

LETTER TO THE EDITOR

Avoiding errors in the diagnosis of (CAG)_n expansion in the huntingtin gene

Huntington's disease (HD) is an autosomal dominantly inherited disorder with nearly complete penetrance but variable age at onset. The disease affects 1 in 10 000 people of European origin.¹ Typically, first symptoms occur in middle age but the disease may begin at any time between early childhood and old age. The disease manifests with psychiatric or neurological signs or both. Underlying the clinical symptoms of HD is a selective loss of neurons that occurs throughout the brain, most importantly in the caudate and putamen. So far there is no effective treatment to stop the neurodegenerative process leading to progression of the disease over 10 to 20 years until death. Recently, an unstable (CAG)_n trinucleotide repeat in a gene of unknown function has been identified, which is expanded in HD patients.² In normal subjects, the number of (CAG)_n repeats varies from 10 to 36, while in HD patients it ranges from 37 to >100.³⁻⁶ A (CCG)_n trinucleotide block lies immediately adjacent to the unstable (CAG)_n repeat and may also vary in size between seven and 12 repeats. However, the (CCG)_n repeat length shows no correlation with the age at onset of HD.⁷

Estimating the size of the (CAG)_n repeat is widely used to determine whether a person at risk for HD has inherited the mutant gene or to confirm or exclude HD as the cause of neurological/psychiatric symptoms in symptomatic patients.⁸ Several PCR based methods were originally developed that amplify a region encompassing both the (CAG)_n repeat and the adjacent (CCG)_n polymorphic tract.⁹ To prevent inaccuracies in estimating the size of the (CAG)_n repeat because of the variable size of the adjacent (CCG)_n repeat, an additional primer pair with one primer annealing between and partially at both repeats has been generated to amplify exclusively the (CAG)_n repeat.¹⁰ Recently, a small deletion in HD chromosomes from two

unrelated families was found to occur in the region of this primer attachment site preventing PCR amplification of the expanded allele.¹¹ Failure to amplify the mutated (CAG)_n repeat would result in misinterpretation of the diagnostic test excluding the diagnosis of HD in affected patients or failing to define the at risk status in unaffected offspring. In consequence, "homozygosity" of the detected "normal" allele would be given as the test result. Furthermore, the mutation might be missed because of insufficient amplification of very long repeat expansions occurring commonly in juvenile cases.¹² We therefore generated a genomic DNA probe from the promoter region of the human huntingtin gene to establish a detection system of the expanded allele by Southern blotting. This procedure is not sensitive to any polymorphisms at primer annealing sites flanking the (CAG)_n repeat and detects even extremely expanded alleles absolutely reliably. Since, however, the (CAG)_n repeat is flanked by the polymorphic (CCG)_n block, the fragment shown by hybridisation includes this variability.

In brief, a 1.4 kb *Pst*I fragment from cosmid 191F1 derived from a flow sorted human chromosome 4 specific genomic cosmid library⁴ containing exon 1 and part of the promoter region of the human huntingtin gene was subcloned into the plasmid vector pCAT-basic (Stratagene). An internal 300 bp fragment was released by restriction enzyme digestion with *Ava*I. This fragment was used to hybridise a Southern blot containing *Pst*I digested genomic DNA from controls (fig 1, lanes 1-3) and HD patients (fig 1, lanes 4-11). In each case, the expanded allele can clearly be distinguished from the normal allele. Polymorphisms of the *Pst*I restriction sites were not observed.

Since introduction of direct mutation detection for HD, approximately 900 patients and persons at risk have been analysed by PCR amplification of the (CAG)_n repeat in our laboratory. Of these, 55 (6%) were homozygous for one of the normal alleles. In these cases, test results should be reported with caution by explaining the limits of the PCR approach. In our laboratory, we routinely test people exhibiting one allele exclusively by Southern blot hybridisation.

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Repeat length	$\frac{17}{9}$	$\frac{15}{15}$	$\frac{17}{17}$	$\frac{36}{26}$	$\frac{39}{15}$	$\frac{42}{20}$	$\frac{46}{19}$	$\frac{48}{17}$	$\frac{54}{17}$	$\frac{58}{20}$	$\frac{65}{17}$
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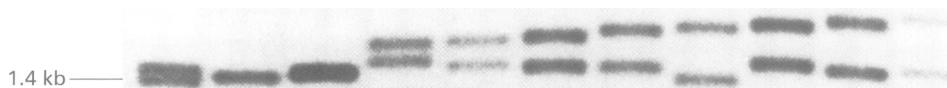


Figure 1 Southern blot analysis of the 5' region of the Huntington's disease gene detecting normal and expanded alleles. DNA from controls (lanes 1-3) and affected HD patients (lanes 4-11) was digested with *Pst*I, separated electrophoretically on a 0.8% agarose gel, and transferred onto a nylon membrane. Probe *h-Cat1* was radioactively labelled by random priming and hybridised in Church buffer¹⁵ at 65°C. Subsequently, filters were washed in 0.1 × SSC, 0.5% SDS for 30 minutes at 65°C and exposed to x ray films. A 1.4 kb fragment is detected for (CAG) repeat lengths of 18 units. The (CAG)_n repeat sizes as estimated by PCR analysis¹⁰ are shown at the top of each lane.

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