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Prevalence of Huntington's disease gene CAG trinucleotide repeat alleles in patients with bipolar disorder

Eliana Marisa Ramos[a,b], **Tammy Gillis**[a], **Jayalakshmi S Mysore**[a], **Jong-Min Lee**[a], **Isabel Alonso**[b], **James F Gusella**[a], **Jordan W Smoller**[a], **Pamela Sklar**[c], **Marcy E MacDonald**[a], **Roy H Perlis**[a]

[a]Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA

[b]UnIGENe, IBMC – Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

[c]Division of Psychiatric Genomics, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Abstract

Objectives: Huntington's disease is a neurodegenerative disorder characterized by motor, cognitive, and psychiatric symptoms that are caused by *HTT* CAG trinucleotide repeat alleles of 36 or more units. A greater than expected prevalence of incompletely penetrant *HTT* CAG repeat alleles observed among individuals diagnosed with major depressive disorder raises the possibility that another mood disorder, bipolar disorder, could likewise be associated with Huntington's disease.

Methods: We assessed the distribution of *HTT* CAG repeat alleles in a cohort of individuals with bipolar disorder. *HTT* CAG allele sizes from 2,229 Caucasian individuals diagnosed with DSM-IV bipolar disorder were compared to allele sizes in 1,828 control individuals from multiple cohorts.

Results: We found that *HTT* CAG repeat alleles > 35 units were observed in only one of 4,458 chromosomes from individuals with bipolar disorder, compared with three of 3,656 chromosomes from control subjects.

Conclusions: These findings do not support an association between bipolar disorder and Huntington's disease.

Keywords

bipolar disorder; depression; Huntington's disease; neurodegenerative disease; polyglutamine expansion; trinucleotide repeat

Huntington's disease (HD) is a dominantly inherited progressive neurodegenerative disorder caused by the expansion of an unstable polymorphic CAG repeat in the *HTT* gene

Corresponding author: Roy H. Perlis, M.D., Center for Human Genetic Research, Massachusetts General Hospital, Simches Research Building, 185 Cambridge Street, Boston, MA 02114, USA, rperlis@mgh.harvard.edu.

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(chromosome 4p16.3), resulting in an expanded polyglutamine tract in the Huntingtin protein (1). HD is a rare disorder, with a prevalence of ~1–7 in 100,000 individuals of European ancestry. Alleles with 35 or less CAGs are considered to produce no overt clinical symptoms of HD and can be grouped in two categories: stable normal alleles (with < 27 CAGs), and high–normal alleles, which have in some cases been reported to expand (27–35 CAGs) upon transmission. A third class of alleles between 36–39 repeats shows incomplete HD penetrance, while alleles with 40 or more repeats are fully penetrant and lead to the onset of HD symptoms.

HD is characterized by motor, cognitive, and behavioral manifestations. The psychiatric symptoms that become manifest in many, but not all, HD patients, tend to be depression and apathy, but mania has also been observed. Individual symptoms observed in HD may include elevated or irritable mood, over activity, decreased need for sleep, hypersexuality, impulsiveness and grandiosity, and delusions and hallucinations (2). Older studies reported hypomanic or manic episodes in up to 10% of HD patients (3, 4), with a more recent study noting that the presentation reported was most commonly irritability rather than expansive or elevated mood (5).

Recently, we found that HD-associated CAG repeat alleles were overrepresented among individuals diagnosed with major depression, estimating that 3.3 in 1,000 individuals diagnosed with major depression carry an expanded *HTT* CAG allele (6). These results suggested that *HTT* CAG allele sizes, in the reduced-penetrant and fully penetrant ranges, may act to sensitize individuals to the effects of other genetic or environmental factors contributing to the manifestation of depressive symptoms both in HD patients and in individuals diagnosed with major depressive disorder. Here we have evaluated whether there may be a similar overlap in the underlying genetic architecture of HD and bipolar disorder. We determined how often individuals diagnosed with bipolar disorder carry *HTT* CAG alleles that are associated with HD, genotyping the *HTT* CAG repeat in 2,529 individuals with a DSM-IV diagnosis of bipolar disorder.

Materials and methods

Bipolar disorder and control sample cohorts

Bipolar disorder cohort.—The patient cohort comprised a total of 2,529 individuals diagnosed with bipolar disorder (62.7% bipolar I disorder) diagnosed by structured interview as previously described (7). In this cohort, 57.1% (1,443) were women and 88.6% had Caucasian, 6.6% African, and 2.2% Asian (self-reported) ancestry.

Control Cohort 1.—This cohort comprised the normal chromosomes 4 of a large cohort of 4,007 HD individuals, utilized previously in studies that assessed the *HTT* CAG repeat allele distribution in major depression and amyotrophic lateral sclerosis patients (6, 8). These chromosomes, with the shorter non-expanded *HTT* CAG repeat alleles, should reflect the distribution of *HTT* CAG alleles in the general population as HD is a true dominant disorder (9) that is rare (i.e., 1–7 in 100,000 Caucasian individuals).

