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Natural Transmission of b2/b3 Subdeletion or Duplication to Expanded Y Chromosome Microdeletions

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Background: Y chromosome microdeletions are usually *de novo* mutations, but in several cases, transmission from fertile fathers to infertile sons has been reported.

Material/Methods: We report 3 cases of infertile patients who inherited expanded Y chromosome microdeletions from their fathers, who carried b2/b3 subdeletion or duplication. The karyotype was analyzed using G-banding. High-throughput sequencing was used to detect AZF region microdeletions.

Results: Cytogenetic analysis showed a normal karyotype 46,XY in patient 1 (P1), patient 2 (P2), and their fathers (F1 and F2). Patient 3 (P3) and his father (F3) presented a karyotype of 46,XY,Yqh-. High-throughput sequencing for the AZF disclosed an identical b2/b3 subdeletion in the F1 and F2. P1 had an AZFc deletion that accounted for 3.5 Mb, and P2 had an AZFa+b+c microdeletion that accounted for 10.5 Mb. F3 had a b2/b3 duplication of 1.8Mb, but P3 had an AZFb+c deletion of 6.2 Mb.

Conclusions: Our findings suggest that b2/b3 partial deletion or duplication can lead to structural instability in the Y chromosome and be a risk factor of complete deletion of AZFc or more expanded deletion during transmission.

MeSH Keywords: **Genetics • Infertility, Male • Y Chromosome**

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Background

Male factor infertility is implicated in 40–50% of infertile couples [1]. Y chromosome microdeletions, known as azoospermia factors (AZF) microdeletions, are one of the main causes of male infertility [2]. It occurs in about 1 in 4000 men in the general population, but its frequency is significantly higher among infertile men. According to global estimates, about 10% of cases of idiopathic infertility occur due to deletions in the AZF region. The first-confirmed and best-characterized deletion is complete AZFc region deletion (also known as b2/b4 deletion), which removes all of the AZFc and leads to spermatogenic impairment [3, 4]. In the past few years, 3 types of partial AZFc deletions have been identified: b1/b3, gr/gr, and b2/b3 deletions (also referred to as g1/g3 deletion or u3-gr/gr deletion) [5]. However, the roles of partial AZFc deletions in spermatogenesis are controversial because the possible susceptibility of the different partial AZFc deletions to spermatogenic failure varies among different populations. Both gr/gr and b2/b3 deletions, the 2 main types of partial AZFc deletions, have been reported to have variable phenotypes among human populations [6]. The b1/b3 deletion is so rare that its effects on spermatogenesis have not been assessed. In addition to deletions, partial AZFc duplication is also a risk factor in the non-deletion patients.

AZF microdeletions are usually *de novo* mutations, but in several cases transmission from fertile fathers to infertile sons has been reported [7]. Zhang et al. reported that partial AZFc deletions were associated with an increased risk of complete deletion in AZFc [8]. The reduction of the distance between recombination targets caused by partial AZFc deletion may consequently increase the risk of complete deletion. However, partial AZFc deletions or duplications leading to more expanded microdeletion than complete AZFc have been rarely reported. Here, we report 3 cases of oligozoospermia or azoospermia patients with an enlarged AZF microdeletion inherited from the father with the b2/b3 subdeletion or duplication though a spontaneous pregnancy. We hypothesized that b2/b3 partial deletion or duplication is a risk factor of complete deletion of AZFc or more expanded deletion during transmission.

Material and Methods

Patients

Patient 1 (P1) was a 31-year-old man who was examined in 2012 for his 2-year history of primary infertility. His height was 169 cm and weight was 89 kg. Physical examination showed normal penis and small bilateral testicular volume of 10 mL. Semen analysis performed on 2 different occasions and showed oligozoospermia according to the World Health Organization

guidelines. The sperm concentration was 5.6×10^6 /mL and 7.2×10^6 /mL, respectively. Reproductive hormone values and ultrasound analysis were normal. His father (F1) was 55 years old when he underwent the AZF microdeletion examination.

