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SHORT COMMUNICATION

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# Microdeletions of a Y-specific marker, Yfm1, and implications for a role in spermatogenesis

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**Abstract** We have detected deletions of a Y-specific microsatellite marker, Yfm1, located on the Y chromosome (Yq) within interval 6 and near the *DAZ* (deleted in azoospermia) genes, in 9/89 oligospermic and 17/68 azoospermic Japanese men. No Yfm1 deletions were detected in the 150 normal fertile males examined as controls. Yfm1 deletions in the oligo- and azoospermic males were associated with other deletions that removed entire DAZ genes in those infertile men. These deletions indicated that all Yfm1 loci are located within azoospermia factor c (AZFc) in interval 6 on the long arm of the Y chromosome. Mapping Yfm1 on the Y chromosome using the draft sequence of the human genome revealed that at least three Yfm1 loci are located within about 25–30 kbp of the *DAZ* genes. Moreover, the Yfm1 marker showed the least number of copies in Japanese males derived from a Y chromosomal lineage called haplotype II, defined by having the Y Alu polymorphism (YAP) insertion. Males from this haplotype II lineage are known from our previous studies to have lower spermatogenic abilities, with higher rates of oligo- and azoospermia than other haplotypes. The least number of Yfm1 loci, whose copy number may correspond to that of the *DAZ* genes, may be a risk factor predisposing an individual to azoospermia or oligospermia.

**Key words** Y chromosome · Spermatogenesis · Yfm1 · Microdeletions · Haplotypes

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## Introduction

Approximately 20% of men who seek help at infertility clinics present with non-obstructive oligo- or azoospermia. Most of these men are chromosomally normal but may have a microdeletion of an azoospermia factor (AZFa, AZFb, or AZFc) located on the long arm of the Y chromosome. Deletions of these AZF sequences are an important cause of male infertility, and they may involve germ-cell-specific genes or ubiquitously expressed genes (Moro et al. 2000; Foresta et al. 2001). Cytogenetic studies in sterile men suggest the localization of a gene controlling spermatogenesis, referred to as azoospermia factor (*AZF*), on interval 6 of the Y chromosome, Yq11.23 (Andersson et al. 1988; Chandley et al. 1989). Using probes from interval 6 of the Y chromosome, some evidence for deletions were found in some such sterile patients (Johnson et al. 1989). In the screening of DNA from 21 patients with structural abnormalities in Yq, Ma et al. (1992) constructed a detailed map of interval 6. Then Vogt et al. (1992) postulated that the Yq microdeletions disrupted the functional DNA structure of the *AZF* gene (Ma et al. 1992; Vogt et al. 1992). Kobayashi et al. (1994) analyzed DNA from 63 Japanese men with either azoospermia or severe oligospermia with otherwise cytogenetically normal Y chromosomes. They examined 15 loci on the long arm between the *DYS7E* and *DYZ1* loci, and the *YRRM1* locus, a candidate gene for AZF. They detected microdeletions in 10 of the men; the *YRRM1* gene was involved in only 3 of them. The remaining 7 patients showed a deletion between *DYS7C* and *DYS239* in common, indicating the presence of at least one additional gene, the deletion of which causes azoospermia (Kobayashi et al. 1994). In a blind study, Krausz et al. (1999) screened DNA from 131 infertile males (46 idiopathic and 85 nonidiopathic) for Y chromosome microdeletions. Of idiopathic males with an apparently normal 46, XY chromosome complement, 19% had microdeletions in the AZFa, AZFb, or AZFc region. There was no strict correlation between the extent or location of the deletion and the phenotype (Krausz et al. 1999).

In 1999, we reported that sperm concentrations vary among males from different Y chromosome lineages, and the male populations with oligospermia and azoospermia were overrepresented among males from one specific chromosome lineage: those with haplotype II (defined by the presence of Y Alu polymorphism (YAP) insertions on their Y chromosomes), (Kuroki et al. 1999). Recently, a similar report by Krausz et al. identified one particular haplogroup (hpg 26+) having lower sperm counts than males with other haplotypes in the Danish population (Krausz et al. 2001). Hence, it is likely that genetic and structural differences on the Y chromosome contribute to variations in spermatogenic abilities.

