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# TAA repeat variation in the *GRIK2* gene does not influence age at onset in Huntington's disease

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ABSTRACT

Huntington's disease is a neurodegenerative disorder caused by an expanded CAG trinucleotide repeat whose length is the major determinant of age at onset but remaining variation appears to be due in part

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to the effect of genetic modifiers. *GRIK2*, which encodes GluR6, a mediator of excitatory neurotransmission in the brain, has been suggested in several studies to be a modifier gene based upon a 3' untranslated region TAA trinucleotide repeat polymorphism. Prior to investing in detailed studies of the functional impact of this polymorphism, we sought to confirm its effect on age at onset in a much larger dataset than in previous investigations. We genotyped the *HD* CAG repeat and the *GRIK2* TAA repeat in DNA samples from 2,911 Huntington's disease subjects with known age at onset, and tested for a potential modifier effect of *GRIK2* using a variety of statistical approaches. Unlike previous reports, we detected no evidence of an influence of the *GRIK2* TAA repeat polymorphism on age at motor onset. Similarly, the *GRIK2* polymorphism did not show significant modifier effect on psychiatric and cognitive age at onset in HD. Comprehensive analytical methods applied to a much larger sample than in previous studies do not support a role for *GRIK2* as a genetic modifier of age at onset of clinical symptoms in Huntington's disease.



## 1. Introduction

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder that presents with motor dysfunction, cognitive decline and psychiatric disturbances due to expansion of a trinucleotide CAG repeat encoding a polyglutamine tract in the huntingtin protein [1,2]. There is a strong inverse correlation between age at diagnosis by onset of motor signs and the CAG repeat length, which accounts for up to 67% of the overall variance [3–5]. The remaining variation is strongly heritable, indicating a contribution of modifier genes to determining age at onset [6–8].

*GRIK2* (Glutamate receptor, ionotropic kainate 2), encoding the GluR6 subunit of the predominant excitatory neurotransmitter receptor family in the human brain, is an attractive candidate as an HD modifier because of its potential role in excitotoxic cell death [9]. A TAA trinucleotide repeat polymorphism in the 3'untranslated region (3'UTR) of *GRIK2* mRNA was reported to be associated with age at onset of diagnostic motor signs in small studies from the United Kingdom, New England, France, India, and Italy [10–14], the largest of which comprised less than 300 subjects. The two initial studies suggested that the modifier effect was due to genetic variation on chromosomes carrying a 16 TAA repeat allele. The genetic variation responsible for the earlier than expected age at onset was implicated as the TAA repeat itself by subsequent haplotype analysis [15]. However, the mechanism by which this polymorphism could act is not certain and might conceivably include effects on alternative splicing, editing, stability or translational regulation of the *GRIK2* mRNA. Consequently, before embarking on extensive molecular analyses to define the mechanism of action, we carried out a comprehensive analysis of a much larger set of HD subjects than has previously been examined to confirm *GRIK2* as a genetic modifier of HD pathogenesis.

## 2. Methods and materials

### 2.1. HD samples

HD patient DNA samples from individuals with known age at motor, psychiatric and/or cognitive onset were obtained from ongoing genetic studies at the MGH HD Center Without Walls, members of the HD-MAPS collaboration, post-mortem brain specimens (Harvard Brain Tissue Resource Center and the UCLA Brain Bank), and two large observational studies: the Huntington Study Group's COHORT project and the European Huntington Disease Network's REGISTRY study. In total, 2,911 HD heterozygote subjects with one expanded HD allele and known age of motor onset (2,362 individuals), psychiatric onset (547 individuals) and/or cognitive onset (210 individuals) were genotyped for the *GRIK2* TAA repeat polymorphism and for the *HD* CAG repeat as described previously [9,16]. The mean age at onset was 42.6 (range, 4–92) and the mean expanded allele *HD* CAG repeat length was 45.1 (range, 36–98). This study used de-identified DNA samples and was approved by the Institutional Review Board of the Partners HealthCare System.

