, XXY	Not detected
3	28	46, Y, t(X;7) (q26; q11.22)	Not detected
5	26	46, XY, t (13;17) (p10; q10)	Not detected
29	24	46, XY, t (2;9) (q13; p24)	Not detected
30	29	47, XYY	Not detected
49	33	47, XXY	Not detected
69	32	46, XY, t (8;12) (q22; q24.1)	Not detected
92	39	46, X, inv (Y) (p11.2; q11.23)	Not detected
102	31	46, X, del (Y) (q12) [32] / 45, X [18]	Not detected
109	30	46, XY, t (3;14) (p10; q10)	Not detected
113	30	47, XXY	Not detected
117	44	47, XYY	Not detected
122	27	47, XXY	Not detected
127	29	47, XXY	Not detected
14	16	46, XY	Detected AZFa (SY82, SY83, SY88 & SY1065), AZFb (SY153, SY121, SY105 & SY143) AZFc gr/gr (SY1191 & SY1291)
41	37	46, XY	Detected AZFc (SY254 & SY255), AZFd (SY152 & SY153)
61	8	46, X, del(Y) (q11.21)	Detected AZFa (M259, SY84, SY86 & SY625), AZFb (SY127, SY130, SY131 & SY134), AZFc (SY157, SY254 & SY255), AZFd (SY152 & SY153).
80	33	46, X, der(Y)	Detected AZFc (SY157, SY254 & SY255), AZFd (SY152 & SY153).
86	36	46, XY	Detected AZFc (SY157, SY254 & SY255), AZFd (SY152 & SY153).

(47,XXY; KFS) was identified in five cases (3.7%) and represented the most common anomaly among all cases.

Y chromosome microdeletions were detected in 3.7% of cases overall, with the AZFc region being the most frequently deleted. Based on molecular screening of the AZF region, five AZF microdeletions were identified: one involving AZFa+b+c, one involving AZFa+b+c+d, and three involving AZFc+d (Table 1). Representative karyotype images and Y chromosome deletion images are shown in Figures 1–3.

DISCUSSION

Male infertility is caused by genetic factors among many other etiologic contributors. Numerous genes on the Y chromosome that function at

various stages of germ cell development regulate spermatogenesis.^[13] In the present study, the frequency of chromosomal abnormalities was determined to be 5.2%. These results closely resemble those reported in previous studies.^[14] Numerous studies investigating the rate of chromosomal abnormalities from different countries have been published, with reported frequencies ranging from 6.2% to 12.6%.^[15] KFS was identified as the most prevalent abnormality in this study, which is consistent with prior clinical findings.^[16] In 50–60% of cases, the extra X chromosome is of paternal origin, whereas in 40–50% of cases it is maternal.^[17] Epidemiological studies have shown that the incidence of KFS is gradually increasing and is thought to be associated with advanced paternal age.^[18] In recent years, however, patients with KFS have been reported to experience

