CAG repeat expansion in Huntington disease determines age at onset in a fully dominant fashion

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Supplemental data at www.neurology.org

Supplemental Data

ABSTRACT

Objective: Age at onset of diagnostic motor manifestations in Huntington disease (HD) is strongly correlated with an expanded CAG trinucleotide repeat. The length of the normal CAG repeat allele has been reported also to influence age at onset, in interaction with the expanded allele. Due to profound implications for disease mechanism and modification, we tested whether the normal allele, interaction between the expanded and normal alleles, or presence of a second expanded allele affects age at onset of HD motor signs.

Methods: We modeled natural log-transformed age at onset as a function of CAG repeat lengths of expanded and normal alleles and their interaction by linear regression.

Results: An apparently significant effect of interaction on age at motor onset among 4,068 subjects was dependent on a single outlier data point. A rigorous statistical analysis with a well-behaved dataset that conformed to the fundamental assumptions of linear regression (e.g., constant variance and normally distributed error) revealed significance only for the expanded CAG repeat, with no effect of the normal CAG repeat. Ten subjects with 2 expanded alleles showed an age at motor onset consistent with the length of the larger expanded allele.

Conclusions: Normal allele CAG length, interaction between expanded and normal alleles, and presence of a second expanded allele do not influence age at onset of motor manifestations, indicating that the rate of HD pathogenesis leading to motor diagnosis is determined by a completely dominant action of the longest expanded allele and as yet unidentified genetic or environmental factors. *Neurology*® 2012;78:690–695

GLOSSARY

HD = Huntington disease.

Huntington disease (HD) is a neurodegenerative disorder with motor impairment, cognitive decline, and psychiatric manifestations[1,2] caused by an expanded CAG trinucleotide repeat (>35 CAGs) in the gene encoding huntingtin. The expansion shows a very strong negative correlation with age at onset of motor signs.[3–7] Although the dominant inheritance pattern of HD indicates that one expanded allele is sufficient to trigger the disorder, polymorphism of the normal allele CAG repeat (i.e., the number of consecutive CAGs in the nonexpanded allele; <36 CAGs) has been suggested in some but not all studies to influence the timing of onset of disease manifestations.[6–9] Recently, an interaction of the expanded and normal allele was reported in a large study to modify age at onset based upon motor signs, cognitive change, and behavioral manifestations.[10] Counterintuitively, longer normal alleles (e.g., 30 CAGs) seemingly delayed age at onset of subjects with longer expanded CAG alleles (e.g., >50 CAGs).[10] This finding would have important implications for the precise molecular mechanism that initiates HD pathogenesis, as it would suggest that individual huntingtin molecules might physically interact, with a resultant alteration of the pathogenic potential of the expanded repeat. Similarly, if the normal allele can modify HD pathogenesis, it would provide a potential

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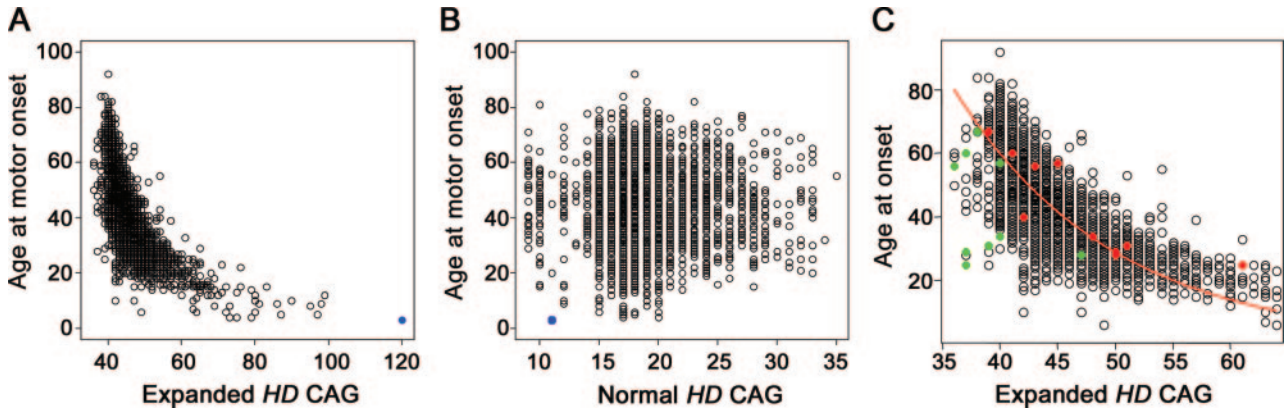
Figure 1 Relationship of expanded allele CAG repeat length and age at onset of motor manifestations in individuals with Huntington disease



