Journal of Assisted Reproduction and Genetics (2021) 38:941–948
https://doi.org/10.1007/s10815-020-02031-x

GENETICS

Y chromosome structural variation in infertile men detected by targeted next-generation sequencing

Xiangyin Liu[1] · Han Zhang[1] · Xinyue Zhang[1] · Hongguo Zhang[1] · Yuting Jiang[1] · Ruizhi Liu[1] · Jia Fei[2] · Ying Wang[2] · Yang Yu[1]

Received: 17 June 2020 / Accepted: 8 December 2020 / Published online: 16 January 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

Purpose To provide a validated method to identify copy number variation (CNV) in regions of the Y chromosome of infertile men by next-generation sequencing (NGS).

Methods Semen analysis was used to determine the quality of semen and diagnose infertility. Deletion of the azoospermia factor (AZF) region in the Y chromosome was detected by a routine sequence-tagged-site PCR (STS-PCR) method. We then used the NGS method to detect CNV in the AZF region, including deletions and duplications.

Results A total of 326 samples from male infertility patients, family members, and sperm donors were studied between January 2011 and May 2017. AZF microdeletions were detected in 120 patients by STS-PCR, and these results were consistent with the results from NGS. In addition, of the 160 patients and male family members who had no microdeletions detected by STS-PCR, 51 cases were found to exhibit Y chromosome structural variations by the NGS method (31.88%, 51/160). No microdeletions were found in 46 donors by STS-PCR, but the NGS method revealed 11 of these donors (23.91%, 11/46) carried structural variations, which were mainly in the AZFc region, including partial deletions and duplications.

Conclusion The established NGS method can replace the conventional STS-PCR method to detect Y chromosome microdeletions. The NGS method can detect CNV, such as partial deletion or duplication, and provide details of the abnormal range and size of variations.

Keywords Y chromosome microdeletion · Copy number variation (CNV) · Next generation sequencing (NGS) · Male infertility · Spermatogenic failure

Introduction

For infertile men, microdeletion in the Y chromosome is one of the common genetic factors leading to spermatogenic disorders. The incidence of microdeletions in the azoospermia factor (AZF) region of the Y chromosome is about 10–15% in patients with azoospermia and severe oligozoospermia [1]. The deletion region involves three loci: AZFa, AZFb, and AZFc. The Y chromosome is not necessary for an individual's life, but it is required for male sexual differentiation, and Y chromosome disruptions cause male infertility. The Y chromosome has a complex structure that includes repeated elements [2], which are associated with various genomic rearrangements, including involvement in proposed mechanisms of structural rearrangements, such as non-allelic homologous recombination [3].

Because of the structural complexity of the Y chromosome, it took 30 years to accurately detect the AZF region. Structural rearrangements involving these repetitive sequences have been difficult to detect. Current chromosome screening methods are mostly dependent on the diploid genome and may not allow detailed investigation of the haploid state of the Y chromosome. To date, several methods have been employed to detect copy number variation of Y chromosome. The choice of method largely depends on the resolution and throughput provided by each method, as well as the inherent characteristics of the information generated.

✉ Yang Yu
yuyang0351@126.com

[1] Center for Reproductive Medicine, Center of Prenatal Diagnosis, First Hospital, Jilin University, 71th. Xin-min Street, Changchun 130021, Jilin Province, People's Republic of China

[2] Peking Medriv Academy of Genetics and Reproduction, Peking 102629, China

Cytogenetic methods can show altered structures down to 5 Mb in size and aneuploidies. However, chromosome karyotyping analysis has a low throughput. The first report of the AZF locus on the Y chromosome used cytogenetic analysis. For higher resolution analysis, fluorescence in situ hybridization (FISH) techniques can be utilized on interphase nuclei or metaphase chromosomes. The FISH method can provide more detailed information, from ~ 100 kb down to a few kilobases. Moreover, it can be used to reveal inversions, by the use of multiple probes, mainly targeting specific variants. In comparison, the PCR method is ideal for screening a limited number of specific variants, as well as multiplexing and the simultaneous analysis of multiple regions. The PCR approach has been used to design tests for AMELX /Y [4] and AZF microdeletions [5]. One study reported using the sequence-tagged-site PCR (STS-PCR) method to evaluate the AZFc structure in more than 20,000 individuals [6]. Multiplex STS-PCR assays are currently considered the standard protocol according to the best practice guidelines of the European Molecular Genetics Quality Network (EMQN: http://www.emqn.org). Compared with the two methods mentioned above, microarray technologies can yield more information and offer the advantages of high-throughput analysis. However, array methods have critical limitations in the detection of long identical repeats. Although genome-wide or Y chromosome copy number variation (CNV) studies have been reported using array methods [7–9], the method could not specify clear signal for the long, nearly identical repeated units, for which array-based methods are based on shorter probes and the similarity of sequence between a probe and a target genomic region; in particular, if there is a small change in copy number, the method cannot tell which one has mutated [10].