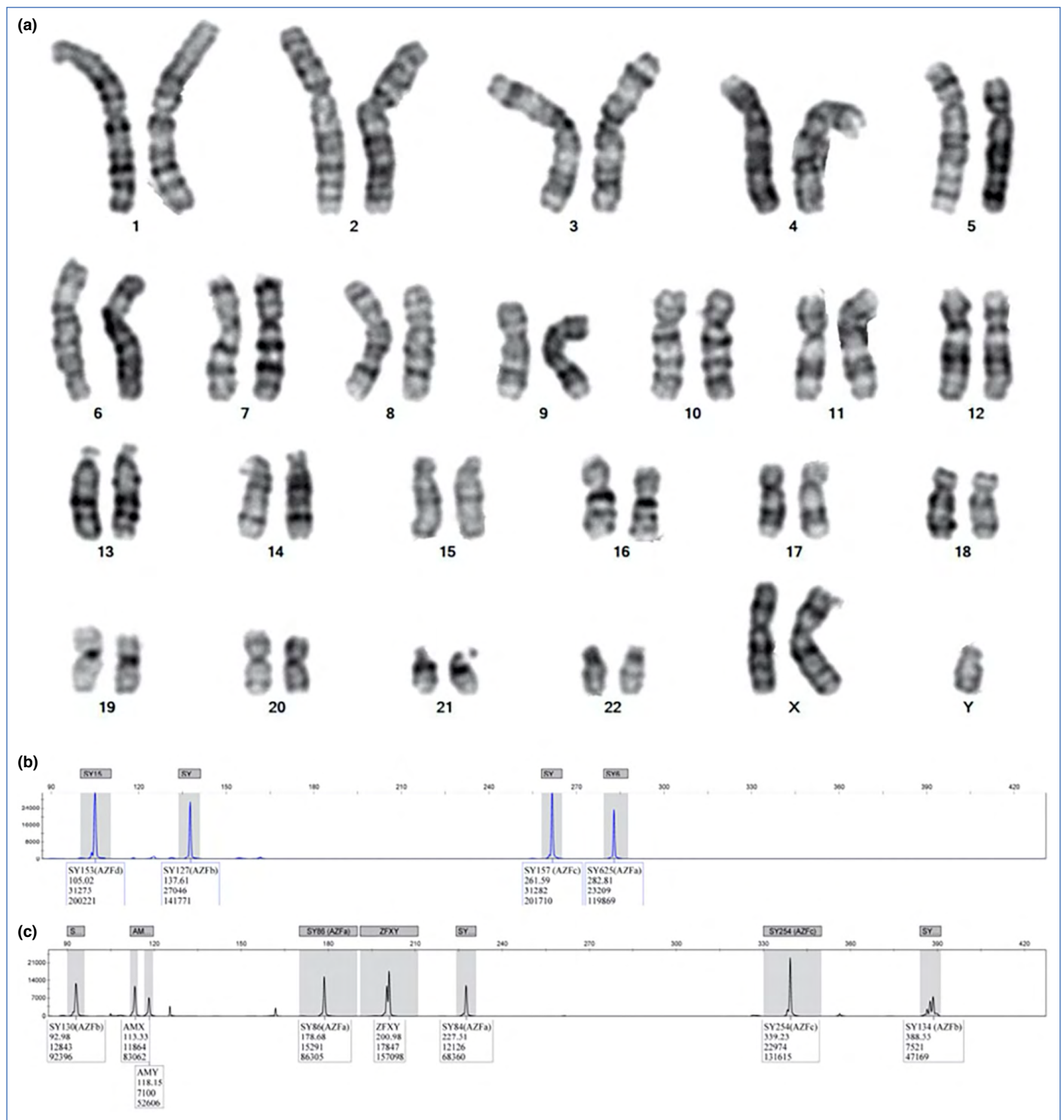


Figure 1: Case 113 (a) karyotype image (47, XXY), (b) Y microdeletion image (No deletion was detected), (c) Internal control DNA.

serious health problems, including substantial morbidity (70%) and mortality (50%), in addition to infertility.^[19] Early diagnosis and timely initiation of disease management are therefore crucial, particularly during puberty. Testosterone replacement therapy promotes the development of secondary sexual characteristics in patients with KFS, alleviates depressive symptoms, and improves self-confidence.

Although patients with KFS are generally considered infertile, assisted reproductive techniques may enable fertilization in selected cases.

In addition, these patients may achieve parenthood through intracytoplasmic sperm injection (ICSI), testicular sperm extraction (TESE), micro-TESE, and sperm cryopreservation. Testicular tissue preservation and spermatogonial stem cell cryopreservation remain