An initial regression model was generated using all 4,068 subjects with Huntington disease (HD), each heterozygous for 1 expanded allele (>35 CAG) and 1 allele in the normal range (<36 CAG). (A) The relationship between expanded allele CAG repeat length and age at onset of motor signs. (B) The relationship between normal allele CAG repeat length and age at onset of motor signs. Filled blue circle: a subject with 120 CAGs with a disproportionate influence on the model. (C) Subjects with 2 expanded alleles (>35 CAGs) were plotted (filled red circle: longer expanded allele; green: shorter expanded allele) over the heterozygote subsample conforming to statistical assumptions underlying regression analysis (3,674 HD subjects based upon expanded CAGs from 40–53 and statistical exclusion of outliers; see figures e-3, e-4, and e-5). One homozygous subject has 2 equal alleles of 42 CAGs. The minimal adequate model for this heterozygote data set is shown as a red line.

route to therapeutic intervention to delay or prevent onset via a genetic modifier validated to act via a mechanism that operates in humans with HD. Consequently, we re-explored this critical issue using a much larger dataset. Specifically, we aimed to test whether the normal allele CAG repeat, either alone or in interaction with the expanded allele, or the presence of a second expanded allele modifies age at onset of HD motor signs.

METHODS

Subjects. We analyzed DNA of 4,078 patients with HD (1,864 men, 1,845 women, and 369 unreported) with known age at onset of overt motor manifestations, including 10 subjects with 2 expanded *HD* alleles ("HD homozygote"). DNA samples were from subjects involved in long-term genetic studies in the Massachusetts HD Center Without Walls, either from the local region or from collaborating investigators (HD-MAPS), and from 3 large HD observational studies (COHORT, PREDICT-HD, and REGISTRY). The *HD* CAG repeat length was determined using a modification of the PCR amplification assay reported by Warner et al.,[9] with a fluorescent oligonucleotide primer pair flanking the repeat for automated allele calling after capillary electrophoresis on an ABI3730XL DNA Analyzer, using a set of HD CAG allele standards determined by DNA sequencing, as reported.[11] The means of mutant and normal CAG repeat lengths of HD heterozygote subjects (4,068) were 44.86 (range, 36~120; median, 44) and 18.48 (range, 9~35; median, 18), respectively (figure e-1 on the *Neurology*® Web site at www.neurology.org).

Standard protocol approvals, registrations, and patient consents. This study used only deidentified, previously collected DNA samples and phenotypic data in a manner approved by the Institutional Review Board of Partners HealthCare, Inc.

Statistical analysis. Primarily, this study utilized samples from 4,068 HD heterozygote subjects with 1 expanded *HD* allele. Natural log-transformed age at onset of motor signs was modeled as a function of mutant *HD* CAG repeat length, normal *HD* CAG repeat length, and interaction of mutant and normal allele as independent variables using a linear regression analysis. For models involving interaction terms, all continuous variables were centered around the mean value of each variable. Initially, statistical models were fitted to data including all subjects. Subsequently, as described in the supplemental data, we built models using only normally distributed data points in the restricted range of 40 to 53 CAG repeats, where there is no evidence of heteroscedasticity. All statistical analyses were performed using *R* (version 2.7.2).

