Non-chimerism and chimerism pseudo dicentric Y chromosome: two case reports about azoospermia and cytogenetic/molecular genetic analysis in the Chinese population

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Abstract

Objective To report the cytogenetic and molecular genetic analysis of the first two cases of non-chimerism and chimerism karyotype in Chinese male patients who suffer from azoospermia, which may be caused by pseudo dicentric Y chromosomes.

Design Case study.

Setting Academic reproductive medicine center.

Patients Two male patients with azoospermia, carrying pseudo dicentric Y chromosome.

Capsule Pseudo dicentric Y may lead to a male phenotype by duplicating SRY and spermatogenesis of individuals may be affected. However, AZF deletions may be the key factor that causes azoospermia.

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Interventions Review the records of inquiry, testicular biopsy, pathological examination, semen routine examination, endocrine evaluation, cytogenetic chromosomal analysis, and FISH detection of peripheral blood to evaluate Y chromosome deletion.

Main outcome measures To investigate the possible association among pseudo dicentric Y, chimeric status and azoospermia.

Results Two patients were both diagnosed with azoospermia by a variety of andrology inspections. Further chromosomal analysis and FISH indicated their pseudo dicentric Y chromosome and different chimerism status. PCR confirmed simultaneous deletions of AZFb and AZFc regions in the Y chromosome of both patients.

Conclusions Pseudodicentric Y chromosome affecting the long arm may lead to a male phenotype by duplicating the sex-determining region of Y chromosome (SRY) fragment and chimeric status may further impact patient's hormone levels, which obstruct spermatogenesis. However, the deletion of the azoospermia factor (AZF) is likely the key factor that causes azoospermia.

Keywords Pseudodicentric Y chromosome · Azoospermia · SRY · AZF region

Introduction

Genetic mutation is one of the main causes of male infertility and is closely related to the occurrence of azoospermia or severe oligozoospermia. Previous study demonstrate that 10 to 15 % of couples of childbearing age are unable to bear children, with half due to male infertility [1]. Spermatogenesis is under the control of many genes; hence, various

abnormalities of autosomal or sex chromosomes, including changes in number or distortion of structure can extensively influence spermatogenesis. The Y chromosome is particularly important in this process, and a variety of abnormalities are known to occur (deletions, inversion, translocation, dicentric Y, isochromosome Y, ring Y) and result in varying degrees of clinical outcome: testicular hypoplasia or developmental malformations, severe oligozoospermia and even azoospermia [2]. Currently the most related azoospermia case types that have been reported include 47,XXY Klinefelter's syndrome [3, 4], 45,XO Turner's syndrome [5], and 46,XX Male syndrome [6].

A “dicentric chromosome” is a type of structural chromosome aberrations. Two chromosomes are broken separately, then their chromosome arms with their own centromeres form one “new chromosome”, resulting in two centromeres in the final structure by the way of “stump connection”. This causes the formation of dicentric X chromosomes or dicentric Y chromosomes [7]. Sometimes, the cells of the same individual may have different genetic composition, chromosome structure or number. This kind of phenomenon is referred to as mosaicism [8]. The phenotypes of individuals with mosaic cell lines that exhibit structural aberrations of the X or Y chromosome are difficult to predict, varying from females with Turner's syndrome to males with infertility, and may even include individuals with ambiguous genitalia. In general, however, the severity of clinically adverse phenotype in chromosomal chimeric individuals is usually less than that in non-chimeric people, whose chromosomes in their cells are all abnormal [9].

There are still many issues worth exploring about the clinical and cytogenetic aspects in non-chimerism or chimerism patients with pseudo dicentric Y chromosomes, yet few reports are available in the Chinese population [10–12]. In this paper we report two Chinese cases of azoospermia caused by pseudo dicentric Y chromosomes, and discuss the cytogenetic and molecular genetics involved in the cases.

Materials and methods

We report here two patients (1 and 2), both suspected of azoospermia and referred for further diagnosis to our tertiary care hospital.

Inquiry

Patient 1 was a 30 year old male (height: 156 cm, weight: 68 kg) and patient 2 was 25 year old (160 cm, 70 kg). They came to our hospital respectively for infertility evaluation, following >24-month histories of unsuccessful conception after unprotected sexual intercourse. They had always been healthy and their social and family histories were unremarkable. Their marriages were not consanguineous and their wives

had regular menstrual cycles and engaged in regular intercourse with their husbands. The fertilities of these two patients' siblings were all normal.

Andrology inspections

A variety of andrology inspections were conducted including testicular biopsy, pathological examination, semen routine examination and endocrine evaluation. Among them, testicular biopsy was followed by pathological examination with H&E staining. The “SCA automated semen quality analyzer” from Spain was used to determine the semen routine indexes and the results were assessed according to the 《WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction》 (the fourth edition). The detection of sexual hormone levels was conducted by the chemiluminescence method using the ACCESS System (Beckman Coulter).