Next-generation sequencing (NGS) allows the detection of variants or mutations by DNA or RNA sequencing, and the potential of base-pair resolution, and was recently the primary source of data for studying CNVs on the Y chromosome [3, 11] and other parts of the genome [12, 13]. However, the genomic context of Y chromosome amplifies the inherent limitations of NGS.

At present, the STS-PCR method is often used to detect Y chromosome microdeletions, but it cannot be used to detect previously undescribed deletion types. In this study, we applied a high-performance, accurate, and low-cost method suitable for clinical use, where the classical PCR method was used to detect STS loci on the Y chromosome.

Materials and methods

Patients

The present study selected cases from men seeking fertility consultation, from January 2011 to May 2017, at the Center for Reproductive Medicine, First Hospital of Jilin University. Medical history, physical examination, and questionnaire counseling were required for all subjects, and clinical examination results, such as hormone levels and semen analysis, were also collected. All subjects included in the present study did not exhibit known clinical causes of infertility, such as infection and varicocele.

A total of 326 subjects were recruited, in which 222 were infertile patients with abnormal semen results, 58 were male family members of these infertile men, and 46 were sperm donors with normal semen (from the Jilin Province sperm bank). In the present study, all subjects were selected for NGS analysis from the STS-PCR database that all infertility cases (excluding those with infection or varicocele) with microdeletion detected by STS-PCR. The inclusion criteria were to ensure that most carriers of AZF microdeletions, from STS-PCR assays, were included in the study. Therefore, clinical statistics of incidence are for reference only.

Semen analysis

Ejaculates were obtained after 3–5 days of sexual abstinence. Semen analysis was performed according to the protocol of the World Health Organization (5th edition) (http://www.who.int/en/). Patients were diagnosed as exhibiting azoospermia when no sperm was found in at least three ejaculates after sample centrifugation, or having oligozoospermia when sperm concentrations of $< 20 \times 10^6$/mL were obtained for the last three semen samples, taken at intervals of at least 1 week.

Y chromosome microdeletions analysis by STS-PCR

The STS-PCR analysis was performed following the European Academy of Andrology and the European Molecular Genetics Quality Network (EAA/EMQN) guidelines [14]. All patients and control samples were tested by multiplex PCR using classical AZF markers; STS markers were AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255). The internal controls were sY14 and ZFX/ZFY markers. The PCR analysis was performed twice to confirm any deletion.

Confirmation of detected partial AZFc deletions using STS-PCR

To verify partial AZFc deletions, the subject samples were tested by STS-PCR using established STS markers sY1191 and sY1291 [14]. DNA was then assessed by gel electrophoresis. Sample DNA was replaced with water for a blank control, and a normal male sample was used as a negative control.

The partial deletion termed gr/gr was identified by the absence of marker sY1291 and presence of marker sY1191. The

b2/b3 deletions were characterized by the absence of marker sY1191 and presence of marker sY1291. The b1/b3 deletion was characterized by the absence of both sY1291 and sY1191 markers [14].

High-throughput sequencing of the Y chromosome AZF region

Specific oligonucleotides were designed as markers for high-throughput sequencing of the AZF region on the long arm of the Y chromosome. All 138 loci in the AZF region and 10 loci of housekeeping genes on the Y chromosome were selected based on the human genome database (http://genome.ucsc.edu/). The oligonucleotides had uniform melting temperatures and minimal complementarity with universal amplification sequences. Each of the 138 AZF loci had confirmed repeat numbers (denoted the theoretical copy number) and each locus was not found in other regions of the human genome. Six traditional STS markers used for multiplex PCR were also represented. Three oligonucleotides per locus were used: a left locus-specific oligo, a 5′-phosphorylated middle locus-specific oligo, and a 5′-phosphorylated right locus-specific oligo. All oligonucleotides were pooled together to create an AZF microdeletion assay oligo pool.

End-repair, acetylation, and adapter ligation were performed for library preparation following standard personal genome machine protocols, with sequencing performed on an Ion PGM Analyzer (Life Technologies, Carlsbad, CA), according to the manufacturer's protocol [15].

For each selected locus, sequencing reads were aligned to the expected sequence using TMap software (Karolinska Institute, Solna, Sweden), and reads with fewer than three mismatches were counted. The read count for each locus was normalized to the percentage value using the total sequencing read count and housekeeping loci value. Next, the copy number of each locus was then compared with its theoretical copy number. Specific experimental details were described previously [16].

Results

Flowchart of the present study

A flowchart of the study is shown in Fig. 1.

