



Universiteit
Leiden
The Netherlands

Huntington's disease : hypothalamic, endocrine and metabolic aspects

Aziz, N.A.

Citation

Aziz, N. A. (2010, March 31). *Huntington's disease : hypothalamic, endocrine and metabolic aspects*. Retrieved from <https://hdl.handle.net/1887/15183>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/15183>

Note: To cite this publication please use the final published version (if applicable).

Normal and mutant *HTT* interact to affect clinical severity and progression in Huntington's disease

N. A. Aziz, MSc¹; C.K. Jurgens, MSc¹; G.B. Landwehrmeyer, MD² on behalf of the EHDN Registry Study Group³; W.M.C. van Roon-Mom, PhD⁴; G.J.B. van Ommen, PhD⁴; T. Stijnen, PhD⁵; R.A.C. Roos, MD¹

Neurology (2009); 73(16):1280-1285

¹ *Departments of Neurology, ⁴Human Genetics and ⁵Medical Statistics, Leiden University Medical Center, Leiden, the Netherlands*

² *Department of Neurology, Ulm University, Ulm, Germany*

³ *A complete list of all European Huntington Disease Network (EHDN) Registry Study Group investigators can be found on <http://www.euro-hd.net/html/registry>*

ABSTRACT

Objective: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in the HD gene (*HTT*). We aimed to assess whether interaction between CAG repeat sizes in the mutant and normal allele could affect disease severity and progression. *Methods:* Using linear regression and mixed-effects models, the influence of mutant and normal CAG repeat sizes interaction was assessed on: (1) age of onset in 921 HD patients, (2) clinical severity and progression in 512 of these patients with follow-up data available, and (3) basal ganglia volume on magnetic resonance images in 16 premanifest HD mutation carriers. *Results:* Normal and mutant CAG repeat sizes interacted to influence: (1) age of onset ($p=0.001$), (2) severity or progression of motor, cognitive and functional, but not behavioral, symptoms in HD patients (all $p<0.05$), and (3) in premanifest subjects, basal ganglia volumes ($p<0.05$). In subjects with mutant CAG expansions in the low range, increasing size of the normal repeat correlated with more severe symptoms and pathology, whereas for those subjects with expansions in the high range, increasing size of the normal repeat correlated with less severe symptoms and pathology. *Conclusions:* Increasing CAG repeat size in normal *HTT* diminishes the association between mutant CAG repeat size and disease severity and progression in HD. The underlying mechanism may involve interaction of the polyglutamine domains of normal and mutant huntingtin (fragments) and needs further elucidation. These findings may have predictive value and are essential for the design and interpretation of future therapeutic trials.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion in exon 1 of the *HTT* gene, resulting in a long polyglutamine tract in the N-terminus of the encoded protein huntingtin.¹ The disease is characterized by motor impairment, cognitive deterioration and behavioral disturbances.² The size of the CAG repeat sequence in the mutant allele is inversely related to age of onset, accounting for up to 73% of the variance.³ Moreover, larger mutant CAG repeat sizes correlate with an increased rate of deterioration on motor, cognitive and functional domains as well as weight loss, whereas behavioral symptoms appear not to be related to repeat size.⁴⁻⁶

It has been suggested that the polyglutamine stretch in normal huntingtin could bind to the expanded polyglutamine stretch, and thereby modulate the effects of mutant huntingtin or result in loss of function of the normal protein.⁷⁻⁹ Indeed, interaction between CAG repeat sizes in the mutant and normal allele is reported to influence age of onset, with larger sizes of the normal repeat being associated with a delayed age of onset in persons having large mutant CAG repeat sizes.¹⁰⁻¹³ However, it is not known whether interaction between mutant and normal huntingtin could also influence clinical signs and progression of the disease in HD patients, or affect indices of immanent phenocconversion in premanifest HD mutation carriers.

Therefore, this study was undertaken to test the hypothesis that CAG repeat sizes in the mutant and normal allele interact to influence not only age of onset, but also clinical severity and progression in HD patients, as well as brain pathology in premanifest HD mutation carriers.

METHODS

HD patients

We used monitored data from the European Huntington Disease Network (EHDN) Registry study collected prior to December 31st 2007. Registry – a multi-centre, prospective, observational study – is EHDN's core study. It was established in 2004 to collect phenotypical data and biomaterials from a large group of HD patients. The study aims to enlist one third of the European HD population by 2010. More information about the Registry cohort can be found at '<http://www.euro-hd.net/html/registry>'. In order to assess interactive effects of mutant and normal CAG repeat sizes on age of onset, we included all Registry participants with a clinical diagnosis of HD whose ages of onset and CAG repeat numbers in both alleles (mutant allele ≥ 36 repeats) were available (n=921). To assess the effects on clinical severity and disease progression, only those patients with two or more measurement occasions were included (n=512). The clinical characteristics of all HD patients are summarized in Table 1.

