

High Frequency of 45,X/46,XY mosaicism carrying a structurally abnormal Y chromosome in patients with Y chromosome microdeletions: a 8-year period retrospective study

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Research

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Abstract

Background: Structural abnormalities of Y chromosome are commonly described in associated with the occurrence of a 45,X/46,XY chromosomal mosaicism. In recent years, evidences of an association between Y chromosome microdeletions and 45,X/46,XY mosaicism had been investigated, and 45,X/46,XY mosaicism was found to be particularly common in patients with Y chromosome microdeletions. The aim of our study was to investigate the presence of 45,X/46,XY mosaicism carrying a structurally abnormal Y chromosome in patients with Y chromosome microdeletions.

Results: A 8-year retrospective study was conducted on 6545 infertile men with nonobstructive azoospermia or oligozoospermia. A total of 19 patients with 45,X/46,XY mosaicism or its variants were found, of which 78.95% (15/19) had a structural Y chromosome abnormalities in 46,XY cell line. Thirteen of 19 (68.42%, 13/19) patients with 45,X/46,XY mosaicism had Y chromosome microdeletions, and 12/13 (92.31%) of them exhibited a structurally abnormal Y chromosome.

Conclusions: Our results were consistent with previous studies that a high frequency of 45,X/46,XY mosaicism or its variants were detected in patients with Y chromosome microdeletions. Different from previous studies, we found that 45,X/46,XY mosaicism in patients with Y chromosome microdeletions almost all exhibited a structurally abnormal Y chromosome.

Background

Chromosomal abnormalities and Y chromosome microdeletions are considered to be the two more common genetic causes of spermatogenic failure. The mosaic 45,X/46,XY karyotype is a common sex chromosomal abnormality associated with male infertility. Structural abnormalities of Y chromosome are estimated to affect 10–20% of men with non-obstructive azoospermic infertility[1], which are commonly described in associated with the occurrence of a 45,X/46,XY chromosomal mosaicism due to a probable mitotic instability of the abnormal chromosome[2]. A previous report showed that more than half of the patients with Y chromosome structural abnormalities might present with 45,X mosaic cell lines [3]. Y chromosome microdeletions are associated with severe spermatogenic failure and represent the most frequent molecular genetic cause of azoospermia and severe oligozoospermia [4]. Azoospermia Factor (AZF) region is responsible for the Y chromosome microdeletions. This region located on the long arm of Y (Yq11.23) and was mapped to three non-overlapping regions described as AZFa, AZFb, and AZFc[5]. The frequency of Y chromosome microdeletions was reported to be in the range of 3–55% according to previous studies with an average frequency of 7% [6].

In recent years, evidences of an association between Y chromosome microdeletions and the formation of 45,X/46,XY mosaicism had been investigated, and found that 45,X/46,XY mosaicism to be particularly common in patients with Y chromosome microdeletions. The aim of our study was to investigate the presence of 45,X/46,XY mosaicism carrying a structurally abnormal Y chromosome in patients with Y chromosome microdeletions. Here, we retrospectively reviewed a single center experience over a 8-year period (2012–2019) at a university teaching hospital in southern China. Our study included 19 clinically well-characterized mosaic

patients whose karyotype consisted of a 45,X cell line and a second cell line containing a normal or an abnormal Y chromosome.

Material And Methods

Subjects

We conducted a retrospective study of infertile men with nonobstructive azoospermia or oligozoospermia from male outpatients of the Women's Hospital of Zhejiang University between January 1st 2011 and December 31st 2019. A total of 6,545 cases of infertility men were recruited in the study. The patients' ages ranged from 17 to 63 (mean±SD, 31.45±5.29) years, and infertility lasted from 2 to 21 years old. Karyotype analysis and Y chromosome microdeletions were performed in all patients. This study was approved by the Scientific Research Ethics Committee of the Women's Hospital of Zhejiang University. This was a retrospective study of the clinical database with no intervention and no informed consent was required. All the methods used in the study followed the current approved guidelines.

Karyotype Analysis

Metaphase chromosomes with targeted 400-band level were performed as previously described [7]. Briefly, about 1 mL peripheral blood was inoculated aseptically into culture bottle containing of 5 mL lymphocyte culture solution in 5% CO₂ incubator at 37°C for 72 h. Then 20 µg ml⁻¹ colchicine was added to the culture system half an hour before the termination of cell culture to arrest the chromosomes at metaphase. G-banding of metaphase chromosomes were obtained by hypotension, fixation, trypsinization, Giemsa staining, and so forth. At least 30 metaphases were counted and 5 metaphases were analyzed for each sample by two certified physicians according to the International System for Human Cytogenetic Nomenclature guidelines (ISCN, 2016, 5th edition) [8].

