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LETTER TO THE EDITOR

Male Health

A fertile male with a single sY86 deletion on the Y chromosome

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Dear Editor,

Y chromosome microdeletions (YCMs) occur when gene fragments are deleted from the azoospermia factor (*AZF*) gene that is located on chromosomal band Yq11. YCMs are the second most common genetic cause of male infertility following Klinefelter syndrome.[1] Genetic screening of *AZF* is widely used in the diagnosis of male infertility. YCMs are primarily identified by screening six sites as indicated in the European Academy of Andrology and the European Molecular Genetics Quality Network (EAA/EMQN) guidelines, including *AZFa* (sY84 and sY86), *AZFb* (sY127 and sY134), and *AZFc* (sY254 and sY255).[1] The *AZFa* region is mainly involved in spermatocyte proliferation and is considered as the most important region of *AZF*.[2] Gene deletions in *AZFa* are rare, account for 0.5%–4.0% of all YCMs, and include complete as well as partial deletions.[1] Patients with a complete *AZFa* deletions are azoospermic, and thus standard biopsy examination is futile.[1] The proximal end of the *AZFb* region contains numerous palindromic sequences, and deletions in this region often cause the loss of large gene fragments. Patients with *AZFb* deletions are incapable of generating mature sperm cells due to the arrest of spermatogenesis at the spermatocyte stage.[3] Among all deletion types, *AZFc* deletions result in the mildest clinical symptoms, which usually manifest as oligospermia. Some patients with *AZFc* deletions can have progeny through assisted reproductive surgery.[1] Here, we report a case involving a partial deletion of the *AZFa* region yet possessing normal fertility.

The patient was a 29-year-old male whose daughter died at the age of 7 months due to congenital bile duct dysplasia. The couple visited the Reproductive Medicine Center of Shanghai Changhai Hospital (Shanghai, China) because his wife was diagnosed with oviduct adhesion. The couple accepted a prepregnancy physical examination. His past medical history was unremarkable, and he had a family history of cryptorchidism (nephew). Clinical examination and genitalia ultrasound imaging generated normal results. As required by the Institutional Review Board of Changhai Hospital, the patient and his father provided written informed consent for participation in this study.

Semen examination results showed normal ranges, which included semen standard analysis, sperm morphology examination, and semen biochemistry. Peripheral blood karyotyping indicated a 46,XY male karyotype.

As routine examination of infertile couples in our hospital, the husband underwent YCM testing. A total of six classic sites (sY86, sY84, sY127, sY134, sY254, and sY255) in the basic set were checked using the multiplex polymerase chain reaction (PCR) method (Y chromosome microdeletions detection kit, Tegen Biotechnologies Inc., Shanghai, China). The primary test showed the absence of sY86, indicating an *AZFa* partial deletion (**Supplementary Figure 1**). Following the EAA/EMQN guidelines, we performed an extended analysis of the *AZFa* region by single-site PCR amplification to confirmation of the deletion and exclude false-positive results. The primers were redesigned, and eight markers (sequence-tagged sites, STS) in the *AZFa* region were screened, which included sY82, sY83, and sY1064 at the proximal border; sY86 and sY84 as the basic sites; and sY1065, sY1182, and sY88 at the distal border. The analysis showed that sY83, sY1064, and sY86 were absent, whereas sY82, sY84, sY1065, sY1182, and sY88 were present (**Figure 1a**). The primer sequences used in the extended analysis are shown in **Supplementary Table 1**. We performed the same test on the patient's father, and the same results were obtained (**Supplementary Figure 2**). The other members of the family refused to undergo any testing.

We also used another method to verify the absence of sY86 in the patient and his father, namely, Agilent high-density oligonucleotide array-based comparative genomic hybridization (aCGH), which is an efficient and precise approach to discover copy number variations (CNVs) for clinical diagnoses.[4] Genomic DNA was isolated from peripheral blood samples using an AxyPrep™ Blood Genomic DNA Miniprep kit (Axygen, Union City, CA, USA). The DNA was then tested for CNVs using aCGH. Analysis revealed a 208-kb YCM (chrY:14,415,452-14,623,795 [GRCh37/h19]). This missing fragment does not contain any known protein-coding genes and was located upstream (about 200 kb) of the ubiquitin specific peptidase 9 Y-linked (*USP9Y*) and DEAD-box helicase 3 Y-linked (*DDX3Y*) genes (**Figure 1b**). By querying the "MSY breakpoint mapper," we confirmed that the deleted sY86 site (chrY:14,596,277-14,619,148 [GRCh 37/h19]) was included in the missing fragment.