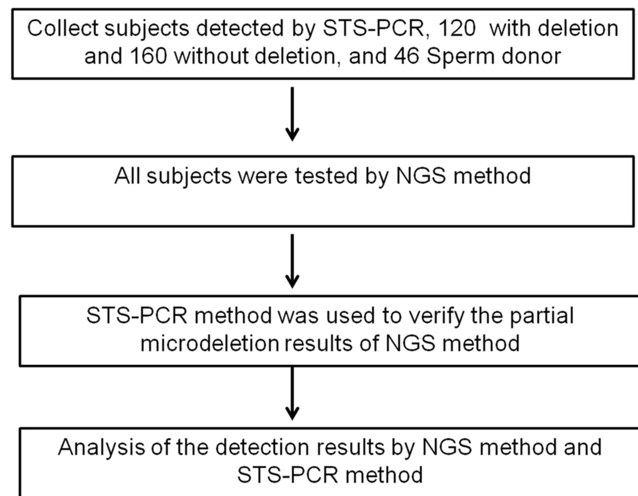

Fig. 1 Flowchart on detection of Y chromosome microdeletion with STS-PCR and NGS method

Comparison of AZF microdeletions detected by the STS-PCR and NGS methods

We selected 326 subjects for analysis by the STS-PCR and NGS methods. Microdeletions were found in 120 patients with male infertility by the STS-PCR, and all these microdeletions were also detected by NGS method. In the 160 patients and male family members with no detectable microdeletions by STS-PCR, we found 51 cases exhibiting structural variations of the AZF region by NGS analysis, with a detection rate of 31.88% (51/160). In the 46 sperm donors without detected microdeletions by STS-PCR, 11 were found to have structural variations by NGS analysis, and the detection rate was 23.91% (11/46) (Table 1).

NGS results for 120 patients with AZF microdeletions

The 120 patients with AZF microdeletions detected by STS-PCR and NGS analysis, the most frequent structural variation shown by NGS was the AZFc microdeletion (64.17%, 77/120), followed by the AZFb+c microdeletion (22.5%, 27/120). There were also three novel structural variations: AZFa+partial c microdeletion (Table 2, No. 4), AZFb+c microdeletion combined with a g1/g3 inversion (Table 2, No. 16), and AZFc microdeletion combined with a b1/b2 fragment repeat (Table 2, No. 18).

Table 1 Overall results of 326 subjects screening for AZF microdeletions

STS-PCR method		NGS method		
Number (case)	Results	Number (case)	Results	Detection rate
120	deletions	120	deletions	100%(120/120)
160	no deletions	51	structure variations	31.88%(51/160)
46	no deletions	11	structure variations	23.91%(11/46)

Table 2 Results of NGS method in 120 patients with AZF microdeletions detected by STS-PCR method

Serial number	STS-PCR method	NGS method			Number of samples
		region	result	range and size	
1	sY86,sY84,sY127,sY143,sY254,sY255	AZFa+b+c	AZFa+b+c deletion	del(14487582-15980851; 19732230-28365090)(10.1Mb)	4
2	sY86 or sY84	AZFa	AZFa partial deletion	del(14469266-14607672)(0.14Mb)	2
3	sY86,sY84	AZFa	AZFa deletion	del(14469266,15195932)(0.7 Mb)	5
4	sY86,sY84	AZFa+c	AZFa,+b2/b3 deletion	del(14469266-15195932) (0.7Mb)del(24653016-25206639; 25875460-27122017)(1.8Mb)	1
5	sY127,sY143	AZFb	b6/u1 deletion	del(20891683-23633975)(2.7M)	1
6	sY127,sY143, sY254,sY255	AZFb+c	b-AZF terminal deletion	del(15980792-28365090)(12.4 Mb)	1
7	sY127,sY143,sY254,sY255	AZFb+c	P5 distal /P1 proximal deletion	del(19968400-25911138)(5.9Mb)	7
8	sY127,sY143,sY254,sY255	AZFb+c	P5/P1 distal deletion	del(20099846-27708415)(7.6Mb)	4
9	sY127,sY143,sY254,sY255	AZFb+c	b5-AZF terminal deletion	del(20638109-28365090)(7.7Mb)	1
10	sY127,sY143,sY254,sY255	AZFb+c	y3-AZF terminal deletion	del(19732230-28365090)(8.6Mb)	3
11	sY127,sY143,sY254,sY255	AZFb+c	y4-AZF terminal deletion	del(20099846-28365090)(8.3Mb)	6
12	sY127,sY143,sY254,sY255	AZFb+c	u1-AZF terminal deletion	del(22554722-28365090) (5.8Mb)	2
13	sY127,sY143,sY254,sY255	AZFb+c	b1/b4 deletion	del(24164717-28188363) (4.0Mb)	1
14	sY127,sY143,sY254,sY255	AZFb+c	b6-AZF terminal deletion	del(20891683-28365090) (7.5Mb)	1
15	sY127,sY143,sY254,sY255	AZFb+c	t2-AZF terminal deletion	del(24571236-28365090) (3.8Mb)	1
16	sY127,sY143,sY254,sY255	AZFb+c	g1/g3 inversion,y4/y1 deletion	del(20099846-24875662, 24943305-25835359) (5.7Mb)	1
17	sY254,sY255	AZFc	b2/b4 deletion	del(24653016-28127011) (3.5Mb)	77
18	sY254,sY255	AZFc	b1/b2 repeat,b2/b4 deletion	dup(24164717-24370081) (0.2Mb)del(24653016-28127011) (3.5M)	2

