Letters to the Editor

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Methylation at the Huntington Disease–Linked D4S95 Locus

To the Editor:

In a paper published in *Nature* last year, Wasmuth et al. (1988) reported the identification of a highly polymorphic locus, *D4S95*, which they demonstrated to be tightly linked to the Huntington Disease (HD) gene. The probe (pBS674E-D) for the locus they described detects five polymorphic sites with four different restriction enzymes. *Acc*I is th. most informative of the four enzymes, as the probe detects two polymorphic sites, one of which is a two-allele system (site A) and the other of which is a multiallele system with at least five different allelic fragments (site B; fig 1). Because of the high PIC and heterozygosity value of this allele system (fig. 1), Wasmuth et al. (1988) recommended its use in predictive testing for HD. Two reports have now appeared, one describing the use of *D4S95 Acc*I polymorphisms in the analysis of a family showing a distal recombination event with HD (Hayden et al. 1988*b*) and one in which the allele system is used for presymptomatic diagnosis (Hayden et al. 1988*a*).

Recently, we utilized the pBS674E-D probe and *Acc*I allele system to analyze 56 DNA samples isolated from lymphoblastoid cell lines of members of the Venezuelan HD pedigree. To our surprise, we discovered three individuals with three alleles of the B type, each of which contained a B5 allele. Analysis of blood DNA from 12

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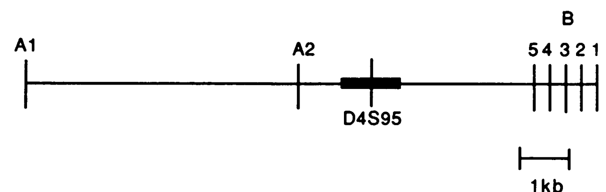

Figure 1 *Acc*I alleles at the *D4S95* locus. An *Acc*I site is present in the probe and reveals polymorphic sites in both directions. Site A is a two-allele system with a PIC of .33. Site B has a large number of alleles (at least five) and a PIC of .67 (Wasmuth et al. 1988). More than 90% of Caucasian individuals are heterozygous for one or both allele systems. For most DNA samples the B5 site is methylated, so that only the other, larger B alleles are seen. If the B5 site is not methylated, as is the case for some DNA samples, then the B5 allele appears. Three alleles are seen if the B5 site is partially methylated and if the individual is heterozygous for two other, larger B alleles.

non-HD individuals also demonstrated the presence of three B alleles in five samples, with the B5 allele being present in each case. As the appearance of the extra band could not be explained easily by mosaicism or allele duplication in all of the cell lines and blood DNA, we attributed this finding to partial cleavage of the B5 site by *Acc*I. This seemed the most likely explanation, since the intensity of the B5 allele was variable in each of the eight samples. We further predicted the B5 site to have the recognition sequence 5′-GTCGAC-3′, rather than one of the other three possibilities for *Acc*I (5′-GTPyPuAC-3′), such that it would be subject to cytosine methylation at an internal CpG dinucleotide. Methylation at the CpG site would then prevent the appearance

of the B5 allele, as seen for most DNA samples, whereas variable levels of methylation would produce the B5 band as well as an additional, larger B allele (fig. 1). To confirm this prediction, we grew several of the cell lines in the presence of the demethylating agent 5-azacytidine and were able to increase the intensity of the B5 allele in each of the three cases. 5-Azacytidine also induced the appearance of the B5 allele in cell lines previously not showing the presence of this allele. As an independent test, we treated DNA samples with *Taq*I methylase, which adds a methyl group to the A residue of the sequence 5′-TCGA-3′, and we were able to prevent the appearance of the B5 allele on digestion with *Acc*I, confirming indeed that the B5 *Acc*I site is of the type 5′-GTCGAC-3′. Only one of the other two B alleles was changed in intensity by the 5-azacytidine and *Taq*I methylase treatments, suggesting that the B5 site is polymorphic as well as being subject to cytosine methylation.

Our findings have important implications regarding the use of the *Acc*I allele system in predictive testing for HD. The B5 allele is not inherited in a Mendelian fashion, as its appearance depends on the methylation status of the cells from which DNA samples are obtained. This clearly creates the potential for a misinterpretation of data, which, in turn, could lead to an error in the diagnosis of HD. Such a mistake could be made if, for example, an affected mother with the B5 HD haplotype does not apparently pass on the disease to her son because his B5 allele is completely methylated and appears as B4. This B4 allele would then have to be mistaken either for her normal allele or for an allele inherited from his unaffected father. Although these types of errors are likely to be rare, the accuracy of diagnosis with the *Acc*I allele system is reduced by the methylation problem. Methylation at the B5 locus could also complicate the analysis of *D4S95* inheritance in family linkage studies and may lead to the identification of false recombination events. We therefore suggest great care be taken in the use of the *Acc*I allele system and recommend that the other polymorphic sites described for *D4S95*, although less informative, are used to check the haplotypes for this locus.

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Glasnost and Perestroika in Human Genetics

To the Editor:

Last June I accepted an invitation from Dr. Nikolai P. Bochkov, director of the Institute of Medical Genetics in Moscow, to spend 10 d visiting his institute and traveled with my wife to Russia, a country I had never visited before. As we all know, since 1940 Russian genetics has been forced into almost complete dormancy, and it is only during the past 20 years, approximately, that it has been allowed to function again. The process of resurrection was long, but glasnost and perestroika gave promise that contacts can become as frequent and friendly as is desirable. This can only help Russian genetics to again take its eminent place in the international world of science.

The strong desire to resume exchanges, not only of information but also of people, and a clear interest in establishing collaborations is witnessed by a letter I have recently received from Dr. Tatiana Lomova, who is in charge of international relations at the Institute of Medical Genetics in Moscow. I think some firsthand information I can supply could serve to facilitate this aim.

The Institute of Medical Genetics is located on the periphery of Moscow, fairly close to the Oncology Center, a huge building with hospitals and with research laboratories. We received gracious and comfortable hospitality in the "house of scientists" of the Oncology Center and were given daily escort and transportation. This is a blessing in a city of the size of Moscow, in which the English language is not spoken by many and in which taxis are rare. The program of our visit was