VIEWPOINT

Genetic Testing of *HTT* Modifiers for Huntington's Disease: Considerations for Clinical Guidelines

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After decades of genetic testing for Huntington's disease (HD), research is beginning to provide clues about genetic modifiers.[1-4] These include modifiers of age of onset and disease symptoms, including psychiatric manifestations and cognitive impairment.[3-6] These modifiers have yet to be incorporated into clinical testing, but critical questions are emerging about if, when, and how they should be included in clinical care.

The *HTT* gene was identified in 1993 and found to have a CAG repeat region coding for a polyglutamine protein chain. Forty or more repeats are causative of HD.[7] Alleles of 26 and fewer repeats produce a normal phenotype and are stable in the next generation. Alleles with 27–35 CAG repeats (the intermediate range where phenotype is generally normal) and 36–39 (the reduced penetrance range in which some people will manifest disease usually later in life) are unstable and can expand in subsequent generations[8] (Fig. 1). Genetic testing cannot accurately predict the age of onset, severity, or disease course. Furthermore, there is significant inter- and intrafamilial variation in age of onset and symptoms. Given this variability and predictive limitations, having the ability to provide a more refined risk estimate is important for counseling at-risk individuals on when to anticipate symptoms, which may impact their decision-making and potentially their eligibility for clinical trials.

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Relevant conflicts of interest/financial disclosures: Jill Goldman, Wendy Uhlmann, Ali B. Naini, Robert Klitzman, and Karen Marder have no conflicts of interest or financial disclosures to report.

This Viewpoint is unfunded.

Received: 11 July 2023; **Revised:** 24 September 2023; **Accepted:** 16 October 2023

Published online 17 November 2023 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.29650

Identification and Use of HD Modifiers

Currently, genetic counseling for HD predictive testing is guided by the CAG repeat number and family history but leaves a great deal of uncertainty about the future of at-risk individual. Results in the reduced penetrant (RP) range are particularly limiting as people are left wondering whether they will develop symptoms. A study using the largest available dataset for the reduced penetrance range (Enroll-HD) demonstrates the difficulty in predicting phenoconversion.[9] The likelihood of anticipation in the next generation is also unknown. Therefore, the identification and potential use of genetic modifiers may make more refined HD risk information possible.

The age of onset of motor symptoms is highly associated with the number of uninterrupted CAG repeats rather than the number of glutamines in the protein.[1-3] Both *cis*-acting (CAA interruptions in the CAG tract) and *trans*-acting modifiers (other genes described below) that impact the *HTT* gene and phenotypic expression have been identified. Studies have shown that CAA interruptions (which also code for glutamine) stabilize the CAG expansion. Loss of a CAA leads to an earlier onset by as much as 10 years and duplication of a CAA delays onset by as much as 5 years.[1,3] The loss of interruptions (LOI) is found in approximately 1% of those manifesting HD. About 32% of manifesting individuals within the reduced penetrance range have a LOI allele, with 86% of those having 36 and 37 repeats.[4] Without sequencing for CAA interruptions, LOI carriers receive an incorrect CAG repeat number erring by two repeats (eg, 38 CAGs with LOI will act like 40 repeats).[4] As opposed to the current short-fragment length analysis used in most clinical laboratories, long-fragment sequencing is required to accurately assess the loss or duplication of CAA interruptions.[2,10]

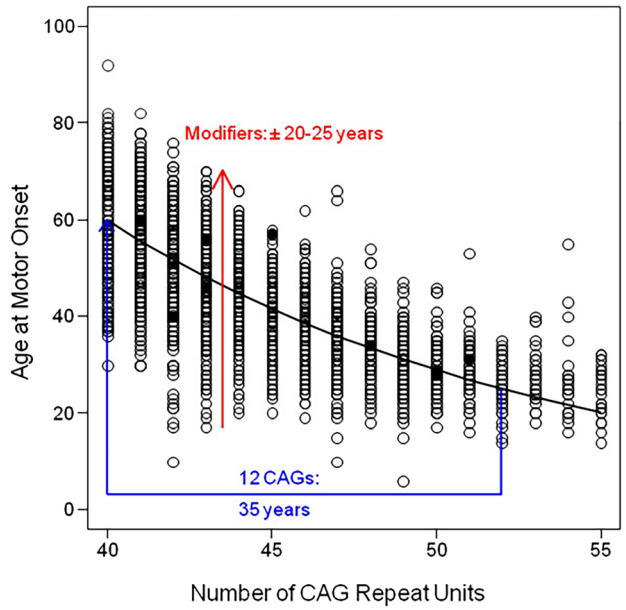


FIG. 1. Reproduced from Gusella JF, MacDonald ME, Lee JM. Genetic Modifiers of Huntington's Disease. Movement Disorders 2014; 29 (11) 1359–1365. [Color figure can be viewed at wileyonlinelibrary.com]

