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Polymorphisms associated with the *DAZ* genes on the human Y chromosome

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Abstract

The human Y chromosome is unique in that it does not engage in pairing and crossing over during meiosis for most of its length. Y chromosome microdeletions, a frequent finding in infertile men, thus occur through intrachromosomal recombination, either within a single chromatid or between sister chromatids. A recently identified polymorphism associated with increased risk for spermatogenic failure, the gr/gr deletion, removes two of the four *Deleted in Azoospermia* (*DAZ*) genes in the *AZFc* region on the Y-chromosome long arm. We found the likely reciprocal duplication product of gr/gr deletion in 5 (6%) of 82 males using a novel DNA-blot hybridization strategy and confirmed the presence of six *DAZ* genes in three cases by FISH analysis. Additional polymorphisms identified within the DAZ repeat regions of the *DAZ* genes indicate that sister chromatid exchange plays a significant role in the genesis of deletions, duplications, and polymorphisms of the Y chromosome.

Keywords: DAZ; Y chromosome polymorphism; sister chromatid exchange

The male-specific region of the Y chromosome (MSY) does not engage in pairing and crossing over with the X chromosome during male meiosis and was once described as the nonrecombining region of the Y [1]. It was realized recently that MSY is active in recombination, as indicated by abundant gene conversion within the region [2,3]. In addition, several large Y-chromosome deletions that are frequently found in infertile men could have occurred only through nonallelic recombination between direct repeats [4–12]. The *AZFc* region on the long arm of the Y chromosome offers a unique opportunity to investigate the mechanisms underlying Y-chromosome rearrangements. This region consists mainly of very long repeats, making it prone to deletion [2,8]. The b2/b4 deletion (aka the *AZFc* deletion), which removes a 3.5-Mb segment between the b2 and the b4 repeats, occurs in one in 4000 men and impairs their fertility [8,13]. The gr/gr and

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b2/b3 (g1/g3) deletions that remove about half of the *AZFc* region are more common and represent new Y-chromosome polymorphisms [10–14]. These deletions, as well as the *AZFa* and *AZFb* deletions, could be the results of recombination between two repeats on the same chromatid or on two different sister chromatids. For Y chromosome rearrangements resulting in gene duplication, sister chromatid exchange (SCE) appears to be the most likely mechanism [10,13–15].

DAZ belongs to a family of germ-cell-specific RNA-binding proteins that are essential for gametogenesis [16,17]. Most men have four *DAZ* genes, arranged in two head-to-head arrays 1.6 Mb apart within the *AZFc* region [8,18]. The *DAZ* genes are almost identical in sequence except that they encode 1–3 copies of an RNA-binding motif (RBM; encoded by exons 2–6) and 7–24 copies of a unique DAZ repeat of 24 amino acid residues (encoded by exon 7, Fig. 1a) [16,18,19]. There are nine types of DAZ repeats that share 90–96% of nucleotide sequence similarity [16,20]. Arrays of the 2.4-kb DAZ repeat unit that contains exon 7 are interrupted at different locations in the various *DAZ* genes by the insertion of a LINE [18,19]. So far the four *DAZ* genes could be distinguished only by the use of sequence family variants, which was considered by some unreliable due to gene conversion [11,19,21–25]. We designed a novel Southern hybridization scheme to characterize the *DAZ* genes in 82 individuals. Our results provide strong evidence that SCE plays an important role in generating Y chromosome rearrangements and polymorphisms.