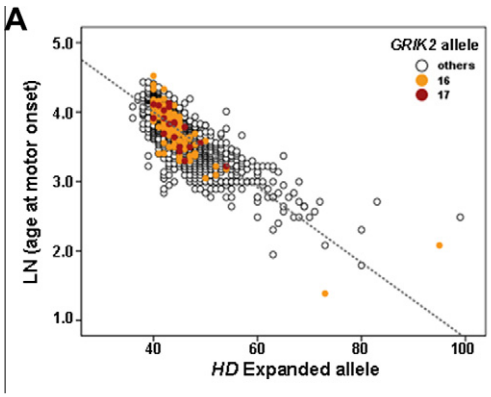
### 2.2. Data analysis

This study utilized samples from 2,911 HD heterozygote subjects with one expanded HD allele and known age of motor onset (2,365 individuals), psychiatric onset (547 individuals) and/or cognitive onset (210 individuals). Potential modifier effects were explored by adding the factor to a linear regression model relating the natural log-transformed age at onset to *HD* CAG repeat length and determining the degree of improvement in goodness-of-fit. Since the regression plots for onset of symptoms in the three different domains show quite different relationships between of natural log-transformed age at onset and *HD* CAG repeat length, each category of age at onset was analyzed separately. SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

## 3. Results

The *GRIK2* TAA repeat displays 7 alleles, ranging from 12 to 17 TAAs. The 16 TAA allele was reported previously to be associated with earlier age of onset in HD [10,11]. We initially tested potential modifier effects by determining whether adding the *GRIK2* genotype (dominant model) had a significant impact on a linear regression model relating the natural log-transformed age at onset to HD CAG repeat length. Separate tests were performed for 1) presence of at least one 16 TAA allele, 2) presence of at least one of the two longest alleles (16 or 17 TAAs), and 3) the *GRIK2* TAA repeat as a continuous trait, based upon the larger of the two alleles present in each individual. Fig. 1A shows the relationship in 2,362 HD subjects of age at onset of diagnostic motor signs with CAG repeat length and Fig. 1B reveals a lack of any significant impact of adding *GRIK2* to the model on improving the $R^2$ value.

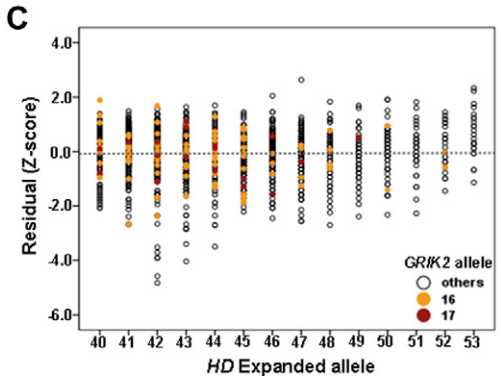
To avoid the potential disproportionate impact of subjects with extreme CAG lengths reported by Lee et al. 2012 [17], we calculated standardized residuals for the 2,205 subjects with CAG repeats in the 40–53 range, relative to the regression line generated in that report. This analysis (Fig. 1C–D) again shows no evidence of an effect of *GRIK2* genotype on motor onset. We also performed a number of analyses in which we subdivided these samples into HD subjects with extremes of age at onset associated with each CAG repeat length using different cut-offs for inclusion or exclusion and in no case did comparison of *GRIK2* genotypes between the two extreme groups reveal a significant difference. Thus, our findings in this large dataset indicate that the *GRIK2* TAA repeat polymorphism, previously thought to be an HD modifier, is not significantly associated with deviation in HD age of motor onset from that expected based upon CAG repeat length.