Y Chromosome Microdeletions

Y chromosome microdeletions were performed by multiplex PCR amplification as previously described [7]. Six specific sequence-tagged sites (STS) that recommended by the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMGQ) were used for the detection of Y chromosome microdeletions, including sY84 and sY86 for AZFa, sY127 and sY134 for AZFb, sY254 and sY255 for AZFc. The Human Y Chromosome Microdeletion Gene Detection Kit was used for the detection of Y chromosome microdeletion. Multiplex PCR amplification was performed on the LightCycler 480 II thermocycler. PCR was performed with the following thermal cycling conditions: 95°C for 3 min, then 10 cycles at 95°C for 15 s, 63°C for 20 s, 72°C for 20 s, followed by 30 cycles at 95°C for 15 s, 63°C for 32 s, 72°C for 20 s, and a final extension at 72°C for 10 min. An STS marker was considered to be deleted only after at least two failed PCR amplification attempts with single primer pairs.

Results

A total of 19 patients with 45,X/46,XY mosaicism or its variants were detected in 6545 male patients, with an incidence rate of 0.29% (19/6545). The results of karyotype analysis and AZF microdeletions for the 19

patients are summarized in Table 1. Of these 19 patients with 45,X/46,XY mosaicism, 78.95% (15/19) had a structurally abnormal Y chromosome in the 46,XY cell line and 21.05% (4/19) had pure 45,X/46,XY mosaicism. All of these patients (100.00%, 15/15) with a structurally abnormal Y chromosome and three patients (75.00%, 3/4) with pure 45,X/46,XY mosaicism exhibited azoospermia, while the remaining one patient with pure 45,X/46,XY mosaicism exhibited oligozoospermia. Thirteen of 19 patients (68.42%, 13/19) with 45,X/46,XY mosaicism had AZF microdeletions, including 11 patients (84.62%, 11/13) with AZFb + c microdeletions and 2 patients (15.38%, 2/13) with AZFc microdeletions. In the present study, 92.31% (12/13) of 45,X/46,XY mosaicism in patients with Y chromosome microdeletions exhibited a structural Y chromosome abnormalities in 46,XY cell line; six with Yqh-, five with del(Y) (q12), and one with Y ≤ 21. All patients (100.00%, 11/11) with AZFb + c microdeletions and one of two patients (50%, 1/2) with AZFc microdeletions exhibited a structurally abnormal Y chromosome. Moreover, a total of 315 patients with AZF microdeletion were identified in this study. The pattern/number and characteristic of the patients showing these deletions were shown in Table 2. Only the deletions of AZFc region and AZFb + c region involved 45,X/46,XY mosaicism, including eleven of 60 (18.33%, 11/60) men with the AZFb + c deletion and two of 208 (0.96%, 2/208) men with the AZFc deletion.

Table 1

The results of AZF microdeletions in men with 45,X/46,XY mosaicism or its variants reported in the literature.

No. of Patients	Age	Karyotype Results	Microdeletions Results	Clinical diagnosis
Case 1	24	45,X[12]/46,X,del(Y)(q12)[8]	AZFb + c	azoospermia
Case 2	36	45,X[25]/46,X,del(Y)(q12)[5]	AZFb + c	azoospermia
Case 3	31	45,X[26]/46,XY,Y ≤ 21[14]	AZFb + c	azoospermia
Case 4	30	45,X[34]/46,X,Yqh-[16]	AZFb + c	azoospermia
Case 5	27	46,X,del(Y)(q12)[41]/45,X[10]	AZFb + c	azoospermia
Case 6	36	46,X,del(Y)(q12)[17]/45,X[13]	AZFb + c	azoospermia
Case 7	27	46,X,del(Y)(q12)[8]/45,X[7]	AZFb + c	azoospermia
Case 8	33	46,X,Yqh-[21]/45,X[9]	AZFb + c	azoospermia
Case 9	30	46,X,Yqh-[23]/45,X[7]	AZFb + c	azoospermia
Case 10	34	46,X,Yqh-[44]/45,X[6]	AZFb + c	azoospermia
Case 11	27	46,X,Yqh-[45]/45,X[5]	AZFb + c	azoospermia
Case 12	29	45,X[6]/46,XY[16]	AZFc	azoospermia
Case 13	28	46,X,Yqh-[14]/45,X[6]	AZFc	azoospermia
Case 14	29	45,X[6]/46,XY[44]	Normal	oligozoospermia
Case 15	28	46,X,+mar[22]/46,X,del(Y)(q12)[8]/45,X[2]	Normal	azoospermia
Case 16	31	46,X,+mar[36]/45,X[11]/46,XY[3]	Normal	azoospermia
Case 17	28	46,X,idic(Y)(p11.3)[45]/45,X[4]	Normal	azoospermia
Case 18	40	46,XX[16]/47,XXY[8]/45,X[3]/46,XY[2]/48,XXXY[1]	Normal	azoospermia
Case 19	24	46,XY,1qh+[28]/45,X,1qh+[22]	Normal	azoospermia

