New molecular diagnostic kit to assess Y-chromosome deletions in the Japanese population

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Title

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Running title

Kit for Y-chromosome deletions

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Abstract

Objectives:

Deletions in the azoospermia factor (AZF) regions are the most common known molecular-genetic cause of human male infertility involving spermatogenetic failure. Testing for these deletions in Japanese DNA samples using conventional sequence-tagged site (STS) probes occasionally led to considerable nonspecific or faint products in Japanese population. To improve the sensitivity and specificity of the detection of AZF microdeletions in the Japanese population, we developed a new molecular diagnostic kit.

Methods: STS probes were reselected and the Luminex suspension array assay was performed. Validation was retrospectively carried out with 2,014 DNA sequences with known microdeletions, which were divided into four categories.

Results: Category-1 deletions corresponded to the conventional classification of AZF deletion are present in 83 men (4.2%), which can result in intrachromosomal homologous recombination. Kit data confirmed the presence of deletions of this type in DNA sequences known to harbor AZF deletions. Category-2 deletions involved cytogenetic abnormalities in 28 (1.4%), while category-3 deletions in 759 (37.7%) were atypical classifications including gr/gr deletion. Since these deletions are thought to be due to palindromic units and non-homologous recombination, these microdeletions may impact the interpretation of some clinical findings. The rest of 1145 cases (56.8%) were assigned to category 4 as normal variants (polymorphism/ no deletion).

Conclusions: Our findings demonstrate that this new kit exhibits good sensitivity and specificity in Japanese men plus saving money and time.

Key words

AZF, Y-chromosome, microdeletion, male infertility, detection kit

Introduction

Male factors leading to infertility may account for up to 40-50% of total infertile couples.[1] The origin of male-factor infertility remains largely unexplained. In healthy males, infertility may have a number of unknown causes, including genetic disorders. Approximately 7% of infertile men harbor microdeletions of the Y chromosome that are not detectable with routine karyotype analyses.[2] Cytogenetic studies in infertile men have revealed a gene that controls spermatogenesis; this AZF is located on the Yq.[3] Three spermatogenesis loci in Yq11 have been classified into three regions: AZFa, AZFb, and AZFc.[4] The euchromatic region of the Y chromosome is currently characterized by three structurally distinct features, the X-degenerated, X-transposed, and ampliconic sequences. The ampliconic region includes eight palindromes with pairs of duplicated amplicons in which the DNA sequences share more than 99.9% homology.[5] These amplicons and sub-amplicons can serve as substrates for structural genomic rearrangement. Thus, AZF deletions can result from intrachromosomal homologous recombination events between non-reciprocal homologous sequences.[6]

From the clinical point of view, deletions in the AZF regions are the most common known molecular-genetic cause of human male infertility involving spermatogenetic failure.[7] Thus, the molecular diagnosis of Y-chromosomal microdeletions should be routinely performed worldwide in the workup of male infertility in men with azoospermia or severe oligozoospermia. The importance of a molecular-genetics approach that includes the evaluation of AZF deletions must be emphasized for men considering assisted reproductive techniques including TESE, because this genetic defect is transmitted to their

sons, affecting their fertility.[8] Furthermore, this information is useful for the avoidance of unnecessary surgical therapy.

Nowadays, the gold standard for the diagnosis of microdeletions in the AZFa, AZFb, and AZFc regions utilizes PCR primers, including primers sY84, sY86, sY127, sY134, sY254, and sY255.[9] When we previously tested Japanese patients using these eight probes, considerable nonspecific or faint bands were occasionally observed in the PCR products, particularly following the use of the sY254 and sY255 probes in the AZFc region. Re-evaluation frequently necessitated the use of several adjacent STS markers.[10]

To improve the sensitivity and specificity of the detection of microdeletions in the Y chromosome, we developed a new kit for the detection of molecular Y-chromosome deletions by re-selecting STS probes and carrying out multiplex target detection on the Luminex suspension array platform. Here we report the results of a retrospective evaluation of the new molecular Y-chromosome deletion kit using DNAs with previously determined genetic phenotypes.

Materials and methods

DNA sources and samples

This study was approved by the Ethics Committee of the Kanazawa University Graduate School of Medical Science. All participants had granted informed consent for a previous study. Genomic DNA was sampled in a male infertility clinic and stored after diagnosing the presence or absence of Y-chromosomal microdeletions between 1999 April and 2012 December. This genetic diagnosis included the identification of AZF deletions. The samples were

anonymized and made available for research following approval by the appropriate ethics committee.