B

**Model: LN(AO) ~ Expanded + *GRIK2* Genotype**

| *GRIK2* genotype mode | $R^2$ | P value |
|---|---|---|
| Expanded CAG alone | 0.6362 | <.0001 |
| 16 vs others | 0.6360 | 0.7760 |
| 16 or 17 vs others | 0.6361 | 0.6874 |
| continuous | 0.6360 | 0.9972 |




D

**Model: Residual (Z-Score) ~ *GRIK2* Genotype**

| GRIK2 genotype mode | Adjusted $R^2$ | P value |
|---|---|---|
| 16 vs others | 0.0004 | 0.3549 |
| 16 or 17 vs others | 0.0005 | 0.2920 |
| continuous | 0.0001 | 0.7371 |

**Fig. 1.** *GRIK2* modifier analysis in 2,362 HD subjects with known age at motor onset. For the *GRIK2* TAA repeat polymorphism, subjects with at least one 16 or 17 TAA allele are displayed as closed orange and red circles, respectively, with all other subjects being represented by open black circles. (A) The relationship between *HD* expanded allele and log transformed age at onset of diagnostic motor signs. (B) Summary statistics for linear regression analysis of data shown in A, in each dominantly encoded *GRIK2* allele mode. The *P* value for each *GRIK2* allele mode tests for significant improvement in the model over use of the expanded CAG repeat length alone. (C) The distribution of the standardized residual (Z-Score) for subjects with 40–53 CAG repeats, calculated for each subject based upon the standard curve generated from 3,674 HD subjects in [17], is plotted against expanded CAG repeat length. Subjects with a 16 or 17 TAA allele are displayed as closed orange and red circles, respectively. (D) Summary statistics for global linear regression analysis of data shown in C, for each *GRIK2* genotype mode. The P value for each *GRIK2* allele mode tests for association with the residual variance in age at onset after accounting for the contribution of the CAG repeat length. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Although HD is typically diagnosed based upon onset of motor signs, the age at onset for cognitive and/or psychiatric symptoms is available in a subset of this sample. Directly comparable to Fig. 1, Fig. 2 shows no significant effect of the *GRIK2* TAA repeat polymorphism on age at onset of either psychiatric (Fig. 2A–B; 547 subjects) or cognitive (Fig. 2C–D; 210 subjects) symptoms.

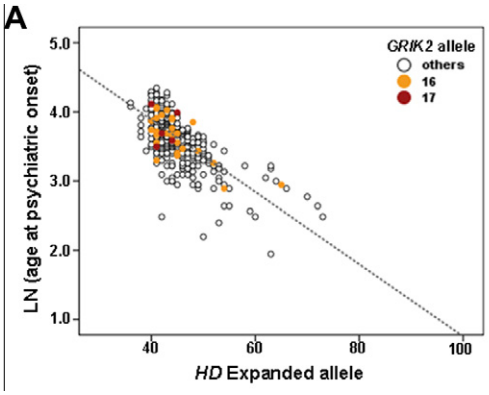
## 4. Discussion

It is well established that the expanded CAG repeat in the *HD* gene is the primary determinant of age of onset of clinical symptoms, but that the mutation alone does not explain all of the variation observed. The remaining variance displays a high degree of heritability, supporting the existence of genetic modifiers, factors whose polymorphic variation influences the course of HD. Consequently, identification of genetic modifiers from human patients represents a potential route to discovering validated targets whose modulation would be expected to alter HD pathogenesis. To date, candidate gene strategies have produced a number of reports of potential modifiers from pathways postulated to be involved in HD pathogenesis [7,12,18–21], including several in which the *GRIK2* TAA repeat polymorphism was associated with altered onset age [10,11,15]. *GRIK2* encodes a protein integrally involved in mediating excitatory neurotransmission in the brain and its status as a genetic modifier would bolster the excitotoxicity model of HD. However, detailed investigation of its mode of action would entail a major investment of labor and research funds.

As genetic analysis of functional variants has argued strongly against other attractive modifier candidates, such as *BDNF* which showed no effect on age at onset in two large studies [22,23], we felt it important to confirm the *GRIK2* modifier effect in a much larger study sample than was previously tested. The increased power afforded by the much larger sample size and more comprehensive analytical methods in our current study argue strongly that *GRIK2* has no significant effect on HD pathogenesis leading to age of onset of motor symptoms. Albeit with smaller sample sizes, our study also does not support modifier effects on age of onset psychiatric or cognitive symptoms. These findings argue against the pursuit of detailed molecular and biological analysis of any putative functional effect of this *GRIK2* polymorphism as a clue to how to alter HD pathogenesis. In this regard, they are consistent with negative findings by Metzger et al. [24] in a German HD population and by Andresen et al. [25] in the Venezuela HD population, although the latter had relatively few 16 TAA alleles.

