Proceed with Care: Direct Predictive Testing for Huntington Disease

C. M. Benjamin,[1] S. Adam,[1] S. Wiggins,[1] J. L. Theilmann,[1] T. T. Copley,[1] M. Bloch,[1] F. Squitieri,[1]
W. McKellin,[1] S. Cox,[1] S. A. Brown,[1] H. P. H. Kremer,[1] M. Burgess,[2] W. Meshino,[4] A. Summers,[4]
D. Macgregor,[4] J. Buchanan,[4] C. Greenberg,[5] N. Carson,[5] E. Ives,[6] M. Frecker,[6] J. P. Welch,[7] A. Fuller,[7]
D. Rosenblatt,[8] S. Miller,[8] S. Dufrasne,[8] M. Roy,[8] E. Andermann,[8] C. Prevost,[9] M. Khalifa,[10] K. Girard,[10]
S. Taylor,[10] A. Hunter,[11] C. Goldsmith,[11] D. Whelan,[12] D. Eisenberg,[12] H. Soltan,[13] J. Kane,[14]
M. H. K. Shokeir,[14] A. Gibson,[14] S. Cardwell,[14] S. Bamforth,[15] S. Grover,[15] O. Suchowersky,[3]
M. Klimek,[3] T. Garber,[3] H. A. Gardner,[16] P. MacLeod,[17] and M. R. Hayden[1]

[1]Departments of Medical Genetics and Anthropology and Sociology, University of British Columbia, Vancouver; [2]Department of Bioethics, University of Calgary, and [3]Alberta Children's Hospital, Calgary; [4]North York General Hospital, North York, Ontario; [5]Children's Hospital, Winnipeg; [6]Memorial University, St. John's, Newfoundland; [7]Dalhousie University, Halifax; [8]McGill University, Montreal; [9]Department of Genetics, Chicoutimi Hospital, Chicoutimi, Quebec; [10]Queen's University, Kingston, Ontario; [11]Children's Hospital of Eastern Ontario, Ottawa; [12]McMaster University Medical Centre, Hamilton; [13]Children's Hospital of Western Ontario, London, Ontario; [14]Royal University Hospital, Saskatoon; [15]University of Alberta Hospital, Edmonton; [16]Oshawa General Hospital, Oshawa, Ontario; and [17]Royal Victoria Hospital, Victoria, British Columbia

Summary

The cloning of the Huntington disease (HD) gene allows highly accurate predictive testing using direct analysis of the CAG repeat. This new test provides results with almost complete certainty but poses unique counseling issues related to direct testing for an adult-onset disease. These include testing individuals who are at 25% risk, without the need for blood from a 50% at risk relative; the assessment of symptomatic individuals; the need for ongoing counseling despite simplification of laboratory procedures; and counseling of persons from families who represent a new mutation for HD. This paper describes protocols for direct predictive testing for adult and prenatal assessment, on the basis of the experience of the Canadian Collaborative Study on Predictive Testing (CCSPT). Over the past 8 years, we have provided >400 results by using linked markers and, more recently, 416 results by using direct assessment of CAG expansion in the HD gene. The vast majority (86%) of requests for direct predictive testing have been from persons who have not previously received results by using linked markers. Despite the ability to now directly assess for the mutation associated with HD, we still recommend assessment of DNA from an affected relative, as this may significantly enhance the accuracy of information to be provided to the at-risk individual. Distance from a genetics center has previously limited the availability of testing, and therefore we have developed approaches to providing predictive testing in the patient's own community.

Introduction

Huntington disease (HD) is an autosomal dominant neuro-psychiatric disorder that usually presents in adult life, with mood and personality changes, clumsiness, and chorea. The disease is progressive, with cognitive decline and worsening of the movement disorder, resulting in death ~15–20 years after onset (Hayden 1981; Harper 1991). At present there is no effective treatment for this disease. After the discovery of the first DNA marker linked to HD (Gusella et al. 1983) and of additional markers that enhanced the informativeness of testing (Wasmuth et al. 1988), predictive testing became an option for some families.

In 1986 a pilot project on predictive testing was established to assess the feasibility of a long-term, prospective study of the impact of predictive testing on individuals and families with HD, by using quantitative and qualitative psychosocial assessment (Bloch et al. 1989; Fox et al. 1989). In 1988 the research program expanded to include 14 centers from across Canada. Over the past 7 years, >600 individuals have entered the program and >400 individuals have received results. Prediction was based on the inheritance of the linked DNA markers and required blood samples from multiple family members. From its inception, this study was multidisciplinary, involving physicians, geneticists, counselors, psychologists, neurologists, psychiatrists, ethicists, and representatives of the lay groups, and, more recently, an anthropologist and a sociologist have joined the research team. Preliminary results suggest that predictive testing may have benefits for the psychological health of persons who have received results through a structured predictive testing program (Wiggins et al. 1992). However, in ~10% of individuals who received an increased or decreased risk result, serious psychiatric difficulties were encountered (Bloch et al. 1992; Huggins et al. 1992).

Prior to the discovery of the gene, predictive testing us-

ing linked markers had become a regular clinical service in 19 countries (Brandt et al. 1989; Quaid 1992; Tyler 1992; Bennett et al. 1993; Harper et al. 1993). By the end of 1991, despite prior estimates of 50%–70% (Mastromauro et al. 1987; Meissen and Barchek 1987), only an estimated 5% of persons at risk worldwide had participated in predictive testing (Harper et al. 1993). Specific factors limiting participation included concern about an increased risk result and the absence of any treatment to halt the progression or reduce the severity of the illness (Quaid and Morris 1993; Babul et al. 1993).

The discovery of a novel gene (IT15) containing a trinucleotide repeat (CAG) that is expanded on HD chromosomes (Huntington's Disease Collaborative Research Group 1993) now allows a highly accurate predictive test using direct analysis of the CAG repeat. This trinucleotide repeat is located in the 5' region of the gene, and expansion of the CAG is associated with HD in >99% of affected individuals who were given the clinical diagnosis of HD (Andrew et al. 1993; Kremer et al. 1994). Numerous issues relating to the sensitivity and specificity of the test have now been examined. In particular, specific CAG repeat ranges seen in affected persons have been determined, and the overlap with a large number of normal chromosomes has been examined. Furthermore, the CAG expansion appears to be highly specific for HD and is not seen in numerous other neuropsychiatric disorders that may have clinical overlap with HD (Kremer et al. 1994).

More centers are offering direct mutation analysis of the HD gene as the laboratory analysis required has become less complex. However, at present, little is known as to either how individuals will react to more definitive results or what role the previous level of uncertainty played in maintaining hope, optimism, and positive psychological health.