We retrospectively analyzed genomic DNA samples from 2,014 anonymized subjects who visited the clinic with male infertility as their chief complaint. Deletions in these DNA samples were confirmed using in-house detection methods. DNA samples from patients with Klinefelter syndrome or hypogonadotrophic hypogonadism were not included in the present investigation.

In-house detection probes

All DNA samples were screening for Y-chromosomal microdeletions according to the guidelines of the EAA and the EMQN. These guidelines recommend the following first-choice STS primers: two STSs in AZFa (sY86 and sY84), two in AZFb (sY127 and sY134), and two in AZFc (sY254 and sY255).[9] These STS probes previously yielded reproducible results.

All DNA samples were evaluated for deletions using the following in-house STS probes: for AZFa, probes sY82, sY84, and sY86; for AZFb, probes sY1264, sY1235, sY1227, sY1228, sY117, sY280, sY127, sY134, sY135, sY258, sY142, and sY143; for AZFc, probes sY1161, sY1191, sY1197, sY1291, sY1125, sY1054, sY1206, sY1201, sY255, and sY254.[11]

These probes were designed according to EAA/EMQN guidelines and produced with the UCSC Genome Bioinformatics and the GenBank database. The human reference sequence (UCSC version hg16, 17 and 18) was based on National Center for Biotechnology Information Build 34 (July 2003), 35 (May 2004), 36 (Mar. 2006) produced by the International Human Genome Sequencing Consortium (http://genome.ucsc.edu/index.html).

All DNA samples were subjected to STS testing to confirm the presence or absence of deletions.

STS probes in the GENOSEARCH™ AZF Deletion kit

STS probes for the Y chromosome were chosen as markers of either single or double sequence copies using MSY Breakpoint Mapper (http://breakpointmapper.wi.mit.edu/) and UniSTS (http://www.ncbi.nlm.nih.gov/unists). Twenty-one STS probes were located on the Y chromosome. Probe sY757 (SOX3) served as a control probe for the X chromosome. The controls for the Yp were sY14 (SRY) and sY3118, and the controls for Yq were sY1251 and sY3159. Probes sY1251 and sY3159 were controls for the proximal and distal Y chromosome, respectively. Probes sY1324, sY1316, and sY1714 were used for the detection of AZFa deletions (Fig. 1). Fig. 2 depicts the locations (proximal to distal) of the following probes in the AZFb and AZFc regions: sY1024, sY1967, sY1309, sY3199, sY1233, sY3010, sY2990, sY1197, sY1191, sY1307, sY1291, sY2858, and sY1206. All of these probes are single-copy probes, with the exceptions of sY1967, sY1307, sY2858, and sY1206, which are present in two copies on the Y chromosome.

Multiplex PCR

Genomic DNA was prepared from patient peripheral blood lymphocytes with several commercial extraction kits according to the manufacturers' protocols. PCR was performed in 25 µL of PCR buffer (20 mM Tris-HCl (pH 8.3), 30 mM KCl, 2.5 mM $MgCl_2$) containing 200 µM of each deoxyribonucleoside triphosphate, with 0.625 U TaqDNA Polymerase (Roche Diagnostics, Mannheim, Germany), 50-100 ng of genomic DNA, and each primer at a final concentration of 0.2 µM. All PCR primers were biotinylated. The PCR profile consisted of one cycle of 2 min at 93 °C, followed

by 45 cycles of 1 min at 93 °C, 30 s at the annealing temperature of 59 °C, and 60 s at 70 °C, with a final cycle of 5 min at 70 °C. Amplifications were carried out on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA).