Table 2
The pattern/number and characteristic of patients with AZF microdeletions in the study.

AZF Microdeletions			Karyotype Results	
Deletion Patterns	No. of Patients	Percentage	Mosaic 45,X/46,XY	Other Karyotype
sY127	1	0.32%	0 (0.00%)	1 (100.00%)
sY84	1	0.32%	0 (0.00%)	1 (100.00%)
sY134	2	0.63%	0 (0.00%)	2 (100.00%)
AZFc + sY134	3	0.95%	0 (0.00%)	3 (100.00%)
AZFa	11	3.49%	0 (0.00%)	11 (100.00%)
AZFa + b + c	12	3.81%	0 (0.00%)	12 (100.00%)
AZFb	17	5.40%	0 (0.00%)	17 (100.00%)
AZFb + c	60	19.05%	11 (18.03%)	49 (81.67%)
AZFc	208	66.03%	2 (0.96%)	206 (99.04%)
Total	315	100.00%	13 (4.13%)	302 (95.87%)

Discussion

The prevalence of 45,X/46,XY mosaicism or its variants in this male infertility study was 0.29% (19/6545) (approximately 29/10,000), which was consistent with previous studies (0.27%)[9]. However, this is much higher than its incidence (1.5/10,000) in newborns [10], indicating that 45,X/46,XY mosaicism is a common chromosomal aberration associated with male infertility. In this study, we analysed 6545 infertile men and found that 68.42% (13/19) of patients with 45,X/46,XY mosaicism had AZF microdeletions. In previous studies, Li et al investigated 5269 cases of infertility men and found that 71.43% (10/14) of patients with 45,X/46,XY mosaicism exhibited AZF microdeletions [9]. Pan et al detected 5235 male patients with primary infertility and reported that 83.3% (5/6) patients with mosaic karyotype 45,X/46,XY had AZF deletions [11]. dos Santos AP et al studied 15 patients with mosaicism and found that approximately 40% (6/15) patients with AZF deletions had mosaic karyotype 45,X/46,XY [12]. The prevalence of Y chromosome microdeletions in patients with 45,X/46,XY mosaicism or its variants was different from other reports, main cause of which might be the sample size. However, our results were consistent with previous studies that a high frequency of Y chromosome microdeletions was detected in patients with 45,X/46,XY mosaicism or its variants.

In the study, 78.95% (15/19) patients with 45,X/46,XY mosaicism had a structurally abnormal Y chromosome in the 46,XY cell line, while 21.05% (4/19) had pure 45,X/46,XY mosaicism. Different from previous studies, we found that 45,X/46,XY mosaicism in patients with Y chromosome microdeletions almost all exhibited a structurally abnormal Y chromosome. In this study, 92.31% (12/13) of 45,X/46,XY mosaicism in patients with Y chromosome microdeletions exhibited a structural Y chromosome abnormality in the 46,XY cell line. Li et al found 10 patients with 45,X/46,XY mosaicism had AZF microdeletions, and 40.00% (4/10) of them exhibited a structurally abnormal Y chromosome [9]. dos Santos AP et al reported a group of six patients with 45,X/46,XY

