tions in *SRY*, one of which was also present in the patient's father and brother. To explain the difference in phenotype between male and female carriers of the same mutation in this pedigree and in two similar pedigrees described elsewhere (Berta et al. 1990; Jäger et al. 1990), the authors invoke pleiotropic effects of other genes, variation in the *SRY* target site, late action of the mutated *SRY* allele, and a threshold required to trigger testis determination. The increasing variety of subsidiary hypotheses put forward to prop up the concept of a single sex-determining gene demonstrates the weakness of this hypothesis in explaining the observed facts of sex differentiation. In accordance with Occam's razor, the concept of the hypothetical "testis-determining gene," *TDF*, thought at one time to produce the "testis-determining factor," should be discarded and be replaced by the more realistic working hypothesis based on a multifactorial system with threshold dichotomy (Mittwoch 1973, 1992). This would facilitate the task of understanding the role played by *SRY* and other genes in the process of sexual development.

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Reply to Mittwoch

To the Editor:

The testis-determining factor (*TDF*) was the name given to the genetic material on the Y chromosome that is normally required for testis determination. We and others have presented evidence that the gene *SRY* is equivalent to *TDF* and that *SRY* is the only Y-located gene required for testis formation.

It is obvious that *SRY* cannot operate in isolation and that it must interact directly or indirectly with other genes to bring about sex determination. *SRY* is "the sex-determining gene" only in the sense that the Y chromosome is the sex-determining chromosome. These observations make no predictions about how the sex-determination pathway works, although, like Professor Mittwoch, we have our own working hypotheses.

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Sensitivity of Single-Strand Conformation Polymorphism (SSCP) Analysis in Detecting p53 Point Mutations in Tumors with Mixed Cell Populations

To the Editor:

Mutations in the p53 tumor-suppressor gene are commonly found in human cancers of diverse origin (Nigro et al. 1989). One of a number of methods developed to analyze large numbers of DNA samples for specific mutations is the single-strand conformation polymorphism (SSCP) analysis (Orita et al. 1989). This method is particularly well suited for analysis of tissues, such as brain tumors, with mixed cell populations. It takes advantage of the fact that, in a mixed cell population con-

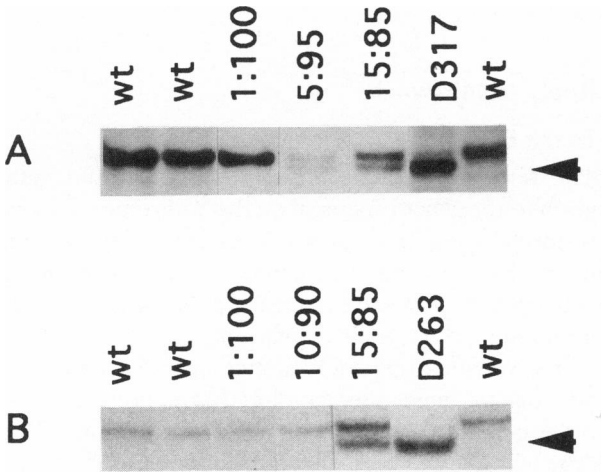

Figure 1 *A*, SSCP analysis of mixture samples containing different ratios of tumor D317 with a G-to-A point mutation in codon 272 of the human p53 gene and wild-type (wt) DNAs. The area of interest on the autoradiograph with the mutant allele is shown. Lanes 1, 2, and 7 contain wild-type leukocyte DNA from three different patients, demonstrating that the electrophoretic pattern of the wild-type allele is consistent in different individuals. Lane 3 shows the tumor-to-wild-type-DNA ratio of 1:100; lane 4 shows the tumor-to-wild-type-DNA ratio of 5:95; and lane 5 shows the tumor-to-wild-type-DNA ratio of 15:85. Lane 6 contains only the tumor DNA. The mutant p53 allele is indicated by the arrowhead, which is seen even when the tumor-to-wild-type-DNA ratio is 5:95 but which is not seen at 1:100. *B*, SSCP analysis of mixture samples containing different ratios of tumor D263 with a G-to-A point mutation in codon 175 of the human p53 gene and wild-type (wt) DNAs. Lanes 1, 2, and 7 contain wild-type leukocyte DNA from three different patients. Lane 3 shows the tumor-to-wild-type-DNA ratio of 1:100; lane 4 shows the tumor-to-wild-type-DNA ratio of 10:90; and lane 5 shows the tumor-to-wild-type-DNA ratio of 15:85. Lane 6 contains only the tumor DNA. The mutant p53 allele is indicated by the arrowhead, which is seen even when the tumor-to-wild-type-DNA ratio is 15:85 but which is not seen at 10:90 or 1:100. A total of 100 ng of DNA was amplified using a 10-μl two-step PCR protocol consisting of 30 cycles at 94°C (30 s) and 63°C (4 min). The PCR contained 1.25 mM magnesium chloride, 10 mM Tris-HCl (pH 8.3), 50 mM potassium chloride, 70 μM deoxynucleoside triphosphates, 0.05 μl α [^{32}P]dCTP (3,000 Ci/mmol; Amersham), 10 pmol of each of the amplifying primers (for tumor D317, 5′-ATAGAATTCCCTCGCTTAGTGC-TCCCTGG-3′ and 5′-TGAGTAGTGGTAATCTACTGGGACGG-3′; and, for tumor D263, 5′-CATCGCTATCTGAGCAG-3′ and 5′-TACTCCCCTGCCCTCAA-3′), and 0.5 units of AmpliTaq (Perkin Elmer Cetus). A 2-μl aliquot of the PCR product was added to 8 μl of sequencing stop solution (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol) and was boiled for 2 min to denature the sample DNAs. Three microliters of each denatured aliquot were then loaded onto a 5%–7% nondenaturing *bis*-acrylamide/TBE gel and electrophoresed in the cold room at 10–25 W for 4–6 h. The gels were dried and autoradiographed using an intensifying screen and exposure of 12–48 h at −70°C.