Luminex xMAP technology

Results from the GENOSEARCH^{TM} AZF Deletion kit (MBL, Nagoya, Japan) of development stage were confirmed via Luminex xMAP technology. To detect deletions in the AZF regions, amplification by sequence-specific probes and hybridization of each solid phase-binding STS were performed. The probes were 15-30mers that encompassed the sequence in the amplification region for each set of biotinylated primers. PCR products were mixed with the microbeads; the probes bound these beads, enabling multiple hybridization reactions in the same tube. Each 30 µL hybridization reaction consisted of 5 µL PCR product, 20 µL 1x hybridization buffer (75 mM Tris-HCl (pH 8.0), 0.6 mM ethylenediaminetetraacetic acid (pH 8.0), 0.15% surfactant, 4.5 M tetramethylammonium chloride), and 5 µL probe-binding beads. The hybridization reactions were carried out at 95 °C for 2 min and 52 °C for 30 min. After the incubation, the beads were washed twice with 70 µL of phosphate-buffered saline (PBS) -Tween with centrifugation for 1 min at 2,000 x *g*. After washing, the samples were labeled with streptavidin-phycoerythrin and incubated at 52 °C for 15 min. This hybridization between the PCR- amplified product and the probes on the fluorescently labeled beads allowed the quantification of the number of probes corresponding to each STS marker following flow cytometry based on the Luminex system (Luminex Corp., Austin, TX, USA). Each measurement encompassed at least 50 beads; the median phycoerythrin intensity was considered. To determine the presence of deletions, we set individual cutoff values for the fluorescence intensity of each bead-bound probe; if the intensity was below the cut-off, the sequence was defined as harboring a deletion. Samples from healthy female

subjects and a negative control of distilled water were used to determine the individual cutoffs.

Validation

We tested 2,014 samples with our in-house kit. Our laboratory performed validation with external control DNA samples dispatched from the EMQN every year.

Results

General results and probe characteristics

The current investigation evaluated 2,014 DNA samples. The mean subject age was 35.0±7.0 years (range 19-72 years). All DNA samples that had previously been determined to harbor deletions associated with a chief complaint of male infertility were analyzed. The deletions were classified into 40 patterns according to differences in adjoining patterns (Supplementary Fig. 1).

Here we present broad genetic information about microdeletions of the Y chromosome that are pertinent for clinicians or researchers investigating Japanese populations. For convenience, we grouped the results from our kit into four categories. Probe sY1291 is especially pertinent for Japanese populations; this marker was previously used for the detection of both the gr/gr (green-red sub-amplicon) deletion and Y haplogroup marker D.[12 13] One-third of Japanese males carry the deletion probed by sY1291.[14-16] Therefore, the deletion of the sequence probed by sY1291would not be taken into account during the evaluation of all but gr/gr deletions (pattern 39).

Furthermore, a pair of palindromes was duplicated in the set of DNA samples included in this investigation, yielding double hybridization of STS probes, for example for probes sY1967, sY1307, sY2858, and sY1206. These probes may not always detect the deletion of the duplicated region when implemented in a PCR-based evaluation. When noncontiguous deletions were observed, such as in patterns 7, 8, 11, 14, 15, 18, 25, 26, 31, 32, 35, and 36, we avoided non-sequential regions when designating the deletion location (Supplementary Fig. 1, 1*).

Categorization of deletion patterns and number in a Japanese population

We classified the detected deletion patterns into the following four categories: category 1, classical AZF microdeletions; category 2, Y chromosome long-arm terminal deletions, including heterochromatin deletion; category 3, subclassification of Yq microdeletions; category 4, miscellaneous deletions (polymorphisms).

Category 1 deletions in 83 men (4.2%), category 2 deletion in 28 (1.4%) and category 3 deletion in 759 (37.7%) including gr/gr deletion were indicated. The rest 1145 cases (56.8%) were assigned to category 4 in this study.

For category-1 deletions, we divided the conventional concept of the AZF region into regions AZFa, AZFb (P5/proximal P1), AZFb+c (P5/distal P1; recombination between palindrome 5 and distal palindrome 1), and AZFc (b2/b4) (Fig. 2a, d). The P5/distal P1 recombination eliminated the intervening homologous sequences. The ‘b2/b4’ categorization captures a recombination event between sub-amplicons b2 and b4 (Fig. 2b, d). This deletion category is based on the EAA/EMQN guidelines.[17] This conventional classification

encompasses the probes indicated in Fig. 2c, and yielded the corresponding patterns reported in Fig. 3. There were 7, 1, 12, and 63 samples that harbored deletions in AZFa, AZFb, AZFb+c, and AZFc, respectively (Fig. 3). All AZF deletions were confirmed by in-house STS probes. Therefore, the sensitivity and the specificity of our new kit were 100% for the detection of category-1 AZF deletions.

Category-1 deletions result in the recombination of the intervening homologous sequences between duplicated palindromes (Fig. 2d). Thus, the term ‘microdeletion’ implies that a part of the deletion of the inner euchromatin region of Yq is due to homologous recombination. We suggest differentiating microdeletion from partial Yq terminal deletion like following category-2 deletions.