Clinical evaluations

Demographic data included age, gender and age of onset. Age of onset was defined as the age at which, according to the rater, the first motor, cognitive or behavioral signs of HD appeared. Visit data were collected annually (+/- 3 months). Clinical severity was assessed using the Unified Huntington Disease Rating Scale (UHDRS), and was recorded at all visits.¹⁴⁻¹⁶ In addition, body weight was recorded at every visit.

Premanifest HD mutation carriers

Sixteen premanifest subjects were included whose characteristics are summarized in Table 1. Premanifest status was defined as the absence of unequivocal HD signs by a neurologist specialized in movement disorders (R.A.C.R.).¹⁷ In all premanifest subjects, magnetic resonance imaging (MRI) was performed to assess caudate, putamen and globus pallidus volumes, using a 3.0 Tesla scanner (Philips Medical Systems, Best, The Netherlands) as described previously.¹⁷

Standard Protocol Approvals, Registrations, and Patient Consents

Full ethical approval has been obtained for each European country contributing to the Registry study. The MRI study was approved by the medical ethics committee of the Leiden University Medical Center. All subjects gave written informed consent.

Table 1. Characteristics of the study populations

	Group I^{† a}	Group II^{† b}	Group III^{† c}
<i>No. of subjects (n)</i>	921	512	16
<i>Male (%)</i>	47.9	47.5	37.5
<i>Age of onset, y</i>	42.9 (12.0; 6–79)	42.9 (11.8; 9–79)	-
<i>Age at MRI, y</i>	-	-	42.4 (10.1; 24–59)
<i>Disease duration, y*</i>	7.1 (5.3; 0–40.3)	7.0 (4.9; 0–29.5)	-
<i>UHDRS total motor score*</i>	35.3 (21.4; 0–106)	34.7 (20.6; 0–105)	-
<i>UHDRS TFC score*</i>	8.2 (3.7; 0–13)	8.2 (3.6; 0–13)	-
<i>Mutant CAG repeat size</i>	44.9 (5.0; 36–90)	44.9 (4.5; 36–90)	42.4 (2.7; 40–49)
<i>Normal CAG repeat size</i>	18.7 (3.2; 9–37) [‡]	18.6 (3.1; 9–32)	17.8 (3.2; 9–23)

*Disease duration, total motor score and total functional capacity (TFC) score are indicated at the first study visit.

[†] All data are indicated as means (standard deviation; range).

[‡] There was one homozygous subject in this cohort with 37 and 42 repeats; exclusion of this subject did not change the results.

^{a)} The total Registry cohort with data available on age of onset and CAG repeat sizes. ^{b)} All the patients from the total Registry cohort with two or more follow-up measurements; patients with and without follow-up data did not differ with respect to age, sex, body mass index, age of onset, mutant and normal CAG repeat size, and disease duration, total motor score and total functional capacity at first visit (all $p \geq 0.223$; unpaired t-tests and χ^2 -test). ^{c)} Premanifest HD gene carriers. MRI = Magnetic Resonance Imaging, TFC = Total Functional Capacity, UHDRS = Unified Huntington Disease Rating Scale.

Statistical analyses

To assess whether age of onset is influenced by the interaction between mutant and normal CAG repeat sizes, we used multiple linear regression. As the relation between age of onset and mutant CAG repeat size is known to be exponential³, we used the logarithmic transform of age of onset as the dependent variable. The sizes of the mutant and normal CAG repeats, and their interaction, were used as predictor variables. To examine whether clinical scores during the follow-up period were influenced by the interaction between mutant and normal CAG repeat number, we used linear mixed-effects models¹⁸ with the clinical scores as dependent variables. We used random effects to account for the correlation between the repeated measurements on each individual. In all models, both random intercepts and slopes were used for disease duration to reflect the heterogeneity in terms of baseline levels and evolution with time. Age of onset was always included as a covariate, since it can confound the relation between clinical phenotype and CAG repeat number.⁴ Other explanatory variables included mutant CAG repeat number and its interaction with disease duration, normal CAG repeat number and its interaction with disease duration, an interaction term between mutant and normal CAG repeat sizes, and an interaction term between disease duration and mutant and normal CAG repeat sizes. Thus, an interaction term in which disease duration is not included assesses whether two variables interact on the dependent variable at the mean time of follow-up, whereas an interaction term in which disease duration is included assesses whether an independent variable (or the combination of two independent variables in case of a three-way interaction) influences the *rate* of progression. In order to visualize the results, we drew regression lines based on the predictions of the mixed-effects models for different groupings of mutant and normal CAG repeat sizes, representing the association between clinical features and disease duration. In premanifest subjects, multiple linear regression was applied to assess whether CAG repeat sizes interact to affect basal ganglia volumes. Volumes of the caudate, putamen, globus pallidus, and their sum were used as dependent variables, while the sizes of the mutant and normal alleles, and their interaction, were used as predictor variables. In order to reduce multicollinearity, particularly with respect to the interaction terms, and simplify the interpretation of results, disease duration, age of onset, and mutant and normal CAG repeat sizes were centered around their respective means. We did not apply a specific correction for multiple testing because (1) based on previous literature we could formulate an a priori hypothesis presuming a relation between CAG repeat sizes interaction and disease severity and progression, and (2) there is a high degree of interdependence between various measures of clinical severity, i.e. the UHDRS scores cannot be regarded as independent of one another. Therefore, all tests were two-tailed and values of $p < 0.05$ were considered to be significant. Programming was performed in SPSS version 14.0 for Windows (SPSS Inc, Chicago, Ill, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