In 2000, Matsuki et al. reported the development of a new Y-specific microsatellite marker, Yfm1, which is a polymorphic, multicopy Y-specific CA repeat. They mapped this marker to the long arm of the Y chromosome and showed that it is transmitted in a patrilineal manner from father to son. They concluded that Yfm1 is a useful marker for various human genetic and forensic studies (Matsuki et al. 2000).

We typed the Yfm1 marker in a set of DNA samples from a normal Japanese population. Our results confirmed that Yfm1 is a Y-specific marker located in the nonrecombining part of the Y chromosome that is transmitted exclusively from fathers to sons. This Yfm1 marker was consistently associated with other Y-specific biallelic polymorphic markers, indicating its usefulness for population and forensic studies (Ewis et al. unpublished).

In the present study, we show that the Yfm1 loci are located near the *DAZ* (deleted in azoospermia) genes in the AZFc region, with the possibility that the copy numbers of the Yfm1 loci reflect those of the *DAZ* genes. Furthermore, we discuss the possible relations between Yfm1 and spermatogenic ability in Japanese men.

## Subjects and methods

### DNA samples

Semen and blood samples were obtained from 68 azoospermic, 89 oligospermic (defined by using the World Health Organization (WHO) standard as $<20 \times 10^6$ sperm/ml) patients, and 150 normal fertile controls who had fathered at least one child. These samples were collected at hospitals in Kanagawa and Osaka prefectures, Japan. All patients and controls were recruited from the same areas to eliminate sampling bias and geographical or environmental confounders. Informed consent was obtained from all participants prior to collecting samples.

Genomic DNA was extracted from subjects' blood samples according to the standard method (Sambrook et al. 1989). Semen samples were obtained by masturbation after sexual abstinence for at least 48 hours. Samples were examined within one hour of ejaculation. For all the collected ejaculates, sperm concentrations were assessed using a hemocytometer. Only spermatozoa with tails were counted under a microscope at 400× magnification, and the average of at least two chambers was calculated.

### Development of Yfm1 marker

The development of the Yfm1 microsatellite marker on the Y chromosome was described previously (Matsuki et al. 2000).

### Yfm1 microsatellite genotyping

Yfm1 microsatellite genotyping was done as described previously by Ewis et al. (unpublished). Genotyping of Y chromosomes from different populations for the Yfm1 marker was done using an ABI 377 (Applied Biosystems, Foster City, CA, USA) sequencer. The polymerase chain reaction (PCR) contained a primer pair, one of which was end-labeled with the fluorescent dye FAM. DNA samples were amplified by PCR in a volume of 10 µl containing 66 ng genomic DNA, 67 mM Tris–HCl (pH 8.3), 3 mM $MgCl_2$, 16.6 mM $(NH_4)SO_4$, 0.1 mM dNTPs, 0.25 µM of each primer and 1 U *Taq* Gold DNA polymerase. Thermocycling was performed using a PE9600 (PerkinElmer, Shelton, CT, USA) thermocycler under the following conditions: initial denaturation at 95°C for 12 min, followed by 30 cycles of denaturation at 95°C for 30 s, ramping slowly to 60°C within 2 min, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The final extension step was at 72°C for 30 min. PCR products were resolved with urea denaturing polyacrylamide gels on the ABI sequencer by using an internal size standard in each lane. Raw genotype data were collected using GeneScan software (Applied Biosystems), and gel files were analyzed with the Genotyper software package (Applied Biosystems).

The following primer set was used for PCR; the forward primer was labeled with FAM: CA-F: 5′-CACCATTT GTTGAAAAGAC-3′; CA-R: 5′-TATAGAGAGCCC AGAAAGAG-3′.