NGS analysis of 160 patients and male family members with no detectable AZF microdeletions by STS-PCR

Of the 160 patients and male family members with no detectable AZF microdeletions by STS-PCR, the NGS analysis found that 51 subjects had structural variations in the AZF region (31.88%, 51/160). The remaining 109 cases had no structural variations detected by NGS. Of the 51 subjects with AZF structural variations, 31 cases had four types of deletions (19.38%, 31/160), nine cases exhibited six types of fragment repeat (5.63%, 9/160), and 11 cases had nine types of deletion and repeat (6.88%, 11/160). Inversion structural variation also occurs with deletion or repeat (Table 3).

NGS analysis of 46 sperm donors without AZF microdeletions detected by STS-PCR

Of the 46 sperm donors with no detectable microdeletions by the STS-PCR method, NGS analysis revealed 11 cases with structural variations, with a detection rate of 23.91% (11/46). Of these 11 subjects, five cases had three types of deletion (10.87%, 5/46), two cases had two types of fragment repeat (4.35%, 2/46), and four cases had three types of deletion and repeat (8.70%, 4/46). Inversion structural variation also occurs with deletion or repeat (Table 4).

STS-PCR confirmed of AZFc partial deletions detected by NGS

Fifteen cases with AZFc partial deletions revealed by NGS were selected for STS-PCR analysis, including three cases of b1/b3 deletions, five cases of b2/b3 deletions, and seven cases of the gr/gr deletion.

The deletions present in these cases were detected by STS-PCR using the sY1291 and sY1191 markers. The absence of sY1291 alone indicated deletion of the gr/gr region. The absence of sY1191 indicated deletion of the b2/b3 region. The absence of both sY1191 and sY1291 indicated deletion of b2/b3 region. The detection results are shown in Fig. 2.

Discussion

Y chromosome microdeletions have clinical significance for the diagnosis of infertile men [17]. The Y chromosome contains a high proportion of segmental duplications, and CNVs generated from these structural features are usually challenging to detect. At present, STS-PCR analysis is usually used to screen Y chromosome AZF deletions. However, the limited STS markers do not allow for comprehensive detection of duplications or rare deletions on the Y chromosome. The detection of such variants requires analysis with more throughput and higher resolution of the Y chromosome.

Table 3 Results of NGS method in the 51 subjects without AZF microdeletion detected by STS-PCR method

Region	Result	Range and size	Number of cases
AZFb	gr/gr repeat,b1/b3 deletion	dup(24943305-26577055)(1.6Mb) del(24164717-25665550)(1.6Mb)	1
AZFb+c	b2/b3 inversion,g1/g3 repeat,b1-AZF terminal deletion	dup(24653016-25206639;25875460-27122017)(1.8Mb)del(24164717-28188363)(4.0Mb)	1
AZFc	b2/b3 inversion,g1/g3 deletion,gr1/r2 repeat	dup(25451167-25597876)(0.2Mb) del(24653016-25206639;25875460-27122017)(1.8Mb)	3
AZFc	b2/b3 inversion,g1/g3 deletion,b3/b4 repeat	del(24653016-25206639;25875460-27122017)(1.8Mb) dup(25208858-25835359;27122017-28297359) (1.8Mb)	1
AZFc	g1/g3 inversion,b2/b3 deletion,gr1/b4 repeat	del(24653016-24875662;25665550-25835359) (1.8Mb)dup(24943305-25597876;27385353-28297359)(1.6Mb)	1
AZFc	gr/gr deletion,b2/b4 repeat	del(24943305-26577055) (1.6Mb) dup(24653016-24875662;26577055-28127011)(1.8Mb)	2
AZFc	gr/gr deletion,b2/b4 repeat*2	del(24943305-26577055) (1.6Mb)dup(24653016-24875662;26577055-28127011) (1.8Mb)*2	1
AZFc	b2/b3 deletion,b3/b4 repeat	del(24653016-25206639;25875460-27122017) (1.8Mb)dup(25208858-25835359;27122017-28297359) (1.8Mb)	1
AZFc	b2/b3 inversion,g1/g3 deletion	del(24653016-25206639;25875460-27122017) (1.8Mb)	11
AZFc	b2/b3 inversion,g1-b4 deletion	del(24653016-25206639;25875460-28297359) (3Mb)	2
AZFc	gr/gr deletion	del(24943305-26577055) (1.6Mb)	17
AZFc	u3/g1 deletion	del(24873940-25206639) (0.3Mb)	1
AZFb	b1/b3 repeat	dup(24164717-25665550) (1.6Mb)	1
AZFc	y1/y2 repeat	dup(26254019-27708415) (1.5Mb)	1
AZFc	b2/b4 repeat	dup(24653016-28127011) (3.5Mb)	2
AZFc	gr/gr repeat	dup(24943305-26577055) (1.6Mb)	3
AZFc	u1-AZF terminal repeat	dup(22626334-28365090) (5.7Mb)	1
AZFc	b2/b3 inversion,g1/g3 repeat	dup(24653016-25206639;25875460-27122017) (1.8Mb)	1

