Table 1

Plus/Minus STS Results Distinguishing Different Types of Deletions Involving *AZFc*

	Result at STS[a]					
Deletion	sY142	sY1197	sY1191, sY1192, and/or 50f2/C	sY1291	sY1206	sY1201
b2/b3[b]	+	+	−	+	+	+
gr/gr	+	+	+	−	+	+
b1/b3	+	−	−	−	+	+
b2/b4[c]	+	+	−	−	−	+
None	+	+	+	+	+	+

Note.—See Kuroda-Kawaguchi et al. (2001), Repping et al. (2003), Skaletsky et al. (2003), Fernandes et al. (2004), Repping et al. (2004), and GenBank for STSs.

[a] + = present; − = absent.

[b] Termed the "g1/g3" deletion by Fernandes et al. (2004).

[c] "Classical" *AZFc*.

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Are Sequence Family Variants Useful for Identifying Deletions in the Human Y Chromosome?

To the Editor:

We read with interest the report of a novel deletion of part of the azoospermia factor c (*AZFc* [MIM 415000]) region of the human Y chromosome (Fernandes et al. 2004). This article reported that the deletion is found only in branch N of the Y-chromosome genealogical tree, occurs through one mutational pathway, is ~2.2 Mb in size, and has no effect on spermatogenesis. We, too, recently reported this deletion, which Fernandes et al. termed the "g1/g3" deletion and which we termed the "b2/b3" deletion (Repping et al. 2004). Our findings, however, differed from those of Fernandes et al. in several important particulars: (1) our screening of 1,563 men demonstrated that this deletion is not confined to branch N and that it has at least four independent origins; (2) our analysis revealed two mutational pathways, rather than one, that can generate the deletion, and we confirmed the existence of the inverted *AZFc* organizations that are the intermediate steps in these pathways; (3) on the basis of the reference sequence of the Y chromosome, we concluded that the size of the deletion is 1.8 Mb, rather than ~2.2 Mb; (4) using interphase FISH, we confirmed the amplicon organization that was postulated in the deletion and also identified three instances of duplication subsequent to the deletion; and (5) because of the possibility of a compensatory factor on Y chromosomes in branch N and because of the limited number of deletions outside this branch, we concluded that a possible effect of this deletion on risk of spermatogenic failure cannot be excluded (Repping et al. 2004).

Beyond these differences, however, the characterizations of this and other partial deletions of *AZFc* (Repping et al. 2003) highlight a more important question. At issue is the relative utility of sequence family variants (Saxena et al. 2000), compared with that of plus/minus STSs, for identification and differentiation of deletions involving *AZFc*. *AZFc* is composed entirely of amplicons—repeat units 115–678 kb in length that only differ by ~1 nt per 3,000 bp. These rare differences are called "sequence family variants" (SFVs). We previously relied on SFVs to map and sequence the *AZFc* region of one man's Y chromosome (Kuroda-Kawaguchi et al. 2001). The report by Fernandes et al. (2004) emphasized the use of SFVs in identification of the novel deletion, whereas our analysis relied on plus/minus STSs for identification of the deletion, followed, in most instances, by confirmation with FISH.

Two observations led us to ask whether SFVs, as opposed to plus/minus STSs, offer the simpler and more robust means of detecting and distinguishing deletions in *AZFc*. First, figures 1 and 4 in the report by Fernandes et al. (2004) indicated that negative results at the plus/minus STS sY1192 or 50f2/C combined with positive