With regards to category-2 deletions, probe sY3159 is located the end of the Yq euchromatin (Fig. 1). Deletion of this sequence may be accompanied by elimination of the distal Y chromosome end, including the heterochromatin. Patterns 3 (Yq I), 6 (Yq II), 13 (Yq III), 17 (Yq IV), 19 (Yq V) and 29 (Yq VI) were deleted from the distal Yq (Fig. 4). These deletions were also detected by evaluating karyotype analyses (Supplementary Table 1). Yq terminal deletions I-VI appeared in 1, 4, 6, 5, 3, and 3 samples respectively. Interestingly, all patients carrying pattern 2 were diagnosed as XX male. According to deletions of sY3159, patterns 37 indicated a normal karyotype. One sample displayed pattern 38, deletion of sY3159. This karyotype was 46, XY (Fig. 6). Deletion of sY3159 alone was classified as a polymorphism present in the Japanese population; therefore, pattern 25 was classified as Ym-8 (Fig. 5).

Category 3 included atypical Y microdeletions (Fig. 5). These deletions most likely involved palindromic units, unlike the intra-chromosomal non-homologous recombination that underlies category-1 deletions. Although few category-3 deletions were detected (Fig. 5; Ym-1 to Ym-7, Ym-9, Ym-10 and Ym-13 deletions occurred in 2, 1, 2, 1, 1, 5, 1, 2, 1 and 2 samples, respectively), these deletions are of clinical significance and are the focus of a separate investigation.

In contrast, patterns 25 and 26 (Ym-8), pattern 28 and 32 (Ym-11), and pattern 39 (Ym-12) indicate partial deletions of AZFc due to homologous recombination between sub-amplicons b1/b3, b2/b3, and gr/gr, respectively (Fig. 5). These deletions have already been investigated.[18-20] There were 18, 33, and 690 samples in this study with Ym-8, Ym-11, and Ym-12 deletions, respectively.

For category-4 deletions, patterns 12, 22, 23, 35, 36, 37 and 38 occurred in 5, 1, 1, 1, 2, 1, and 1 samples, respectively (Fig. 6). Although probe sY1291 is neglected absence or presence of band, these group have only one deletion. Therefore, we classified these deletions as polymorphisms specific to Japanese populations.

Discussion

In Japan, all available genetic testing for AZF deletions is based on the Promega Y Chromosome AZF Analysis System (version 2.0; Promega, Madison, WI, USA). Previously, numerous nonspecific bands were occasionally observed with these PCR products, probably because this system was optimized for Caucasian DNAs or different thermocyclers.

In this study, we evaluated the performance of the GENOSEARCH™ AZF Deletion kit, which is based on Luminex xMAP technology and identifies samples harboring deletions of sequences represented by STS probes. An automated multiplex bead-array system provided high-throughput identification of PCR products.[21]

Currently, up to 100 spectrally distinct fluorescence-labeled beads are available for multiplex target detection on the Luminex suspension microarray platform. This array system saves labor, money, and time[22, 23] and can be implemented daily with acceptable sensitivities and specificities. Its routine use may underlie the development and implementation of assays to detect genetic deletions in a timely manner.

Our molecular assays were performed on DNA samples that had undergone several freeze/thaw cycles. Although deletion assays might be improved by further probe optimization, changes in the probe sequences could have deleterious effects on other targets in the multiplexed reaction. Therefore, the GENOSEARCH™ AZF Deletion kit provides a practical solution in the clinical setting for rapidly screening and detecting AZF deletions.

We detected excellent specificities and favorable sensitivities in comparison to the use of conventional STS probes for the detection of AZF deletions. Moreover, this kit is compatible with rapid, automated, high-throughput procedures; simultaneous detection strategies reduced the manpower, reagent volumes, and specimen volumes required for use of the GENOSEARCH™ AZF Deletion kit.

From the clinical point of view, microdissection TESE via microscopy is currently recognized as the best way of retrieving sperm in men with non-

obstructive azoospermia. Compared with conventional TESE, microdissection TESE has a higher sperm retrieval rate and is safer.[24] Genetic testing for Y-chromosome microdeletions is of prognostic significance for TESE.

Men with complete deletions of the AZFa or AZFb region or absence of the AZFb+c region have no chance of sperm retrieval during microdissection TESE[25] and are not recommended to undergo this procedure. Many unnecessary TESE procedures are carried out, motivating the need for reliable genetic testing.

