and subdividing by seven or eight age strata (35–37, 38, 39, 40, 41, 42, 43, and ≥44 years for 47,+21 and the same except for pooling of those aged 42 and 43 in analysis of the other abnormalities). For 47,+21 the age-adjusted summary relative risk for 47,+21 in Milan compared with the other two centers was 0.87 (95% confidence interval 0.44–1.69); for all other abnormalities, excluding lethals, the age-adjusted summary relative risk was 1.74 (95% confidence interval 0.82–3.70); and for all other abnormalities, including lethals, the summary relative risk was 1.67 (95% confidence interval 0.85–3.28). There is still a suggestive trend to an increase of non-47,+21 abnormalities in Milan, but it is now not nominally significant.

Dr. Simoni also informs us that the mosaics from the Milan report in fact *were* "discrepancies," so this explains the apparent difference between Milan and the two other laboratories, at which there were very few (nondiscrepant) mosaics and about which we puzzled in our Discussion. He also noted some amplifications of the original data set that we received. (These were not typographical errors in the publication but errors in the original summary we received for analysis.) In brief, the case at age 36 years is 46,+11, +18 (not +8); the case at age 39 years is +der13 (not +der1?); and, at age 43 years, the mosaic case with +21 also has a mosaic +11 line (it was denoted as +?). Fortunately, these corrections at least have no bearing on the main analysis.

Last, we note that the cytogenetic diagnoses cited were made by Dr. Simoni in his laboratory on specimens provided by Dr. Brambati, coauthor of the paper. Dr. Brambati forwarded the original data summary to Dr. Pergament, who had recruited Dr. Brambati into the study, and Dr. Pergament then forwarded these data to us for analysis. We thank Dr. Simoni for calling our attention to these matters.

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Am. J. Hum. Genet. 45:477, 1989

Re: Pellestor et al. on Sperm Chromosomes

To the Editor:

It is well known that language barriers can create confusion. This is the case with the article by Pellestor et al. (*Am J. Hum. Genet.* 44:464–473, 1988) on sperm chromosomes. In a footnote to table 6 they refer to one of our papers (Templado et al. 1988) and state textually: "The t(2;5) translocation has been considered despite the fact that most of its adjacent 1 and alternate segregations have been misidentified."

Of course, anyone will understand that we were unable to correctly identify these segregations. What they meant to say is exactly what we said in our paper—i.e., that, owing to the similar banding patterns of the translocated segments, only 22 of 55 alternate or adjacent 1 segregations could be exactly identified. The remaining 33 were described by us as "alternate or adjacent 1." Unfortunately, what tried to be an explanatory note unwillingly became a defamation.

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Am. J. Hum. Genet. 45:477–479, 1989

Methylation at the D4S95 Locus and Predictive Testing

To the Editor:

We have described a variable number of tandem repeat (VNTR) and a single-site-variation polymorphism detected with D4S95 that is closely linked but centromeric to the Huntington disease (HD) gene (Wasmuth et al. 1988; Robbins et al. 1989). Pritchard et al. (1989) have shown that there is variable methylation occurring within the locus recognized by D4S95 and have suggested that this is occurring at the B5 *Acc*I allele at 4.1

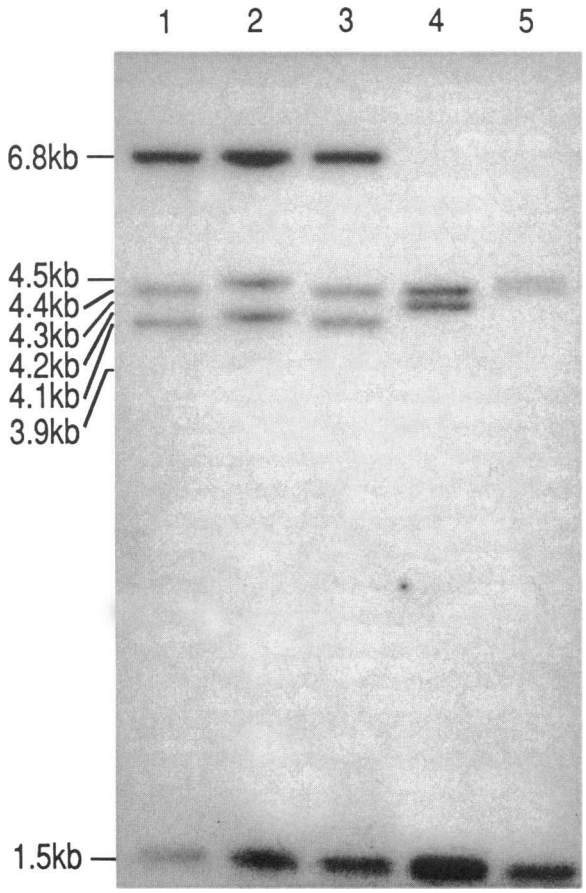

Figure 1 Autoradiograph of *Acc*I-digested DNA from blood of five individuals that was hybridized with D4S95. All alleles of the VNTR polymorphism are indicated, from 4.1 kb to 4.5 kb. The single-site *Acc*I polymorphism results in the 6.8-kb and/or 1.5-kb bands. A lighter band appears at 3.9 kb whenever the 6.8-kb band is present in either the homozygous or heterozygous state (lanes 1–3) but is not present when the person is homozygous for the 1.5-kb band (lanes 4, 5).