Direct testing is likely to be more frequently requested than was the case for testing using linked markers. In a study that assessed the potential use of a highly accurate test for HD, 80% of persons who were not able to have predictive testing using DNA markers and 72% of individuals who had previously received a result in a predictive testing program indicated that they would request more accurate testing, once it became available (Babul et al. 1993). Many centers with little previous experience of predictive testing using linked DNA markers may underestimate the ethical dilemmas, family issues, and laboratory complexities that may become apparent when predictive testing for HD by using direct assessment for the mutation is offered (Huggins et al. 1990; European Community Huntington's Disease Collaborative Study Group 1993; Simpson and Harding 1993). Here we present our approach and initial results of direct predictive testing for HD in Canada.

Methods

The Clinical Protocol

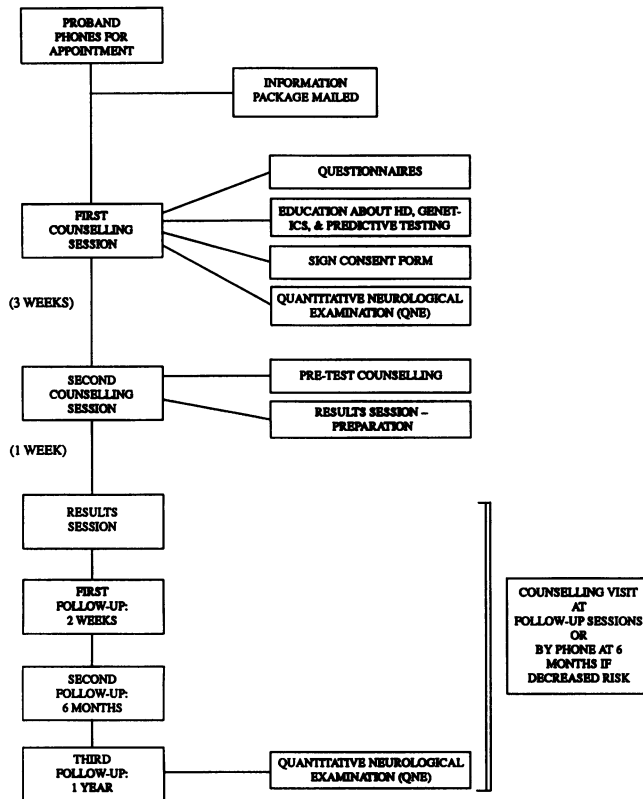To assist the modification of our research protocol to clinical service, we sent a questionnaire to patients who had participated in the predictive testing program, asking which aspects of the research protocol should be retained in the service guidelines. The purpose of the questionnaire was to determine which aspects patients felt were important (Babul et al. 1993). A questionnaire was also mailed to the clinicians involved in the research (Copley et al. 1993). Response to the mailed questionnaire was good (257/354, or 73%). Patients indicated that the pretest counseling, including the self-assessment questionnaires, was of great value. They felt that a minimum of two sessions should be offered prior to results and that follow-up counseling should be available at regular intervals. Although professionals and patients generally agreed on components of the program, there were some significant differences of opinion. For instance, significantly more participants than professionals thought it essential that a 24-h contact phone number be provided as part of a service program ($P = <.001$). In addition, more participants (23%) than professionals (6%) thought that it was not at all important that the family physician be informed of the patient's involvement in a predictive testing program (Copley et al. 1993). These two factors influenced the decision to consider including a contact phone number and to follow the patient's direction as to when and if to contact the family physician, as part of the program.

Protocol for direct predictive testing—adult.—The aim of adult predictive testing is to provide asymptomatic individuals with access to information that can reduce the uncertainty of living at risk for HD and to ensure provision of support to promote healthy adaptation to their changed status. It is crucial that predictive testing programs be designed with adequate safeguards to facilitate a positive outcome and to minimize possible harm.

The guidelines presented here are not intended to be prescriptive but to create a framework for the delivery of adult direct predictive testing (fig. 1) and prenatal testing. They are designed to be flexible, as each clinical situation is unique and many centers may not have all the recommended services available. Furthermore, for some persons who have already participated in predictive testing, modification of the protocol may be warranted.

General guidelines.—Participation in our predictive testing programs is guided by the following eligibility criteria: the individual must (1) have a confirmed family history of HD and an a priori risk of 50% or 25%, (2) be able to provide informed consent, and (3) not have been given an established clinical diagnosis of HD (as predictive testing is for persons who consider themselves to be at risk).

We recommend that predictive testing continue to be coordinated through a genetics center. Within the genetics center the predictive testing team ideally would consist of two professionals, including a geneticist and, if possible, a genetic counselor or other qualified professional (e.g., psychologist or social worker). A multidisciplinary approach is ideal but may not be feasible in some centers. Involvement of family physicians is recommended, as they

Figure I   Summary of the predictive testing guidelines

counseling are to provide the patient with information about predictive testing and to explore the benefits and potential harm of receiving a test result. We present a balanced clinical description of HD, the limited treatment options, and the potential developments emanating from future research. The patients' decision-making processes are explored and the motives behind the decision to take the test are clarified.

The educational component of this session consists of information about the nature of the predictive test and the limitations of direct testing. We discuss the possible test results. Patients are encouraged to review life- and disability-insurance policies and to be aware of the possible implications that the test results have for future health care and employment (Quarrell et al. 1989; Billings et al. 1992). We also provide written information about direct predictive testing, which the patient can take home and review at leisure.

We take a medical, psychiatric, and family history, with particular emphasis on the occurrence of any major affective disorders or suicide attempts on the part of the patient or family members. The patient's psychological status is assessed, as adverse reactions to predictive testing results have been noted in some individuals with a psychiatric history (Kessler 1987; Lam et al. 1990; Huggins et al. 1992). In some centers the psychosocial assessment includes both an interview and questionnaires. The questionnaires (table 1), take ~30 min to complete, are easy to score, and provide the counselors with an independent useful assessment of the patient's psychosocial status. If the combined results of psychosocial and clinical assessment and psychiatric history raise concerns about the psychiatric status of the patient, a separate counseling session and/or referral to an appropriate mental health professional is offered. At this point, possible postponement of predictive testing may be discussed.

We inform people that they may withdraw from testing at any time. Furthermore, we specifically inform persons that, despite significant progress in research, there is still no treatment to halt or retard progression of this illness. Once the patient has decided to proceed with testing, a written consent for predictive testing is obtained.

Individuals at 25% risk.—For individuals with a 25% a priori risk, the test result may confer a change of risk status for other family members, such as an at-risk parent who may not wish to know this information and may not have access to appropriate counseling. The ethical and social implications of this testing are complex. If the 50%-at-risk parent is alive, involvement of this parent in counseling is recommended, as results of direct testing of an offspring at 25% risk may result in a predictive test for the parent. If the parent wishes to have predictive testing, he or she is enrolled in a program and receives results before the 25%-at-risk patient does. If, after counseling, the parent declines to be tested or is unavailable, then the rights of the 25%-at-risk patient to receive results need to be consid-

can provide valuable local support for some individuals (Mennie et al. 1990). However, physician participation is not a requirement for receiving results and is only established after specific discussion with the participant.

