Huntington disease (HD) is a neurodegenerative disorder caused by an expanded trinucleotide repeat (CAG)\textsubscript{n} located at the 5' end of the novel \textit{IT15} gene. Discovery of this expansion allows the molecular diagnosis of HD by measuring repeat length. We applied a simple nonisotopic method to detect (CAG)\textsubscript{n} repeats, avoiding both radioactive and Southern transfer analysis. The assay is based on direct visualization of electrophoresed PCR products, after silver nitrate gel staining. Its accurate sizing of HD alleles allows presymptomatic diagnosis of at-risk persons. By avoiding isotopic manipulations, the method is safe and accurate, with no radioactive background bands. Furthermore, because it permits direct allele visualization after gel staining, the method is simple and rapid, allowing allele sizing within hours rather than days.

INDEXING TERMS: molecular genetics • expanded trinucleotide repeats • polymerase chain reaction • electrophoresis, polyacrylamide gel

Huntington disease (HD) is a progressive neurodegenerative disease of midlife onset, inherited in an autosomal dominant manner, that affects 1:10 000 individuals [1]. The clinical picture is characterized by involuntary movements, psychiatric changes, intellectual and cognitive decline, and dementia. The symptoms of HD appear to be caused by marked neuronal death, most notably in the caudate nucleus and putamen [2].

The mutation responsible for HD has recently been discovered as an expansion of a CAG trinucleotide repeat located at the 5’ end of a novel \textit{4p16.3} gene, named \textit{IT15} (interesting transcript 15) [3]. The repeat is polymorphic in the normal population, varying between 8 and 36 units on normal chromosomes, but is expanded to at least 37 copies on HD chromosomes [3–7]. A significant inverse correlation between the size of the CAG repeat and the age of onset of symptoms has been observed in HD, especially when the repeat is >50 [8–10].

The discovery of the defect causing HD allows the direct presymptomatic diagnosis of the disease through measuring the number of CAG repeats in the DNA of a person at risk. Until now, the procedures used to detect the length of this trinucleotide repeat required radioactive analysis—radiolabeled polymerase chain reaction (PCR) and Southern transfer [3, 11, 12] or chemiluminescent detection of biotied PCR products [13].

We have applied a simple and rapid method for HD diagnosis avoiding both radioactivity and Southern transfer analysis. The system involves sample PCR, separation of alleles on polyacrylamide gels, and staining with silver nitrate. The new PCR conditions we describe improve the yield of the product, allowing direct visualization of HD alleles on silver nitrate-stained polyacrylamide gels.

**Materials and Methods**

\textit{Subjects}. Seven families affected by HD, including 13 affected and 20 unaffected individuals, were analyzed. Diagnosis of HD on the basis of clinical symptoms was made either by private neurologists or at the Hospital of Calabria.

\textit{PCR assay}. Genomic DNA was extracted with an automated DNA extractor (Applied Biosystems, Foster City, CA). A double PCR profile, using a total of 35 cycles, was carried out in a 9600 DNA thermal cycler (Perkin-Elmer, Norwalk, CT). After an initial denaturation of 2 min at 96 °C, there were 12 cycles at 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 2 min, followed by 23 cycles at 92 °C for 30 s, 65 °C for 30 s, and 72 °C for 2 min; final extension was at 72 °C for 10 min.