Category-1 deletions correspond to the AZF deletions referred to by the 2004 EAA/EMQN guidelines and are sufficient to make a diagnosis for male infertility. Our results indicate that the GENOSEARCH™ AZF Deletion kit may be a suitable replacement for conventional STS probes for Japanese populations. When deletions in category 2 are detected, cytogenetic assays should be performed for determining the patient's karyotype.

It should be emphasized that microdeletions, excluding the Ym-8, Ym-11 and Ym-12 microdeletions in category 3, are atypical classifications that have not been rigorously investigated. Therefore, the presence of these microdeletions may impact the interpretation of some clinical findings, although this scenario is expected to occur rarely. The accurate detection of these microdeletions was the most striking feature of the GENOSEARCH™ AZF Deletion kit.

In conclusion, we have described the development of the GENOSEARCH™ AZF Deletion kit for the detection of a panel of AZF deletions; this technology includes the use of Luminex xMAP arrays. This new kit provided a routine tool for the diagnosis of AZF deletions in patients accessing a male infertility clinic

in Japan. This kit would also be useful for the detection of atypical microdeletions.

Acknowledgements

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Conflict of interest

None declared.

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Figure Legends

Fig. 1. Location of STS probes for control regions and for the AZFa region for use with the GENOSEARCH® AZF Deletion kit. Black, pseudoautosomal region; light grey, euchromatin; dark grey, the heterochromatic region of the Y chromosome. Probes SRY and sY3118 were Yp controls. Probe sY1251 was a control for the Yq centromere, and probe sY3159 was a control for the Yq euchromatin.

Fig. 2. AZFb and AZFc regions in the Y chromosome. (a) Palindrome (P) structure in the Y chromosome. Rightward-facing arrows denote the 3' to 5' direction. (b) Sub-amplicon structure of the AZFc region. Identical pairs of amplicons appear in the same color. yel, yellow sub-amplicon; b, blue sub-amplicon; t, tan sub-amplicon; r, red sub-amplicon; gr, grey sub-amplicon; g, green sub-amplicon. (c) Locations of the probes used with the GENOSEARCH® AZF Deletion kit. (d) The AZFb and AZFc regions. Microdeletions in AZFb and AZFc result from nonreciprocal intrachromosomal recombination events between homologous sequences.

Fig. 3. Classical AZF microdeletions (category 1). Within the body of the figure, '0' denotes 'absence,' '1' denotes 'presence,' and '1*' denotes duplicated copies of a DNA sequence. Black arrows highlight data from the same probe. Possible deletions are colored grey. AZF; azoospermia factor, P; palindrome

Fig. 4. Y chromosome long-arm terminal deletions, including heterochromatin (category 2). Yp, Y chromosome long arm; Yq, Y chromosome short arm. Other notation is as in Fig. 3.

Fig. 5. Y chromosome microdeletion (subclassification; category 3). Ym, Y chromosomal microdeletions. Other notation is as in Fig. 3.

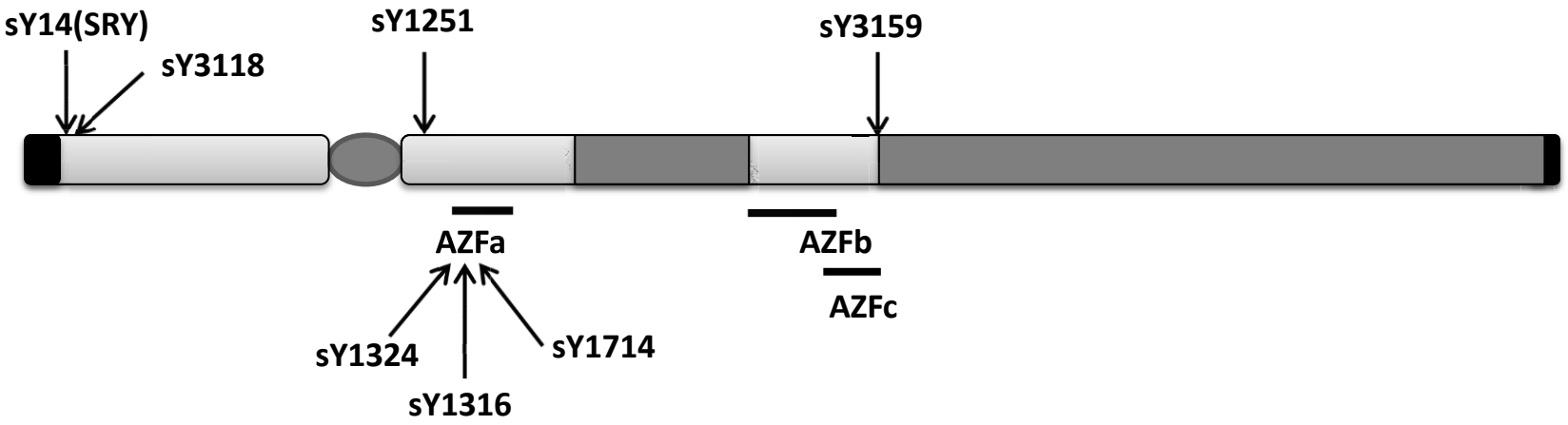
Fig. 6. Miscellaneous deletions (category 4). Notation is as in Fig. 3.