Patients are encouraged to bring a support person (e.g., spouse or close friend) to as many counseling sessions as possible. Involvement in the counseling process provides the support person with an understanding of the issues involved in testing and enables him or her to provide informed emotional support to the patient throughout the testing process. However, it is recognized and accepted that some persons choose to go through testing without a designated support person in attendance at the counseling sessions.

If the patient has never received counseling on the natural history, genetics, and clinical features of HD, we provide this information in a session prior to formal enrollment in predictive testing. Individuals who have only recently been informed of their risk status or who have had no previous experience of HD may need more time to adjust to their new situation, prior to initiating testing. Patients are assured that participation in predictive testing is completely voluntary and that all information obtained through predictive testing is strictly confidential. Results are only to be disclosed only to other parties with the express written consent of the patient.

Pretest Counseling—Session 1.—The aims of pretest

Table I

Psychological Assessment Questionnaires Used in Predictive Testing

Name of Questionnaire	Description	Reference
Demographics	Covers areas of employment, relationship status, drug use, and previous psychiatric history	Available from authors
Symptom Check List (SCL-90R)	Measure of global distress	Derogatis 1975
Beck Depression Inventory (BDI)	Measure of clinical depression	Beck et al. 1961

ered (World Federation of Neurology Research Committee Research Group on Huntington's Chorea 1990). One option would be to offer exclusion testing, using linked markers, in the hope of excluding the risk of HD in the individual at 25% risk, without disclosing the risk to the parent. If HD cannot be excluded, predictive testing using direct analysis is offered.

Neurological examination and testing of symptomatic individuals.—Prior to the neurological examination, the counselor should ascertain if the patient is aware of any symptoms or has concerns that he or she may be showing signs of HD. Individuals are allowed to choose if they would want to know if they are at present showing clinical signs of HD (Bloch et al. 1993). We assess the patient's readiness to receive a clinical diagnosis, as obvious clinical signs do not necessarily indicate that the patient wishes to be diagnosed; if the patient does not so wish, he or she may choose to continue with the predictive test rather than proceed to diagnosis.

Preparation for predictive testing results—Session 2.— A session is scheduled ~1–2 wk prior to results and is designed to answer any questions regarding predictive testing and to provide additional counseling. Individuals are again given the opportunity to decline testing at this session. Previous experience has shown that ~25% of individuals who enter a program decide not to receive results (Wiggins et al. 1992; Quaid and Morris 1993). The professionals who are involved in the care of the patient at this point have no knowledge of the DNA results, to enable unbiased counseling. To enable this, the laboratory results are not revealed to the counseling team until shortly before the latter is ready to provide results. Counseling focuses on personal feelings about the test and on preparation for different possible test outcomes. Details concerning the delivery of test results, which include the time and the people to be present, are shared. A date is set for delivery of results, and the date for the first follow-up is arranged, to avoid having to do this at the results session. A card with a phone number for telephone contact with members of the predictive testing team is provided.

Results.—Results are presented in a clear, direct, unambiguous, nonjudgmental fashion. Even with adequate preparation, the results often precipitate shock in both the candidate and the support person. It is sometimes helpful to create an opportunity for the patient to spend some time alone with the support person during this session.

Follow-up sessions.—These are designed to provide continuing support and contact for individuals who are adjusting to their new risk status. Support networks are mobilized, and follow-up continues for ≤1 year following results, in both the increased- and decreased-risk groups (Bloch et al. 1992; Huggins et al. 1992). In general, persons with an increased risk more frequently have adverse events in the first few months after testing, while persons receiving a decreased risk may have adverse events many months after receiving results (Huggins et al. 1992).

A planned counseling session ≤2 wk after receipt of results is offered for all patients. For individuals who have inherited the HD mutation, we recommend personal interviews at 6 mo and 1 year. If they have not inherited the HD mutation, we recommend follow-up phone contact at 6 mo and a personal interview at 1 year. All patients are invited to contact the predictive testing team, as they feel necessary, at any other times.

Testing individuals who live a significant distance away from a genetics center—a community-based protocol.— Many individuals live a significant distance away from centers providing predictive testing programs. Physical distance from a genetics center is a significant barrier preventing some individuals from participating in predictive testing. In 1987, a community-based predictive testing program was established in British Columbia to provide genetic counseling and to offer predictive testing to these individuals. The program combines the services of a genetics center and utilizes the available professional support counseling within the local community.

Because of the complex nature of the predictive test, the initial session (session 1) is conducted at a genetics center, while all other sessions are conducted in the patient's community, with a designated health professional. This professional (e.g., family doctor, nurse, social worker, or psychologist) is chosen by the patient and works with the predictive testing team to provide information, results, and counseling to the patient in the community. The genetics center provides the professional with information about HD and predictive testing (e.g., video or written information) and the results of the psychological assessment based on the individual's questionnaire responses. In the patient's community, a counseling session is undertaken by the professional before results are given, and follow-up is arranged. The patient is given the option of visiting the genetics center at any time.
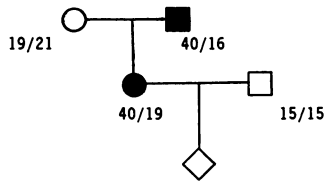
Figure 2 Direct prenatal testing. The parent is clinically affected with HD and has been confirmed to have CAG expansion in the HD range. If CAG repeat length in the fetus is 40/19, the fetus has inherited the HD mutation; if it is 19/15, the fetus has not inherited the HD mutation.

## The Protocol for Prenatal Testing

Prenatal testing is currently available to individuals and their partners who either are at 50% a priori risk or are affected with HD. Counseling is available regarding different options, including prenatal diagnosis, discussion of the different procedures available (amniocentesis and chorionic villus sampling), and the impact of possible outcomes. Prenatal testing for HD may be offered in the following situations:

Parent has a clinical diagnosis of HD (fig. 2).—The parent is affected with HD and shows CAG expansion. The status of the fetus can be assessed by direct gene testing.

Parent at increased risk (fig. 3).—The parent has had predictive testing using linked markers and was determined to be at increased risk. Where possible, we would recommend that the status of the at-risk parent be clarified by direct gene testing prior to pregnancy. If the parent is confirmed to have CAG expansion in the HD gene, the status of the fetus can be assessed by direct gene testing.