The application of NGS provides the possibility of a low-cost and high-throughput screening method. We have developed a novel capture sequencing method that utilizes unique STSs on the Y chromosome and offers the potential to detect established and unknown structural variations.

We selected 326 subjects, including 222 infertile patients with abnormal semen results, 58 family members of these infertile male patients and 46 sperm donors (Fig. 1). The 46 sperm donors had normal semen, and no deletion of the AZF region was detected by STS-PCR. In the remaining 280 subjects, STS-PCR analysis found that 120 patients had AZF region microdeletions, and 160 patients and male family members had no detectable AZF microdeletions (Table 1).

All patients with male infertility and microdeletions in the present study were selected after initial STS-PCR analysis, so the cases examined by NGS do not reflect the overall clinical incidence of Y chromosome variations.

All the microdeletions identified in 120 patients by STS-PCR were also detected by the NGS method. The exhibited structural variations included partial deletions, inversions, and

Table 4 Results of the 46 sperm donors on AZF region revealed by NGS method

Region	Result	Range and size	Number of cases
AZFc	b2/b3 inversion,g1/g3 deletion	del(24653016-25206639;25875460-27122017) (1.8Mb)	2
AZFc	gr/gr deletion	del(24943305-26577055) (1.6Mb)	2
AZFb	t2/g1 deletion	del(24571236-25206639) (0.6Mb)	1
AZFc	b2/b3 inversion,g1/g3 repeat	dup(24653016-25206639;25875460-27122017) (1.8Mb)	1
AZFc	b2/b4 repeat	dup(24653016-28127011) (3.5Mb)	1
AZFc	gr/gr repeat,b1/b3 deletion	dup(24943305-26577055) (1.6Mb)del(24164717-25665550) (1.6Mb)	1
AZFc	gr/gr repeat*2,b1/b3 deletion	dup(24943305-26577055) (1.6Mb)*2 del(24164717-25665550) (1.6Mb)	2
AZFc	b2/b3 inversion,gr1/r2/r1 repeat,g1/g3 deletion	dup(25208858-25597876) (0.4Mb) del(24653016-25206639;25875460-27122017) (1.6Mb)	1

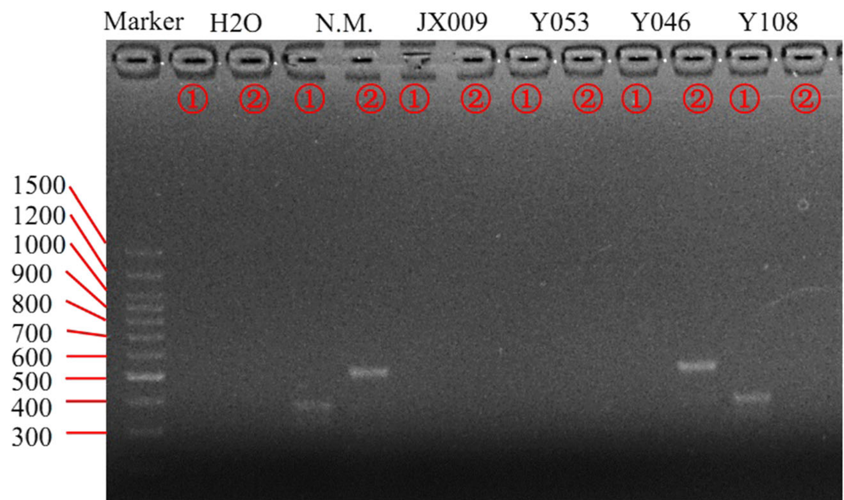

Fig. 2 Results of sY1191 and sY1291 by STS-PCR method. Note: ① represent detection of marker sY1191; ② represent detection of marker sY1291. JX009 and Y053 are cases with b1/b3 deletion. Y108 is a case of gr/gr deletion. Y046 is a case of b2/b3 deletion

duplications in the AZF region. To verify the repeatability and stability of the technique, two researchers independently analyzed each batch.