## Results and discussion

Cytogenetic and deletion analyses using polymerase chain reaction (PCR) amplification of Y chromosomal genes in DNA from azoospermic and oligospermic patients have led to the identification of at least four azoospermia factor (AZF) regions, namely AZFa, AZFb, AZFc, and AZFd. Beginning with the cloning of functional copies of the RNA-binding motif (RBM) and DAZ gene families from AZFb and AZFc, respectively, researchers have described numerous Y-linked genes whose microdeletion detection rates range from 3% to almost 30% among infertile males (Kent-First et al. 1999).

For the present study, we selected a newly developed marker, Yfm1, which is located near the *DAZ* genes in the AZFc critical region for spermatogenesis, to study its relation to the spermatogenic process. Using PCR and

GeneScan software, we typed the Yfm1 marker in a series of DNA samples from 68 nonobstructive azoospermic and 89 oligospermic males and from 150 normal fertile male controls. We found that the Yfm1 loci were completely deleted (Fig. 1a) in 17 (25%) azoospermic and 9 (10.1%) oligospermic patients, but none of the normal fertile males showed such deletions (Table 1). All Yfm1 deletions were accompanied by deletions of other markers on the long arm of the Y chromosome, mainly *DAZ* genes, implying that these microdeletions possibly shared in the disruption of the function of the AZFc region. However, among the tested group of oligo- and azoospermic males, Yfm1 loci were not deleted in the 3 patients with deletions in the AZFa region (2 with a *DBY* deletion and 1 with a *USP9Y* deletion), or in the other 4 patients with AZFb deletions (2 with *RBMY* and 2 with *SMCY* deletions) (data not shown). These findings also confirm the localization of the Yfm1 marker to the critical region of azoospermia near to *DAZ* genes, i.e., AZFc. To know the detailed locations of the Yfm1 loci, we searched the draft sequence of the human genome deposited on the U.S. National Center for Biotechnological Information (NCBI) web site. We found that there are three Yfm1 loci in contig NT011903.8 on the Y chromosome, and all loci are located within about 25–30 kbp of the *DAZ* genes (Fig. 2).

