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Molecular microdeletion analysis of infertile men with karyotypic Y chromosome abnormalities

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Abstract

Objectives: To investigate azoospermic factor (AZF) microdeletions in infertile men from northeastern China with karyotypic Y chromosome abnormalities.

Methods: G-banding of metaphase chromosomes and karyotype analysis were performed in all infertile male patients. Genomic DNA was isolated and used to analyze classical AZF microdeletions by PCR. The regions and sequence-tagged sites of AZFa (SY86, SY84), AZFb (SY127, SY134, SY143), and AZFc (SY152, SY254, SY255, SY157) were sequenced by multiplex PCR.

Results: A total of 190 Y chromosome abnormality carriers were found, of whom 35 had AZF microdeletions. These were most common in 46,X,Yqh− patients, followed by 45,X/46,XY patients. Most microdeletions were detected in the AZFb + c region, including 48.57% of all AZF microdeletion cases. AZF partial deletions were also seen in these patients. Overall, AZF microdeletions were detected in 38.5% Y chromosome abnormality carriers, and most were observed in 46,X,Yqh− individuals. Loss of SY152 was seen in all 35 patients, with SY254/SY255 detected in 34 of 35 patients.

Conclusions: AZF microdeletions were detected in 38.5% of Y chromosome abnormality carriers. This indicates that AZF microdeletion screening is advisable for individuals with karyotypic Y chromosome abnormalities.

Keywords

Male infertility, Y chromosome, karyotype, AZF microdeletion, oligozoospermia, azoospermia

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Introduction

Genetic factors including Y chromosome microdeletions are responsible for around 10% of cases of male infertility,[1,2] and are particularly associated with azoospermia or

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severe oligozoospermia.[3] The azoospermic factor (AZF)-a, b, and c regions on the long arm of the Y chromosome are considered important for spermatogenesis.[4] These regions may be lost in microdeletions, causing problems with spermatogenesis. Numerical and structural chromosomal abnormalities are other important genetic factors associated with male infertility that can be detected cytogenetically.[2,5] Guidelines from the American Urological Association and European Academy of Andrology recommend cytogenetic analysis in all men with a total motile sperm count <5 million, and who are thought to have non-obstructive azoospermia;[6] however, Y chromosome microdeletions cannot be detected cytogenetically. Although intracytoplasmic sperm injection (ICSI) enables infertile men with Y chromosome microdeletions to become fathers, the potential risks of infertility being transmitted from infertile fathers to their offspring should be considered.[7]

Carriers of chromosomal disorders may also have AZF microdeletions. Indeed, patients with Klinefelter syndrome (KFS) may harbor Y chromosome microdeletions to varying degrees.[8–10] We previously reported[11] that AZF microdeletions are not found in azoospermia cases diagnosed with KFS, and that their frequency varies among populations because of genetic drift or selective pressure. Lee et al.[12] reported that carriers with 45,X/46,X,idic(Y) mosaicism have breakpoints between SY161 and SY121 on Yq11.221–q11.222, and Aydemir et al.[13] documented a rare case of 45,X/46,XY mosaicism with deletion of the AZFb + c region. Further more, Kim et al.[14] reported eight male patients with AZF deletions among 26 cases of structural Y chromosome abnormalities. However, Clark et al.[15] described a male patient with mosaicism between the 45,X karyotype and ring Y chromosome, but without AZFa, b, and c deletions.

Although Y chromosome microdeletions cannot be detected cytogenetically, karyotypic Y chromosome abnormalities may imply their presence, but this has rarely been reported. In the present study, therefore, we aimed to examine the occurrence of AZF microdeletions with karyotypic Y chromosome abnormalities in infertile men from northeastern China.