Chromosomal analysis

Cytogenetic chromosomal karyotype analysis and fluorescent in situ hybridization (FISH) detection of patients' peripheral blood were conducted for further examination. Karyotype analysis was carried out following peripheral blood lymphocyte culture. A total of 20~100 metaphase cells was analyzed by the G-banding method and the chromosome length included approximately 450~550 subbands. FISH analysis was carried out with the SRY combination and DYZ3 probes.

Evaluation of Y chromosome deletions

A method developed previously in our center is used to evaluate Y chromosome deletions [13]. Briefly, DNA was extracted from peripheral blood using a commercial kit following manufacturer instructions (Ugene company, Korea), and the detection of Y chromosome microdeletions was processed by real-time PCR method. Three selected sequence-tagged sites (STSs) within specific AZFa, AZFb, and AZFc regions were chosen as targets according to European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) guidelines. SRY region was also involved. Primers were designed using Primer Premier 5.0 (Premier Biosoft Int.) and four hydrolysis probes were designed for detection of four amplicons respectively. The assay was carried out in a quadruplex reaction.

Results

Bilateral vas deferentia were present in both patients 1 and 2, and their bilateral spermatic veins were not varicosed by

palpation. Their bilateral testicular volumes were only about 10 ml. The testis texture of patient 1 was slightly soft, while that of patient 2 was unremarkable. No sperm could be found in the centrifugal sediment of either of their semen after routine examinations.

The results of patient 1's hormone levels were as followed: follicle-stimulating hormone (FSH) was 22.31 mIU/mL (reference value: 1.27~18.96 mIU/mL), luteinizing hormone (LH) was 12.93 mIU/mL (1.24~8.62 mIU/mL), testosterone (T) was 3.08 ng/mL (4.14~7.26 ng/mL), estradiol (E2) was 27 pg/mL (20~75 pg/mL) and prolactin (PRL) was 11.27 ng/mL (2.64~13.13 ng/mL). With the same reference values, the results of patient 2's hormone levels were as followed: FSH was 21.30 mIU/mL, LH was 11.15 mIU/mL, T was 2.41 ng/mL, E2 was 28 pg/mL and PRL was 17.83 ng/mL. Thus for both patients FSH and LH exceeded the upper limit of the reference value, which may reflect poor gonadal function and a low capacity of spermatogenesis. In addition, the PRL level of patient 2 also exceeded reference range, which may indicate hypogonadism.

For patient 1, pathological examination showed that the hierarchy of the seminiferous epithelium in the seminiferous tubules decreased, germ cells were rare while supporting cells were relatively abundant, neither spermatogenic cell nor sperm were found but part of interstitial cells were visible, and a few seminiferous tubules showed fibrosis (Fig. 1a). For patient 2, the pathology results showed that the number of supporting cells and germ cells in the seminiferous tubules were reduced, cellular hierarchy became thinner, and the remaining germ cells were spermatogonia while neither spermatocytes nor sperm cells could be found, which suggest a complete failure of spermatogenesis. In addition mild fibrosis was seen in the interstitium of the walls of the seminiferous tubules (Fig. 1b).

Combining the results of cytogenetics and FISH, the karyotype of patient 1 was eventually described as 46,X,psu dic (Y)(pter→q11.2::q11.2→pter), with the presence of two copies of SRY and DYZ regions in his Y chromosome (SRY++, DYZ++) (Fig. 2a, b, c, d). For patient 2, two cell lines with different chromosome complements were found within his lymphocytes (Fig. 3a, b, c, d). His karyotype was eventually described as 45,X [5]/46,X,psu dic (Y)(pter→q11.2::q11.2→pter)[49], of which the proportion of 45,X cells was about 9 %. In addition, there also existed two copies of SRY and DYZ regions in his Y chromosome (SRY++, DYZ++) (Fig. 3e, f).

PCR detection of Y chromosome microdeletions showed that the AZFb and AZFc regions were simultaneously deleted in both of the two patients' Y chromosomes, while the AZFa region was retained (Fig. 4a, b).