Several DNA repair genes (*FAN1*, *MLH1*, *MSH3*, *MLH3*) are also associated with modifications in age of onset and severity of symptoms, apparently by affecting somatic expansion.[2] The use of high-throughput ultra-deep sequencing with long-fragment sequencing instead of fragment length analysis can detect both CAA interruptions and variants in the DNA repair genes; therefore for better risk prediction, a risk modification model theoretically could be generated (similar to a polygenic risk score [PRS]) that incorporates CAA interruptions and variants in the DNA repair genes.[2]

What Can We Learn from the Experience with Other Neurological Conditions?

Genetic testing for Fragile X syndrome may serve as an example of a methodologic and ethical approach to HD testing. AGG interruptions in the *FMR1* gene influence the probability of expansion of a premutation to a full mutation in the next generation. Previously, guidelines were created about the need for AGG interruption assessment, but this was hampered by the lack of adequate technology.[11,12] Recently, the use of long-range PCR together with long-range sequencing provides a comprehensive assay to detect the number of CGG repeats and AGG interruptions.[13] Reporting these interruptions in genetic counseling has allowed carrier women to better assess their reproductive options, including transfer of embryos in preimplantation genetic testing (PGT). This research study demonstrates clinical utility.[14]

The use of a modifier panel is being explored in other diseases such as SCN1A-related epilepsy where several modifiers significantly influence the severity of disease.[15] These modifiers have been explored only in large cohort research studies, and before individual or panel testing of modifiers can be used for diagnostic and predictive purposes, clinical validity needs to be proven.

Questions Regarding Incorporating Modifiers

Before using a PRS-like model for HD, many questions must be answered including the following: Will labs be willing to report modified risk information? Will modifier results be reliable and consistent across labs? What will be the process of incorporating new modifiers? Is there sufficient evidence that modifier results robustly alter the age of onset for HD? Will insurance cover the modifiers? How will the information be conveyed to at-risk individuals? Will the information be understood and beneficial to these individuals? And what will they want to know?

PRS scores in common multifactorial diseases such as cancer, heart disease, or Alzheimer's disease can guide screening and prevention strategies and, therefore, have clinical utility.[16-18] At the present time, with no proven interventions, genetic testing for HD has personal and research but not clinical utility. Additionally, unlike the common diseases, it is not clear whether lifestyle changes will have a significant effect on the onset or phenotype of HD.[19–22] Until effective treatment/prevention exists, many people may continue to avoid genetic testing or may wish to learn their status without refinement.

Moreover, the efficacy of PRS depends on the sample used to generate scores. Most scores are derived from a largely white, western European patient pool, limiting the clinical utility for other ethnic groups and creating inequitable use of the PRS.[21,22] For HD, the determination of the relative risk of the DNA repair gene variants in different populations will have to be elucidated before using them in any testing protocol.[23,24] Enroll-HD, a worldwide platform with more than 28,000 participants, might be used to develop a PRS.[25]

Another important consideration is how to convey the information. In the early days of HD predictive testing, CAG numbers were generally not communicated because individual CAG repeat length variability had not been assessed. At present, genetic test results are given by reporting CAG number and the range that conveys the likelihood of developing the disease and transmitting it to children. With refined testing, these predictions should improve but will never be perfect. Clinicians will need to explain how the number of CAG repeats is influenced by the modifier genes and CAA interruptions, and that at the present time, this information will not influence medical decisions.

The way the information is conveyed and understood will influence the benefits and harms of its use.