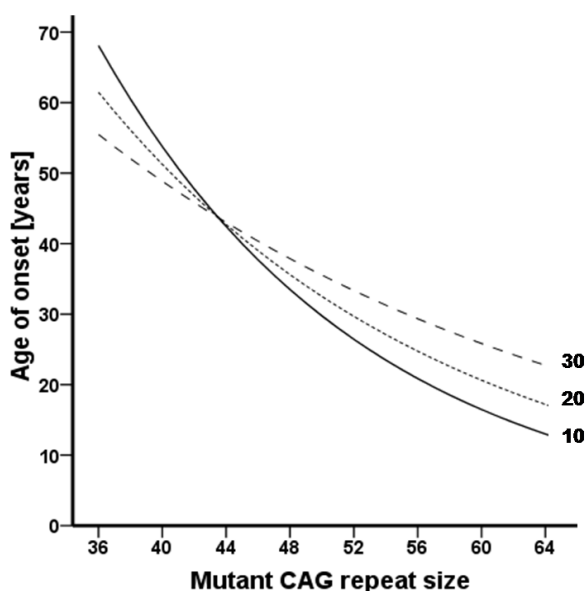
RESULTS

Age of onset

The clinical characteristics of this cohort of HD patients (group I) are summarized in the second column of table 1. The interaction term between mutant and normal CAG repeat sizes was highly significant (table E-1). The inclusion of the interaction term raised the adjusted R^2 from 0.529 to 0.534, indicating that the model with the interaction term included, can account for 53.4% of the variance in the age of onset. Model predicted best fit lines (figure 1), revealed that in the low range of mutant CAG repeat size, higher normal CAG repeat sizes

are related to a lower age of onset, while in the high range of the mutant repeat size, higher values of the normal repeat size are related to a higher age of onset. Thus, the association between mutant CAG repeat size and age of onset progressively weakens for higher normal CAG repeat sizes (figure 1).

Figure 1. Normal and mutant CAG repeat sizes interact to affect age of onset in HD patients. The relation between age of onset and mutant CAG repeat size progressively weakens with increasing normal CAG repeat size. Solid line, line with small dashes, and line with large dashes represent the relation between CAG repeat size in the mutant allele and age of onset for, successively, 10, 20 and 30 CAG repeats in the normal allele.