Targeted NGS has detected copy number variation in the field of pre-implantation diagnosis and screening of birth defects, and had the same sensitivity as examples of array-based methods, which are the gold standard for the detection of CNV [18, 19]. The NGS method had good sensitivity and specificity for detection of Y chromosome microdeletions. Eighteen different deletion types were detected by the NGS method, while seven of these were detected by the STS-PCR method, demonstrating that the NGS method is more accurate and it detected more types of variation. Another advantage of the NGS method was that it detected the detailed the specific range and fragment size of structural variations, such as a partial deletion (Table 2, No. 2), structural inversion (Table 2, No. 16), and fragment repeat (Table 2, No. 18). A graph of the pattern of normal male NGS results is shown in Fig. 3.

In the present study, the NGS method identified structural variations of the AZF region in 31.88% of subjects that had no detectable microdeletions by STS-PCR analysis (Table 3).Four different deletion patterns, six fragment repeat patterns, and nine deletions with repeat patterns were detected by NGS analysis. These structural variations were concentrated in the AZFc region, and the size of the altered regions ranged from 0.2 to 5 Mb, including a 5.7 Mb repeat.

For sperm donors with no microdeletions detected by the STS-PCR method, 23.91% of donors were found to exhibit structural variations by NGS analysis (Table 4). Of these variations, five cases were deletions (10.87%, 5/46), two cases were fragment repeats (4.35%, 2/46), and four cases were a deletion with a repeat (8.70%, 4/46). There were three types of deletion, two types of fragment repeats, and three types of deletion with repeat variations. These structural variations were also concentrated in the AZFc region, and the size of the variations ranged from 0.4 to 3.5 Mb.

It should be pointed out that the partial deletion types detected above, such as b1/b3 deletion and gr/gr deletion, can also be detected by extended analysis of STS-PCR method [14].

The effects of AZF structural variations upon fertility have been previously summarized by diagnostic infertility screening studies [20]. Microdeletions in the AZFa, AZFb, AZFb+c, and AZFc regions were associated with oligospermia or

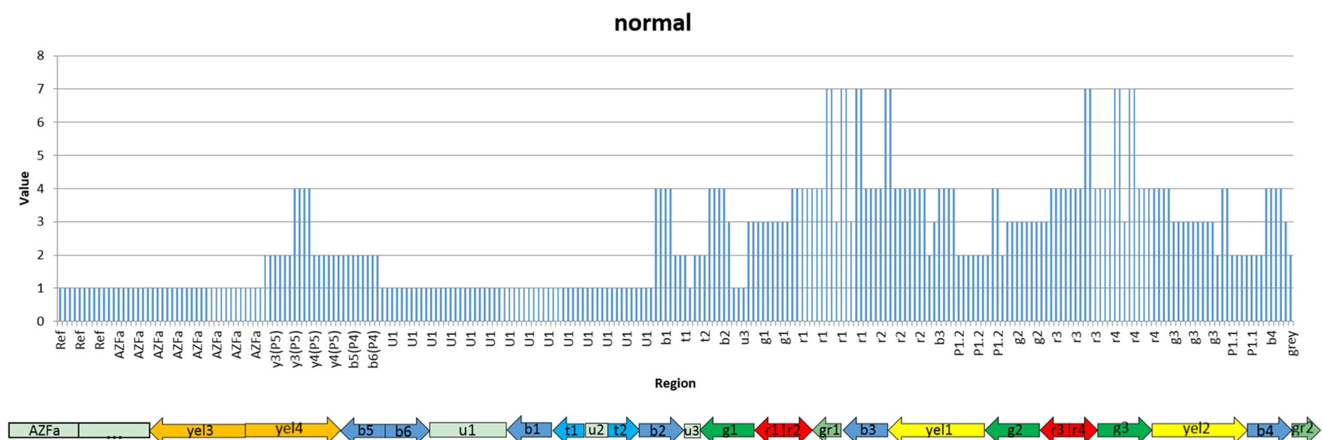

Fig. 3 The pattern graph of normal male NGS method results

azoospermia. Patients with complete deletions of AZFa, AZFb, or AZFb+c regions typically have no testicular sperm, so intracytoplasmic sperm injection is usually not possible for these subjects.

AZFc deletions are the most frequent types of Y chromosome deletion and produce various clinical phenotypes for carriers [21]. Patients with AZFc deletions and oligospermia may exhibit a progress decrease in sperm concentration over time [22]. Sperm cryopreservation is recommended for assisted reproductive treatment of these carriers [14, 23].

Partial AZFc deletions involving three subregions, gr/gr, b1/b3, and b2/b3, were significantly more frequent among sterile males, although frequencies varied widely across different populations [24]. A common partial deletion within AZFc involves a 1.6 Mb gr/gr deletion, which removes four genes and is also associated with the risk of spermatogenic failure. Nevertheless, this deletion has only minor phenotypic effects in some haplogroups, suggesting that compensating variants may exist elsewhere. It was reported that the gr/gr deletion may lead to complete AZFc region deletions [25]. These couples should be aware that such deletions provide genetic risk factors for impaired spermatogenesis in male offspring and that there is a high risk of transmitting complete AZFc deletions.