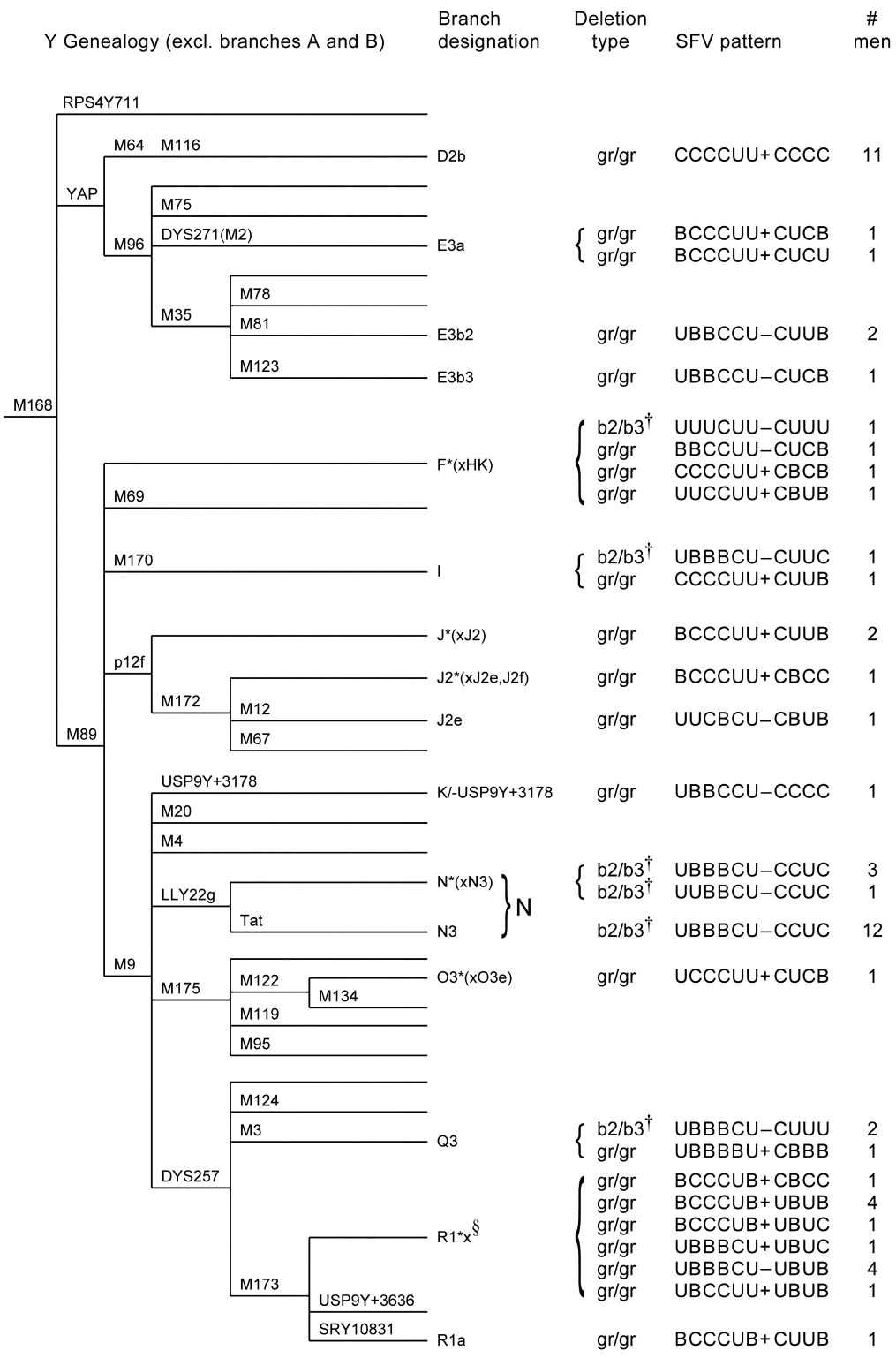

Figure 1 Genealogical analysis of SFV patterns associated with b2/b3 and gr/gr deletions. In the SFV patterns, "C" indicates the cut variant described by Fernandes et al. (2004), "U" indicates the uncut variant, "B" indicates both variants, and + and − indicate the presence or absence, respectively, of the Y-DAZ3 variant. The order of SFVs is as shown in table 2 in the work of Fernandes et al. (2004): DAZ-SNV I, DAZ-SNV II, sY586 (DAZ-SNV III), DAZ-SNV IV, sY587 (DAZ-SNV V), DAZ-SNV VI, *AZFc* SFV 18 (assayed by Y-DAZ3), TTY4-SNV I, BPY2-SNV, GOLY-SNV I, and *AZFc* SFV 20 (AZFc-P1-SNV I) (Saxena et al. 2000; Kuroda-Kawaguchi et al. 2001 [Web table E]; Fernandes et al. 2002, 2004). The genealogical tree of extant human Y chromosomes and the branch designations are from the studies by Underhill et al. (2000) and the Y-Chromosome Consortium (2002). §, R1*x is an abbreviation for R1*(xR1a,R1/-USP9Y+3636). †, Termed "g1/g3" by Fernandes et al. (2004).

results at flanking STSs are sufficient to detect the deletion (table 1). Moreover, the b2/b3 deletion and other types of deletions involving *AZFc* can be distinguished by their plus/minus signatures, without the use of SFVs (table 1).