Our findings present a cautionary note for HD modifier studies in general since an apparent *GRIK2* effect was noted previously for motor onset in multiple studies [10–15]. These apparent positive associations may have been due to small, possibly unrepresentative samples and may have been disproportionately influenced by genotypic or phenotypic outliers.

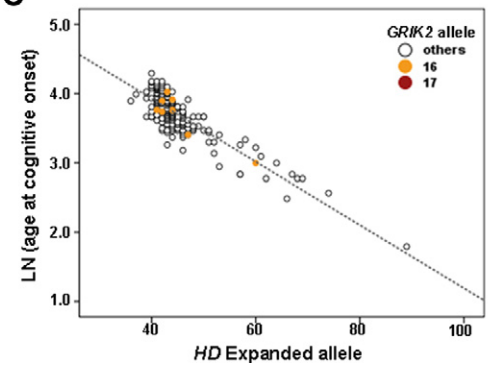
In conclusion, our study results showed that the previously studied TAA repeat variation in the *GRIK2* gene does not influence age at onset in Huntington's disease using the largest dataset assembled to date and suggest that similarly large-scale investiga-




B

**Model: LN(AO) ~ Expanded + *GRIK2* Genotype**

| *GRIK2* genotype mode | $R^2$ | P value |
|---|---|---|
| Expanded CAG alone | 0.4900 | <.0001 |
| 16 vs others | 0.4901 | 0.8059 |
| 16 or 17 vs others | 0.4901 | 0.7842 |
| continuous | 0.4935 | 0.0548 |




D

**Model: LN(AO) ~ Expanded + *GRIK2* Genotype**

| *GRIK2* genotype mode | $R^2$ | P value |
|---|---|---|
| Expanded CAG alone | 0.7447 | <.0001 |
| 16 vs others | 0.7447 | 0.9512 |
| continuous | 0.7447 | 0.9342 |

**Fig. 2.** *GRIK2* modifier analysis in 547 HD subjects with known age at psychiatric onset and in 210 HD subjects with known age at cognitive onset. For the *GRIK2* TAA repeat polymorphism, subjects with at least one 16 or 17 TAA allele are displayed as closed orange and red circles, respectively, with all other subjects being represented by open black circles. (A) The relationship between *HD* expanded allele and log transformation of age at onset of psychiatric signs. (B) Summary statistics for linear regression analysis for data shown in (A), in each *GRIK2* allele mode. The near-significant result for analysis of the TAA repeat as a continuous trait is skewed by the small number of extreme CAG repeat samples, as identical analysis of the bulk of subjects with CAG repeats of 40–53 yielded $P = 0.503$. (C) The relationship between *HD* expanded allele and log transformation of age at onset of cognitive signs. (D) Summary statistics for linear regression analysis for data shown in (C), in each *GRIK2* allele mode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tions may be required to adequately assess the potential modifier effects of other reported candidates.

## 5. Competing interests

The authors declare that they have no competing interests.

## 6. Authors' contributions

JHL participated in the design of the study, analyzed the data statistically and drafted the manuscript. JML assisted in statistical analysis of the data and critical revision of the manuscript. EMR, TG, JSM and SK generated the molecular data while TH, AEH, MRH, PM, MN, CAR, RLM, FS, CG, EGT, CA, OS, RJT, EM, AN, MF, RJ, TA, SF, MHSH, SMH, HDR, DL, MBH, AZ, RKA, KM, JS, GBL and IS participated in design of the study and helped to generate the clinical data. RHM, MEM and JFG conceived the study, participated in its design and critically revised the manuscript. All authors have read and approved the final manuscript.

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