Materials and methods

Study population

Between July 2010 and December 2015, 5235 men with infertility or receiving counseling for infertility were recruited from outpatients of the Center for Reproductive Medicine (the First Hospital of Jilin University, Changchun, China). All patients underwent a thorough physical examination and semen analysis, and completed a detailed questionnaire regarding their smoking, drinking, marital status, childbearing history, spontaneous abortion status, medical history, and working conditions. Semen analysis was performed according to World Health Organization guidelines.[16] If no sperm was found, semen samples were centrifuged ($3000 \times g$ for 15 min), and the sediment was reexamined.

Patients were defined as having oligozoospermia if sperm densities of their last three semen samples were $<15 \times 10^6$/ml (taken at intervals of 1–3 weeks). Azoospermia, oligozoospermia, and severe oligozoospermia were defined according to our previous descriptions.[17] Cytogenetic analysis was performed for all patients. In men with karyotypic Y chromosome abnormalities and azoospermia, oligozoospermia, or severe oligozoospermia, detection of AZF microdeletions was also performed. The process is shown in Figure 1. Men with normal sperm densities were excluded from the study. Detection of the AZF gene was performed in men with abnormal sperm densities. The study was approved by the Ethics Committee of the First Hospital

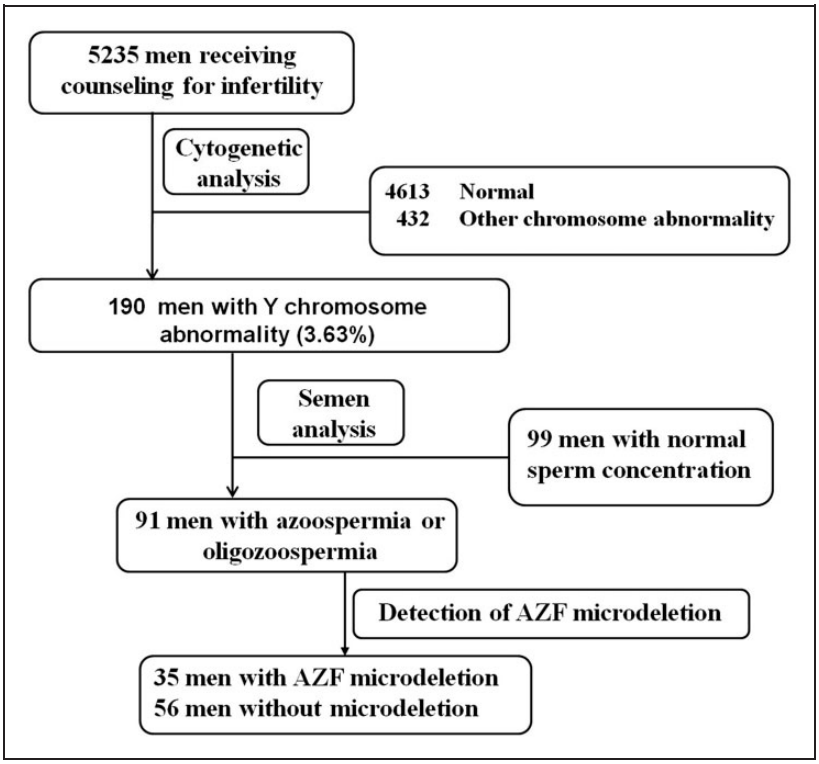

Figure 1. Flow chart of cytogenetic analysis and detection of AZF microdeletions.

of Jilin University, and all participants provided written informed consent.

Cytogenetic analysis

Peripheral blood (0.5 mL) was cultured for 72 h in sterile tubes with Yishengjun culture media (Guangzhou Baidi Biotech, Guangzhou, China) containing 30 U/mL heparin, and subsequently treated with 20 μg/mL colcemid for 1 h. G-banding of metaphase chromosomes and karyotype analysis were performed using our previously published methods.[17] Images of karyotype analysis were acquired using the Leica CW-4000 system (Leica, Wetzlar, Germany). Karyotypes were described in accordance with the International System for Human Cytogenetic Nomenclature 2009. A total of 20 metaphases were counted with six metaphases analyzed on two slides. The analysis was performed by two technicians.