The ultimate clinical diagnosis for both patients 1 and 2 was azoospermia, and as spermatogenic dysfunction was

Fig. 1 Pathological findings from H&E stained testicular biopsy sections **a** the pathological examination of patient 1 showed that the hierarchy of the seminiferous epithelium in the seminiferous tubules decreased, germ cells were rare while supporting cells were relatively abundant (Arrow 1), a few seminiferous tubules showed fibrosis (Arrow 2), and neither spermatogenic cell nor sperm were found but part of interstitial cells were visible (Arrow 3). **b** the pathology results of patient 2 showed that the number of supporting cells and germ cells in the seminiferous tubules were reduced, cellular hierarchy became thinner (Arrow 1), and the remaining germ cells were spermatogonia while neither spermatocytes nor sperm cells could be found (Arrow 2), which suggests a complete failure of spermatogenesis. Besides, some interstitium in the walls of the seminiferous tubules showed mild fibrosis (Arrow 3)

due to genetic mutation we recommended artificial insemination by donor (AID).

Discussion

In the two cases in this report, the testicular volumes of two male patients were relatively small (reference value >15 ml), implying testicular hypoplasia or atrophy. No sperm was found in their semen. The levels of FSH and LH in both patients exceeded the upper limits of the reference range, whereas the T levels were below the lower limit of the reference value. Thereby, the patients might have the pathological symptoms of spermatogenic dysfunction. The diagnosis of “azoospermia” was made by testicular biopsies (no spermatogenesis). Further testing included karyotype analysis which showed that patient 1 and 2 were both pseudodicentric Y chromosome individuals, with psu dic

Fig. 2 The chromosome karyotype and FISH analysis of patient 1. **a** and **b** The stretched preparation of peripheral blood and the pairing of homologous chromosomes of patient 1; **c** The FISH result of patient 1, using SRY combination probe; **d** The FISH result of patient 1, using DYZ3 probe

(Y)(pter→q11.2::q11.2→pter). In addition, 45, X chromosome mosaicism existed in patient 2 at the same time. Since the breakpoint of Y chromosome was at Yq11.2, the expression of the SRY area (located near the pseudoautosomal region of Y chromosome) and the DYZ3 clip (located near the centromere of Y chromosome) were doubled (SRY++, DYZ3++). Due to the fact that the patients were unable to produce sperm, we speculated that there might be a partial deletion in the Y chromosome, which may lead to spermatogenic failure apart from *psu dic(Y)*. Through PCR, we identified simultaneous microdeletions at the AZFb and AZFc regions in both patients' Y chromosomes.

In human, the genes that decide testis differentiation on the Y chromosome are called testis-determining factor (TDF). TDF is positioned on the short arm of the Y chromosome (Yp). The sex-determining region of Y chromosome (SRY) is the PY53.2 section on the Yp, about 2.1 kb, nears the pseudoautosomal region. *Sinclair et al.* discovered the SRY in 1990 and they considered that SRY is the best candidate gene for the TDF [14]. Lots of studies have shown that the proteins encoded by the SRY gene play a crucial role in sex determination and differentiation [15–17]. DNA hybridization experiments have shown that a single hybridization band of SRY can appear in humans, chimpanzees, and some other male mammals, but does not appear in female animals.

Interestingly the two patients reported here had normal external genitalia development despite the pseudodicentric distortion accompanied by microdeletions on the Y chromosome.

This may be due to the two SRY (SRY++). Apart from *psu dic(Y)*, patient 2 was also a chromosomal chimeric individual, with a 45, X karyotype cell line in 9 % of cells (5:49). Male development was reported in a similar case with mosaicism [18]. However, 45, X chimeric karyotype may still affect the individual's clinical presentations, such as stature or endocrine status. Parts of those clinical manifestations may be close to the phenotypes of Turner's syndrome patients. In our report, compared with patient 1, whose level of PRL was within the reference range, the PRL level of patient 2 was up to 17.83 ng/mL, which exceeded the reference range. This phenomenon was likely due to the effect of the 45, X karyotype. Some research studies have shown that a high level of PRL may have adverse effects on male spermatogenesis or sperm quality [19–21]. Therefore, for patient B, the 45, X chimeric cells may have aggravated his spermatogenic failure. In addition, a previous study has reported that once there is a 45, X cell line, regardless of whether there is Yp, Yq, or both Yp and Yq, or even a free Y chromosome in the other cells, there is an increased chance for that individual to be a phenotypic female [22]. In our mosaic case, the patient has a male phenotype. Again, we speculate that the two SRY (SRY++) copies might be the important factor leading to the phenotype.