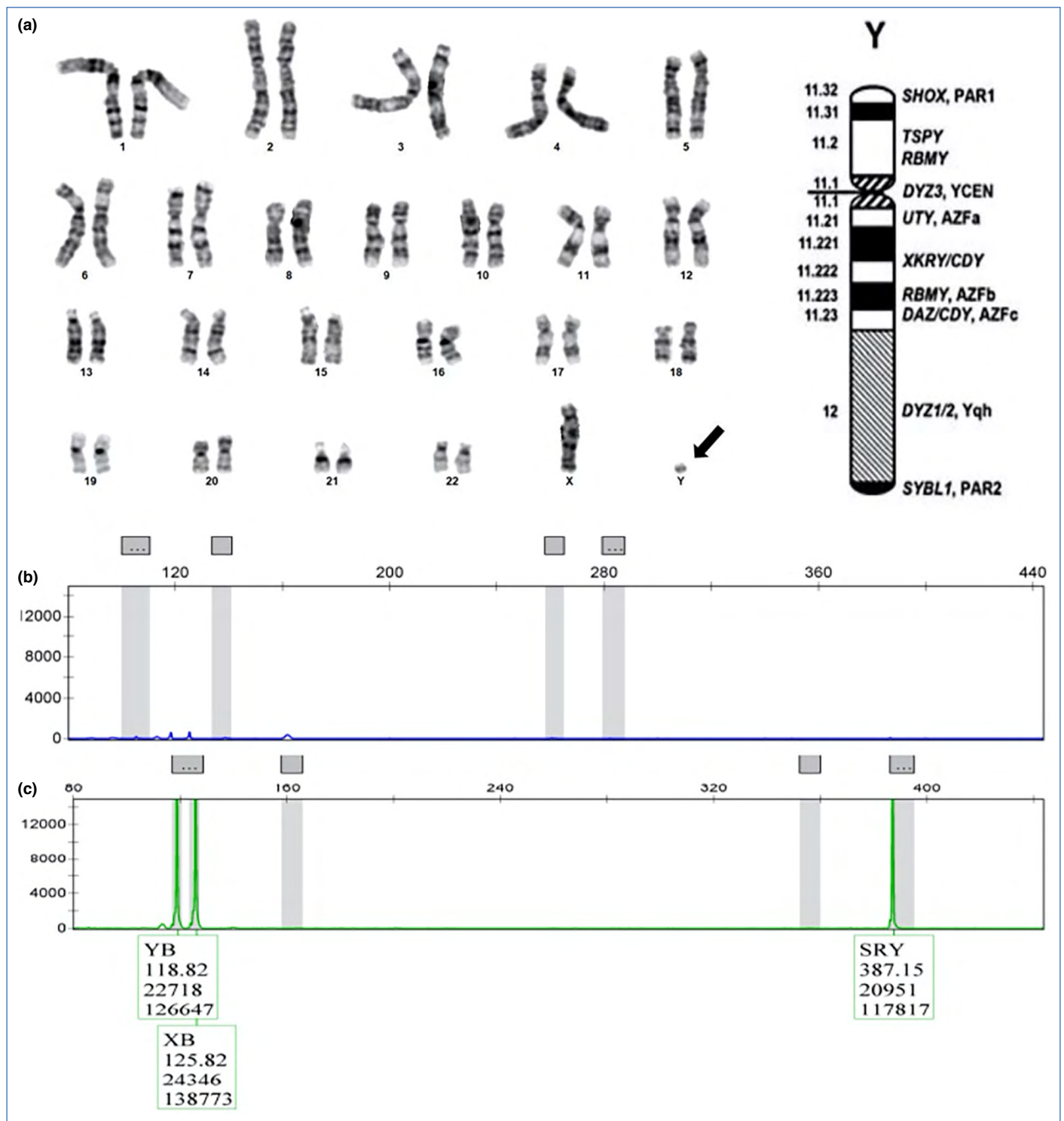


Figure 2: Case 61 (a) karyotype image [46, X, del(Y)(q11.21)], Y chromosome ideogram image on the right side. (b) Y microdeletion image (Deletions were detected in the AZFa, AZFb, AZFc, and AZFd regions). (c) Internal control DNA.

experimental approaches.^[20] Y chromosome microdeletions are among the most common genetic causes of male infertility.^[21] Ethnic and regional characteristics have been identified as major factors influencing the variability and prevalence of these microdeletions. An international study based on a large dataset reported Yq

microdeletion rates ranging from 7% to 10%.^[22] Several studies have investigated the incidence of Y chromosome microdeletions in the Türkiye population, reporting frequencies between 1.3% and 9.6%.^[23]

In the present study, the prevalence of Y chromosome microdeletions was found to be 3.7%. Variations in reported