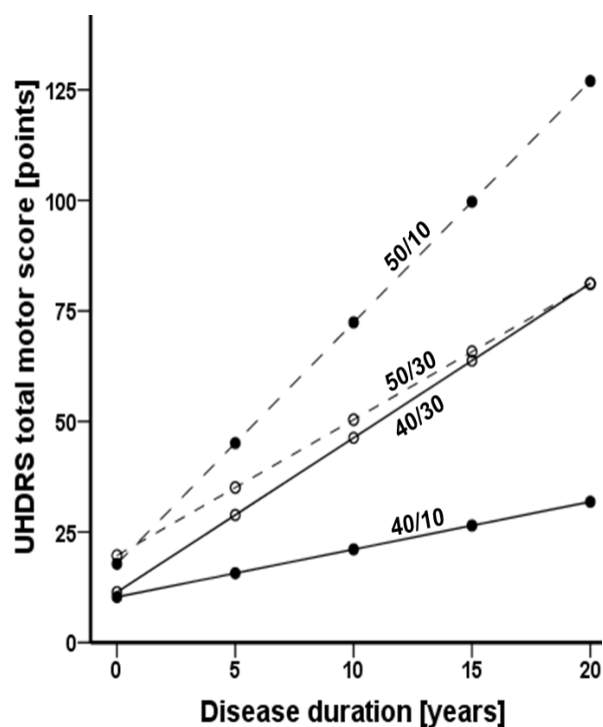
Clinical severity and progression

The clinical characteristics of the HD patients of whom follow-up data were available (group II) are summarized in the third column of table 1. Interaction between mutant and normal CAG repeat sizes significantly influenced the mean — at average disease duration — of the UHDRS total motor score, total cognitive score, and total functional capacity (TFC), but not the mean total behavioral score or body mass index (table E-2). The regression coefficients in table E-2 indicate that increasing normal CAG repeat size was associated with more severe symptoms in the low range of the mutant CAG repeat size, but with less severe symptoms in the high range of the mutant CAG repeat size. For example, in case of 40 CAG repeats in the mutant allele, for each unit increase in the normal CAG repeat size the mean motor score increased with 0.80 points, the mean cognitive score decreased with 3.05 points, and the mean TFC score decreased with 0.10 points. However, in case of 50 CAG repeats in the mutant allele, for each unit increase in the normal CAG repeat size the mean motor score decreased with 0.74 points, the mean cognitive score increased

with 2.16 points, and the mean TFC score increased with 0.08 points. In addition, the rate of progression of the UHDRS total motor score was influenced by the interaction between the two CAG repeat sizes (table E-2). Model predictions of total motor score during follow-up revealed that, in the lower range of the mutant CAG repeat sizes, increasing normal CAG repeat size was associated with a faster rate of motor progression, while in the upper range of mutant CAG repeat size, increasing normal CAG repeat size was associated with a slower rate of motor progression (figure 2, appendix E-1). Similar plots were generated for the total cognitive score, total behavioral score, total functional capacity, and body mass index. Except for the total behavioral score, these graphs showed a similar pattern as the total motor score, although the differences were generally less pronounced (data not shown).

Figure 2. Normal and mutant CAG repeat sizes interact to affect disease progression in HD patients.

The relation between motor progression and mutant CAG repeat size is different for various values of normal CAG repeat size. The graph shows model predicted best fit lines based on the median age of onset of 43 years for four different combinations of mutant and normal CAG repeat sizes: 40/10 (solid line and filled circles), 40/30 (solid line and open circles), 50/10 (dashed line and filled circles), and 50/30 (dashed line and open circles). Note that for large normal CAG repeat sizes — e.g. 30 in this case — the effect of the mutant CAG repeat size on disease progression is diminished, i.e. disease progression rate is very similar for 40/30 and 50/30 CAG repeat combinations. UHDRS = Unified Huntington Disease Rating Scale.



Basal ganglia volumes in premanifest subjects

The clinical features of this cohort of premanifest HD gene carriers (group III) are displayed in the last column of table 1. Volumes of the basal ganglia as a whole, as well as the putamen alone, were significantly affected by the interaction between the mutant and normal CAG repeat sizes, while the interaction effect was borderline significant in case of the caudate nucleus ($p = 0.091$) and globus pallidus ($p = 0.057$) volumes (table E-3). Again, when accounting for the centering of the data, the direction of the interaction effect was opposite to that of the effect of the mutant CAG repeat size. This indicates that, for a given mutant repeat size, larger sizes of the normal repeat weaken the association between mutant repeat size and basal ganglia volume.

DISCUSSION

We found that normal CAG repeat size interacts with mutant CAG repeat size to affect both clinical severity and progression in HD patients, as well as brain atrophy in premanifest HD mutation carriers. These findings represent a major extension upon previous reports on CAG repeat sizes interaction, all of which used age of onset as the sole outcome measure.^{10,11,13} Here we demonstrate that, in addition to age of onset, many signs and symptoms of HD, including motor, cognitive and functional indices, are also influenced by the interaction between CAG repeat sizes in the normal and mutant allele. As the effect of the interaction on basal ganglia volume could already be detected in premanifest subjects, our data suggest that the interplay between normal and mutant huntingtin (fragments) directly influences neuronal atrophy or loss and is thus an inherent feature of HD pathogenesis.