Parent at 50% risk.—In the situation where a parent is at 50% a priori risk and has had no prior clarification of risk status, two options are available if the couple wish to proceed with prenatal testing:

a. *Direct gene testing.*—The status of the fetus can be assessed by direct gene testing. If the fetus has no CAG expansion, then HD has been excluded and no information is learned about the parent. If, however, the fetus is shown to have CAG expansion in the HD gene,
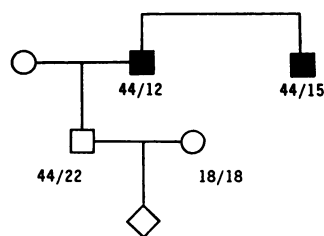
Figure 3 Direct prenatal testing. The parent is clinically unaffected and has chosen to have predictive testing, which has indicated the presence of CAG expansion in the HD gene. If CAG repeat length in the fetus is 44/18, the fetus has inherited an HD gene; if it is 22/18, the fetus has not inherited an HD gene.
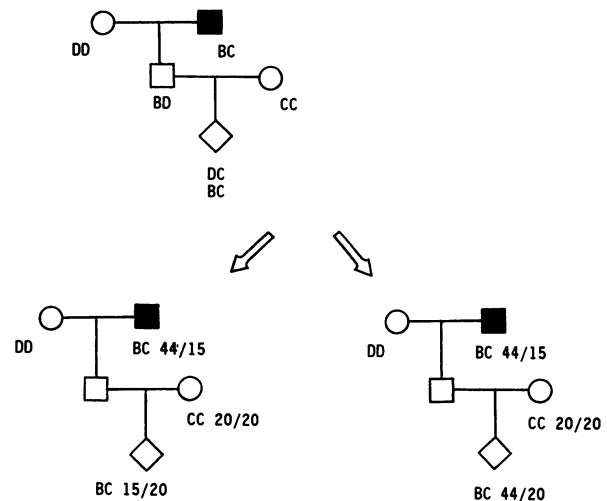
Figure 4 Prenatal diagnosis by exclusion testing. Parent is at 50% a priori risk for HD and has chosen not to have a predictive test. If the family structure is appropriate, linked markers could be used to exclude HD from the pregnancy. If HD is not excluded, then direct gene testing to clarify the fetus's risk could be offered. However, if the fetus was shown to have CAG expansion in the HD range, this results in a predictive test for the at-risk parent. In the upper pedigree, DC = low risk; and BC = 50% risk, linked to risk for the parent. Direct testing (*arrows*) produces one of two results—i.e., that the fetus has not inherited an HD gene (*lower-left pedigree*) or that it has not (*lower-right pedigree*).

then the parent simultaneously receives a predictive test result, as well as learning the status of the pregnancy. This situation, however, results in the added stress of the parent receiving a personal predictive test at the same time as he or she learns that the fetus has inherited the HD gene.

b. *Exclusion testing using linked DNA markers (fig. 4).*— The parent is at 50% a priori risk and prefers not to have predictive testing for him- or herself. In this scenario, the fetus's a priori risk can be changed from 25% to low risk or to a risk similar to that of the at-risk parent (i.e., close to 50%). No information is learned about the at-risk parent. Exclusion testing can still be offered in some families, as an alternative to direct gene testing of the fetus. However, prior to the testing, the counselor discusses with the parent(s) the possibility that the fetus may be found to be at 50% risk. If their decision in this situation would be to elect to continue with direct gene testing, we suggest that direct gene testing be the first option, thus avoiding the laboratory costs and time involved in linked-marker analysis.

Prenatal pretest counseling.—Where possible, preconception counseling is preferred, as this provides sufficient time to assimilate the complex information and to explore the different reproductive options available. If the parent(s) is definitely not considering termination, the reasons for having prenatal testing are explored. Issues such as the child's right to make his or her own decision about receiv-

ing predictive testing results, as well as the potential impact that this knowledge will have on raising a child, are discussed (Bloch and Hayden 1990). In our experience, couples do not choose to proceed with prenatal testing if the procedure will have no influence on the pregnancy. If however the parent(s) elects to continue with a pregnancy when testing has shown that the fetus has inherited the HD gene, it is important to offer continuing counseling to the family.

In previous years the number of couples choosing to use prenatal testing in HD has been small (Adam et al. 1993; Quaid and Morris 1993). Parents' feelings about their ability to cope with the stress of learning of an increased risk for the fetus should be discussed. In this situation a parent may also simultaneously receive results of predictive testing. Professional and social support networks should be explored. This is especially important during prenatal testing, as the parent(s) may avoid telling people about the pregnancy, for fear of having to proceed with a termination of pregnancy, thus isolating themselves from their usual sources of support when they are in most need of them.

Prenatal results and follow-up.—The availability of counseling following prenatal testing is important, especially in the first month. A common misconception about exclusion testing is that the risk to the at-risk parent has also been altered. For couples contemplating another pregnancy, other issues to be addressed include recognition of the option of not feeling obliged to use prenatal testing in subsequent pregnancies.

Laboratory Protocol for Direct Predictive Testing

Variations in the specific ranges of CAG repeats designating the normal, HD, and intermediate ranges have been reported (Duyao et al. 1993; Snell et al. 1993; Andrew et al. 1994b). It may therefore be necessary to clarify the ranges with the laboratory providing the clinical testing for each center. To ensure accurate determination of the number of CAG repeats, it is important that the length of an adjacent variable CCG polymorphism (7–12 repeats) be accurately assessed (Andrew et al. 1993; Rubensztein et al. 1993) or that CAG size be directly assessed using primers flanking only the CAG repeats (Andrew et al. 1994b). This is especially important for CAG expansions of 42 or less. In a series of 995 affected persons, the lower range of CAG repeats was found to be 37 repeats, except for a single affected individual who has 36 repeats (Kremer et al. 1994). A test result of CAG repeats in the range 30–36 is therefore indicative of an intermediate allele (IA). While this means that this person at risk will in all likelihood not develop HD, there may still be a risk of HD to that person's offspring, particularly if the transmitting parent is a male (Goldberg et al. 1993a, 1993b).

The critical issue for at-risk persons is whether they will develop signs and symptoms similar to those seen and diagnosed as HD in the parent. To clarify the analysis, where

possible, DNA from one affected family member is requested. Table 2 shows the potential alterations in risk provided by the Vancouver group, based on different DNA results. Finding an expanded CAG repeat in the HD range in an affected relative confirms that HD is associated with the CAG expansion in that family. Therefore, the absence of the CAG expansion in the proband is evidence that he or she has not inherited the mutation and will not develop signs and symptoms of HD. On one occasion in a family with established HD and CAG expansion, there is a report of a symptomatic person with a presentation similar to HD who does not have CAG expansion. While this remains unexplained at the present time, this is most likely due to another illness (Andrews et al. 1994a) (table 2).