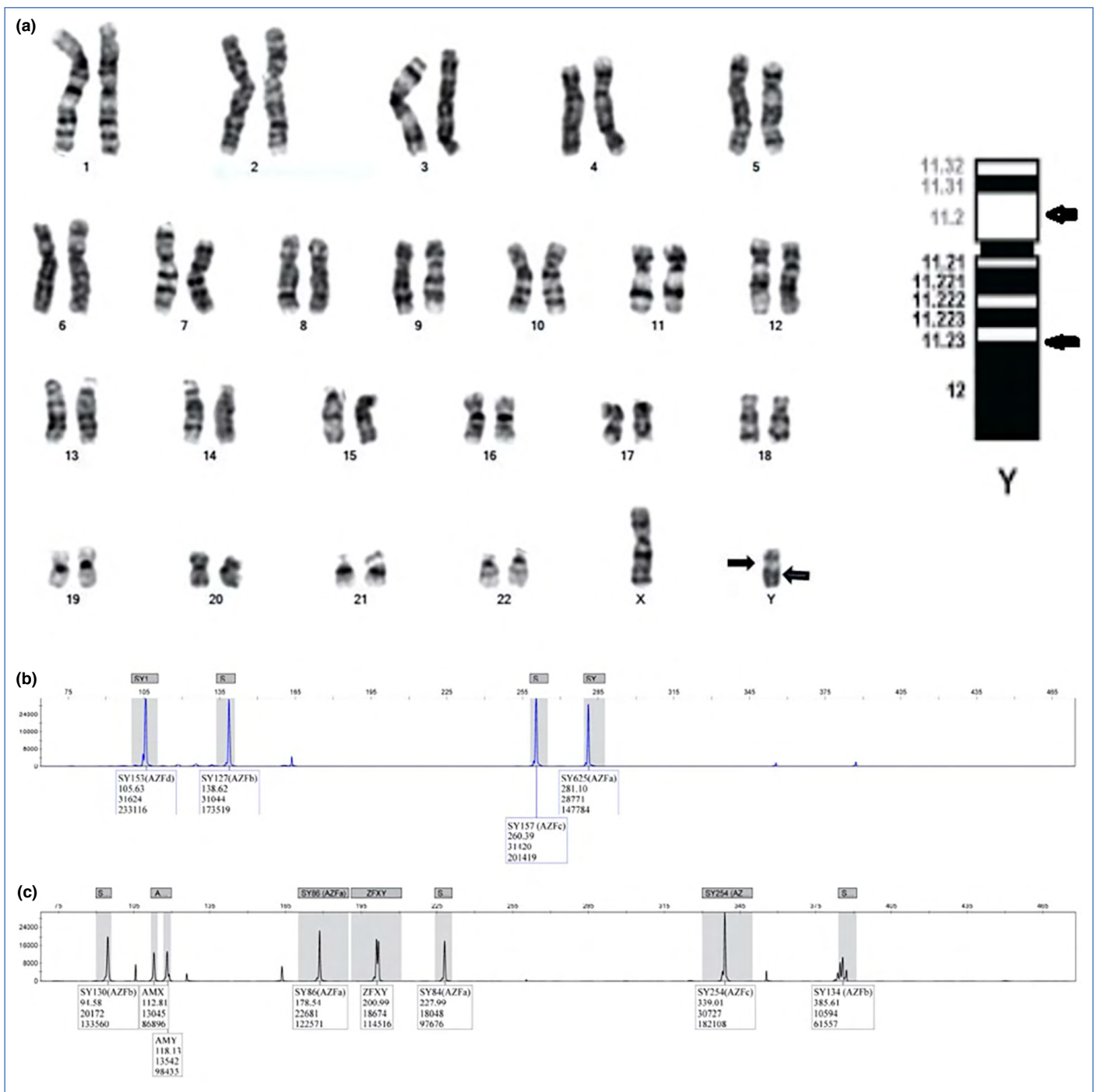


Figure 3: Case 92 (a) karyotype image (46, X, inv[Y] [p11.2 q11.23]), Y chromosome ideogram image on the right side (b) Y microdeletion image (No deletion was detected). (c) Internal control DNA.

frequencies may be attributed to differences in genetic background, environmental factors, primer sets used for AZF microdeletion analysis, and genetic heterogeneity among populations, particularly with respect to Y chromosome-specific haplotypes. Population size, regional differences, and patient selection based on the etiology and severity of spermatogenic impairment may also contribute to these discrepancies. In the Iranian population, Akbarzadeh et al.^[24] reported a higher frequency of AZFb microdeletions (66.67%)

compared with AZFc deletions (41.67%). It has been suggested that although AZFc deletions impair spermatogenesis, they do not invariably result in complete infertility.

Sperm retrieval by TESE may be possible in azoospermic men with AZFc deletions, allowing fertilization. In contrast, complete deletions of the AZFa and AZFb regions lead to total germ cell loss, rendering TESE and ICSI ineffective. Reports indicate that sperm retrieval by TESE is possible in approximately 50% of cases with partial AZFb deletions.

[25] Couples with Yq microdeletions who undergo assisted reproductive procedures should be informed that male offspring may also develop spermatogenic abnormalities due to transmission of the microdeletion.

Comprehensive genetic screening plays a crucial role in the diagnostic evaluation of azoospermic men, as it enables identification of underlying genetic abnormalities that directly affect clinical management and reproductive counseling. Karyotype analysis is essential for detecting chromosomal aneuploidies and structural rearrangements, such as KFS, which are major contributors to male infertility. In parallel, Y chromosome microdeletion analysis provides critical information on deletions within the AZF regions that are strongly associated with spermatogenic failure.^[2] The combined use of these diagnostic modalities allows more accurate etiological classification of azoospermia, facilitates assessment of the feasibility of sperm retrieval for assisted reproductive techniques, and supports informed genetic counseling regarding inheritance risks and reproductive options. The limitations of this study include its retrospective design and the lack of data on patient treatment strategies and paternity outcomes during follow-up.

CONCLUSION

Incorporating both karyotype analysis and Y chromosome microdeletion testing into the diagnostic workflow for azoospermic men ensures a comprehensive genetic evaluation. This integrated approach not only clarifies the underlying cause of infertility but also provides essential guidance for personalized clinical management and accurate genetic counseling of affected individuals.

Statement

Ethics Committee Approval: The Umraniye Training and Research Hospital Ethics Committee granted approval for this study (date: 30.09.2025, number: B.10.1.TKH.4.34.H.GP.0.01/329).

Informed Consent: Written informed consent was not required due to the retrospective design and ethics committee regulations.

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