Several models could account for our findings, including competitive polyglutamine length dependent interaction of normal and mutant huntingtin with numerous protein binding partners,^{19,20} mitochondrial energy production²¹ or transcriptional mechanisms.²² However, as mutant N-terminal huntingtin fragments can promote the fibrillogenesis and co-aggregation of normal huntingtin fragments, with an increasing rate for larger polyglutamine stretches in either the normal or the mutant range,^{23,24} the simplest model would be to assume that the rate of co-aggregate formation is proportional to the polyglutamine stretch in either the normal or the mutant protein.^{23,24} This could explain the observation that the association between mutant CAG repeat size and clinical and pathological severity was weakened with larger normal CAG repeat sizes, since increasing stretches of polyglutamine in normal huntingtin could lead to a stronger association with mutant protein fragments, promoting their co-aggregation and preventing them from aberrantly interfering with other proteins.^{20,25} Indeed, a number of studies have found that normal huntingtin can reduce the cellular toxicity of the mutant protein both *in vitro* and *in vivo*.^{7,26,27} However, a stronger interaction between normal and mutant huntingtin could also result in a greater degree of loss of normal huntingtin function.²³ There is now considerable evidence indicating that normal huntingtin is essential for neuronal function and survival, that it can protect cells from a host of toxic stimuli, and that loss of normal huntingtin function is likely to be involved in HD pathogenesis.^{8,9,27-30} Therefore, the interaction between mutant and normal huntingtin could have both beneficial (i.e. mitigation of mutant protein toxicity) and detrimental (i.e. loss of normal huntingtin function) effects.^{23,25} The finding that in subjects with mutant CAG expansions in the low range, increasing size of the normal repeat correlated with more severe symptoms and pathology, suggests that in the low range of mutant polyglutamine stretches, the net effect of this interaction is likely to be detrimental due to loss of normal huntingtin function. Conversely, as in subjects with expansions in the high range increasing size of the normal repeat correlated with less severe symptoms and pathology, the net effect of this interaction in the high range of the mutant polyglutamine sequences is likely to be beneficial due to mitigation of mutant protein toxicity.

As we show that the normal *HTT* allele can also influence disease severity, our findings are in line with recent studies in transgenic mouse models of HD³¹ and indicate that HD displays an intermediate dominant phenotype in humans as well. These findings challenge the classical common view of HD as a disorder with complete dominance, which is largely based on the clinical evaluation of small groups of potential homozygotes prior to the discovery of the genetic mutation.^{32,33} More recent clinical, imaging and neuropathological assessment of genetically confirmed homozygote patients with HD, however, did indeed reveal an increased rate of disease progression.³⁴ Additionally, our findings are supported by recent studies in transgenic HD mice with super-long CAG repeat expansions which show that homozygous transgenic mice with CAG repeat expansions of around 400 in both transgenes do not exhibit a noteworthy acceleration of phenotype compared to mice with only one 400 CAG transgene, whereas homozygous mice with one transgene in the 350 CAG range and one in the 400 CAG range, do show an accelerated phenotype.^{35,36} Together these findings underscore that the exact as well as the relative sizes of the CAG repeat tract in both *HTT* alleles are important determinants of pathology in HD.

Potential limitations of our study include the lack of follow-up data on a subset of HD patients and the relatively short follow-up period. However, patients with and without longitudinal data did not differ in any particular

way (table 1), indicating that lack of follow-up in some cases is unlikely to have biased the results. As the Registry cohort continues to accrue, more precise estimates of the clinical effects of the CAG repeat sizes interaction will be possible in the future.

ACKNOWLEDGEMENTS

We express our gratitude to all the European Huntington Disease Network investigators for collecting the data, and would also like to thank all the participating patients for their time and efforts. The EHDN's Registry study is sponsored by the High Q Foundation. N.A.Aziz is supported by The Netherlands Organization for Scientific Research (grant #017.003.098).

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993; 72(6):971-983.
2. Bates G, Harper PS, Jones L. Huntington's Disease. Third edition ed. New York: Oxford University Press, 2002.
3. Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. *Clin Genet* 2004; 65(4):267-277.
4. Ravina B, Romer M, Constantinescu R, Biglan K, Brocht A, Kiebertz K et al. The relationship between CAG repeat length and clinical progression in Huntington's disease. *Mov Disord* 2008; 23(9):1223-1227.
5. Rosenblatt A, Liang KY, Zhou H, Abbott MH, Gourley LM, Margolis RL et al. The association of CAG repeat length with clinical progression in Huntington disease. *Neurology* 2006; 66(7):1016-1020.
6. Aziz NA, van der Burg JM, Landwehrmeyer GB, Brundin P, Stijnen T, EHDI Study Group et al. Weight loss in Huntington disease increases with higher CAG repeat number. *Neurology* 2008; 71(19):1506-1513.
7. Leavitt BR, Guttman JA, Hodgson JG, Kimel GH, Singaraja R, Vogl AW et al. Wild-type huntingtin reduces the cellular toxicity of mutant huntingtin in vivo. *Am J Hum Genet* 2001; 68(2):313-324.
8. Rigamonti D, Bauer JH, De Fraja C, Conti L, Sipione S, Sciorati C et al. Wild-type huntingtin protects from apoptosis upstream of caspase-3. *J Neurosci* 2000; 20(10):3705-3713.
9. Cattaneo E, Zuccato C, Tartari M. Normal huntingtin function: an alternative approach to Huntington's disease. *Nat Rev Neurosci* 2005; 6(12):919-930.
10. Djousse L, Knowlton B, Hayden M, Almqvist EW, Brinkman R, Ross C et al. Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. *Am J Med Genet A* 2003; 119(3):279-282.
11. Farrer LA, Cupples LA, Wiater P, Conneally PM, Gusella JF, Myers RH. The normal Huntington disease (HD) allele, or a closely linked gene, influences age at onset of HD. *Am J Hum Genet* 1993; 53(1):125-130.