taining DNA with and without a mutation (e.g., the p53 gene mutation), both molecular species will be amplified by PCR. A mutation within a PCR-amplified DNA fragment will alter the secondary structure of the amplified fragment and affect its electrophoretic mobility in a nondenaturing gel. The DNA fragments with the mutation are detected as an aberrantly migrating allele that can be seen concurrently with the wild-type allele. Although many studies have used this technique to screen for p53 mutations in tumors, with detection of a number of different mutations (Mashiyama et al. 1991; Ohgaki et al. 1991; Fults et al. 1992; J. K. Wu, unpublished data), the limit of detection of point mutations in a background of wild-type DNA is not known. To test this, mixtures of mutant DNA from tumor D317 (with a G-to-A point mutation in codon 272 of the p53 gene; see Nigro et al. 1989, table 1) or from tumor D263 (with a G-to-A point mutation in codon 175 of the p53 gene) and wild-type DNA from leukocytes, in ratios of 1:100, 5:95, 10:90, 15:85, 50:50, and 30:70, were prepared. The mixtures containing 100 ng of DNA were amplified using standard PCR technique (Saiki et al. 1988). After the double-stranded DNAs were denatured, the DNA samples were loaded and electrophoresed on a nondenaturing acrylamide gel (for a detail of the methods, see fig. 1). Figure 1*A* shows a composite of the experimental results with tumor D317, and figure 1*B* shows a composite of the experimental results with tumor D263. The mutant allele was detectable even when the ratio of mutant to wild-type DNA was 5:95 in tumor D317 (fig. 1*A*, lane 4). When the ratio was dropped to 1:100 (fig. 1*A*, lane 3), only the wild-type pattern was seen. For tumor D263, the mutant allele was detectable when the ratio of mutant to wild-type DNA was 15:85 (fig. 1*B*, lane 4), and it was not detectable at 10:90 (fig. 1*B*, lane 3). These results demonstrate that SSCP is a sensitive technique useful in screening tumor samples with mixed cell populations containing both the mutant and wild-type p53 alleles, even when the background normal wild-type allele is as much as 85%–95% of the total population of the p53 alleles amplified. This estimate of sensitivity might, in fact, be a conservative one, since the tumor DNA samples (i.e., D263 and D317) utilized in the study contain only one 17p allele (Nigro et al. 1989), whereas the wild-type leukocyte DNA contains two 17p alleles. In most tumor-tissue specimens used for DNA extraction, the degree of contamination with normal cells is usually less than 50%; therefore, mutant alleles should be easily detectable by SSCP analysis.

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On the Use of Excess Homozygosity for Subpopulation Detection

To the Editor:

The National Research Council's (1992) report "DNA Technology in Forensic Science" expresses concern about potential population substructures in racial data bases, leading to erroneous conclusions from genotypic frequencies calculated under the "product rule" (Budowle et al. 1991). Critics have argued that instances of excess homozygosity (Lander 1989; Cohen 1990) by the Wahlund test (Wahlund 1928) are the evidence for such substructuring.

The Wahlund test assumes that identical alleles are so by descent as well as in state. We present data to show that this requirement is not necessarily fulfilled. Devlin et al. (1990, 1992) and Chakraborty et al. (1992) have shown that an excess of apparent homozygotes need not be due to population substructuring but could be a consequence of analytical artifacts—namely, band coalescence and "covert" alleles. Our results with modified electrophoretic conditions and different restriction enzymes confirm their predictions.

The probe YNH24 (Promega) for the VNTR locus D2S44 (Nakamura et al. 1987) was used to reexamine 23 African-American samples with single-band patterns (SBP) in *Hae*III-restricted DNA, as determined by stan-

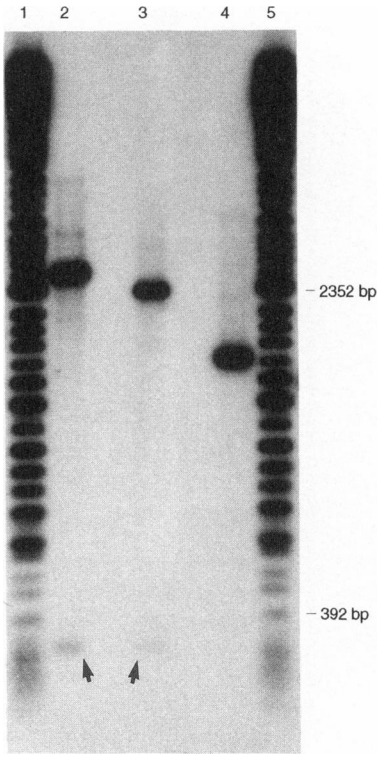

Figure 1 Covert alleles in *Hae*III-digested DNA. Electrophoretic conditions were as follows: 1.5% agarose, 17-cm gel, 2 V/cm, and 5.5 h. Shown are size markers (lanes 1 and 5), covert alleles (lanes 2 and 3, marked by arrows), and SBP (lane 4). Two covert alleles are shown, of seven found.