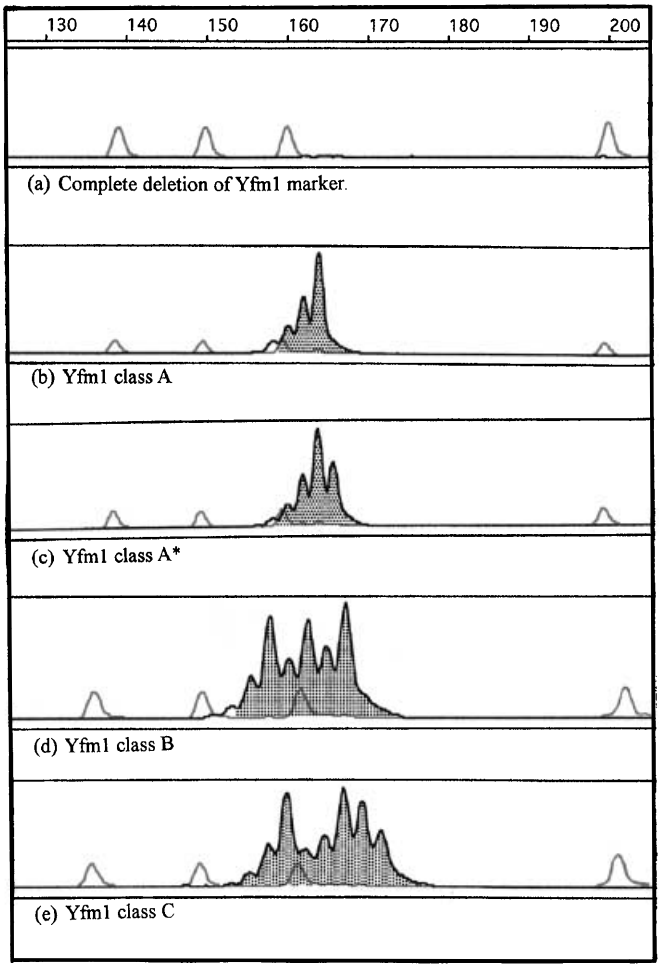


**Fig. 1.** **a** Yfm1 peaks are completely deleted along with Y chromosome deletions of the AZFc region including DAZ genes. **b, c, d,** and **e** show *Yfm1 classes A*, *A**, *B*, and *C*, respectively. Yfm1 is always intact in males having no deletions in the AZFc region. The true Yfm1 peaks are *shaded*. The *unshaded peaks* are those of the standard size marker. The horizontal scale indicates the size (bp) of the Yfm1 peaks

In our previous study, we reported that males from different Y chromosomal lineages have different spermatogenic abilities. We demonstrated high rates of azoospermia and oligospermia in a certain Y chromosomal lineage called haplotype II, defined by the YAP insertion (Kuroki et al. 1999). These findings led us to study the relationship between Y chromosomal haplotypes and the Yfm1 polymorphic microsatellite marker. In a separate study (Ewis, et al. unpublished data), we demonstrated that Japanese males could be classified into four main classes with respect to peak patterns and the number of copies of Yfm1. Class A was very characteristic in that it had the smallest number of copies ranging from 160 to 164 bp (Fig. 1b). Some males belonging to Yfm1 class A showed an extra peak (copy) at 166 bp that we named class A* (Fig. 1c). Class B was unique in having a fixed pattern with a fixed number of copies ranging from 154 to 164 bp (Fig. 1d). All others were described as belonging to class C, which included any other patterns of Yfm1 with different copy numbers and not fitting into classes A, A*, or B (Fig. 1e). The class C pattern showed copies that ranged from 154 up to 172 bp (Ewis et al. unpublished).

There were constant relationships between these Yfm1 classes and the four Japanese haplotypes constructed by using three Y chromosomal biallelic polymorphic markers [DYS287 (YAP), DXYS5Y (47z/*Stu*I), and SRY] as reported by Shinka et al. (1999). After we combined the Yfm1 classes with the haplotyping system of Shinka et al. (1999),

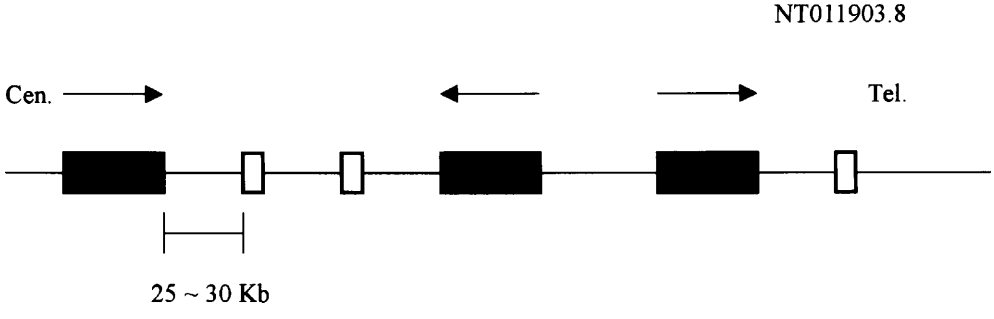


**Fig. 2.** A schematic diagram showing the location of Yfm1 loci near each *DAZ* gene. *Black boxes* indicate the DAZ genes. *Open boxes* indicate the Yfm1 loci. The direction of *arrow* indicates the direction of the *DAZ* gene transcript. The distance between each *DAZ* gene and the Yfm1 locus ranges from 25–30 kb. The contig number is *NT011903.8*. *Cen.*, centromere; *Tel.*, telomere