12. Kehoe P, Krawczak M, Harper PS, Owen MJ, Jones AL. Age of onset in Huntington disease: sex specific influence of apolipoprotein E genotype and normal CAG repeat length. *J Med Genet* 1999; 36(2):108-111.
13. Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P et al. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat Genet* 1993; 4(4):393-397.
14. Djousse L, Knowlton B, Cupples LA, Marder K, Shoulson I, Myers RH. Weight loss in early stage of Huntington's disease. *Neurology* 2002; 59(9):1325-1330.
15. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. *Mov Disord* 1996; 11(2):136-142.
16. Siesling S, van Vugt JP, Zwinderman KA, Kieburts K, Roos RA. Unified Huntington's disease rating scale: a follow up. *Mov Disord* 1998; 13(6):915-919.
17. Jurgens CK, van de WL, van Es AC, Grimbergen YM, Witjes-Ane MN, van der GJ et al. Basal ganglia volume and clinical correlates in 'preclinical' Huntington's disease. *J Neurol* 2008; 255(11):1785-1791.
18. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis. Hoboken, New Jersey: John Wiley & Sons, Inc., 2004.
19. Kaltenbach LS, Romero E, Becklin RR, Chettier R, Bell R, Phansalkar A et al. Huntingtin interacting proteins are genetic modifiers of neurodegeneration. *PLoS Genet* 2007; 3(5):e82.
20. Li XJ, Friedman M, Li S. Interacting proteins as genetic modifiers of Huntington disease. *Trends Genet* 2007; 23(11):531-533.
21. Seong IS, Ivanova E, Lee JM, Choo YS, Fossale E, Anderson M et al. HD CAG repeat implicates a dominant property of huntingtin in mitochondrial energy metabolism. *Hum Mol Genet* 2005; 14(19):2871-2880.
22. Benn CL, Sun T, Sadri-Vakili G, McFarland KN, DiRocco DP, Yohrling GJ et al. Huntingtin modulates transcription, occupies gene promoters in vivo, and binds directly to DNA in a polyglutamine-dependent manner. *J Neurosci* 2008; 28(42):10720-10733.
23. Busch A, Engemann S, Lurz R, Okazawa H, Lehrach H, Wanker EE. Mutant huntingtin promotes the fibrillogenesis of wild-type huntingtin: a potential mechanism for loss of huntingtin function in Huntington's disease. *J Biol Chem* 2003; 278(42):41452-41461.
24. Slepko N, Bhattacharyya AM, Jackson GR, Steffan JS, Marsh JL, Thompson LM et al. Normal-repeat-length polyglutamine peptides accelerate aggregation nucleation and cytotoxicity of expanded polyglutamine proteins. *Proc Natl Acad Sci U S A* 2006; 103(39):14367-14372.
25. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 2004; 431(7010):805-810.
26. Ho LW, Brown R, Maxwell M, Wyttenbach A, Rubinsztein DC. Wild type Huntingtin reduces the cellular toxicity of mutant Huntingtin in mammalian cell models of Huntington's disease. *J Med Genet* 2001; 38(7):450-452.
27. Van Raamsdonk JM, Pearson J, Rogers DA, Bissada N, Vogl AW, Hayden MR et al. Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. *Hum Mol Genet* 2005; 14(10):1379-1392.
28. Rigamonti D, Bolognini D, Mutti C, Zuccato C, Tartari M, Sola F et al. Loss of huntingtin function complemented by small molecules acting as repressor element 1/neuron restrictive silencer

element silencer modulators. *J Biol Chem* 2007; 282(34):24554-24562.

29. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 2001; 293(5529):493-498.
30. Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat Genet* 2003; 35(1):76-83.
31. Graham RK, Slow EJ, Deng Y, Bissada N, Lu G, Pearson J et al. Levels of mutant huntingtin influence the phenotypic severity of Huntington disease in YAC128 mouse models. *Neurobiol Dis* 2006; 21(2):444-455.
32. Myers RH, Leavitt J, Farrer LA, Jagadeesh J, McFarlane H, Mastromauro CA et al. Homozygote for Huntington disease. *Am J Hum Genet* 1989; 45(4):615-618.
33. Wexler NS, Young AB, Tanzi RE, Travers H, Starosta-Rubinstein S, Penney JB et al. Homozygotes for Huntington's disease. *Nature* 1987; 326(6109):194-197.
34. Squitieri F, Gellera C, Cannella M, Mariotti C, Cislighi G, Rubinsztein DC et al. Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. *Brain* 2003; 126(Pt 4):946-955.
35. Dragatsis I, Goldowitz D, Del Mar N, Deng YP, Meade CA, Liu L et al. CAG repeat lengths \geq 335 attenuate the phenotype in the R6/2 Huntington's disease transgenic mouse. *Neurobiol Dis* 2009; 33(3):315-330.
36. Morton AJ, Glynn D, Leavens W, Zheng Z, Faull RL, Skepper JN et al. Paradoxical delay in the onset of disease caused by super-long CAG repeat expansions in R6/2 mice. *Neurobiol Dis* 2009; 33(3):331-341.

Table E-1. Effect of interaction between mutant and normal CAG repeat sizes on natural log-transform of age of onset in Huntington disease patients.

	Regression coefficients [†] (95% CI)	p-values
<i>Mean value</i>	3.711 (3.697, 3.725)	<0.001
<i>mCAG[‡] effect</i>	-0.047 (-0.050, -0.044)	<0.001
<i>nCAG[‡] effect</i>	0.002 (-0.002, 0.006)	0.394
<i>mCAG × nCAG[‡] interaction effect</i>	0.001 (0.001, 0.002)	0.001

[†]) All values and effect sizes are centered around the mean mutant CAG repeat size of 44.901, and mean normal CAG repeat size of 18.673. CI = Confidence Interval.

[‡]) mCAG = mutant CAG repeat size; nCAG = normal CAG repeat size.

Table E-2. Mutant and normal CAG repeat sizes interact to influence clinical severity and progression in Huntington disease patients

	Mean value (SE) ^a	Age of onset effect (SE) ^{a,b} points/y	Time effect (SE) ^{a,c} points/y	mCAG effect (SE) ^{a,d} points/repeat	mCAG × time effect (SE) ^{a,e} points/repeat/y	nCAG effect (SE) ^{a,f} points/repeat	nCAG × time effect (SE) ^{a,g} points/repeat/y	mCAG × nCAG effect (SE) ^{a,h} points/repeat	mCAG × nCAG × time effect (SE) ^{a,i} points/repeat/repeat/y
<i>Total motor score</i>	37.182 (0.772)	0.620 (0.083)***	3.242 (0.157)***	2.401 (0.236)***	0.231 (0.042)***	0.102 (0.256)	0.004 (0.050)	-0.164 (0.046)***	-0.024 (0.012)*
<i>Total cognitive score</i>	159.472 (3.570)	-3.132 (0.397)***	-8.270 (0.755)***	-7.645 (1.056)***	-0.553 (0.194)**	-0.517 (1.163)	-0.189 (0.233)	0.521 (0.191)**	0.035 (0.055)
<i>Total behavioral score</i>	11.418 (0.517)	-0.062 (0.060)	0.035 (0.119)	-0.116 (0.173)	-0.037 (0.032)	0.117 (0.163)	0.002 (0.037)	0.003 (0.031)	-0.009 (0.009)
<i>TFC score</i>	7.792 (0.137)	-0.108 (0.016)***	-0.559 (0.028)***	-0.314 (0.043)***	-0.034 (0.007)***	-0.009 (0.045)	-0.003 (0.009)	0.018 (0.008)*	0.001 (0.002)
<i>BMI</i>	23.817 (0.164)	0.027 (0.619)	-0.017 (0.033)	-0.136 (0.051)**	-0.008 (0.008)	0.009 (0.053)	0.001 (0.010)	0.016 (0.010)	0.002 (0.003)

^a) All values and effect sizes are centered around the mean age of onset of 42.853 years, mean disease duration of 6.996 years, mean mutant CAG repeat size of 44.861, and mean normal CAG repeat size of 18.633. BMI = Body Mass Index; mCAG = mutant CAG repeat size; nCAG = normal CAG repeat size; SE = Standard Error, Time = disease duration; TFC = Total Functional Capacity; y = year. * p < 0.05; ** p < 0.01; *** p < 0.001.