The finding that all affected persons in a family suspected of having HD do not show CAG expansion in the HD range means that the HD phenotype is not linked to CAG expansion in that family. In this case, the failure to detect CAG expansion in the proband does not mean that he or she has not inherited the disorder found in the parent. The person at risk may still have a 50% chance of developing the same illness seen in the affected parent. In the absence of both DNA from an affected relative diagnosed with HD and no CAG expansion in the at-risk person, we counsel that there is a <1% risk of developing signs and symptoms similar to HD. This is based on the finding that a very small number of persons with a clinical diagnosis of HD do not have CAG expansion (Andrew et al. 1994b).

On rare occasions, persons with repeats in the affected range (36–38) may not yet have manifested signs and symptoms even after living to an advanced age (>80 years). In addition, very rarely (1 in 1,595 chromosomes), asymptomatic persons not knowingly related to anyone with HD will have repeats in the affected range (Kremer et al. 1994). One unaffected, unrelated spouse had a CAG repeat length of 39, with no history of HD over two generations. At this stage, we do not know whether such persons without a family history of HD who are found to have expanded CAG repeats in the HD range will develop HD, as studies to date have only focused on affected persons. It is conceivable that, if these persons lived long enough, they would manifest signs and symptoms of HD. For these reasons and until there is additional information, in the absence of both blood from an asymptomatic affected relative and the finding of CAG expansion in an at-risk person, we counsel that there is a >99% likelihood that the person will develop HD. In this situation, although unlikely (this has occurred in 1 of 593 families studied), the expanded CAG triplet could have unexpectedly come from an unaffected spouse and not from the affected parent. Caution still needs to be exercised in counseling persons for whom DNA from an affected relative is not available.

At the present time our laboratory does not routinely report the size of the CAG expansion. There is a well-established inverse correlation between age at onset and re-

Table 2

Interpretation of CAG Repeat Lengths in Persons at Risk for HD

Situation	CAG Size (no. of repeats)	Interpretation
Affected family member	>36	
At-risk person	>36	Will develop signs and symptoms of HD in the future
Affected family member	>36	
At-risk person	<30	Will not develop signs and symptoms of HD in the future
Affected family member	<30	
At-risk person	<30	May still have a 50% chance of developing signs and symptoms similar to those seen in parent
Affected family member	DNA unavailable	
At-risk person	>36	>99% chance of developing signs and symptoms of HD
Affected family member	DNA unavailable	
At-risk person	<30	<1% chance of developing signs and symptoms of HD

peat length (Andrew et al. 1993; Duyao et al. 1993; Snell et al. 1993). However, for the majority of individuals (those with repeat sizes 36–50) the 95% confidence intervals for prediction of age at onset are very broad. This information, therefore, cannot generally be used to predict an accurate age at onset, although it might be useful for those with significantly expanded CAG repeats (>50). Furthermore, for persons with CAG repeats <40, information that the predicted age at onset is extremely broad is important. For example, the small number of persons with CAG sizes in this range have not manifest, even having lived >80 years.

Testing Relatives of Persons Who Represent Probable New Mutations for HD

Patients with suspected HD who do not have a confirmed clinical family history of this illness are not rare and, in one large study, represented 11% of a cohort of >1,000 patients. This study also demonstrated that the mutation rate for HD may be much more frequent than previously recognized, accounting for ≥3% of families with HD (Goldberg et al. 1993*b*).

New mutations have been shown to be caused by expansion of an IA of 30–36 CAG repeats in the HD gene in the parents of the sporadic case, to CAG repeats >36 that are associated with the phenotype of HD (Goldberg et al. 1993*b*; Myers et al. 1993). This expansion occurs only during transmission through the male germ line and is associated with advanced paternal age (Goldberg et al. 1993*b*). These IAs may undergo expansion in subsequent meioses, resulting in the presentation of more than one affected sibling without any other family history of HD and presenting a pseudorecessive pattern of inheritance (Goldberg et al. 1993*a*).

Offspring of new mutations for HD have a 50% likelihood of inheriting the HD gene, similar to that of any other persons who have an affected parent. However, there is a previously unrecognized genetic risk to siblings of sporadic cases. The precise estimates of the likelihood of expansion of the IA during transmission through the germ line is not yet ascertained. However, we have previously shown that 10 (42%) of 24 meioses do show expansion, which would indicate that the risk to a sib of a sporadic case is likely not to be >25% (Goldberg et al. 1993*a*). This reflects the likelihood that they have inherited the chromosome containing the IA and that the CAG has undergone expansion.

At the present time, it is also not known whether all IAs, when transmitted through the male germ line, have similar susceptibility to expansion. IAs occur randomly in the general population, on ~0.75% of normal chromosomes (Kremer et al. 1994). Preliminary evidence suggests that advanced paternal age may be one factor contributing to expansion of this IA in families where the IA has a susceptibility to expansion. If confirmed, this would be important information for family members. At the present time, we would offer predictive testing to siblings of sporadic cases and counsel that they have an a priori risk of ≤25% if it can be demonstrated that the sporadic case has risen as a result of expansion from an IA. However, if the parents are not available but have lived to an advanced age, we would offer predictive testing to a sibling of a sporadic case. The a priori risk to such a sibling would be either ≤25% if the sporadic case had expansion of an IA or 50% if this represented transmission of an expanded allele from a parent who had not yet manifested signs and symptoms.

If siblings of the sporadic case of HD have a CAG size of <30 repeats, we would suggest that it is unlikely that

Table 3

Results of Direct Testing for HD Mutation in Canada

	Consultand Has CAG >36	Consultand Has CAG 30–36	Consultand Has CAG <30	Total
A. At-risk individuals (*n*=209):				
Prior participant in testing using linked markers (*n*=29):				
Prior result was increased risk, and affected family member has CAG >36	11	...	...	11
Prior result was decreased risk, and affected family member has CAG >36	...	1	7	8
Prior result was uninformative, and DNA from affected family member unavailable	3	...	5	8
Prior result was uninformative, and affected family member has DNA unavailable	...	...	2	2
New consultand (*n*=180):				
Affected family member has CAG >36	53	1	70	124
Affected family member has DNA unavailable	25	...	31	56
B. Symptomatic individuals (*n*=207):				
Positive family history, and affected family member has CAG >36	70	...	2	72
Positive family history, and affected family member has DNA unavailable	73	...	7	80
No family history	38	1	17	56
Total no. of tests	273	3	141	417

this allele would be susceptible to further expansion in future generations. If the allele size in the sibling of the sporadic case is in the affected range, we would explain to this person that the expansion was similar to that seen in the affected sibling, and we would counsel that this person was likely to manifest signs and symptoms of HD in the future. However, if the sibling who inherited an IA is a male, we would counsel that there is a risk of expansion of this IA in future generations. However, the precise risk to future generations is currently not known, but it is not likely to be >25%. If the sibling who has inherited the IA is a female, we would counsel that at the present time there is no evidence for instability of the IA or for expansion into the affected range, in any such cases.