Patient 2 (P2) was a 26-year-old male, with normal phenotype and intelligence. He was referred for cytogenetic analysis for infertility. His height was 170 cm and weight 60 kg. An andrological examination revealed that both testicular volumes were 6 mL (normal reference: >15 mL). Semen analysis performed on 3 different occasions showed azoospermia. Reproductive hormone values and ultrasound analysis were normal. His father (F2) was 51 years old when he underwent the AZF microdeletion examination.

Patient 3 (P3) was a 25-year-old healthy male admitted to our center in 2015 due to primary infertility. His height was 182 cm and weight was 69 kg. Clinical examination of the genitals revealed a normal penis and both testes located in the scrotum, with a volume of approximately 16 mL and 18 mL, respectively. He had azoospermia as shown by 3 semen analyses. Reproductive hormonal profile was normal for serum concentration of luteinizing hormone and testosterone, but the level of follicle-stimulating hormone was a little higher (14.1 mIU/mL; normal range 1.5–12.4 mIU/mL). His father (F3) was 53 years old when he was recalled to undergo the AZF microdeletion examination.

The study was approved by the Ethics Committee of the First Hospital of Jilin University. Written informed consent was obtained from all study participants. Screening for microdeletions was performed in the patients and their fathers using high-throughput sequencing for the AZF region.

Karyotype analysis

Peripheral blood samples (0.5 mL, from patients and their fathers) were collected.

Karyotyping of Giemsa (GTG)-banded chromosomes from lymphocytes culture was performed. The protocol for lymphocytes culture, chromosome preparation procedures, and karyotype analysis were performed using previously described methods [9].

Molecular analysis of the Y chromosome

Genomic DNA was extracted by conventional methods from peripheral blood [3]. Multiplex PCR amplification of 9 sequence-tagged site (STS) markers was used to detect AZF region microdeletions on the Y chromosome as suggested by the European Academy of Andrology and European Molecular Genetics Quality Network recommendations. These markers

Table 1. Patient informations and the results of Cytogenetic analysis and Molecular analysis of the Y chromosome.

Sample ID	Age (years)	Semen analysis	Left testicular volume (mL)	Right testicular volume (mL)	Karyotype	The type of AZF microdeletion	Size of deletion/duplication
F1	31	Oligozoospermia	10	10	46,XY	AZFc deletion	3.5 Mb
P1	55	N/A	N/A	N/A	46,XY	b2/b3 subdeletion	1.8Mb
F2	26	Azoospermia	6	6	46,XY	AZFa+b+c deletion	10.5Mb
P2	51	N/A	N/A	N/A	46,XY	b2/b3 subdeletion	1.8Mb
F3	25	Azoospermia	16	18	46,XY,Yqh-	AZFb+c deletion	6.2Mb
P3	53	N/A	N/A	N/A	46,XY,Yqh-	b2/b3 duplication	1.8Mb

in the AZFc. P1 had an AZFc deletion that accounted for 3.5 Mb and P2 had an AZFa+b+c microdeletion that accounted for 10.5Mb. In contrast to the above 2 pedigrees, F3 had a b2/b3 duplication (b2/b3 inversion followed by g1/g3 duplication) of 1.8 Mb, but his son (P3) had an AZFb+c deletion of 6.2Mb (Figure 2). Patient information and the results of cytogenetic analysis and molecular analysis of the Y chromosome are shown in Table 1.

Multiplex PCR screening for the STS of the AZFa region (SY84, SY86), AZFb region (SY127, SY134, SY143), and AZFc region (SY152, SY157, SY254, and SY255) in the AZF region was performed to examine the classical AZF microdeletion before high-throughput sequencing was performed. We found that in P1 the classical STS of sY152, sY157, sY254, and SY255 in the AZFc region were absent. In P2 we found that the whole STS detected were all absent. P3 also presented an AZF microdeletion for STS of sY127, sY134, sY143, sY152, sY157, and sY254, and sY255 was absent. The fathers (F1, F2, and F3) were all not detected and STS was absent. The results shown by multiplex PCR were consistent with high-throughput sequencing.