Second, table 2 in the report by Fernandes et al. (2004) showed that the SFV patterns of undeleted chromosomes vary considerably among different branches of the Y-chromosome genealogy and that the patterns also vary among individuals within branches. These observations suggested that the link between SFV patterns and particular types of deletions would likely not be consistent across the worldwide diversity of Y chromosomes.

The diversity of SFV patterns in undeleted chromosomes is not surprising, since *AZFc* is subject to large inversions, deletions, and duplications caused by ectopic homologous recombination between amplicons (Kuroda-Kawaguchi et al. 2001; Repping et al. 2003, 2004). Such events would rearrange the locations of particular variants and would blur the association between SFV patterns and particular types of deletions. The association would likely be further blurred by gene conversion, which frequently erases small sequence differences (i.e., SFVs) between amplicon copies on the Y chromosome (Rozen et al. 2003).

We experimentally investigated the consistency of SFV patterns in different types of deletions involving *AZFc*. First, using the SFVs employed by Fernandes et al. (2004), we typed 20 men reported elsewhere to have the b2/b3 deletion (Repping et al. 2004) (see GenBank Web site for SFV assays). These men represented branch N and three other branches of the Y-chromosome genealogy (fig. 1). Second, using the same SFVs, we typed 40 men reported elsewhere to have the gr/gr deletion, the other common partial *AZFc* deletion (Repping et al. 2003). These men represented 14 branches of the Y-chromosome genealogy (fig. 1).

The b2/b3 deletions outside branch N showed diverse SFV patterns, and the gr/gr deletions showed even greater diversity (fig. 1). This greater diversity was likely due to the larger number of independent gr/gr deletions studied. Two branches, F*(xHK) and R1*x, contained numerous deletions and a high diversity of SFV patterns (fig. 1). In these branches, multiple independent deletion events probably account for the high diversity. By contrast, two other branches, D2b and N, contained numerous deletions but uniform SFV patterns. This uniformity is explained by the fact that all chromosomes in these branches descended from deleted founders (Repping et al. 2003, 2004; Fernandes et al. 2004). Thus, the chromosomes in each of these branches represent a single deletion event.

Our data also showed that the SFV patterns of b2/b3 and gr/gr deletions are not distinct from each other. For example, the b2/b3 pattern UUUCUU−CUUU (branch F*[xHK]) is more similar to the gr/gr pattern UUCCUU+CBUB (branch F*[xHK], four differences [*underlined*]) than to the b2/b3 pattern UBBBCU−CCUC (branch N, six differences). In another example, the gr/gr pattern UBBBCU−UBUB (branch R1*x) is more similar to the b2/b3 pattern UBBBCU−CUUC (branch I, three differences) than to the gr/gr pattern BCCCUB+CBCC (branch R1*x, 10 differences).

In conclusion, the SFV patterns of b2/b3 and gr/gr deletions vary widely and are not clearly distinct. SFVs can offer insight only if one knows the common SFV organizations in the genealogical branches represented by the Y chromosomes being tested. However, SFV organizations across the Y-chromosome genealogical tree are largely unknown, and SFV patterns vary even among individuals in the same branch. Just as important is that a large number of two-step assays are needed for SFV typing and for determining the Y-chromosome branch. By contrast, six simple plus/minus STSs distinguish between the deletions involving *AZFc* (table 1). Thus, plus/minus STSs provide a straightforward means of identifying and distinguishing the deletions of part of *AZFc,* whereas, in most situations, SFVs do not.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for STSs 50f2/C [accession number Y07728], sY142 [accession number G38345], sY1191 [accession number G73809], sY1192 [accession number G67166], sY1197 [accession number G67168], sY1201 [accession number G67170], sY1206 [accession number G67171], and sY1291 [accession num-

ber G72340] and for SFV assays DAZ-SNV I [accession number G73167], DAZ-SNV II [accession number G73166], sY586 [accession number G63907], DAZ-SNV IV [accession number G73168], sY587 [accession number G63908], DAZ-SNV VI [accession number G73169], Y-DAZ3 [accession number G73170], TTY4-SNV I [accession number BV012731], BPY2-SNV [accession number BV012732], GOLY-SNV I [accession number BV012733], and *AZFc* SFV 20 [AZFc-P1-SNV I] [accession number G73351])

Nature Genetics, http://www.nature.com/ng/journal/v29/n3/extref/ng757-S6.doc (for *AZFc* SFVs 18 and 20 in Web table E in Kuroda-Kawaguchi et al. 2001)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *AZFc*)

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