Although the SRY gene is required for the gonad to differentiate to a testis, normal fertility requires additional genetic material. It is postulated that in addition to SRY on Yp, there may exist a few other functional fragments (genes) in other parts of the Y chromosome (e.g., Yq) which may be involved in sex determination or spermatogenesis. In the

Fig. 3 The chromosome karyotype and FISH analysis of patient 2. **a** and **b** The stretched preparation of peripheral blood and the pairing of homologous chromosomes of patient 2 (45,X cell line); **c** and **d** The stretched preparation of peripheral blood and the pairing of homologous chromosomes of patient 2 (46, X,Y cell line); **e** The FISH result of patient 2, using SRY combination probe; **f** The FISH result of patient 2, using DYZ3 probe

past few years, the azoospermia factor (AZF) region on the Y chromosome has become a focus in the infertility field. Yet the understanding of its comprehensive functions is still limited by its own complexity. Additionally, the sequence structure of the AZF region has only been identified in few of primate lineages. AZF was first reported by *Tiepolo et al.* in 1976. The entire AZF region is located in the Zone 1 band 1 of Y chromosome (Yq11). Its length is about 8.73 Mb and it is reported to be closely related to spermatogenesis [23]. In 1999, *Kent-First et al.* divided AZF into three regions: AZFa, AZFb and AZFc. Among them, AZFb and AZFc possess an overlapping area of about 1.5 Mb, only the AZFa region is relatively independent [24–26]. Microdeletion in the AZF region is closely related to the male infertility, as it is one of the most common molecular genetic causes of azoospermia or severe oligozoospermia.

The microdeletions of AZFb often occur between the palindromic structures and can lead to the lack of seminiferous

epithelial cells after meiosis and cause spermatogenesis arrest at the spermatocyte stage. Only spermatogonia and primary spermatocytes can be found in the testis, whereas spermatogenesis fails [27], which is consistent with the situation described in our case. The incidence of complete deletion of AZFb is equal to the incidence of AZFa deletion or slightly higher, accounting for 3~10 % of Yq microdeletions [28, 29]. The AZFc is located within the distal deletion interval 6 of the Y chromosome and its length is about 3.5 Mb. The function of AZFc in the germ cells includes apoptosis of germ cell [30], ubiquitination of protein [31], transcriptional regulation of chromosome [32], adjustment of the transcripts' transport or storage and activation of translation, etc. Studies have shown that the deletion of AZFc can hinder the seminiferous process [33]. In clinical practice, deletion of the AZFc region accounts for more than 60 % of AZF deletions, and the sperm concentration of patient with AZFc deletion is usually less than one million/ml (reference value >20 million/ml) [29].

Fig. 4 Y chromosome microdeletion analysis of patients. **a** Y chromosome microdeletion of patient 1, both SRY and AZFa are detected (*elevated curve*), however, AZFb and AZFc curves are missing; **b** Y chromosome microdeletion of patient 2, both SRY

and AZFa are detected (*elevated curve*), however, AZFb and AZFc curves are missing; **c** an unaffected male control sample, SRY, AZFa, AZFb, and AZFc curves are all elevated

The AZFc region, however, is not indispensable for spermatogenesis. It is possible for patients who suffer from complete absence of AZFc to produce mature sperm. But in the two cases reported here, both microdeletions of AZFb and AZFc were present simultaneously. Therefore,

we speculate that this kind of microdeletion might be the reason for the two patients' infertility.

As previously mentioned, chromosomal abnormalities can affect spermatogenesis; as a result, patients with chromosomal abnormalities may try to conceive by assisted

reproductive techniques (ART). In such cases it is very easy to form gametes with unbalanced chromosome, leading to pregnancy failure. Therefore, it is necessary for patients who are diagnosed as severe oligozoospermia or azoospermia to accept further chromosome screening. So far, the technology of FISH, combined with high resolution-comparative genomic hybridization (HR-CGH), can diagnose the patient's karyotype accurately [34, 35], and they are widely used to provide a good genetic basis for clinical diagnosis and treatment. In the future, it is important to further clarify the relationship between some of the important functional regions (such as the AZF region) and the clinical phenotypes of oligospermia or azoospermia. In order to have a better guide to clinical diagnosis and treatment, clinicians also need to select more detection methods and sites that have better specificity, higher sensitivity and wider range of applications to accurately detect individual chromosomal abnormalities (such as pseudo dic Y reported in this paper). Last but not least, medical staff should be aware of the genetic risks of performing intracytoplasmic sperm injection (ICSI) for oligozoospermia patients with chromosomal abnormalities and determine whether it is necessary to prevent abnormal genetic propagation by methods such as preimplantation genetic diagnosis (PGD).

In summary, we report the first two Chinese cases of non-chimerism and chimerism male patients with pseudo dicentric Y chromosome abnormality on the long arm, leading to microdeletions of AZF region and hence azoospermia. Compared to patients with pseudo dicentric Y chromosome abnormality on the short arm, whose SRY gene is often affected directly and develop phenotypes mimicking those seen in Turner's syndrome, the patients in our cases developed male phenotype but suffered from azoospermia. A real-time PCR method developed in our center was applied to detect the AZF microdeletions in the Y chromosome specifically.

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committee of Women and Children Health Care Hospital of Xiamen City and is in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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