Need for Updates to Guidelines

Laboratory guidelines for HD genetic testing were developed by the American College of Medical Genetics and Genomics, and there are international HD predictive genetic testing guidelines.[26-28] In view of the recent advances in HD genetics, updated guidelines are needed. Such guidelines should revise laboratory methodology (high-throughput ultra-deep sequencing with long-fragment sequencing), interpretation and reporting of test results including CAA interruption information, a modified protocol for predictive genetic counseling, and informed consent documents with information and choices on CAA interruptions.

However, before initiating modifier testing, it is important to recognize that all at-risk individuals may not want the added information. Currently, less than 20% of the at-risk population for HD and other untreatable autosomal dominant neurodegenerative diseases chooses to test.[29] These individuals wish to learn their genetic status for reproductive options, life planning, elimination of uncertainty, family information, and clinical trial candidacy. Most people do not wish to learn their status for fear of adverse psychological reactions, discrimination, lack of treatment, fatalism, loss of hope, and other reasons. Genetic testing does not always end uncertainty and may aggravate stress.[30]

In considering updating guidelines for HD, basic principles of medical ethics should be followed with some added considerations as noted here.

1. Autonomy:
   a. Individuals have a right to their own genetic results.
   b. Individuals have a right to choose to hear or not hear different results (eg, no results, CAG repeat numbers with/without CAA or modifier genes' results).

a. At-risk individuals should have full information (eg, what is known/not known about the significance of results) before deciding to test.

2. Beneficence/non-maleficence:
   a. Individuals should receive information to help them with healthcare/life decisions.
   b. Individuals should not be given possibly inaccurate information, and the implications of results should be clearly communicated.
   c. Individuals should be counseled about possible psychological impact of test results so they can opt in/out of hearing them.
3. Justice:
   a. Clinical laboratories should standardize HD testing.
   b. Modifier genes need to be studied in multi-ethnic cohorts before being part of clinical testing.
   c. Results should be reported at an appropriate educational level so that they can be understood by all individuals.
   d. Cost of testing should be affordable with equal access as a goal.

Recommendations

Based on these considerations, we would make several recommendations, as follows.

Recommendations:

1. Additional larger detailed studies are needed to better predict absolute effect sizes on HD outcomes for each specific *HTT* gene structure and other modifiers in diverse populations.
2. Clinical testing of modifiers other than CAA interruptions should be delayed until enough data are collected with results that will have clinical utility.
3. HD clinical trial design could benefit from genetic stratification: incorporation of *HTT* gene structure (including LOI or CAA duplication).
4. Clinical testing laboratories should move toward developing standardized genetic testing using long-fragment sequencing or PCR plus fragment analysis to detect both CAG repeats and CAA interruptions.

When this becomes available and if it will be offered clinically, the following recommendations will also become applicable:

1. Informed consent documents should be written to explain test results based on CAG repeats and the effect of LOI or CAA duplication. Individuals should have the option of opting in/out of CAA reporting.
2. Test reports should be carefully constructed to give CAG repeat numbers (as is standard now) in addition to an explanation of how LOI or CAG duplication influences the interpretation of the CAG repeat number (eg, "You carry *HTT* genes with 39 and 17 CAG repeats. Although 39 CAGs is in the reduced penetrant range, your gene lacks a CAA sequence. The absence of this CAA will cause your gene to act as though it had 41 repeats which is in the full-penetrant range for HD. Please talk with your healthcare provider for a more complete explanation").
3. Patient education material should be developed and made available in multiple languages.
4. Updated education of clinicians will be essential to offer at-risk individuals appropriate counseling about the limitations of testing with and without modifier testing, choices, and test interpretation. Predictive genetic testing should be conducted by a team of healthcare providers with expertise in HD, including a genetic counselor.

5. Studies should be conducted with at-risk individuals to determine interest in learning these expanded results, knowledge/comprehension of results, psychological impact, and communication to family members.

Conclusions

The discovery that CAA interruptions in the CAG repeat region of the *HTT* and several other modifiers can influence age of disease onset offers the possibility of refining prediction through genetic testing. Technology now exists to be able to do this; however, several key questions must be posed and addressed. This Viewpoint presents some challenges that will likely be involved, considerations that should underline guidelines, and suggestions of what these guidelines might contain. Given the ongoing advance of genetics and therapeutics for similar neurological diseases, timely development of guidelines incorporating these promising genetic testing advances is needed.

Data Availability Statement

There is no data being shared.

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