

## sY116, a human Y-linked polymorphic STS

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### Abstract

During a study of deletions of Y-chromosomal DNA in infertile males, sY116, a Y-linked STS, showed different electrophoretic mobilities in three males, two infertile and one fertile. A study of this STS among 35 other normal males showed that this locus is polymorphic. sY116 has a polyA-rich stretch whose instability appears to be the most likely cause of this polymorphism. The possible usefulness of sY116 polymorphism in the detection of subtle genome-wide instabilities in some types of cancer is discussed.

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### Introduction

In a cytogenetic study of 1170 infertile males, Tiepolo and Zuffardi (1976) found deletions of distal Yq in six azoospermic individuals. In four of these cases the fathers were tested, and all were found to carry an intact Y chromosome. On the basis of these *de novo* deletions in azoospermic males, Tiepolo and Zuffardi proposed the existence of a spermatogenesis gene, or *Azoospermia Factor* (*AZF*). The *AZF* locus has been mapped to deletion interval 6 in Yq11.23 (Vergnaud *et al.* 1986; Andersson *et al.* 1988; Bardoni *et al.* 1991). Systematic refinement of physical maps of the human Y chromosome by identification of sequence-tagged sites (STSs) (Foote *et al.* 1992; Vollrath *et al.* 1992) led to narrowing of the *AZF* region to that lying between DNA markers sY143 and sY159 (Reijo *et al.* 1995; Vogt *et al.* 1995). However, the *AZF* locus is now known to consist of at least three nonoverlapping regions on Yq which are associated with male fertility (reviewed in Vogt *et al.* 1997).

This study deals with the analysis of the human Y chromosome in two cases of male infertility. These individuals were studied by using Y-linked STSs, most of which were distributed in intervals 5 and 6 of the long arm. It was serendipitously discovered that one of the STSs, sY116, is polymorphic. Instability of a polyA-rich stretch in sY116 appears to be the most likely cause of the polymorphism. These observations prompted us to study sY116 in a number of other males. The potential use of sY116 in studying genomic instabilities in some types of cancer is discussed.

### Materials and methods

Chromosome banding and extraction of genomic DNA from blood was done using standard techniques (Ausubel *et al.* 1995; Dracopoli *et al.* 1995). The following Y-linked STSs were used in this study: sY34, sY49, sY62, sY76, sY79, sY81, sY82, sY83, sY84, sY85, sY86, sY87, sY88, sY95, sY101, sY107, sY114, sY116, sY119, sY125, sY127, sY135, sY143, sY145, sY149, sY155, sY158, sY182,

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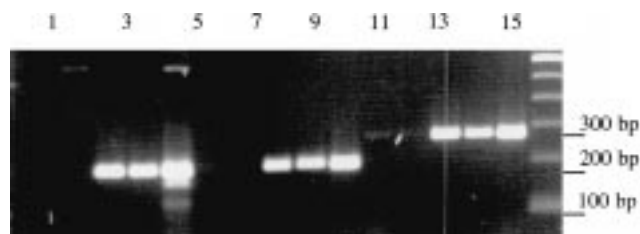
sY183, sY208, sY255 (within *DAZ* locus) and sY269 (Vollrath *et al.* 1992; Reijo *et al.* 1995). There were three types of controls for each primer pair: (i) no DNA—the volume was made up with water, (ii) female DNA, and (iii) normal male DNA. Each amplification was carried out in a reaction mixture (25  $\mu$ l) containing 50 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M of each dNTP, 0.25  $\mu$ M of each primer and 2 units of Taq DNA polymerase (Pharmacia). Initial denaturation was carried out for 3 minutes at 94°C, following which amplification was performed for 35 cycles (1 minute each at 94°C, 55°C and 72°C) and a final extension of 10 minutes at 72°C. After PCR, the reaction mix was electrophoresed on 2% agarose gel in 1  $\times$  TBE buffer at 4 V/cm for 1 hour. DNA was visualized by staining with ethidium bromide, examined under a UV transilluminator, and photographed.

PCR of sY116 using fluorescence-labelled dUTP[R6G] (Perkin-Elmer) was carried out largely as above. The only differences were (i) addition of fluorescence-labelled dUTP, which was 1/600th the concentration of any one dNTP and (ii) the number of PCR cycles was 30. The products of the PCR reactions were run on 6% acrylamide gel (19 : 1 :: acrylamide:bis-acrylamide) under denaturing conditions after mixing with 0.5  $\mu$ l of marker (GeneScan400, Perkin-Elmer) in an automated sequencer (ABI 310, Perkin-Elmer), and the gel image analysed using the GeneScan software supplied by the manufacturer. The software provides a densitometric scan of each lane. Allele lengths were determined with reference to the internal marker (red peaks, see figure 3). The highest point in the sY116 peak (in green) was taken as the allele representative of that individual.