YCMs are the major cause of severe oligospermia and azoospermia. The observed deletion frequency in infertile males ranges from 2% to 11.5 %.[1,5] A partial *AZFa* deletion may result in various clinical symptoms ranging from asthenozoospermia to azoospermia.[1,5,6] Behulova *et al.*[7] reported two cases that had the sY86 deletion only, and the clinical manifestation was azoospermia. In another study, two

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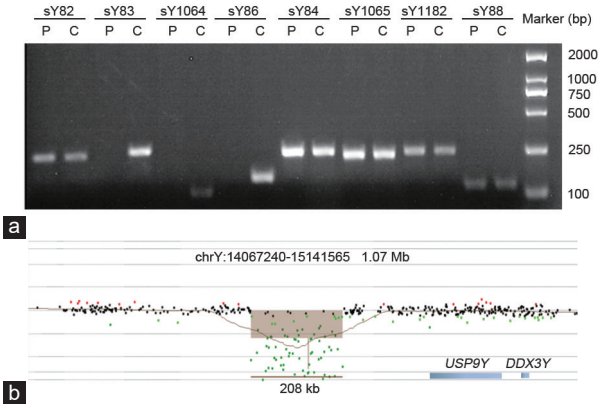

Figure 1: Analysis of the *AZFa* region in patient's Y chromosome. (**a**) Extended analysis of the *AZFa* region (including sY82, sY83, sY1064, sY86, sY84, sY1065, sY1182, and sY88) by single-site PCR amplification. (**b**) High-resolution genome-wide CGH analysis for screening deletions involving the Y chromosome. The brown color indicates the deleted area of the dots to determine the genomic distributions of oligonucleotide probes for CGH assays. Blue bars indicate the gene regions (*USP9Y* and *DDX3Y* genes), and the transcriptional orientation is from blue to light blue. *AZF*: azoospermia factor locus; P: patient; C: control; CGH: comparative genomic hybridization; chrY: human Y chromosome; *USP9Y*: ubiquitin specific peptidase 9 Y-linked; *DDX3Y*: DEAD-box helicase 3 Y-linked.

patients were found to be azoospermic in association with a single sY86 deletion, while another patient with a deletion of the same site was oligozoospermic.[8] In a family survey, Luddi *et al.*[6] found that both the patient and his male family members had Y chromosome deletions, including sY84 and sY86 in the *AZFa* region. These individuals were determined to undergo normal spermatogenesis, although a decline in sperm motility was detected. However, to our knowledge, no males with sY86 defects have been reported to have normal semen and normal fertility thus far.

In the present case, the same gene fragment and sY deletion were detected in the peripheral blood of the patient and his father. On the basis of the normal spermatogenetic phenotype of our patient and the proven fertility of the patient and his father, we infer that sY86 plays a marginal role in spermatogenesis. The deleted region that was detected in this study did not contain the two single-copy genes, *USP9Y* and *DDX3Y*, which play crucial roles in spermatogenesis and are located within the *AZFa* region.[1] *USP9Y* has been reported to regulate sperm production,[9] and mutations in the *DDX3Y* gene could lead to male infertility and a decrease in the number of germ cells.[10] Previous studies have indicated that azoospermia or oligoasthenospermia may not only be caused by the deletion of sY86 and that additional genetic or nongenetic factors may play a more significant role in generating the phenotypic consequences of sY86 deletions.[6–8] If there is no complete deletion of the *AZFa* region, including sY84 and sY86, a more detailed and extensive analysis is necessary to evaluate its impact such as screening for other genetic markers, followed by a testicular biopsy. Similar to the partial deletion of the *AZFa* region, if a partial deletion of *AZFb* is detected in the primary test, then there may be cases in which the patient undergoes normal spermatogenesis and thus is fertile.[1] Furthermore, genetic counseling is recommended.

In summary, our results suggest that partial deletion of the *AZFa* region (sY86 fragment) may not affect normal sperm production and fertility in humans. Even if YCM testing is widely used in the diagnosis and screening of patients with reproductive disorders, additional genetic testing should be conducted to verify and ensure the accuracy of data when various severe microdeletions involving the *AZF* region are observed.