RESULTS

Regression analysis of HD heterozygotes. For all subjects with one expanded *HD* allele (4,068; figure e-1), the relationship with age at motor onset is shown for both the expanded and normal allele CAG repeat lengths in figure 1, A and B, respectively. For this dataset, we modeled natural log-transformed age at onset by 1) expanded allele CAG repeat length (greater than 35 repeats), 2) normal allele CAG repeat length (35 or fewer repeats), and 3) interaction between them using a linear regression analysis, as has been the common practice in investigation of allele length effects in CAG repeat disorders. Although we initially observed an apparent significant association for the interaction of the normal and mutant alleles, we discovered that this result (table 1) relied on a single outlier with a mutant allele of 120 CAGs and a normal allele of 11 CAGs (shown in blue in figure 1, A and B). That one individual sample could have such a profound effect on the final result indicated that in testing for genetic modifiers,

Table 1 Statistical analyses of the regression models incorporating expanded and normal CAG alleles and their potential interaction

Regression model[a] (CAG range)	No. of subjects	Intercept (*p* value)	Expanded CAG allele (*p* value)	Normal CAG allele (*p* value)	Interaction (*p* value)	R^2
Initial model (36–120)	4,068	−0.00023 (0.943)	−0.05256 ($<2\times10^{-16}$)	−0.00106 (0.267)	−0.00061 (0.0006)	0.637
Initial model excluding one 120 CAG subject (36–99)	4,067	−0.00003 (0.992)	−0.05304 ($<2\times10^{-16}$)	−0.00084 (0.376)	−0.00013 (0.506)	0.634
Updated model[b] (40–53)	3,674	−0.00002 (0.993)	−0.07310 ($<2\times10^{-16}$)	−0.00045 (0.548)	−0.00015 (0.559)	0.653

[a] Log_e (age at onset of motor manifestations) ~ expanded + normal + expanded × normal, using centered variables.
[b] See figure e-5.

the analysis of HD age-at-onset data requires a more detailed, rigorous statistical analysis that better conforms with the fundamental assumptions of linear regression analysis (normally distributed data points with constant variance). Consequently, we performed a series of statistical analyses to explore this question (figures e-2, e-3, e-4, and e-5), including analysis of subjects with expanded CAG repeats between 40 and 53, with a formal exclusion of outliers, and a separate analysis of the outlier individuals excluded from the model. In no case did we obtain a statistically significant result supporting either an independent effect of the normal CAG repeat or an interaction of the normal and mutant CAG repeats (table 1; table e-1). We also tested directly whether subjects with shorter normal allele CAG repeat lengths had a different age at onset than subjects with longer normal allele CAG repeat lengths (figure e-6), again finding no significant effect of the normal CAG allele on age at onset. Thus, our results indicate that the expanded allele CAG repeat length is the single most important determinant of the age at onset of motor signs of HD and that the normal CAG allele length does not play a significant modifying role in the pathogenic process leading to motor onset.

HD "homozygotes." Importantly, in addition to the heterozygote subjects analyzed above, there were 10 subjects with 2 expanded alleles in our dataset. Although the sample size is small, these subjects would have the potential to reveal any major additive or synergistic effects of possessing 2 expanded alleles and no normal allele. There is clearly no additive effect, as all of these individuals would be expected to show motor onset at <4 years of age if this were determined by the sum of the 2 expanded CAG repeats. Interestingly, subjects with 2 expanded alleles (figure 1C; red, longer allele; green, shorter allele) did not show a significant deviation from the minimal adequate statistical model (from figure e-5 data) when plotted based upon the longer of their 2 expanded alleles (figure 1C, red line). The ages at onset of these subjects resided within 2 standard deviations of each allele-specific age at onset spectrum. Combined with our findings from HD heterozygotes, these data indicate that age at onset of motor manifestations in HD is primarily determined by a single allelic dose of the mutant gene and is not influenced dramatically by the length of the normal allele, by the presence of a second mutant allele, or by the absence of a normal allele.