Supplementary Material.

Supplemetary Fig. 1

All raw deletion data in accordance with various patterns. Within the body of the figure, ‘0’ denotes ‘absence,’ ‘1’ denotes ‘presence,’ and ‘1*’ denotes duplicated copies of a DNA sequence. Possible deletions are colored grey. Notation is as in Fig. 3

Supplemetary Table 1


sY14(SRY)
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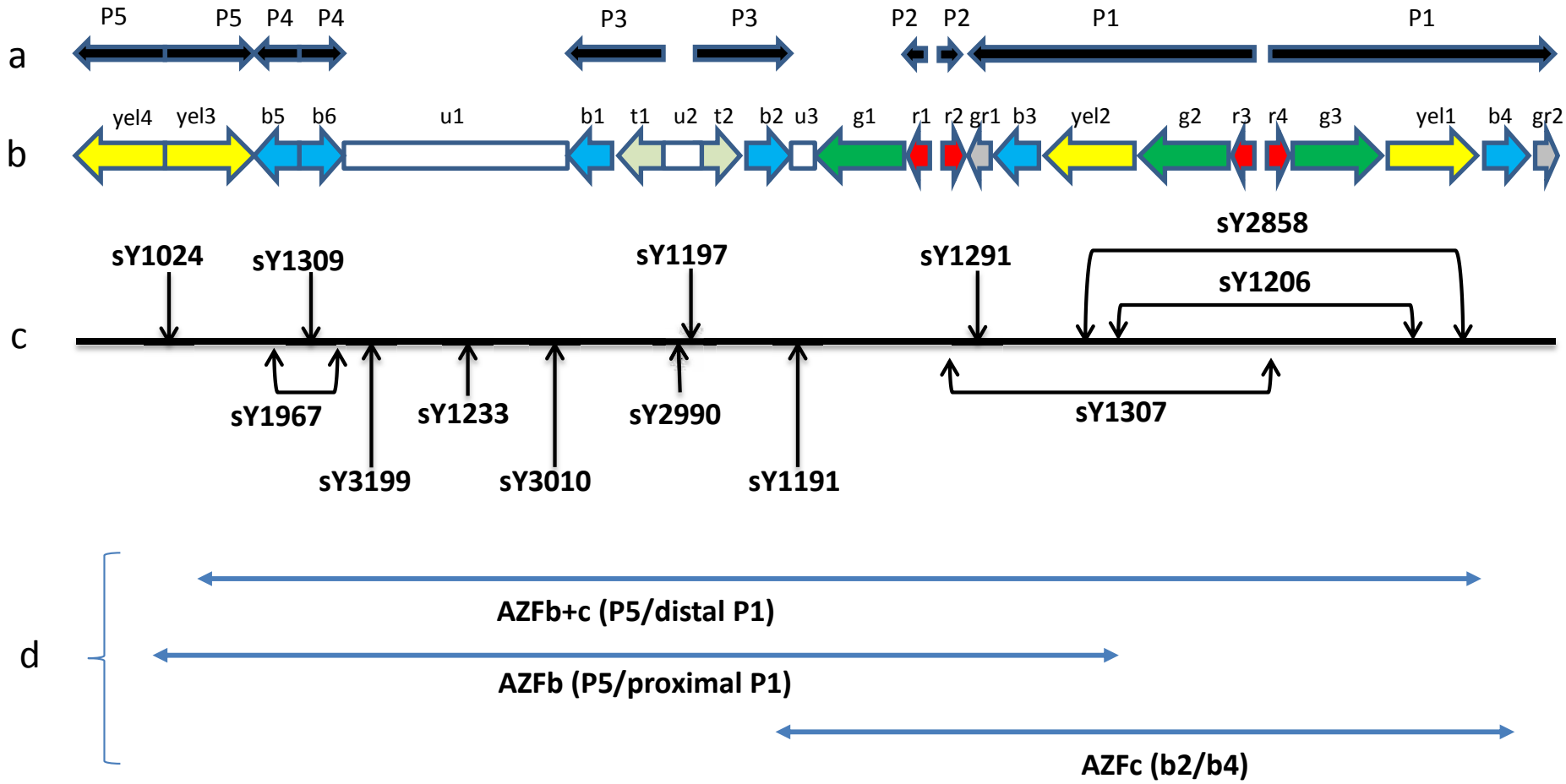
sY1251
sY3159
AZFa
AZFb
AZFc
sY1324
sY1316
sY1714