Molecular analysis

Genomic DNA was isolated using the TIAN amp Blood DNA kit (Beijing Tiangen Biotech Co., Ltd, Beijing, China). PCR analysis of classical AZF microdeletions was performed according to European Academy of Andrology and European Molecular Genetics Quality Network recommendations, using sequence-tagged sites (STS): SY84, SY86, SY127, SY134, SY143, SY152, SY157, SY254, and SY255. SY14 (an STS located within the sex-determining region Y gene) and ZFX/ZFY were used

Table 1. Clinical features of Y chromosome abnormality carriers.

Karyotype	Number of cases	Number of cases with AZF microdeletion	Clinical features
48,XXYY,inv(9)	1	0	Azoospermia
47,XYY	12	0	8 severe oligozoospermia, 4 azoospermia
47,XX,del(Y)(q11)/46,XX	1	1	Azoospermia
47,X,i(Y)(q10),+mar	1	0	Azoospermia
46,X,Yqh+	18	0	3 oligozoospermia, 5 severe oligozoospermia, 10 azoospermia
46,X,Yqh-	34	22	1 oligozoospermia, 5 severe oligozoospermia, 28 azoospermia
46,X,Yqs	1	0	Severe oligozoospermia
46,X,inv(Y)	2	0	Severe oligozoospermia
46,X,der(Y;Y)	1	0	Azoospermia
46,X,del(Y)(q?)	3	3	Azoospermia
46,X,t(Y;12)(p11.2;p11.2)	1	0	Azoospermia
46,X,t(Y;15)(p10;p10)	1	1	Azoospermia
46,X,t(Y;16)(q11;p13)	1	0	Azoospermia
46,XX/46,XY	1	0	Azoospermia
45,X/46,XY	6	5	Azoospermia
45,X/46,X,del(Y)	3	3	Azoospermia
45,X/46,X,dic(Y)(p11)	1	0	Azoospermia
45,X/46,X,i(Y)	1	0	Azoospermia
45,X/48,XYYY	1	0	Azoospermia
45,X/46,X,r(Y)/46,X,r(Y;Y)	1	0	Severe oligozoospermia
	91	35 (38.5%)	

as internal controls. Primer sequences, operating procedures, and analysis of amplification products were performed using our previously published methods.[18]

Results

A total of 190 Y chromosome abnormality carriers were detected in 5235 male patients. Of these, 35 patients (35/190, 38.5%) had AZF microdeletions, which were most common in patients with 46,X,Yqh− (n = 22), followed by those with 45,X/46,XY (n = 5). Karyotypes, cases, and clinical features are summarized in Table 1, and the derivative chromosomes from GTG-banded chromosome analysis are shown in Figure 2. The presence of STS deletions as detected by PCR is shown in Table 2. The most frequent microdeletions were detected within the AZFb + c region, with 17 patients (48.57%) showing deletion of all AZFb + c markers (SY127, SY134, SY143, SY152, SY254, SY255, and SY157). Microdeletions in the AZFa + b + c region were found in three patients (8.57%), with one case of AZFc deletion. AZF partial deletions were also seen in these patients (Table 3). Loss of SY152 was most common, being seen in all 35 patients, followed by SY254/SY255 in 34 of 35 patients (97.14%), and SY143 in 31 of 35 patients (88.57%; Table 3). SY14 and ZFX/ZFY were positive in all patients with Yq microdeletions.

