ACMG/ASHG STATEMENT
Laboratory Guidelines for Huntington Disease Genetic Testing

The American College of Medical Genetics/American Society of Human Genetics
Huntington Disease Genetic Testing Working Group

Introduction

Genetic testing for Huntington disease (HD), by direct analysis of the CAG repeat within the IT-15 gene that encodes the huntingtin protein, has been performed, for clinical purposes, since the summer of 1993. HD was one of the first of what is now a group of "CAG-repeat disorders" for which direct gene analysis is possible; these disorders include spinocerebellar ataxia types 1, 2, 3, and 6; Kennedy disease; and dentatorubropallidoluysian atrophy (LaSpada et al. 1991; Huntington's Disease Collaborative Research Group [HDCRG] 1993; Orr et al. 1993; Kawaguchi et al. 1994; Koide et al. 1994; Nagafuchi et al. 1994; Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996; Zhuchenko et al. 1997). HD remains the most commonly tested CAG-repeat disorder in the United States.

At the October 1996 Workshop of U.S. HD Genetic Testing Centers, held in San Francisco, results of surveys of clinical centers and laboratories performing genetic tests for HD were presented. The total number of IT-15 gene tests performed by 15 laboratories (of 26 surveyed) through mid-1996 was 2,941. Extrapolating from these figures, we can estimate that ~4,700 IT-15 gene tests were performed in the United States from 1993 to mid-1996. A similar number of IT-15 gene tests have been reported in the literature (Nance 1996). The need to standardize the methods and terminology used by clinical laboratories performing these tests was recognized, which led to the formation, in December 1996, of an American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group. The charge to this group was to develop practice guidelines for the laboratory aspects of genetic testing for HD. The following recommendations of the working group are based on a review of the published literature, the results of the 1996 surveys, and formal and informal discussions with representatives of ~25 laboratories and ~75 clinical centers involved in HD genetic testing.

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Address for correspondence and reprints: Dr. Martha A. Nance, Park Nicollet Clinic, 6490 Excelsior Boulevard, Suite E500, St. Louis Park, MN 55426. E-mail: mnance@wavefront.com

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Recommendations

A. Standardization and Interpretation of IT-15 Gene $(CAG)_n$ Repeat Ranges

Both the published literature and unpublished experience support the definition of four diagnostic categories for CAG-repeat lengths in exon 1 of the IT-15 gene, which is shown diagrammatically in figure 1. Laboratories should define CAG-repeat ranges as shown below until additional evidence supports a change or changes in the definitions. The working group supports the standardized use of the CAG category descriptors given below. The CAG-repeat ranges and their descriptions are shown in table 1.

Allele sizes of $\leqslant$26 CAG repeats have never been associated with an HD phenotype in the U.S. survey or in any published study. Allele sizes of $\leqslant$26 CAG repeats have not been demonstrated to show mutability (meiotic instability that results in an HD allele in offspring) (HDCRG 1993; Duyao et al. 1993; Benjamin et al. 1994; Kremer et al. 1994). Although it has not been shown that alleles with $\leqslant$26 repeats cannot be mutable, no examples of mutable alleles of this size have been reported, to date. The shortest reported CAG-repeat length in a human IT-15 gene is nine CAG repeats; there is no evidence that smaller repeat lengths cannot occur or that they would lead to an abnormal phenotype.

Allele sizes of 27–35 CAG repeats have not been associated convincingly with an HD phenotype (Kremer et al. 1994; Rubinsztein et al. 1996). Alleles of 27–35 CAG repeats have demonstrated meiotic instability in sperm (Leeflang et al. 1995; Telenius et al. 1995; Chong et al. 1997). An allele with 27 CAG repeats was reported in the father of an HD-affected individual with 38 repeats (McGlennan et al. 1995), and larger alleles in this range have also demonstrated mutability when transmitted by a male (see Definitions, below) (Goldberg et al. 1993; HDCRG 1993; Myers et al. 1993). Approximately 1.5%–2.0% of alleles reported in the U.S. survey

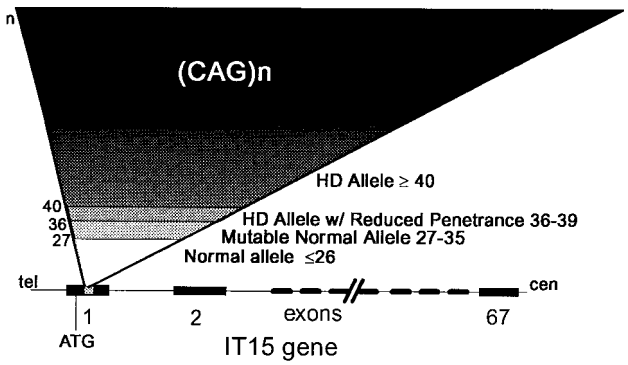

Figure 1 Diagram of the IT-15 gene, showing the location of the CAG-repeat sequence and the boundaries defining the ranges of normal, mutable normal, HD allele with reduced penetrance, and HD allele with full penetrance, based on the worldwide published experience and the experience of the US Huntington Disease Genetic Testing Group.