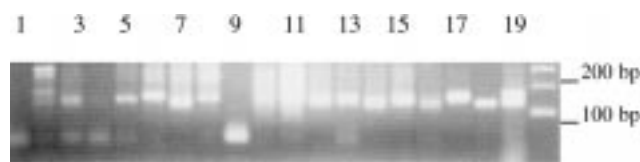
Purification of the sY116 PCR product and its subsequent sequencing were carried out as described previously (Saifi *et al.* 1999).

## Results

The two infertile males were found to be normal for all the 32 Y-linked STSs that were studied (figure 1). These two males and a control male differed from each other in the electrophoretic mobility of one of the STSs, sY116, as seen by agarose gel electrophoresis. In the other 35 males (14 Caucasians, 9 blacks and 12 Arabs) that were studied, this band varied slightly in mobility from individual to individual (figure 2), making unambiguous identification of distinct alleles difficult. To improve resolution, the PCR products were subjected to electrophoresis on denaturing acrylamide-based gels, but these experiments yielded smears instead of the expected sharp bands. Densitometric analysis of these smears was carried out for samples from 15 individuals (figure 3). The distribution of sY116 'alleles' in these 15 individuals is summarized in table 1. Allele sizes ranged from 136 bp to 168 bp.



**Figure 1.** PCR products of Y-linked STS: lanes 1–5 correspond to sY119 (set I), lanes 6–10 to sY125 (set II), and lanes 11–15 to sY127 (set III). In each set, the first lane contained no DNA, the second lane DNA from a female, the third and fourth lanes DNAs from the two infertile males, and the fifth lane DNA from a normal (fertile) male. Lane 16 is a DNA molecular weight marker (100-bp ladder) starting from 100 bp (bottommost band).



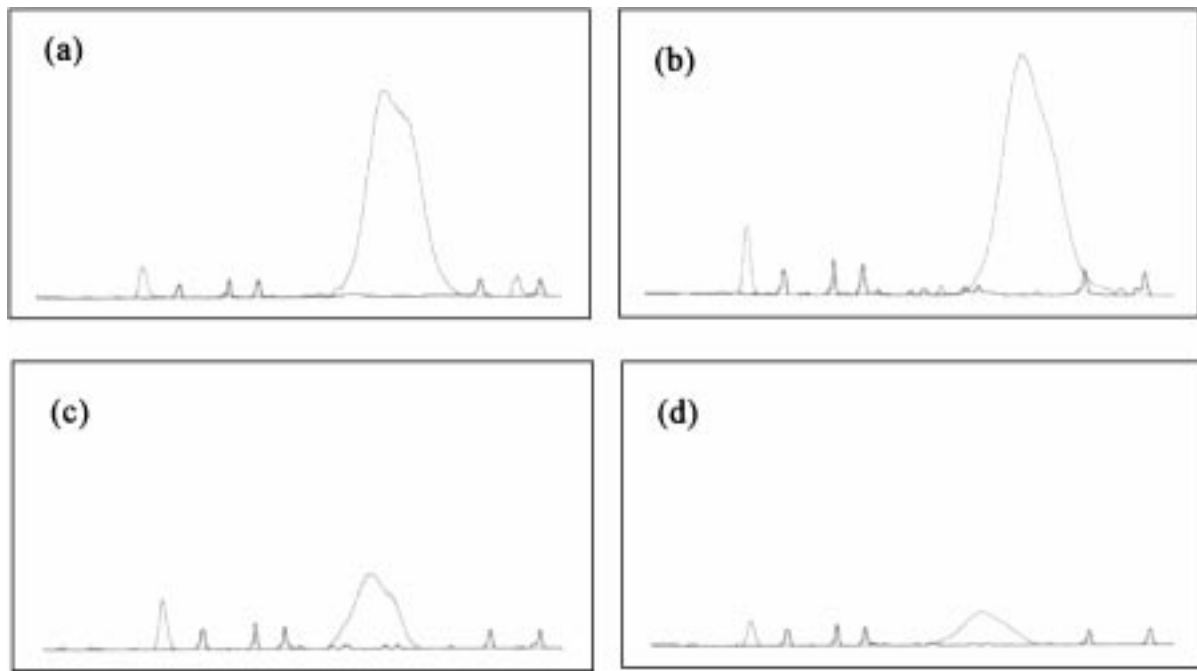
**Figure 2.** Differential electrophoretic mobilities of sY116 from a representative population. Lanes 3–16 and 19 show the PCR products of sY116 in 14 normal unrelated males; lanes 17 and 18 are the two infertile males; lane 1 has no DNA, lane 2 is XX female and lane 20 is a 100-bp ladder.