DISCUSSION It is well known that HD is initiated by a dominant action of an expanded CAG repeat in the *HD* gene and that age at onset strongly correlates with expanded allele CAG repeat length. However, there has been much debate in the field whether age at onset is influenced by the second HD allele, especially the length of the CAG repeat on the normal chromosome.[6–10,12–15] This is a critical question, as the answer determines whether the pathogenic effect of the expanded polyglutamine tract in mutant huntingtin protein can be modulated by expression of an equivalent huntingtin protein with a polyglutamine repeat in the normal size range. A positive finding would clearly have ramifications both for the current effort to develop a therapy by nonselective RNA-mediated suppression of mutant and normal *HD* alleles and also for the potential of using wild-type huntingtin as a therapeutic protein in HD.

Using the largest dataset so far, with stringent statistical analyses, our results have dismissed a significant role for the normal allele CAG repeat length in modifying age at onset of motor manifestations, indicating that the expanded allele CAG repeat length is not only the initial trigger of HD pathogenesis but also the predominant factor determining the rate of the process that leads to motor onset. We found no evidence that an interaction between the expanded allele and the normal allele is an explanation for any portion of the variance in age at onset of motor signs that is not already explained by the expanded allele alone. Furthermore, our findings with a larger series of CAG repeat genotyped HD homozygotes support previous reports that, at lengths over 40 repeats (repeats <40 may show nonpenetrance), a single allelic

dose of the HD mutation is sufficient to cause HD within a typical human lifespan and to determine age at motor onset.[16–21] Though the 10 homozygotes studied are too few to exclude any onset-modifying effect of the second mutant allele on a statistical basis, any such effect would have to be quite small compared to the effect of the single mutant allele discerned in HD heterozygotes. This suggests that the level of mutant huntingtin protein produced from a single allele already exceeds any minimum threshold required to trigger pathogenesis (at a rate determined primarily by its CAG repeat length) and that neither additional mutant protein nor the absence of any normal protein further alters the rate of pathogenesis leading to motor onset.

Although our results clearly showed no effect of the normal allele CAG repeat length on age at onset, other *cis*-factors may play a role in modifying age at onset. For example, a gene closely linked to the *HD* gene has been hypothesized to modify age at onset[14] and, in the HD-MAPS study, suggestive evidence for linkage was found at chromosome 4p16.[22] Therefore, it also will be important to determine a potential role for other *cis*-factors in potentially modifying age at onset of HD manifestations. In addition, although neither the normal *HD* CAG allele repeat length nor its interaction with the expanded allele influenced age at motor onset, the remaining variance has been reported to be highly heritable,[12,15] indicating the presence in the genome of genetic modifiers. Such modifier genes would provide potential therapeutic targets already validated to alter the course of HD in human patients and so their identification is a very active area of investigation. The findings reported here provide the basis for more reliable statistical assessment of the role of individual modifier genes and should contribute to their unequivocal identification.

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AUTHOR CONTRIBUTIONS

Dr. J.-M. Lee conceived of and performed the statistical analysis and interpretation and drafted the manuscript. Drs. Ramos, J.-H. Lee, Gillis, and Mysore generated and interpreted molecular data. Drs. Hayden, Warby, Morrison, Nance. Ross, Margolis, Squitieri, Orobello, Di Donato, Gomez-Tortosa, Ayuso, Suchowersky, Trent, McCusker, Novelletto, Frontali, Jones, Ashizawa, Frank, Saint-Hilaire, Hersch, Rosas, Lucente, Harrison, Zanko, Abramson, Marder, and Sequeiros aided in the conceptualization and design of the study and revised the manuscript for intellectual content. Drs. Paulsen and Landwehrmeyer revised the manuscript for intellectual content. Drs. Myers, MacDonald, and Gusella participated in conceptualization and design of the study and interpretation of the data and revised the manuscript for intellectual content.

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