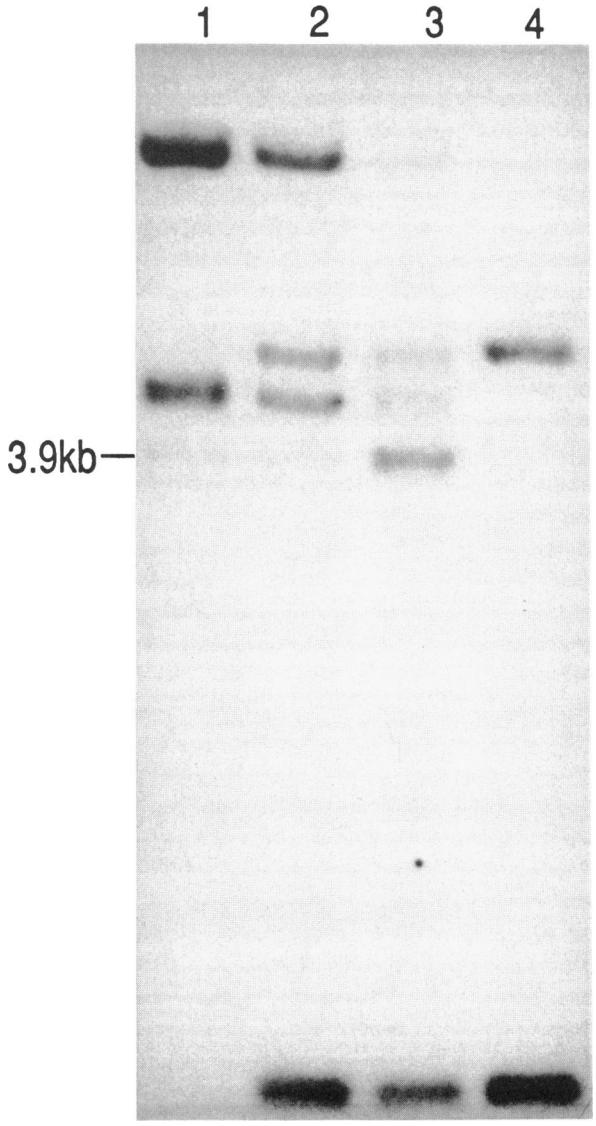

Figure 2 Autoradiograph of *Acc*I-digested DNA from blood (lanes 1, 2, 4) and brain tissue (lane 3) hybridized with D4S95. The intensity of the 3.9-kb band in brain tissue is much greater than that in blood, suggesting variable methylation at this locus.

kb. However, in our experience, after analysis of DNA from white blood cells, brain tissue, and lymphoblastoid cell lines from more than 600 HD and non-HD persons, in more than 50 families, we have always seen the variable band at 3.9 kb beneath the B5 allele. In other words, the variable band is smaller than the smallest allele of the VNTR.

We agree that the fragment showing variability is not inherited in a Mendelian fashion. However, this should not lead to misinterpretation of the data, because this band is not assessed when looking at the restriction map for polymorphisms recognized by D4S95.

Our data suggest that the site of variable methylation in the *Acc*I region is more likely to be 5′ to the D4S95 locus, on the side of the single-site-variation polymorphism. For example, in figure 1 (lanes 4, 5) the 6.8-kb fragment is absent because this person is homozygous for the presence of the *Acc*I site resulting in a 1.5-kb fragment. In this instance the 3.9-kb fragment is also absent, suggesting that this site of variable methylation is *5′ to the Acc*I site that results in a 1.5-kb fragment. Variability in methylation of this particular 3.9-kb frag-

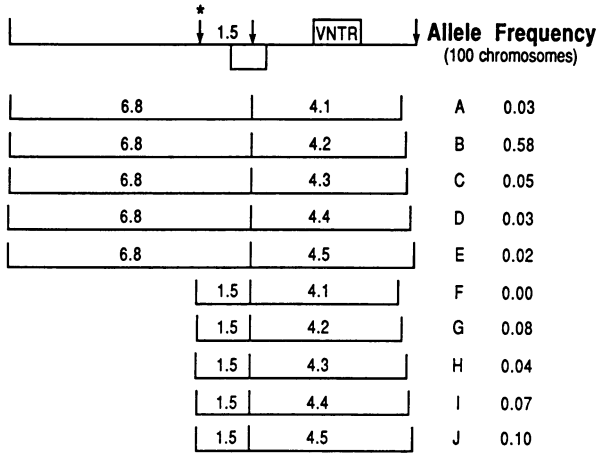

Figure 3 Schematic diagram of the *Acc*I alleles identified by D4S95. There are at least 10 possible combinations when the single-site polymorphism (denoted by the asterisk) and the VNTR are combined. D4S95 is indicated by the unshaded box.

ment in different tissues appears to be related to the intensity of the 6.8-kb fragment. For example, in brain tissue (fig. 2, lane 3), where this restriction site may be undermethylated, the 6.8-kb fragment is barely seen and the 3.9-kb fragment is seen with much greater intensity.

The fragments of the VNTR—including B1, B2, B3, B4, and B5—are always inherited in a Mendelian fashion. Hybridization of *Acc*I-digested DNA with D4S95 detects two polymorphisms that are in equilibrium, and thus they can be combined to form 10 haplotypes as shown in figure 3. This polymorphism is very useful for predictive testing for HD, not only because of its high frequency of heterozygosity and ability to detect a large number of alleles but also because it can be used in a single hybridization experiment with DNA digested by other enzymes such as *Taq*I and *Mbo*I, which also detect polymorphisms around this locus. D4S95 is presently still the most useful single probe for predictive testing for HD.

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