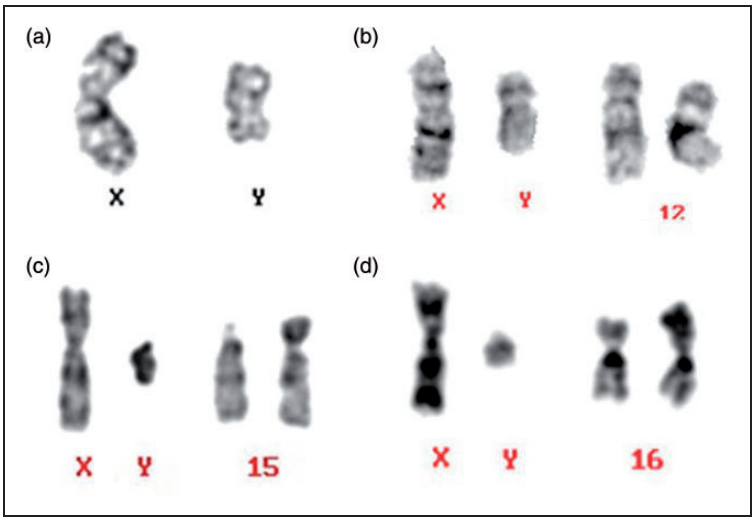

Figure 2. Karyotype of structurally abnormal chromosomes.
a: inv(Y); b: t(Y; 12); c: t(t;15); d: t(Y;16).

Discussion

Y chromosome microdeletions are the most frequent genetic cause of male infertility after KFS.[18] Encouragingly, ICSI and testicular sperm extraction can overcome natural fertilization barriers and help some infertile men become fathers. However, these approaches increase the transmission risk of genetic defects.[19] Accordingly, AZF microdeletions and their associated phenotypes have been extensively studied in infertile men. AZF microdeletions cannot be detected cytogenetically so screening is necessary, and previous AZF microdeletion frequencies have been reported to range from 3% to 55%.[2,18,20] Microdeletions of the AZF region may be a factor involved in absolute azoospermia in men with KFS.[10,21] Loss of the Y chromosome long arm, involving a large part of the AZFb, AZFc, and Yq heterochromatin regions, is associated with the unbalanced translocation, t(Y;22).[22] Further, microdeletions have been detected in the Y chromosome regions AZFb (SY127, SY134) and AZFc (SY254, SY255) in carriers with 45,X/46,XY mosaicism.[13] However, several studies indicated that AZF microdeletions are not detected in carriers with chromosomal abnormalities.[11,15,23] In the present study, we analyzed infertile men with karyotypic Y chromosome abnormalities, and detected AZF microdeletions in 38.5% of carriers with Y chromosome abnormalities and in 22 patients with 46,X,Yqh−.

In carriers with 45,X/46,X,del(Y), 47,XX, del(Y)(q11)/46,XX, or 46,X,del(Y)(q?), del(Y) is the common feature. Generally, the incidence of Y/autosome translocations is low.[23] Here, we found three cases with Y/autosome translocations, with AZF microdeletions detected in the 46,X,t(Y;15) carrier. Translocations involving the Y chromosome have previously been reported in association with male infertility, and could be explained in two possible ways. First, the AZF region may be affected secondary to microdeletions, rearrangements, or complete loss of chromosomes. Second, defective X–Y pairing may cause abnormal sex vesicle formation during meiosis, leading to spermatogenetic arrest.[22] The former explanation is likely when the deletion of AZF loci is observed.

Table 2. Sequence-tagged site deletions.

	AZFa		AZFb			AZFc			
Karyotype	SY86	SY84	SY127	SY134	SY143	SY152	SY254	SY255	SY157
46,X,Yqh−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
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	+	+	+	+	+	−	−	−	+
	+	+	+	+	+	−	−	−	+
45,X/46,XY	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	+
	+	+	−	−	−	−	−	−	+
	+	+	+	+	−	−	−	−	−
45,X/46,X,del(Y)	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	−	−	−
46,X,del(Y)(q?)	+	+	−	−	−	−	−	−	−
	+	+	−	−	−	−	+	+	+
	+	+	+	+	−	−	−	−	−
47,XX,del(Y)(q11)/46,XX	+	+	+	+	+	−	−	−	−
46,X,t(Y;15)(p10;p10)	+	+	+	+	−	−	−	−	−

AZF, azoospermic factor; STS, sequence-tagged site. −, deletion of specific STS; +, presence of specific STS.