Results

Our analyses of published *DAZ* gene sequences identified several convenient restriction sites for the development of a novel Southern blotting scheme to differentiate and characterize the *DAZ* genes present in a genomic DNA sample. We used two restriction enzymes (*Nsi*I and *Bam*HI) and three probes to study separately the RBM repeat region (region A) and the DAZ repeat regions before (region B) and after (region C) the LINE insertion (Fig. 1a). Probe A from intron 1 detected *Nsi*I fragments of 31, 20, and 9 kb with a relative hybridization intensity of 3:2:1 when the genes contain three, two, and one RBM, respectively. *Nsi*I also cut within the LINE and the Y-type DAZ repeats downstream of the LINE insertion, but left the DAZ repeats upstream of the insertion intact. Under stringent hybridization condition, probe B, which contained a D-type DAZ repeat, detected large *Nsi*I fragments from region B of the various *DAZ* genes. And finally, the *Bam*HI sites inside the LINE insertion and intron 10 made it possible to analyze region C with probe C from proximal intron 10.

We used this scheme of genomic Southern hybridization to characterize the *DAZ* genes in 82 DNA samples. They were from 25 subfertile men without the *AZFc* deletion, 10 fertile controls, and 47 individuals with unknown fertility [26–28]. Hybridization patterns of most DNA samples agreed with the current model of four *DAZ* genes on the Y chromosome. Probe A detected the three expected *Nsi*I fragments with a relative signal ratio of close to 3:2:2, consistent with the presence of one *DAZ* gene with three RBMs, one gene with two RBMs, and two genes with a single RBM each, expressed as $1(RBM)_3 1(RBM)_2 2(RBM)$ (Fig. 1b). Individual N2, who is fertile, however, had an altered ratio that is consistent with a gene configuration of $1(RBM)_3 2(RBM)_2 1(RBM)$. Probe B showed that region B was highly polymorphic among the samples (Fig. 1c). The *Nsi*I fragments ranged from 11 to 31 kb in size, corresponding to the presence of 4 to 12 DAZ repeats (including the one in region A) before the LINE insertion. Hybridization of *Bam*HI-digested DNAs with probe C revealed extensive polymorphism in region C (Fig. 1d). The sizes of the fragments ranged from 14.5 kb to approximately 40 kb, corresponding to 1 to 12 DAZ repeats. The number and the relative intensity of the fragments detected by both probes B and C were consistent with the presence of four *DAZ* genes in most of our samples. Each individual had either four fragments with comparable intensity or three fragments with one twice the intensity. Because the fragments were highly polymorphic, assignments to specific genes were made for only a few fragments. Nevertheless, our results are consistent with the model that most men have four *DAZ* genes that are highly polymorphic in the DAZ repeat regions.

Five individuals (S1–S5) appeared to have six *DAZ* genes based on quantitative analyses of the hybridization fragments. Patterns with probe A suggested that individual S1 had $1(RBM)_3 2(RBM)_2 3(RBM)$, and the remaining four had $2(RBM)_3 1(RBM)_2 3(RBM)$ (Fig. 1b). Hybridization patterns with probe B also supported the presence of more than four *DAZ* genes in S1, S2, S3, and S4 (Fig. 1c). The presence of six *DAZ* genes in lymphoblastoid cells of S1, S2, and S4 was further confirmed by interphase FISH analyses (Fig. 2). In a normal control with four *DAZ* genes, probes from the spacer between the *DAZ* gene pair (7A69, red) and from the green (336F2) and yellow (79J10) repeats gave two, three, and two dots, respectively, in the order consistent with the current model. S1, S2, and S4 all gave three red dots, four green dots, and three yellow dots, indicating partial duplication of the *AZFc* region. The orders of the dots suggest that they are the products of gr/gr duplication (Fig. 3b).

We identified an individual (P10) with only two *DAZ* genes among our samples. We also acquired and characterized nine published cases of *DAZ* partial deletion [10,11,21,29,30]. In addition to Southern analysis, we assayed the samples for the presence of eight *AZFc* markers, including sY581, sY586, sY587, sY1161, sY1191, sY1201, sY1206, and sY1291. Based on the hybridization patterns of probe A, P1 and P5 (data not shown) had retained *DAZ3* and probably *DAZ4,* and the remaining eight cases had retained *DAZ1* and likely *DAZ2* (Fig. 1b, Table 1). The