AUTHOR CONTRIBUTIONS

YJ and SRL designed the study. SRL supervised the study. YJ, ZGN, WYL, and FZ analyzed and interpreted the data. YJ and ZGN collected the data. QQ and TTS contributed to the experimental studies. YJ, ZGN, and SRL wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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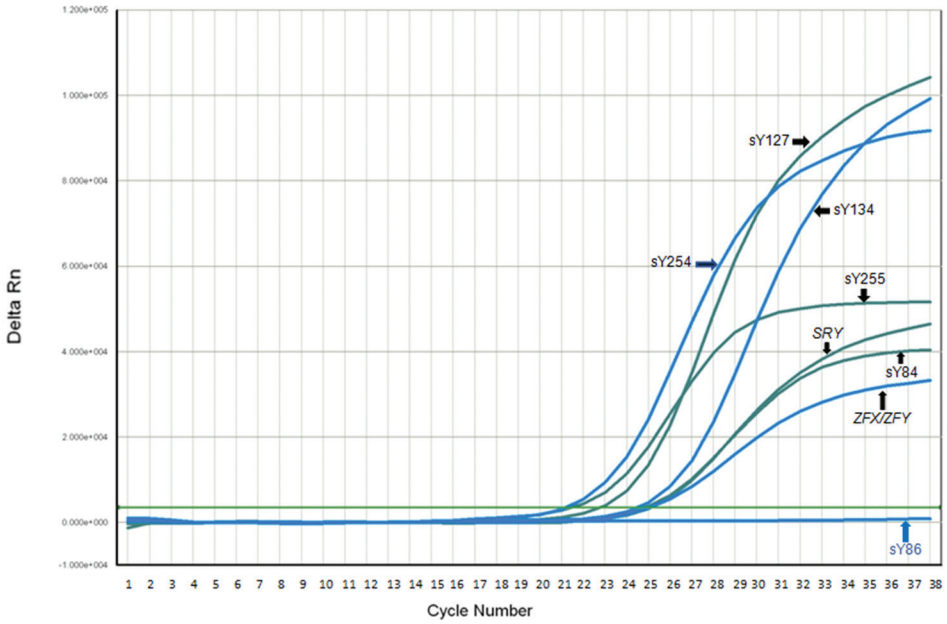
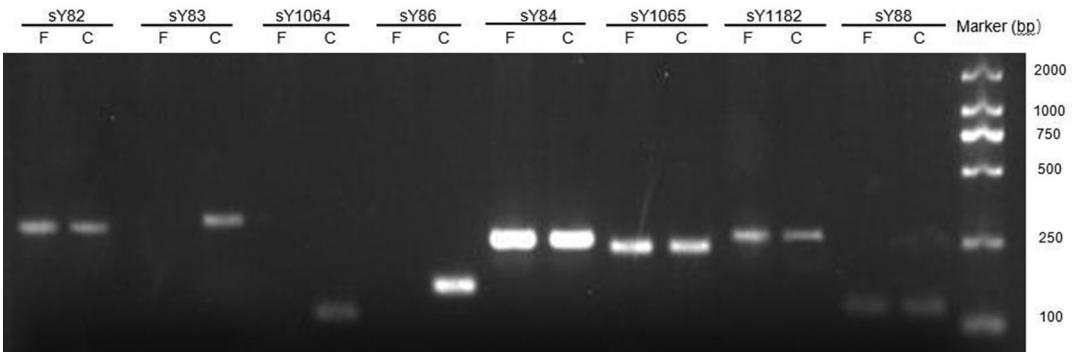


Supplementary Figure 1: Y chromosome microdeletions testing (including sY86, sY84, sY127, sY134, sY254, and sY255) detected by multiple polymerase chain reaction. *SRY* and *ZFX/ZFY*: control fragments.



Supplementary Figure 2: Extended analysis of the *AZFa* region (including sY82, sY83, sY1064, sY86, sY84, sY1065, sY1182, and sY88) by single-site polymerase chain reaction amplification in the patient's father. *AZF*: azoospermia factor locus; F: father; C: control.

Supplementary Table 1: Polymerase chain reaction primer sequences in extended analysis

Supplementary information

Table S1. PCR primer sequences in extended analysis.

Primer	Sequence	Product size [bp]
sY82-F	5′-ATC CTG CCC TTC TGA ATC TC-3′	264
sY82-R	5′-CAG TGT CCA CTG ATG GAT GA-3′	
sY83-F	5′-CTT GAA TCA AAG AAG GCC CT-3′	275
sY83-R	5′-CAA TTT GGT TTG GCT GAC AT-3′	
sY1064-F	5′-GGG TCG GTG CAC CTA AAT AA-3′	110
sY1064-R	5′-TGC ACT AAA GAG TGA TAA TAA ATT CTG-3′	
sY86-F	5'- GTT ACA AAG AAA TCC CAA AGA C -3'	178
sY86-R	5'- ACA CAC AGA GGG ACA ACC CT -3'	
sY84-F	5′-AGA AGG GTC TGA AAG CAG GT-3'	264
sY84-R	5'-ATT AGC CAG ACA CTA AAG AAA GG-3'	
sY1065-F	5′-TCA GGT ACT GTG ATG CCG TT-3′	239
sY1065-R	5′-TGA AGA GGA CAC AAA GGG AAA-3′	
sY1182-F	5′-ATG GCT TCA TCC CAA CTG AG-3′	247
sY1182-R	5′-CAT TGG CCT CTC CTG AGA CT-3′	
sY88-F	5′-TTG TAA TCC AAA TAC ATG GGC-3′	123
sY88-R	5′-CAC CCA GCC ATT TGT TTT AC-3′	

F, Forward primer; R, Reversed primer