Results

A total of 416 results of direct testing for HD have now been obtained in Canada, since direct testing began in September 1993 (table 3). Approximately half of these (209/416, or 50%) have been performed for at-risk individuals, with 44% (92/209) receiving an increased-risk result and 55% (115/209) receiving a decreased-risk result. Only a small proportion of those persons at risk who have now requested direct testing (29/209, or 14%) are individuals who previously have received results of predictive testing using linked markers. To date, there has been only one case where direct predictive testing has contradicted a prior risk result given through predictive testing using DNA-linked markers. In this instance, a prior risk estimate

of 85% using linked markers was reduced to <1% on direct testing.

Direct testing has been requested by a large number of persons who are symptomatic (171 persons, or 49% of the total). In this group, two individuals who clearly had a family history and had signs and symptoms suggestive of HD had a CAG size of <30. Interestingly, for the 56 people who were tested who did not have a family history but were symptomatic, raising suggestions of the diagnosis of HD, 17 (30%) had a CAG size of <30. There were 21 at-risk individuals who previously had requested predictive testing but who, in view of the uninformativeness, which was due either to the linked DNA markers or to the absence of blood from family relatives, could not be tested (Babul et al. 1993). Of these, 10 (48%) have now entered the program and have received results of direct predictive testing.

Discussion

Direct testing began in Canada in September 1993, and to date >400 individuals have received results. The guidelines described here reflect the changes in our clinical practice that are due to the discovery of the CAG expansion associated with HD. These changes result from both the new technology allowing direct molecular analysis of affected families and the counseling concerns raised by the new test. There are significant counseling differences between analyses using linked markers and direct testing. These include new appreciation of the genetic risks, to relatives, of new mutations for HD and the sensitivity and

specificity of CAG expansion for HD. Individuals who have inherited an IA (30–35 repeats) or a CAG repeat length just within the affected range (36–38 CAG repeats) need additional attention.

The protocols presented in this report will pose significant challenges to the resources available for provision of predictive testing. In some countries where there is universal health-care insurance, such as Canada and certain parts of Europe, the ability to follow similar protocols will be greater than it is in other countries, such as the United States, where universal health-care coverage is not yet available. The protocols that have been developed are modified from an intensive research program that clearly showed that predictive testing could be provided in a safe manner without undue psychological harm to the vast majority of patients and with potential for improving the quality of life for many participants (Wiggins et al. 1992). It is not known whether similar outcomes would have been reached if less intense counseling protocols had been followed. However, there is evidence from a prior study (Babul et al. 1993) that the predictive testing participants themselves place major importance on the availability of counseling. As new predictive tests for other genetic disorders become available, it will be difficult for genetic centers to provide all the counseling. Much of the counseling and support will have to be provided through other community services. Consequently, there is an urgent need to assess the impact of approaches to predictive testing when there is less frequent psychological support or when such support is provided in the community by other health-care professionals not directly associated with a genetics center. Assessment of our community-based program, which provides testing for individuals who live a significant distance from a genetic center and who have had only one formal contact with the genetic counseling program, will be important in this regard. In the interim, however, development of protocols for predictive testing should be based on available evidence that the recommended approaches are not harmful and are likely to have humane and advantageous consequences for persons participating in these programs. To date, the only evidence we have is that derived from original research protocols for linkage predictive testing. Consequently, until there are data in support of substantial changes to these guidelines, we plan to follow the protocols as described for the direct predictive test.

To date, the majority (86%) of the requests for direct predictive testing have come from at-risk individuals who did not participate in previous programs using linked DNA markers. Of the individuals who did participate previously, the greatest proportion (10/21, or 49%) of requests have come from those who had received an uninformative result from DNA testing. It is also interesting to note that, for a large proportion (56/181, or 31%) of the new participants, DNA from affected individuals in the participants' families was unavailable. These individuals would have been uninformative under previous testing conditions, and their realization of this may have influenced their decision not to pursue testing at that time. This is consistent with a finding from another study which showed that the vast majority (80%) of individuals for whom testing with linked markers was uninformative would want to return for a direct test (Babul et al. 1993). Clearly, the advent of direct predictive testing will result in a significantly increased demand for testing from such individuals.

In contrast, uptake of direct predictive testing by persons who have already received results of using linked DNA markers has been low. Of 106 individuals who previously received an increased-risk result in this way (Babul et al. 1993), 11 (10.4%) have now requested retesting. This proportion of requests is, however, more than that seen among those who had previously received a decreased-risk result and who have now requested retesting (8/62, or 4.9%) (Babul et al. 1993). These results indicate that it is inappropriate to automatically assess the DNA of prior participants in predictive testing, as many of these persons may not wish refinement of their risk estimate. While this represents early response to availability of this program, these results are consistent with prior findings that the number of persons at increased risk who are likely to participate in a completely accurate predictive test result is greater than the number of individuals in a decreased-risk group who are likely to do so. Persons with a decreased risk may believe that they have more to lose by having a completely accurate test, since there is still a small possibility that their risk status will be reversed and that they thus will learn that they have inherited the mutation associated with HD. On the other hand, individuals who have had an increased-risk result from linked markers might be more likely to see themselves already in a loss situation and to participate in testing, even if an improved outcome is not likely.

Among all of the individuals who request direct predictive testing, a proportion will be symptomatic for HD at the time of their request. Two different studies have found that as many as 15% of individuals requesting predictive testing for HD are symptomatic at the time of their initial neurological exam (Brandt et al. 1989; Bloch et al. 1993). During initial counseling sessions with the patient, it is important to clearly distinguish between clinical diagnosis for HD and predictive testing for HD, so that the patient can seek the information that she or he actually wants. Under our previous testing protocol (using linked DNA markers), all individuals for whom a clinical diagnosis of HD could be made were automatically excluded from predictive testing and were diverted into a process leading to the diagnosis. Under the direct testing protocol, the patient is offered more options. For some individuals it may be psychologically less traumatic to receive the information that they have inherited the mutation of HD and to delay the diagnosis until later, when they are better prepared. For others, it may be preferable to proceed directly

to the diagnosis. Thus, if the neurological examination indicates that the individual is clinically affected with HD, and if the patient has previously expressed a wish to know this information, then an agreement is reached with the patient, to divert the predictive testing process to clarification of neurological status. However, if a patient chooses not to know the results of the neurological examination or chooses not to be examined and still wishes to proceed with direct gene testing, even if she or he is symptomatic, this option is available in our program.

We recommend that individuals who receive a clinical diagnosis through a predictive testing program be followed carefully, especially soon after diagnosis. Referral to appropriate community resources (HD resource center or family doctor) may often assist the patient to establish a local support system for the immediate postdiagnosis period, which can be a particularly stressful time for such persons (Lam et al. 1990).