mosaicism had AZF microdeletions, and 66.67% (4/6) of them an abnormal Y chromosome had been detected in cytogenetic analysis [12]. Pan et al reported 8 patients with mosaic karyotype 45,X/46,XY had AZF deletions, and 37.5% (3/8) of them had an structurally abnormal Y chromosome [11]. The prevalence of 45,X/46,XY mosaicism carrying a structurally abnormal Y chromosome in patients with Y chromosome microdeletions was much higher than other reports. Karyotyping is a reliable technique for the identification of most chromosomal abnormalities, but it cannot detect subtle variations in chromosomal structure, only detecting unbalanced anomalies of at least 5–20 Mb[13]. Patients with the del(Y)(q12) karyotype had a structural deletion on the Y chromosome, which is close to the AZF region. In addition, our previous study confirmed that Y chromosome microdeletions only involved Y chromosome polymorphic variants (especially Yqh- and Y ≤ 21 variants) and had no relationship with other chromosome polymorphisms[8]. There were six patients with Yqh-, five with del(Y) (q12), and one with Y ≤ 21 in the study, which might be the cause of the high frequency of structural Y chromosome abnormalities in patients with Y chromosome microdeletions. In addition, only one of 13 individuals with an apparently normal Y chromosome had AZF microdeletions in this study. This frequency (1/13) was lower than that found by Alvarez-Nava et al (3/11) [14] and lower than that found by Patsalis et al (4/7) [15]. Taken together these findings suggest that 8/31(25.81%) individuals with 45,X/46,XY and apparently normal Y chromosome may have Y microdeletions.

In the present study, the most frequent microdeletions were detected in the AZFc region, followed by the deletion of the AZFb + c region. Consistent with previous researches, we found only the deletions of AZFc region and AZFb + c region involved the 45,X/46,XY mosaicism, and deletion of AZFb + c region might also make men more likely to lose their Y chromosomes. Several studies have found AZFc deletion to be a premutation for 45,X and for the mosaic phenotype 45,X/46,XY [16, 17]. In this study, only 0.96% of men with the AZFc deletion and 18.33% of men with the AZFb + c deletion involved 45,X/46,XY mosaicism, of which AZFb + c microdeletions and AZFc microdeletions accounted for 84.62% (11/13) and 15.38% (2/13), respectively. Hopps et al[18] reported that one out of 25 (1/25, 4.0%) men with AZFc deletion and three out of 12 (3/12, 25.0%) men with AZFb + c deletions had 45,X/46,XY mosaicism. Li et al reported that ten cases (71.43%, 10/14) of 45,X mosaicism exhibiting AZF microdeletions, including two of ten (20.0%, 2/10) with AZFc deletions and the other eight (80.0%, 8/10) with AZFb + c deletions. Aydemir et al[19] documented a rare case of 45,X/46,XY mosaicism with deletion of the AZFb + c region. Pan et al [11] reported all of five male patients (100.0%, 5/5) with a mosaic karyotype 45,X/46,XY had AZFb + c deletions. However, Kleiman SE et al [20] reported that 10.29% (7/68) men with the AZFb + c deletion and 4.76% (1/21) men with AZFb deletion was found to have the 45,X/46,XY karyotype, which was the only report about the deletions of AZFb region involved the 45,X/46,XY mosaicism. Larger sample size and more data are needed to further prove whether AZFb microdeletion will lead to the occurrence of 45,X/46,XY mosaicism.

Conclusions

Our results were consistent with previous studies that high frequency of Y chromosome microdeletions were detected in patients with 45,X/46,XY mosaicism or its variants, supporting the conclusions that microdeletions in the long arm of Y chromosome might be associated with Y chromosomal instability leading to the formation of 45,X cell lines. Different from previous studies, we found that 45,X/46,XY mosaicism in patients with Y chromosome microdeletions almost all exhibited a structurally abnormal Y chromosome.

Declarations

Ethics approval and consent to participate

This study was approved by the Scientific Research Ethics Committee of the Women's Hospital of Zhejiang University. This was a retrospective study of the clinical database with no intervention and no informed consent was required.

Consent for publication

Not applicable

Availability of data and materials

The data supporting the conclusions of this article is included within the article.

Competing interests

The author declares that there are no competing interests.

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Authors' contributions

HGL is fully responsible for this editorial.

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Abbreviations

AZF: Azoospermia Factor; ISCN: the International System for Human Cytogenetic Nomenclature guidelines; STS: Six specific sequence-tagged sites; EAA: the European Academy of Andrology; EMGQ: the European Molecular Genetics Quality Network

References

1. B Peschka , J Leygraaf, K Van der Ven, M Montag, B Schartmann, R Schubert, et al. Type and Frequency of Chromosome Aberrations in 781 Couples Undergoing Intracytoplasmic Sperm Injection. Hum Reprod.