Discussion

In this pedigree study, 2 enlarged AZF deletions were found to be derived from the b2/b3 deletion via natural transmission. This observation suggests that partial AZFc deletion may carry a high risk of complete AZFc deletion or more expansion deletion. Similar results were obtained in the past [11–13], but our study also showed a greater scope of AZF deletion in the transmission from father to son (F2 to P2). Although the mechanism of the susceptibility of partial AZFc deletions to enlarge AZF is ambiguous, Zhang et al. suggested that the reduction of the distance between recombination targets may consequently increase the risk of expansion in the AZF deletion [7]. However, in pedigree 3 (P3 and F3) of our study, the son had an AZFb+c deletion transmitted by his father, who had

a b2/b3 duplication. That conflicts with the hypothesis above and requires further study.

In contrast to classical AZF deletions, the possible susceptibility of the different partial AZFc deletions to spermatogenic failure varies among different populations. In 2004, Repping et al. initially described the b2/b3 subdeletion with a relatively low frequency [14], showing a significant difference between infertile men and healthy controls, which indicated that the b2/b3 deletion may have some effect on male infertility. In 2008 and 2009, based on the Chinese Han population, 2 studies also concluded that b2/b3 deletion was significantly associated with spermatogenic failure [15,16]. In 2012, a survey with a large sample of 20 884 men from 5 populations was carried out; in contrast to previous studies, the b2/b3 deletion was not shown to increase the risk of severe spermatogenic failure [17].

The genotypic and phenotypic characterization of partial AZFc duplications is limited by significant under-reporting of these cases. The prevailing view is that both partial and complete AZFc duplications do not represent any particular risk for spermatogenic failure since the homeostatic mechanisms regulating spermatogenesis can compensate, to some extent, for imbalances associated with gene dosage increases [18]. Nevertheless, in 2007 Lin et al. reported that partial AZFc duplication resulted in increased AZFc gene copies, which was a risk factor of spermatogenic impairment [12]. The b2/b3 duplication, like the b2/b3 deletion, spans 1.8 Mb and involves 12 genes, and the duplication overlaps over 80% of their lengths [19]. Genes in the duplicated regions include the following: DAZ, which encodes RNA-binding proteins, which are involved in regulation of protein synthesis [3]; *CDY1*, which encodes a histone acetyltransferase [20]; *BPY2*, which encodes a basic protein with unknown function [21]; and 5 transcription units – *TTY3*, *TTY4*, *TTY17*, *CSPG4LY*, and *GOLGA2LY* [22]. Some of these genes are likely to be dosage-sensitive, and their increased expression may interfere with normal spermatogenesis.

The b2/b3 partial deletion or duplication resulting in decreased or increased AZFc gene copies has been reported to be associated with impaired spermatogenesis in Han Chinese populations. In this study, our reported cases were Han Chinese, and the fathers who carried with b2/b3 partial deletion or duplication produced offspring naturally. This evidence proves that b2/b3 partial deletion or duplication had little effect on male fertility in these 3 fathers. Sons with a larger deletion in the AZF than their fathers, indicating expansion, have been reported in many studies. This study is the first to reports b2/b3 partial duplication in a father leading to AZFb+c deletion in his son. Our results show that b2/b3 partial deletion and duplication can expand to a complete AZFc deletion or a more expansive deletion in the next generations. This provides new evidence for studying the mechanism of AZF deletion. In our next study we will expand the sample to perform a pedigree study of AZF deletion and to assess the incidence of expanded deletion during transmission. In further research, we plan

to test the AZF partial deletion in the sperm donors (except for the routine detection of AZF deletion) to avoid completed deletion of AZFc or more expanded deletion during transmission in the patients who produce offspring through AID (artificial insemination by donor semen).

Conclusions

Our findings suggest that b2/b3 partial deletion or duplication can lead to structural instability in the Y chromosome and is a risk factor of complete deletion of AZFc or more expanded deletion during transmission.

Conflicts of interest

None.

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