The PCR was carried out in a final volume of 25 mL with the primers HD1 (5’-ATGAGGTCCTGAGTCCCTCAAGTGCTTC-3') and HD3 (5’-GGCGTGCGGCTTGTGTTCGGTCTGCTGCTGCTGCTG-3') [14]. Reaction mixtures contained 2 mmol/L MgCl\textsubscript{2}, 16.6 mmol/L (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 67 mmol/L Tris-HCl, pH 8.8, 67 μmol/L Na\textsubscript{2}EDTA, 35 mL/L formamide, 10 mmol/L β-mercaptoethanol, 2.5 μmol/L bovine serum albumin, 200 mmol/L of each dNTP (with a final ratio of 1:3 dGTP: 7-deaza-dGTP), 12.5 pmol each of HD1 and HD3, 1.25 U of Taq polymerase, and 250–500 ng of genomic DNA. From each
amplified DNA sample, 5 μL was tested on 3% agarose gel with Tris-acetate-EDTA buffer (0.04 mol/L Tris-acetate, 0.001 mol/L EDTA, pH 8.0) containing 0.02 g/L ethidium bromide. After electrophoresis, the DNA was visible under ultraviolet light.

**Allele sizing.** The remaining 20 μL of PCR product was precipitated with cold ethanol and electrophoresed through an 8% nondenaturing polyacrylamide gel (acylamide: bisacrylamide = 19:1) at 500 V for 17 h at 4 °C. For better resolution of normal alleles, we used 10% gel when analyzing DNA from normal subjects. After electrophoresis, the gels were stained with silver, as follows [15]: wash in 4.607 mol/L ethanol solution for 5 min; oxidize in 0.6301 mol/L nitric acid solution for 3 min; rinse in distilled water for few seconds; reduce in a solution of 0.28 mol/L anhydrous sodium carbonate and 6.327 μmol/L formaldehyde, with several changes of the reducing solution (each time the solution turned brown); stop the reducing process with 6.005 mol/L glacial acetic acid for 10 min; and wash in distilled water for 2 min. The size of the polymorphic HD alleles was detected after silver nitrate staining by comparison with both DNA molecular marker V (Boehringer Mannheim, Mannheim, Germany) and previously sequenced alleles.

**Results and Discussion**

Discovery of the gene responsible for HD has had a great impact in the diagnostic field, making it possible to do presymptomatic and prenatal diagnosis of HD by recombinant DNA techniques. In the first published studies on HD alleles, the DNA region containing the HD mutation was amplified with original primers HD1 and HD2 [3], which spanned the CAG trinucleotides as well as an adjacent CCG repeat. When this CCG repeat was found to be polymorphic [16, 17], a new set of primers was designed that selectively amplified the CAG repeat and excluded the CCG polymorphic region [14] (Fig. 1). However, the high repetitiveness of the HD-region, together with its high GC content, make the PCR analysis very difficult, so that the described amplification procedures often fail to detect the upper alleles and radioactive analysis is needed to distinguish between a normal individual and an affected one.

The method described here allows rapid and precise diagnosis of HD. To size the CAG repeat accurately, we used HD1 and HD3 primers (see Fig. 1) that exclude the polymorphic CCG repeat, thus allowing correct HD diagnosis even in borderline cases. The procedure, involving nonradioactive PCR of the

![Fig. 1. 5' Polymorphic region of IT15 gene.](image)

Original HD1 and HD2 primers span both CAG-repeat and CCG repeat, whereas HD3 primer, used together with HD1 primer, selectively amplifies the CAG repeat.

![Fig. 2. PCR analysis of trinucleotide repeats in HD patients.](image)

PCR products are separated on 8% polyacrylamide gel, stained with silver nitrate. N, normal alleles; Exp, expanded alleles.
The accurate detection of the size of CAG repeats is essential for HD diagnosis, and the use of HD1 and HD3 primers is necessary to avoid diagnostic mistakes in individuals carrying borderline numbers of repeats. By optimizing PCR conditions, one can obtain an accurate and rapid sizing of both normal and expanded HD alleles.

In summary, the method described here offers many advantages over the published procedures for sizing HD alleles. No isotopic manipulations are involved, making the method both safe and accurate, because of the absence of radioactive background bands. Previously published nonradioactive assays [13, 21], as performed with the originally recommended primers, were not suitable for detection of borderline-repeat alleles. Furthermore, because it permits direct visualization of alleles after gel staining, our simple and rapid method allows allele sizing within hours rather than days.

References