and in the literature have 27–35 repeats (Goldberg et al. 1995). The biological mechanisms underlying meiotic instability are not known in detail; however, the frequency of CAG-repeat instability in sperm is known to increase with increasing CAG-repeat number (Zühlke et al. 1993; Kremer et al. 1995; Leeflang et al. 1995; Telenius et al. 1995). The likelihood that transmission of an allele in the 27–35-repeat range will result in an HD allele (mutability) is unknown but depends on how close the allele size is to the HD threshold as well as on the frequency and magnitude of expansions, which are dependent on the sex of the transmitting individual and on other unknown factors. More clinical research is required before accurate or specific mutability risk figures can be given to individuals, particularly women, with allele sizes of 27–35 repeats.

Allele sizes of 36–39 repeats have been associated with the HD phenotype in cases documented clinically (Kremer et al. 1994; Legius et al. 1994; Rubinsztein et al. 1996) and pathologically (Persichetti et al. 1994). Alleles in this size range are abnormal. However, the HD phenotype is not always penetrant in individuals with HD alleles in this size range. Alleles of 36–39 CAG repeats have been reported in some clinically unaffected individuals aged >70 years (Legius et al. 1994; Rubinsztein et al. 1996; McNeil et al. 1997; U.S. HD Genetic Testing Group, unpublished data). Empirical penetrance risks for the HD phenotype at CAG-repeat lengths of 36, 37, 38, and 39 repeats have not been published; ascertainment biases preclude the use of current or published data for this purpose.

The absence of HD pathology has not been documented in any individual with an HD allele size of $\geqslant 40$ CAG repeats who died, disease-free, after living up to or past the normal life expectancy. There are, however, individuals who do not develop symptomatic HD until the 9th decade and individuals with repeat sizes of $\geqslant 40$ who die, disease-free, "prematurely."

Table 1

CAG-Repeat Length Categories and Descriptors

Category Description	CAG-Repeat Range	Predicted Phenotype
Normal allele	$\leqslant 26$	Normal
Mutable normal allele	27–35	Normal
HD allele with reduced penetrance	36–39	Normal/HD
HD allele	$\geqslant 40$	HD

The working group *strongly recommends* that laboratories uniformly adopt the above-noted ranges and descriptions in their interpretation of IT-15 gene test results. Reports should reflect what is currently known about the tested individual's risk of developing HD as well as the risk that the individual's offspring could have a similar or larger allele size.

It should be noted that essentially all published data about IT-15 exon 1 CAG-repeat lengths are based on studies of lymphocyte DNA and may not reflect allele sizes in other tissues, such as testis/ovaries, amniocytes, or the CNS. Limited data suggest that CAG-repeat lengths are stable in lymphoblasts, chorionic villus tissues, and somatic tissues other than brain (MacDonald et al. 1993; Zühlke et al. 1993; Benitez et al. 1995). Mosaicism for CAG-repeat length has been reported in sperm and in CNS (MacDonald et al. 1993; Telenius et al. 1994).

B. Definitions

IT-15 gene.—The gene encoding the huntingtin protein. Abnormal expansion of a polymorphic CAG-repeat sequence within exon 1 of the IT-15 gene causes Huntington disease.

HD allele.—An IT-15 gene with $\geqslant 36$ CAG repeats in the polymorphic CAG-repeat region in exon 1. The HD phenotype is not always penetrant for CAG-repeat sizes of 36–39 repeats but appears to be fully penetrant for allele sizes of $\geqslant 40$ repeats.

Normal allele.—An IT-15 gene with $\leqslant 35$ CAG repeats. These alleles do not cause HD. Normal alleles of any size may exhibit meiotic instability, albeit infrequently, but only alleles of 27–35 repeats have been shown to exhibit mutability (see below).

Meiotic instability.—The ability of an allele to increase or decrease CAG-repeat length during meiosis, such that the modal CAG-repeat number present on an IT-15 allele in an individual's somatic cells is not the same as the repeat number(s) present on the same allele in his or her gametes. Meiotic instability for CAG-repeat length in

the IT-15 allele has been demonstrated directly by analysis of sperm DNA (MacDonald et al. 1993; Telenius et al. 1993, 1995; Goldberg et al. 1995; Chong et al. 1997). Meiotic instability of trinucleotide repeat sequences has been demonstrated directly in human oocytes for fragile X syndrome (Malter et al. 1997) but has only been inferred in HD from the analysis of somatic CAG-repeat numbers in mother-child pairs (Andrew et al. 1993).

Mutability.—The ability of a normal allele to change to an HD allele in the next generation. To date, only normal alleles with CAG-repeat sizes of 27–35 repeats have been reported to exhibit mutability.