Clearly symptomatic individuals, who present to their doctor to know the reasons for their symptoms, are not routinely seen in the genetics clinic. DNA testing in these persons will be helpful to confirm that they have indeed inherited the HD gene. However, even with the advent of direct testing, the decision as to whether a patient has HD rests with the clinician's (usually a neurologist) interpretation of the clinical presentation.

It is of interest that, among symptomatic individuals who have no family history but for whom the diagnosis of HD is seriously being considered, ~30% (17/56) do not have CAG repeat length in the affected range. Clearly these data support the fact that there is considerable phenotypic variation in patients suspected of having HD and that, in the absence of a family history, a significant number of individuals who are deemed clinically to have HD are likely to have another disease that may manifest HD-like symptoms (Andrew et al. 1994a; Kremer et al. 1994).

There are numerous benefits of direct predictive testing. The direct test creates greater independence for the individual requesting testing, as multiple family members need not be contacted for DNA samples. Individuals at 25% risk who had previously been uninformative for linkage analysis may now also request testing. Recent debate surrounding the legal and ethical rights of individuals suggests that it is unethical to deny these individuals such testing (Chapman 1990). Representatives from the International Huntington Disease Association suggest that their members are in favor of testing individuals at 25% risk, provided that there is counseling support and that the consequences that testing may have for other family members are discussed (MacMillan and Quinn 1993).

Many research and service laboratories have stored DNA samples from HD families. Since the discovery of the HD gene, our laboratories have received >207 requests for confirmation of diagnosis in symptomatic individuals. The high sensitivity and specificity of CAG expansion for inheritance of the gene for HD (Kremer et al. 1994) suggest

that frequently the test will be used by neurologists to support the diagnosis in a family, especially in one with "atypical" features of HD. This may also result in the confirmation of risk for other family members. While the symptomatic individual will usually be assessed and followed by a clinician, we recommend that the family also be offered genetic counseling in this situation. If a third party (such as a doctor or family member) requests testing of a stored DNA sample from a clinically affected individual, it is our policy to require that informed consent from the patient or legal guardian be obtained. If the individual is deceased, a consent signed by the legal representative of the estate—such as a personal representative, next of kin, or executor of the estate—is obtained.

In the HD national DNA bank located in Vancouver, which now houses DNA from >4,000 persons from families with HD, the original consent permits the use of DNA from affected persons, for predictive testing of a relative, without the need for additional consent. However, if prior consent for testing an affected person permitted DNA to be used only for testing using linked markers, then additional consent would be necessary. To maintain confidentiality, the predictive testing candidate's report does not indicate which affected family members have been used in the analysis. Where it is available, we always use DNA from an affected person, as well as from the at-risk proband.

HD represents the disorder for which predictive testing has been offered for the longest time, to the largest number of people. As new genetic markers and mutations underlying other late-onset disorders continue to be discovered, the lessons learned from predictive testing for HD are having significant impact on the development of research protocols, patterns of practice, and genetic counseling. Furthermore, for other late-onset disorders, such as Alzheimer disease and familial cancer syndromes, predictive testing will eventually shift from the use of linked markers to direct assessment of a mutation, as seen in HD. However, HD may not serve well as a model for all late-onset illnesses, particularly for those where prediction of risk may rapidly be followed by approaches to treatment (Babul et al. 1993). Many of the guidelines for direct predictive testing for HD are consistent with the principles elaborated for genetic testing in medicine, as described in the recent report by the Committee on Assessing Genetic Risks of the Institute of Medicine of the (U.S.) National Academy of Sciences (1994, p. 89), which states that "technological ease at the bench should not diminish the intensive education and counseling both prior to and following testing."

One of the aims of our ongoing study is to identify the impact that cloning the gene has on the demand for predictive testing, from individuals who have already participated in a predictive testing program as well as from those who have not. The effect that predictive testing has on the family is also being closely monitored. Selected family

members are asked to complete a series of questionnaires and interviews, both before results are given and at regular intervals thereafter. The results of these studies will provide additional information on the impact that predictive testing has on the family.

The guidelines presented here and elsewhere (Craufurd and Tyler 1992; Quaid 1992; Bennett et al. 1993) should be considered by those planning to offer direct predictive testing for HD. At the present time there are significant differences between genetics centers across the world, concerning the eligibility criteria (individuals at ≤25% risk and the testing of children) and procedures (counseling availability and laboratory quality-control measures) for predictive testing. As we develop more understanding of the counseling complexities of direct predictive testing, it is likely that these guidelines will need to be revised.

Acknowledgments

References

Adam S, Wiggins S, Whyte P, Bloch M, Shokeir MHK, Soltan H, Meschino W, et al (1993) Five year study of prenatal testing for Huntington disease: demand, attitudes and psychological assessment. J Med Genet 30:549–556

Andrew SE, Goldberg YP, Kremer B, Squitieri F, Theilmann J, Zeisler J, Telenius H, et al (1994a) Huntington disease without CAG expansion: phenocopies or errors in assignment? Am J Hum Genet 54:852–863

Andrew S, Goldberg YP, Kremer HP, Telenius H, Theilmann J, Adam S, Starr E, et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington disease. Nature Genet 4:398–403

Andrew S, Goldberg YP, Theilmann J, Zeisler J, Hayden MR (1994b) A CCG repeat polymorphism adjacent to the CAG repeat in the Huntington disease gene: implications for diagnostic accuracy and predictive testing. Hum Mol Genet 3:65–67

Babul R, Wiggins S, Adam S, Kremer B, Dufrasne S, Huggins M, Theilmann, et al (1993) Attitudes towards predictive testing after the cloning of the Huntington disease gene: relevance for other adult onset disorders. JAMA 270:2321–2325

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:53–63

Bennett RL, Bird TD, Teri L (1993) Offering predictive testing for Huntington disease in a medical genetics clinic: practical applications. J Genet Couns 2:123–137

Billings PR, Kohn MA, de Cuevas M, Beckwith J, Alper JS, Natowicz MR (1992) Discrimination as a consequence of genetic testing. Am J Hum Genet 50:476–482

Bloch M, Adam S, Fuller A, Kremer HPH, Welch JP, Wiggins S, Whyte P, et al (1993) Diagnosis of Huntington disease: a model for the stages of psychological response based on experience of a predictive testing program. Am J Med Genet 47:368–374

Bloch M, Adam S, Wiggins S, Huggins M, Hayden MH (1992) Predictive testing for Huntington disease in Canada: the experience of those receiving an increased risk. Am J Med Genet 42:499–507