In one report, the incidence of partial AZFc duplication was significantly higher in infertile men than in fertile men [26]. Therefore, AZFc structural variants may have variable effects on the risk of spermatogenic failure. If there are no phenotypic consequences of structural variants, should Y chromosome AZF variation be considered neutral? Like as different types of Y chromosome repeats. Further research is required to address this question.

The structural variation of the Y chromosome reflects its special haploid state and high content of repeat sequences. Research into CNV of these repeats has developed over the years, and CNV has been linked to fertility and human evolutionary traits. Microdeletions in the AZF regions are associated with spermatogenic dysfunction and male infertility. Other forms of structural variation have been recognized, such as the inversion/repeat structural variation of the AZF region associated with azoospermia/ oligospermia [27–29]. Therefore, in addition to AZF microdeletions, AZF region structural variation may lead to spermatogenic disorders. The current study designed and developed a NGS detection scheme for the AZF region, and we hope to further detect structural variations of AZF region, improve the detection accuracy of variations, and meet the needs of scientific research and future clinical practice.

In the present report, an extended STS-PCR test with STS markers sY1291 and sY1191 was carried out for AZFc partial deletion patterns detected by NGS but not detected by conventional PCR. The selected samples included three cases of b1/b3 deletions, five cases of b2/b3 deletions, and seven cases of gr/gr deletions. The extended STS-PCR analysis was 100% consistent with the findings of NGS analysis (Fig. 2). One previous study also utilized the NGS method with STS markers to reveal deletions in the male-specific region of the Y chromosome in subjects with non-obstructive azoospermia [30].

The current NGS detection technique provides the possibility of detecting new structural variations. At present, deletions with established clinical significance can be detected by the STS-PCR method, while the clinical significance of other variants identified by the NGS method remains mostly unknown. And the detection of new variations may present a dilemma for the application of assisted reproduction technology. However, from a research perspective, NGS technology provides a useful tool to uncover the structural variability of highly dynamic regions of the human genome, such as the Y chromosome. Understanding the clinical significance of structural variations in AZF regions will require more clinical data and breakthroughs in functional verification.

Conclusion

The established NGS analysis can replace the conventional STS-PCR method to detect Y chromosome microdeletions. The NGS method can detect CNVs, such as partial deletion or duplication, and provide details of abnormal ranges and sizes of variations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10815-020-02031-x.

Acknowledgments This work was supported by the Finance Department Health Special Project of Jilin Province, China (JLSCZD2019-022).

Thanks to our colleagues in the Genetics lab for their work and to Wang Yankun of Peking Medriv Academy of Genetics and Reproduction for his help in the complementary presentation of manuscripts.

Funding The work was supported by the Finance Department Health Special Project of Jilin Province, China (JLSCZD2019–022).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was approved by the medical Ethics Committee of the First Hospital of Jilin University.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee.