C. Recommendations Regarding Laboratory Standards and Procedures Used in IT-15 Gene Analysis

Individual laboratories are responsible for meeting the CLIA/CAP quality control standards for molecular diagnostic laboratories (American College of Medical Genetics 1993). This includes, but is not limited to, appropriate sample documentation, assay validation, proficiency testing, and general quality control. Furthermore, because of the sensitive and disclosing nature of this type of testing, each laboratory should document that informed consent was obtained for both diagnostic (Beresford et al. 1996) and predictive (Guidelines for the Molecular Genetics Predictive Test in Huntington's Disease 1994) testing before IT-15 gene analysis is initiated. Finally, clinicians and laboratories alike must remember that the relevance of a predictive test result to an individual is crucially dependent on the correct diagnosis of HD in the family; whenever possible, the diagnosis should be confirmed by molecular studies in an affected individual before a molecular predictive test is performed. The working group has the following additional comments about laboratory procedures:

1. The Use of Primers that Distinguish the CAG from the Adjacent CCG Repeat.—The CCG repeat that is adjacent to the CAG repeat is polymorphic, with allele sizes of 7–12 repeats. Use of primers that amplify both the CAG repeat and the CCG repeat sequences can therefore lead to diagnostic inaccuracy in the threshold CAG-repeat range (35–40 repeats) (Andrew et al. 1994). Thus, primers that amplify the CAG-repeat sequence only, and not the adjacent CCG repeat, should be a *requirement* of all laboratories that perform IT-15 gene analysis for clinical purposes.

2. Size Standards to be Used in Counting CAG-Repeat Numbers.—The results of an IT-15 gene test are quantitative (i.e., counting repeat numbers), and a standardized method of sizing alleles is required. Laboratories should be particularly sensitive to the need for accuracy in the critical range of 27–40 repeats, where a counting error could lead to a categorical misinterpretation of the results. Nondenaturing assays (i.e., agarose gels with ethidium bromide staining) should *not* be used. As a minimum, internal or external sequencing standards should be selected that allow exact determination of the size of the PCR product. These standards include (but are not limited to) an M13 sequencing ladder *and* appropriate normal and abnormal controls whose sizes have been verified independently in a second laboratory or laboratories. Ideally, cloned universal standards (e.g., a CAG 27 and a CAG 40) should be developed for distribution to all laboratories, but these are not currently available commercially. Laboratories should voluntarily participate in proficiency testing that includes assay of HD samples.

3. The Use of Southern Blot or Other Alternative Methods in IT-15 Gene Analysis.—Occasional samples may yield a single (apparently homozygous) allele according to the standard PCR-based CAG-only assay. Additional laboratory methods may be needed to ensure that a second allele of a different size is not present. These methods can include use of different PCR primer pairs, which may allow expansion and detection of larger alleles (Warner et al. 1993); use of a CCG + CAG primer pair, which can resolve the alleles if the CCG repeat is polymorphic (Andrew et al. 1993); and use of Southern blots to detect very large alleles (Guida et al. 1996). The working group *recommends* that each laboratory either consider the use of additional methods to resolve all apparently homozygous alleles *or* refer such samples to another laboratory experienced in the use of additional assay methods. Laboratory reports should describe the methods that were used to resolve ambiguous or unusual results.

4. Inclusion of CAG-Repeat Number in Test Reports.—The written IT-15 gene test report should include the CAG-repeat numbers of both alleles and an explanation of the significance of these allele sizes to the patient (see section A).

D. Sample Ownership

It has been stressed in a number of recent publications that ownership of DNA research samples resides with the individual from whom the sample was taken, not with the physician or laboratory. Although most of these prior documents concern samples obtained for research, we believe that federal legislation will soon extend this principle to DNA samples obtained for diagnostic purposes. It is *strongly recommended* that each laboratory that performs any gene tests for Huntington disease develop detailed consent forms that specifically address (1) whether the donor permits the DNA sample to be stored after completion of the test(s) ordered, (2) whether the donor permits the sample to be tested in the future for other diagnostically relevant genes without further consent, and (3) under what circumstances a sample taken

for diagnostic purposes may be used in research. Consent forms should be required to accompany all samples prior to testing.

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Martha A. Nance, Hennepin County Medical Center, Minneapolis (cochair); William Seltzer, Athena Diagnostics, Worcester, Massachusetts (cochair); Tetsuo Ashizawa, Baylor College of Medicine, Houston; Robin Bennett, University of Washington, Seattle; Nathalie McIntosh, DIANON Systems, Stratford, Connecticut; Richard H. Myers, Boston University School of Medicine, Boston; Nicholas T. Potter, University of Tennessee, Knoxville; and David K. Shea, Foundation for the Care and Cure of Huntington Disease, Islamorada, Florida.

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