45,X/46,XY mosaicism is a rare chromosomal abnormality,[13] but we identified six cases in our current karyotype analysis, with AZF microdeletions occurring in five of these (83.3%). In carriers of 45,X/46,XY mosaicism, Y chromosome structural anomalies accompany the 45,X cell line, which is thought to emerge with the Y chromosome because of impaired chromosome structure and stability. This can lead to phenotypic variations including the expression of a female phenotype, indefinite genitals, or a male with hypospadias or azoospermia.[13] The 46,X,Yqh− karyotype

Table 3. Type and frequency of specific STS deletions in patients with Y chromosome microdeletions.

Deletion pattern	Number of cases (%)	STS deletion site	Frequency (%)
AZFa + b + c	3 (8.57%)	SY86	3 (8.57%)
AZFb + c	17 (48.57%)	SY84	3 (8.57%)
AZFc	1 (2.86%)	SY127	26 (74.29%)
AZF partials		SY134	27 (77.14%)
b + partial c	5 (14.29%)	SY143	31 (88.57%)
partial b + c	3 (8.57%)	SY152	35 (100%)
partial b + partial c	3 (8.57%)	SY254	34 (97.14%)
partial c	3 (8.57%)	SY255	34 (97.14%)
Total	35 (100%)	SY157	24 (68.57%)

AZF, azoospermic factor; STS, sequence-tagged site.

is a chromosomal polymorphic variant that occurs in the general population and was considered normal for some time.[24] Here, we observed AZF microdeletions in 22 patients (64.7%) with 46,X,Yqh−. This indicates that AZF microdeletion screening may be advisable for 46,X,Yqh− individuals.

Previous studies have shown that AZFb deletions usually result in maturation arrest during meiosis. Deletion of the AZFc region causes variable phenotypes ranging from hypospermatogenesis to spermatogenic arrest to Sertoli cell-only syndrome.[25] Massive deletions in AZFb/b + c are responsible for severe spermatogenic failure.[26] Deletion of the AZFb region is generally associated with a poor prognosis for sperm retrieval, while some patients with AZFc deletions may exhibit retrievable sperm within the testis, although azoospermic men with AZFb + c deletions lack sperm in their testes.[26–28] In the present study, the most frequent microdeletions were detected within the AZFb + c region, which included 17 azoospermia cases (48.57%) with deletions of all AZFb + c markers (SY127, SY134, SY143,SY152, SY254, SY255, and SY157). Hopps et al.[28] reported that three out of 12 men who underwent karyotype analysis had 45,X/46,XY mosaicism and AZFb + c deletions.

We also found that SY152 was the most common marker to be deleted, occurring in all 35 patients, followed by deletion of SY254/SY255 in 34 of 35 patients (97.14%) and SY143 in 31 of 35 patients (88.57%). The SY152 deletion has been shown not to affect sperm quality or clinical outcome for men with oligozoospermia or azoospermia after ICSI treatment, but can decrease the fertilization rate.[29] Recently, we reported that clinical outcomes of ICSI for oligozoospermic patients with SY152 deletions are comparable to those of infertile patients without deletions.[30] Deletion of the AZFc region (including SY152, SY254, SY255, and SY157) results in a variable phenotype ranging from azoospermia to normal semen parameters, whereas the majority of men with AZFc deletions have sperm retrieved successfully.[25,28]

In conclusion, we detected AZF microdeletions in 38.5% of Y chromosome abnormality carriers, and most were observed in 46,X,Yqh− individuals. The most frequent microdeletions were detected in the AZFb + c region, which contained 48.57% of all AZF microdeletion cases. Loss of SY152 occurred in all 35 patients, and SY254/SY255 was lost in 34 of 35 patients (97.14%). Carriers of karyotypic Y chromosome abnormalities have the potential to be at risk from Y chromosome microdeletions, and these microdeletions can be transmitted from infertile fathers to their offspring by ICSI. Therefore, individuals with karyotypic

Y chromosome abnormalities should be screened for AZF microdeletions, and genetic counseling should be considered before couples undergo ICSI.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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