Fig1


a
P5
P5
P4
P4
P3
P3
P2
P2
P1
P1
b
yel4
yel3
b5
b6
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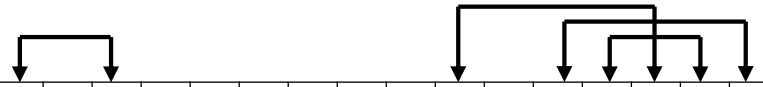
b1
t1
u2
t2
b2
u3
g1
r1
r2
gr1
b3
yel2
g2
r3
r4
g3
yel1
b4
gr2
c
sY1024
sY1309
sY1197
sY1291
sY2858
sY1206
sY1967
sY1233
sY2990
sY1307
sY3199
sY3010
sY1191
d
AZFb+c (P5/distal P1)
AZFb (P5/proximal P1)
AZFc (b2/b4)

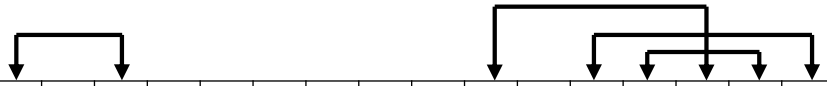
Fig2



		1	2	3	4	5	6	7	8	9	10	9	11	12	13	14	15	16	17	18	19	20	17	20	19	21	
Pattern	sample no.	sy757	sY14	sY3118	sY1251	sY1324	sY1316	sY1714	sY1024	sY1967	sY1309	sY1967	sY3199	sY1233	sY3010	sY2990	sY1197	sY1191	sY1307	sY1291	sY2858	sY1206	sY1307	sY1206	sY2858	sY3159	Deletions
4	5	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	AZFa
5	2	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	AZFa
7	2	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	1	1	AZFb+c (P5/distal P1)
8	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1*	0	1	1	1	1	1	1	AZFb (P5/proximal P1)
14	10	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	1	1	AZFb+c (P5/distal P1)
30	62	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	AZFc (b2/b4)
31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	0	0	1	1	AZFc (b2/b4)

AZF: Azoospermia factor, P: Palindrome, b: b sub-amplicon

Fig.3



		1	2	3	4	5	6	7	8	9	10	9	11	12	13	14	15	16	17	18	19	20	17	20	19	21	
Pattern	sample no.	sy757	sY14	sY3118	sY1251	sY1324	sY1316	sY1714	sY1024	sY1967	sY1309	sY1967	sY3199	sY1233	sY3010	sY2990	sY1197	sY1191	sY1307	sY1291	sY2858	sY1206	sY1307	sY1206	sY2858	sY3159	Deletions
1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Whole Y
2	4	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yp
3	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yq I（distal to AZFa)
6	4	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yq II（distal to AZFb)
13	6	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yq III（distal to AZFb)
17	5	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Yq IV（distal to AZFb)
19	3	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	Yq V（distal to AZFb)
29	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	Yq VI（distal to AZFb)