**Table 1.** Frequency distribution of Yfm1 complete deletion among azoospermic, oliglospermic, and normal fertile Japanese males, classified according to Y chromosome haplotypes of (YAP, 47z/*Stu*I, and SRY) biallelic markers and the Yfm1 microsatellite marker classes

| Haplotypes | Yfm1 class | Normal fertile males | Azoospermic males | Oligospermic males |
|---|---|---|---|---|
| I | A | 5 | 4 | 11 |
| | A* | 8 | 3 | 8 |
| | B | 2 | 1 | **0** |
| | C | 36 | 12 | 26 |
| | Del. | **0** | **7** | **3** |
| II | A | 39 | 16 | 17 |
| | A* | 7 | 0 | 3 |
| | Del. | **0** | **7** | **5** |
| III | B | 15 | 7 | 6 |
| | Del. | **0** | 0 | **0** |
| IV | B | 38 | 8 | 9 |
| | Del. | **0** | **3** | **1** |
| Total Examined | | 150 | 68 | 89 |
| Total with completely deleted Yfm1 | | **0** | **17** | **9** |

Bold numbers are the number of males with completely deleted Yfm1 loci
As shown in Fig. 1., Yfm1 class A has the smallest number of peaks, class A* has one additional peak, class B has a characteristic pattern of six peaks, and class C has peak patterns different from classes A, A*, or B
Del., complete deletion of Yfm1 loci

the Japanese population showed eight haplotypes only: IA, IA*, IB, IC, IIA, IIA*, IIIB, and IVB. The distribution of the azoospermic, oligospermic, and normal fertile males according to these eight haplotypes is presented in Table 1. Interestingly, haplotype II, which was identified by Kuroki et al. (1999) as the male lineage with low spermatogenic abilities, was consistently associated with Yfm1 class A (39/46) or class A* (7/46), which have the least number of copies of Yfm1. It can be inferred that their Y chromosomes with few copies of Yfm1 compromised their spermatogenic abilities. However, the low number of Yfm1 peaks in haplotype II males does not necessarily indicate a deletion of the relevant regions, and an expansion of the low number of repeats might make the loci identical to the higher repeat loci of other haplotypes. This possibility was ruled out, however, when we found that all males with haplotype II have the fewest *DAZ* genes or DAZ repeats compared with those with other haplotypes. Southern hybridization of DNAs from Y chromosomes with different haplotypes using a DAZ-repeat probe revealed the loss of a 15-kb band from all haplotype II chromosomes but not from the other haplotypes (Lee et al., unpublished data). So, haplotype II Y chromosomes may have lost some Yfm1 loci that are closer to the deleted DAZ repeats.

Recently, it was reported that a partial deletion of the *DAZ* gene cluster might cause oligospermia, suggesting that the copy number of *DAZ* genes may influence spermatogenic ability (Bienvenu et al. 2001; de Vries et al. 2002). If the copy number of Yfm1 loci reflects that of the *DAZ* genes, the low copy number found in males with haplotype II may be related to the reduction in sperm concentration.

Therefore, partial deletions of *DAZ* genes as well as the lowest copy number of Yfm1, which implies a low copy number of *DAZ* genes, may increase the risk of spermatogenic failure. It is also possible that the peak pattern of Yfm1 is linked with other unknown Y chromosomal polymorphisms related to the spermatogenic process. The peak patterns of Yfm1 found in males with azoospermia or oligospermia but without apparent deletions of AZFc were the same as those of normal fertile males, suggesting that those males may not have partial deletions of *DAZ* gene clusters and that other genes may be responsible for their abnormal-spermatogenesis phenotype.

To summarize, we showed that multiple copies of Yfm1 are located in interval 6 on the Y chromosome near the *DAZ* genes. Furthermore, we also clarified the possibility that the copy number of Yfm1 may reflect that of *DAZ* genes. An investigation of the genomic structures of the Y chromosome, which are linked to the peak patterns of Yfm1, may provide some clues to understanding the differences in spermatogenic abilities among males with different Y chromosome haplotypes.

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