- 1999;14(9):2257-2263.
2. Hsu LY. Phenotype/karyotype correlations of Y chromosome aneuploidy with emphasis on structural aberrations in postnatally diagnosed cases. *Am J Med Genet.* 1994;53(2):108-140.
 3. Siffroi JP, Le Bourhis C, Krausz C, Barbaux S, Quintana-Murci L, Kanafani S, et al. Sex chromosome mosaicism in males carrying Y chromosome long arm deletions. *Hum Reprod.* 2000; 15(12): 2559–2562.
 4. Krausz C, Forti G. Clinical aspects of male infertility. *Results Probl Cell Differ.* 2000; 28: 1-21.
 5. Liu XH, Qiao J, Li R, Yan LY, Chen LX. Y chromosome AZFc microdeletion may not affect the outcomes of ICSI for infertile males with fresh ejaculated sperm. *J Assist Reprod Genet.* 2013;30(6):813–819.
 6. Yuen RK, Merkoulouvitich A, MacDonald JR, Vlasschaert M, Lo K, Grober E, et al. Development of a high-resolution Y-chromosome microarray for improved male infertility diagnosis. *Fertil Steril.* 2014;101(4): 1079-1085.
 7. Li HG, Fan LH, Liu B, Qian YQ, Chen M, Sun YX, et al. The Association Between the Two More Common Genetic Causes of Spermatogenic Failure: A 7-year Retrospective Study. *Asian J Androl.* 2020;22:1-7.
 8. J McGowan-Jordan, A Simons, M Schmid. *An International System for Human Cytogenetic Nomenclature.* Basel: S. Karger press; 2016. p38.
 9. Li L, Zhang H, Yang Y, Zhang H, Wang R, Jiang Y, et al. High frequency of Y chromosome microdeletions in male infertility patients with 45,X/46,XY mosaicism. *Braz J Med Biol Res.* 2020; 53(3):e8980.
 10. Rosa RF, D'Ecclesiis WF, Dibbi RP, Rosa RC, Trevisan P, Graziadio C, et al. 45,X/46,XY mosaicism: report on 14 patients from a Brazilian hospital. A retrospective study. *Sao Paulo Med J.* 2014;132(6): 332–338.
 11. Pan Y, Zhang HG, Xi QI, Zhang H, Wang RX, Li LL, et al. Molecular microdeletion analysis of infertile men with karyotypic Y chromosome abnormalities. *J Int Med Res.* 2018; 46(1):307-315.
 12. dos Santos AP, Andrade JG, Piveta CS, de Paulo J, Guerra G Jr, de Mello MP, et al. Screening of Y chromosome microdeletions in 46,XY partial gonadal dysgenesis and in patients with a 45,X/46,XY karyotype or its variants. *BMC Med Genet.* 2013;14:115.
 13. Peters DG, Yatsenko SA, Surti U, Rajkovic A. Recent advances of genomic testing in perinatal medicine. *Semin Perinatol.* 2015; 39(1): 44–54.
 14. Alvarez-Nava F, Puerta H, Soto M, Pineda L, Temponi A. High incidence of Y-chromosome microdeletions in gonadal tissues from patients with 45, X/46, XY gonadal dysgenesis. *Fertil Steril.* 2008, 89(2):458–460.
 15. Patsalis PC, Skordis N, Sismani C, Kousoulidou L, Koumbaris G, Eftychi C, et al: Identification of high frequency of Y chromosome deletions in patients with sex chromosome mosaicism and correlation with the clinical phenotype and Y-chromosome instability. *Am J Med Genet A.* 2005, 135(2):145–149.
 16. Jaruzelska J, Korcz A, Wojda A, Jedrzejczak P, Bierla J, Surmacz T, et al. Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion. *J Med Genet.* 2001; 38(11): 798-802.
 17. Patsalis PC, Sismani C, Quintana-Murci L, Taleb-Bekkouche F, Krausz C, McElreavey K. Effects of transmission of Y chromosome AZFc deletions. *Lancet.* 2002; 360(9341): 1222–1224.
 18. Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. *Hum Reprod.* 2003; 18(8): 1660–1665.

19. Aydemir H, Karkucak M, Cimen HI, Halis F, Kumsar S, Sonbahar AE, et al. A rare combination of 45,X/46,XY mosaicism and Y chromosome microdeletion in an Infertile Man with Azoospermia. *Genet Couns.* 2016; 27(1): 95–98.
20. Sandra E Kleiman, Leah Yogev, Ofer Lehavi, Ron Hauser, Amnon Botchan, et al. The likelihood of finding mature sperm cells in men with AZFb or AZFb-c deletions: six new cases and a review of the literature (1994–2010). *Fertil Steril.* 2011;95(6):2005-2012.