Yp: Y chromosome short arm, Yp: Y chromosome long arm, AZF: Azoospermia factor

Fig.4

		1	2	3	4	5	6	7	8	9	10	9	11	12	13	14	15	16	17	18	19	20	17	20	19	21	
Pattern	sample no.	sy757	sY14	sY3118	sY1251	sY1324	sY1316	sY1714	sY1024	sY1967	sY1309	sY1967	sY3199	sY1233	sY3010	sY2990	sY1197	sY1191	sY1307	sY1291	sY2858	sY1206	sY1307	sY1206	sY2858	sY3159	Deletions
9	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	Ym-2 (P5+P4)
10	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	Ym-1 (P5+P4)
11	1	1	1	1	1	1	1	1	0	1*	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	Ym-1 (P5+P4)
15	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1*	0	1	1	1	1	1	1	Ym-3 (AZFb partial)
16	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	Ym-3 (AZFb partial)
18	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1*	0	1	1	1	1	1	1	Ym-4 (AZFb partial)
20	5	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	Ym-6 (P3+P2+P1)
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	Ym-5 (P3)
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	Ym-7 (P3+P2+P1)
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	1	1	1	1	1	0	Ym-8 (b1/b3)
26	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	1	1	1	1	1	1	Ym-8 (b1/b3)
27	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	Ym-9 (P3)
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	Ym-10 (P3)
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1*	0	1	1	1	1	1	1	Ym-11 (b2/b3)
33	32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	Ym-11 (b2/b3)
34	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	Ym-13 (P1)
39	690	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	Ym-12 (gr/gr)

P: Palindrome, AZF: Azoospermia factor

Fig.5

		1	2	3	4	5	6	7	8	9	10	9	11	12	13	14	15	16	17	18	19	20	17	20	19	21	
Pattern	sample no.	sy757	sY14	sY3118	sY1251	sY1324	sY1316	sY1714	sY1024	sY1967	sY1309	sY1967	sY3199	sY1233	sY3010	sY2990	sY1197	sY1191	sY1307	sY1291	sY2858	sY1206	sY1307	sY1206	sY2858	sY3159	Deletions
12	5	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	polymorphism
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	polymorphism
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	polymorphism
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1*	1*	0	1	1	1	polymorphism
36	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1*	0	1	1	polymorphism
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	polymorphism
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	polymorphism
40	1134	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	no deletion

Fig.6

		1	2	3	4	5	6	7	8	9	10	9	11	12	13	14	15	16	17	18	19	20	17	20	19	21
Pattern	sample no.	sy757	sY14	sY3118	sY1251	sY1324	sY1316	sY1714	sY1024	sY1967	sY1309	sY1967	sY3199	sY1233	sY3010	sY2990	sY1197	sY1191	sY1307	sY1291	sY2858	sY1206	sY1307	sY1206	sY2858	sY3159
1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	4	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	5	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
5	2	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	4	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	2	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	1	1
8	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1*	0	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	0	1*	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
12	5	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	6	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	10	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1*	0	0	0	1	1
15	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1*	0	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
17	5	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1*	0	1	1	1	1	1	1
19	3	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
20	5	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1
21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	1	1	1	1	1	0
26	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	1	1	1	1	1	1
27	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1
29	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
30	62	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1

31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1*	0	0	0	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1*	0	1	1	1	1	1	1
33	32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
34	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1*	1*	0	1	1	1
36	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1*	0	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
38	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
39	690	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
40	1134	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Supplementary Table 1 Chromosomal aberrations in category-2 in this st

Yp deletion	Yq I	Yq II	Yq III	Yq IV	Yq V	Yq VI
46, XX male (4)	mos 46X, del(Y) (?q11.23) [24]/ 45, X[6] (1)	mos 46, XYq-? [44]/ 45, X[6] (1)	46, Xdel(Y) (?q11.23) (1)	mos 46, XYq- [44]/ 45, X [6] (1)	mos 45, X [11]/ 46, Xdel(q11.2) [89] (1)	46, Xdel (Y) (q11.2) (1)
		mos 45, X [4]/ 46XY [16] (1)	46, Xidic(Y) (?q11.23) (1)	46, XYq-? (2)	mos 46, X+mar [37]/ 45, X [13] (1)	46, X+mar (2)
		N/A (2)	mos 46, Xidic(Y)(q11.2)/ 46, Xdel(Yq11.2)/ 45, X (1)	mos 46, X+mar [23]/ 46, Xdel (Y) (q11.2) [20]/ 45, X [7] (1)	mos 46, Xdel(Y) (q11.2) [29] / 46, Xidic(Y)(q11.2) [10]/ 45, X	
			n/a (2)	46, Xdel (Y) (q?11.23) (1)		
				mos 46, Xidic(Y) (q11.2)/ 45, X (1)		

karyotype (sample no), n/a, not available