Sequencing of sY116 from two individuals showed the presence of an apparently normal sequence outside the polyA-rich stretch (data not shown). The number of As in the polyA-rich stretch could not be read unambiguously.

## Discussion

The absence of Y-chromosome-specific deletions in the two infertile males is not unexpected because only a small proportion of such males (10–18%) show such deletions (reviewed in Vogt *et al.* 1997; Burgoyne 1998). The proportion of infertile males with detectable Y-chromosomal deletions varies substantially between studies (reviewed in Cooke 1999). The highest proportion reported so far is 37% (Foresta *et al.* 1997). The lower range is estimated to be 5–10%. In one study (Pryor *et al.* 1997), 14 individuals with Y deletions were found in a group of 200 infertile males. In some cases, the same deletion was present in the fathers as well. As a result, it is difficult to associate the deletion with the infertility in the index case. If the deletion is indeed the cause of infertility in the index case, then it was not penetrant in the father. In the two cases we studied, owing to lack of paternal DNA and DNA from other tissues, in particular the gonads, it is not possible to distinguish between mosaicism and variable penetrance.

The appearance of smears rather than a sharp band in sY116 PCRs suggests mitotic instability of the sequence. The sY116 sequence has a polyA-rich stretch (Foote *et al.*



**Figure 3.** Densitometric scans of sY116 PCR products from four normal males. PCR was carried out in the presence of a fluorescent label. The broad peaks in green represent sY116 whereas the sharp red peaks are bands corresponding to the internal molecular weight marker (GeneScan 400).

**Table 1.** Distribution of sY116 alleles in Arabs and blacks.

Allele (bp)	Number of individuals	
	Arabs	Blacks
136	1	0
147	1	0
148	0	2
153	0	2
155	1	4
156	1	0
157	1	1
168	1	0

1992). Detection of an apparently normal sY116 sequence outside the polyA-rich stretch argues in favour of polymerase slippage in this A-rich stretch as the most likely cause of instability.

A germline mutation on the Y chromosome that does not affect reproduction is expected to be passed on from father to son. Markers on the nonrecombining portion of the Y can therefore be used in the reconstruction of paternal lineage and in tracing male-mediated migration (reviewed in Mitchell and Hammer 1996). Several Y-linked microsatellite-based polymorphic markers (see Kayser *et al.* 1997) and biallelic polymorphisms (Underhill *et al.* 1997) have been reported. However, the number of such Y-linked polymorphisms is not large, being about four times lower than that on autosomes (reviewed in Mitchell and Hammer 1996). The polymorphic nature of sY116 reported here makes it a potentially useful marker. However, the presence of smears

that overlap with each other makes identification of distinct alleles at this locus difficult. One way of overcoming the difficulty of assigning allelic status to these variants is by densitometric scanning of each smear. The point in the scan at which the peak begins and that at which it ends can be used to define the range of 'alleles' present in a given individual. Similarly, the highest point on the peak would correspond to the allele occurring most frequently. This allele was taken as being representative of that individual. However, as a result of high variability within populations (table 1), this polymorphism is unlikely to be useful in demographic studies.

Polymorphisms that result in instability of the kind seen in sY116 may prove useful in the detection of subtle genome-wide instabilities that may go undetected when other microsatellites such as dinucleotide repeats are used. For instance, microsatellite instability, seen as length alterations of mononucleotide or dinucleotide repeat sequences, is associated with a nonpolyposis form of colorectal cancer (Claij and te Riele 1999). However, such instability is seen in only a small fraction (10–15%) of such cases (Kinzler and Vogelstein 1996). In the remaining cases, known as RER<sup>-</sup> (replication error rate negative) cases, no genomic instability has been detected when classical microsatellite markers were used. A fraction of these RER<sup>-</sup> cases may have subtle genomic instability and this may be detectable by sY116. This is because sY116 is already unstable in normal individuals and might therefore be expected to have greater instability in this subset of RER<sup>-</sup> cases. If so, one might expect RER<sup>-</sup> cases to have broader peaks or

a wider range of smears (alleles) for sY116 than normal individuals.

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