^b) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per year increase of age of onset (at mean values of disease duration, and mutant and normal CAG repeat sizes).

^c) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per year increase of disease duration (at mean values of age of onset, and mutant and normal CAG repeat sizes).

^d) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of mutant CAG repeat size (at mean values of age of onset, disease duration and normal CAG repeat size).

^e) This column indicates the increase or decrease in the rate of change (points/year) of the Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of mutant CAG repeat size (at mean values of age of onset and normal CAG repeat size).

^f) This column indicates the increase or decrease in average Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of normal CAG repeat size (at mean values of age of onset, disease duration and mutant CAG repeat size).

^g) This column indicates the increase or decrease in the rate of change (points/year) of the Unified Huntington Disease Rating Scale total scores and body mass index per unit increase of normal CAG repeat size (at mean values of age of onset and mutant CAG repeat size).

^h) This column indicates the effect of the mutant and normal CAG repeat sizes interaction on the average Unified Huntington Disease Rating Scale total scores and body mass index (at mean values of age of onset and disease duration).

ⁱ) This column indicates the effect of the mutant and normal CAG repeat sizes interaction on the rate of change of the Unified Huntington Disease Rating Scale total scores and body mass index (at mean value of age of onset).

Table E-3. Effects of interaction between mutant and normal CAG repeat sizes on basal ganglia volume in premanifest subjects

	Mean value [†] (SE)	Age effect [†] (SE)	mCAG effect [†] (SE)	nCAG effect [†] (SE)	mCAG × nCAG interaction effect [†] (SE)
<i>Caudate volume</i>	6.757 (0.296)	-0.066 (0.025)*	-0.016 (0.098)	-0.307 (0.111)*	-0.111 (0.060)
<i>Putamen volume</i>	6.527 (0.286)	-0.069 (0.024)*	-0.091 (0.095)	-0.268 (0.108)*	-0.161 (0.058)*
<i>Globus pallidus volume</i>	1.434 (0.137)	-0.035 (0.011)*	-0.066 (0.045)	-0.106 (0.052)	-0.059 (0.028)
<i>Total basal ganglia volume</i>	14.718 (0.600)	-0.170 (0.050)**	-0.172 (0.199)	-0.681 (0.226)*	-0.331 (0.121)*

[†]) All values and effect sizes are centered around the mean age of 42.445, mean mutant CAG repeat size of 42.438, and mean normal CAG repeat size of 17.813.

* p < 0.05; ** p < 0.01

Appendix E-1. Estimating disease progression

The coefficients provided in table E-2 can be used to estimate the clinical scores of HD patients while accounting for age of onset, as well as mutant and normal *HTT* CAG repeat sizes. For example, the following equation can be used to estimate the UHDRS total motor score:

$$\text{Total motor score} \approx 37.182 + 0.620 \times (\text{age of onset} - 42.853) + 3.242 \times (\text{disease duration} - 6.996) + 2.401 \times (\text{mutant CAG repeat size} - 44.861) + 0.231 \times (\text{mutant CAG repeat size} - 44.861)(\text{disease duration} - 6.996) + 0.102 \times (\text{normal CAG repeat size} - 18.633) + 0.004 \times (\text{normal CAG repeat size} - 18.633)(\text{disease duration} - 6.996) - 0.164 \times (\text{mutant CAG repeat size} - 44.861)(\text{normal CAG repeat size} - 18.633) - 0.024 \times (\text{mutant CAG repeat size} - 44.861)(\text{normal CAG repeat size} - 18.633)(\text{disease duration} - 6.996)$$

The effect of the interaction between mutant and normal CAG repeat size on the total motor score can become substantial over time. This can be illustrated by using the above equation. Suppose individuals A and B both have an age of onset of 43 years and an expansion of 40 CAG repeats in their mutant alleles, while the number of repeats in their normal alleles is different: 10 for person A and 30 for person B. Five years after disease onset, the UHDRS total motor score of person A will equal 16 points ($\approx 37.182 + 0.620 \times (43-42.853) + 3.242 \times (5 - 6.996) + 2.401 \times (40 - 44.861) + 0.231 \times (40 - 44.861)(5 - 6.996) + 0.102 \times (10 - 18.633) + 0.004 \times (10 - 18.633)(5 - 6.996) - 0.164 \times (40 - 44.861)(10 - 18.633) - 0.024 \times (40 - 44.861)(10 - 18.633)(5 - 6.996)$), whereas person B will have a score of 29 points. A considerable difference of 81% after only five years, that, in addition, will continue to increase over time (see also figure 2 in the manuscript).