Bloch M, Fahy M, Fox S, Hayden MR (1989) Predictive testing for Huntington disease. II. Demographic lifestyle patterns, attitudes and psychosocial assessments of first 51 test candidates. Am J Med Genet 32:217–224

Bloch M, Hayden MR (1990) Predictive testing for Huntington disease in childhood: challenges and implications. Am J Hum Genet 46:1–4

Brandt J, Quaid KA, Folstein SE, Garber P, Maestri NE, Abbott MH, Slavney PR, et al (1989) Presymptomatic diagnosis of delayed-onset disease with linked DNA markers. JAMA 261:3108–3114

Chapman MA (1990) Predictive testing for adult-onset genetic disease: ethical and legal implications of the use of linkage analysis for Huntington disease. Am J Hum Genet 47:1–3

Copley TT, Wiggins S, Dufrasne S, Hayden MR, Canadian Collaborative Study for Predictive Testing (1993) Are we all of one mind?: Significant differences in opinion between clinicians and participants regarding the development of a service protocol for predictive testing for Huntington disease (HD). Am J Hum Genet Suppl 53:53

Craufurd D, Tyler A (on behalf of the UK Huntington's Prediction Consortium) (1992) Predictive testing for Huntington's disease: protocol of the UK Huntington's Prediction Consortium. J Med Genet 29:915–918

Derogatis LR (1975) SCL-90R. Clinical Psychometric Research, Baltimore

Duyao M, Ambrose C, Myers R, Novelletto A, Fontali M, Folstein S, Ross C, et al (1993) Trinucleotide repeat length instability and age of onset in Huntington's disease. Nature Genet 4:387–392

European Community Huntington's Disease Collaborative Study Group (1993) Ethical and social issues in presymptomatic testing for Huntington's disease: a European Community collaborative study. J Med Genet 30:1028–1035

Fox S, Bloch M, Fahy M, Hayden MR (1989) Predictive testing for Huntington disease. I. Description of a pilot project in BC. Am J Med Genet 32:211–216

Goldberg YP, Andrew SE, Theilmann J, Kremer B, Squitieri FS, Telenius H, Brown JD, et al (1993a) Familial predisposition to recurrent mutations causing Huntington disease: genetic risk to siblings of sporadic cases. J Med Genet 30:987–990

Goldberg YP, Kremer B, Andrew SE, Theilmann J, Graham RK, Squitieri F, Telenius H, et al (1993b) Molecular analysis of new mutations for Huntington's disease: intermediate alleles and sex of origin effects. Nature Genet 5:174–179

Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306:234–238

Harper PS (1991) Huntington's disease. WB Saunders, London

Harper PS, Ball DM, Hayden MR (1993) Presymptomatic testing for Huntington's disease: a worldwide survey. J Med Genet 30:1020–1022

Hayden MR (1981) Huntington's chorea. Springer, New York

Huggins M, Bloch M, Kanani S, Quarrell OWJ, Theilmann J, Hedrick A, Dickens B, et al (1990) Ethical and legal dilemmas arising during predictive testing for adult-onset disease: the experience of Huntington disease. Am J Hum Genet 47:4–12

Huggins M, Bloch M, Wiggins S, Adam S, Suchowersky O, Trew M, Klimek ML, et al (1992) Predictive testing for Huntington disease in Canada: adverse effects and unexpected results in those receiving a decreased risk. Am J Med Genet 42:508–515

Huntington's Disease Collaborative Research Group, The (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72: 971–983

Kessler S (1987) Psychiatric implications of presymptomatic testing for Huntington's disease. Am J Orthopsychiatry 57:212–219

Kremer HP, Goldberg P, Andrew SE, Squitieri F, Theilmann J, Telenius H, Zeisler J, et al (1994) Worldwide study of the HD mutation: the sensitivity and specificity of CAG expansion. N Engl J Med 330:1401–1406

Lam R, Bloch M, Jones B, Marcus A, Fox S, Amman W, Hayden MR (1990) Psychiatric morbidity associated with early clinical diagnosis of Huntington disease in a predictive testing program. J Clin Psychol 49(11):444–447

MacMillan JC, Quinn N (1993) Conference report. J Med Genet 30:1039–1041

Mastromauro C, Myers RH, Berkman B (1987) Attitudes toward presymptomatic testing in Huntington's disease. Am J Med Genet 26:271–282

Meissen GJ, Berchek RL (1987) Intended use of predictive testing by those at risk for Huntington's disease. Am J Med Genet 26: 283–293

Mennie ME, Holloway SM, Brock DJH (1990) Attitudes of general practitioners to presymptomatic testing for Huntington's disease. J Med Genet 27:224–227

Myers RH, MacDonald ME, Koroshetz WJ, Duyao MP, Ambrose CM, Taylor SA, Barnes G, et al (1993) De novo expansion of a (CAG)n repeat in sporadic Huntington's disease [see comments]. Nature Genet 5:168–173

National Academy of Sciences (1994) Report of the Committee on Assessing Genetic Risks of the Division of Health Sciences Policy of the Institute of Medicine: assessing genetic risks: implications for health and social policy. National Academy Press, Washington, DC

Quaid KA (1992) Presymptomatic testing for Huntington disease: recommendations for counselling. J Genet Couns 1:277–302

Quaid KA, Morris M (1993) Reluctance to undergo predictive testing by those at risk for Huntington's disease. Am J Med Genet 45:41–45

Quarrell OWJ, Bloch M, Hayden MR (1989) Insurance and the presymptomatic diagnosis of delayed-onset disease. JAMA 262:2384–2385

Rubensztein DC, Barton DE, Davison CBC, Ferguson-Smith MA (1993) Analysis of the Huntington gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of Huntington's disease and CAG repeat number. Hum Mol Genet 2:1713–1715

Simpson SA, Harding AE (1993) Predictive testing for Huntington's disease: after the gene. J Med Genet 30:1036–1038

Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, MacDonald M, et al (1993) Relationship between trinucleotide repeat expansion and phenotype variation in Huntington's disease. Nature Genet 4:393–397

Tyler A, Ball D, Crauford D (1992) Presymptomatic testing for Huntington's disease in the United Kingdom. Br Med J 304: 1593–1596

Wasmuth J, Hewitt J, Smith B, Allard D, Haines J, Skarecky D, Partlow E, et al (1988) A highly polymorphic locus very tightly linked to the Huntington disease gene. Nature 332:734–736

Wiggins S, Whyte P, Huggins M, Adam S, Theilmann J, Bloch M, Hayden MR, Canadian Collaborative Study for Predictive Testing (1992) The psychological consequences of predictive testing for Huntington's disease. N Engl J Med 327:1401–1405

World Federation of Neurology: Research Committee. Research Group on Huntington's Chorea (1990) Ethical issues policy statement on Huntington's disease molecular genetics predictive test. J Med Genet 27:34–38