References

1. Moghbeli-Nejad S, Mozdarani H, Behmanesh M, Rezaiean Z, Fallahi P. Genome instability in AZFc region on Y chromosome in leukocytes of fertile and infertile individuals following exposure to gamma radiation. J Assist Reprod Genet. 2012;29(1):53–61.
2. Jain M, Olsen HE, Turner DJ, Stoddart D, Bulazel KV, Paten B, et al. Linear assembly of a human centromere on the Y chromosome. Nat Biotechnol. 2018;36(4):321–3.
3. Poznik GD, Xue Y, Mendez FL, Willems TF, Massaia A, Wilson Sayres MA, et al. Punctuated bursts in human male demography inferred from 1244 worldwide Y-chromosome sequences. Nat Genet. 2016;48:593–9.
4. Sullivan V, Biron KK, Talarico C, Stanat SC, Davis M, Pozzi LM, et al. A point mutation in the human cytomegalovirus DNA polymerase gene confers resistance to ganciclovir and phosphonylmethoxyalkyl derivatives. Antimicrob Agents Chemother. 1993;37(1):19–25.
5. Vogt, Bender. Human Y chromosome microdeletion analysis by PCR multiplex protocols identifying only clinically relevant AZF microdeletions. Methods Mol Biol. 2013;927:187–204.
6. Rozen SG, Marszalek JD, Irenze K, Skaletsky H, Brown LG, Oates RD, et al. AZFc deletions and spermatogenic failure: a population-based survey of 20,000 Y chromosomes. Am J Hum Genet. 2012;91:890–6.
7. Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, et al. Origins and functional impact of copy number variation in the human genome. Nature. 2010;464:704–12.
8. Johansson MM, Van Geystelen A, Larmuseau MH, Djurovic S, Andreassen OA, Agartz I, et al. Microarray analysis of copy number variants on the human Y chromosome reveals novel and frequent duplications overrepresented in specific haplogroups. PLoS One. 2015;10:e0137223.
9. Wei W, Fitzgerald T, Ayub Q, Massaia A, Smith BH, Dominiczak AF, et al. Copy number variation in the human Y chromosome in the UK population. Hum Genet. 2015;134:789–800.
10. Massaia A, Xue Y. Human Y chromosome copy number variation in the next generation sequencing era and beyond. Hum Genet. 2017;136(5):591–603.
11. Espinosa JR, Ayub Q, Chen Y, Xue Y, Tyler-Smith C. Structural variation on the human Y chromosome from population-scale resequencing. Croat Med J. 2015;56:194–207.
12. Hehir-Kwa JY, Marschall T, Kloosterman WP, Francioli LC, Baaijens JA, Dijkstra LJ, et al. A high-quality human reference panel reveals the complexity and distribution of genomic structural variants. Nat Commun. 2016;7:12989.
13. Sudmant PH, Rausch T, Gardner EJ, Handsaker RE, Abyzov A, Huddleston J, et al. An integrated map of structural variation in 2504 human genomes. Nature. 2015;526:75–81.
14. Krausz C, Hoefsloot L, Simoni M, Tüttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology. 2014;2(1):5–19.
15. Parson W, Strobl C, Huber G, Zimmermann B, Gomes SM, Souto L, et al. Evaluation of next generation mtGenome sequencing using the ion torrent personal genome machine (PGM). Forensic Sci Int Genet. 2013;7(5):543–9.
16. Liu XY, Zhang HY, Pang DX, Xue LT, Yang X, Li YS, et al. AZFa microdeletions: occurrence in Chinese infertile men and novel deletions revealed by semiconductor sequencing. Urology. 2017;107:76–81.
17. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril. 2010;93(1):1–12.
18. Liang D, Peng Y, Lv W, Deng L, Zhang Y, Li H, et al. Copy number variation sequencing for comprehensive diagnosis of chromosome disease syndromes. J Mol Diagn. 2014;16(5):519–26.
19. Tan Y, Yin X, Zhang S, Jiang H, Tan K, Li J, et al. Clinical outcome of preimplantation genetic diagnosis and screening using next generation sequencing. Gigascience. 2014;3(1):30.
20. Lo Giacco D, Chianese C, Sánchez-Curbelo J, Bassas L, Ruiz P, Rajmil O, et al. Clinical relevance of Y-linked CNV screening in male infertility: new insights based on the 8-year experience of a diagnostic genetic laboratory. Eur J Hum Genet. 2014;22(6):754–61.
21. Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod. 2002;17:2813–24.
22. Fu L, Xiong DK, Ding XP, Li C, Zhang LY, Ding M, et al. Genetic screening for chromosomal abnormalities and Y chromosome microdeletions in Chinese infertile men. J Assist Reprod Genet. 2012;29:521–7.
23. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod. 2000;15:1431–4.
24. Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, et al. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet. 2003;35(3):247–51.
25. Zhang F, Lu C, Li Z, Xie P, Xia Y, Zhu X, et al. Partial deletions are associated with an increased risk of complete deletion in AZFc: a new insight into the role of partial AZFc deletions in male infertility. J Med Genet. 2007;44:437–44.
26. Lu C, Zhang F, Yang H, Xu M, Du G, Wu W, et al. Additional genomic duplications in AZFc underlie the b2/b3 deletion-associated risk of spermatogenic impairment in Han Chinese population. Hum Mol Genet. 2011;20:4411–21.
27. Shahid M, Dhillon VS, Khalil HS, Sexana A, Husain SA. Associations of Y-chromosome subdeletion gr/gr with the prevalence of Y-chromosome haplogroups in infertile patients. Eur J Hum Genet. 2011;19(1):23–9.
28. Franchim CS, Soares-Junior JM, Serafini PC, Monteleone PAA, Coccuzza MS, Zanardo EA, et al. Efficacy of MLPA for detection of Y-chromosome microdeletions in infertile Brazilian patients. J Assist Reprod Genet. 2020;37(5):1251–9.
29. Vaszkó T, Papp J, Krausz C, Casamonti E, Géczi L, Olah E. Discrimination of deletion and duplication subtypes of the deleted in Azoospermia gene family in the context of frequent interloci gene conversion. PLoS One. 2016;11(10):e0163936.
30. Liu X, Li Z, Su Z, Zhang J, Li H, Xie J, et al. Novel Y-chromosomal microdeletions associated with non-obstructive azoospermia uncovered by high throughput sequencing of sequence-tagged sites (STSs). Sci Rep. 2016;6:21831.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.