Control Cohort 2.—A cohort of 1,288 psychiatrically screened controls (with no diagnosis of bipolar disorder or schizophrenia) and 547 DNA samples derived from anonymous cord blood donors, were used as control Group 2 (7). In this control group, all individuals were Caucasian (self-reported) and 49.0% were women.

In the bipolar disorder and control cohorts, all subjects had provided written informed consent for genetic investigation, approved by participating institutions, with the understanding that no results would be returned to them. DNA was provided to the investigators in anonymized form, such that subject recontact would be impossible in any case.

Genotyping

HTT—CAG repeat allele sizes were determined by a polymerase chain reaction (PCR) amplification assay, using fluorescently labelled primers, as previously described (10). The PCR products were run on an ABI PRISM 3730XL automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and analysed using GeneMapper version 3.7 software. A set of genomic DNA standards for which *HTT* CAG repeat number had been confirmed by DNA sequencing was used to provide CAG allele size standards.

Statistical analysis

Fisher's exact test was used to compare *HTT* CAG repeat frequency between the individuals with bipolar disorder and each of the two different comparison control cohorts. We compared the repeat frequency of normal (with < 27 CAGs) and high–normal alleles (27–35 CAGs), as well as stable normal alleles and alleles with a tendency to expand (with ≥ 27 CAGs). Given their relative rarity, high–normal, and abnormal alleles were combined in secondary analyses. Using the Genetic Power Calculator (11), under a dominant model the power of our study to detect association is 0.88. The *HTT* CAG distribution was also analyzed using the nonparametric Mann–Whitney U-test, since the distribution of allele sizes was highly non-normal. All statistical analyses were performed using PASW Statistics 18 (SPSS, Inc., Chicago, IL, USA).

Results

In the group of individuals with bipolar disorder, the *HTT* CAG repeat range varied between nine to 36 repeats, with two out of 5,032 chromosomes having 35 CAG repeats (end of high–normal range) and only one chromosome carried a reduced-penetrance allele with 36 CAG repeats. The individual carrying a 36 CAG repeat was diagnosed with bipolar II disorder, while the two individuals with 35 CAG repeats were bipolar I and bipolar II disorder cases. In order to characterize the prevalence of *HTT* CAG repeats in bipolar disorder, we used as comparison group for the CAG repeat frequency and distribution, 4,007 non-HD control chromosomes (control Cohort 1) that was also the subject of a prior analysis of major depressive disorder (6). As previously reported, the non-HD control chromosomes HD CAG range varied between nine and 34 *HTT* CAG repeats (6, 8) with none of these chromosomes having 35 or more CAG repeats. The allele frequencies in the individuals with bipolar disorder were not significantly different from the non-HD control chromosomes

(Fisher's exact test: normal versus high–normal alleles, p = 0.086 and normal versus ≥ 27 CAGs, p = 0.099) (Table 1). However, comparison of the distributions of the allele sizes, assessed using the nonparametric Mann–Whitney U-test, revealed nominally statistically significantly *shorter* repeat lengths among the non-HD control compared to bipolar disorder chromosomes (Mann–Whitney U-test, p = 0.036) (Fig. 1).

Since the Cohort 1 control group, comprising the non-HD chromosomes from HD patients (defined as ≤ 35 repeats) may underestimate the true frequency of alleles of 36 or more CAG repeats in the general population, we genotyped a second large control cohort, comprising 1,288 psychiatrically screened controls and 547 cord blood DNA samples. In this control Cohort 2, the *HTT* CAG repeat ranged from nine to 37 CAG repeats, with three alleles having high-end normal alleles (35 CAGs) and three alleles in the reduced penetrance range (one 36 CAG and two 37 CAG repeat alleles) (Table 1). Notably, these six high–normal/reduced-penetrance alleles were all from the psychiatrically screened sample. When comparing allele frequencies of subjects with bipolar disorder with this set of control individuals, there was a significant difference when comparing both normal versus high normal (Fisher's exact test, p = 0.011) and normal versus ≥ 27 HD CAG alleles (Fisher's exact test, p = 0.008) alleles, with *fewer* normal alleles among the control cohort. The nonparametric Mann–Whitney U-test revealed a statistically significantly different distribution of allele sizes between this set of control chromosomes and the bipolar disorder chromosomes (Mann–Whitney U-test, p = 0.004), with *shorter* repeat numbers among the bipolar disorder chromosomes.

To evaluate whether the apparent *under*-representation of high-end normal and HD-associated alleles in the cases with bipolar disorder might reflect population stratification due to ancestry differences between the disease cases and the control samples (all self-reported Caucasian), we excluded from the bipolar disorder cohort all samples of non-Caucasian origin. This yielded a total of 4,458 chromosomes from Caucasian individuals diagnosed with bipolar disorder. In this final set, the *HTT* CAG range and frequency of high-end normal and reduced penetrance alleles remained similar (sizes from nine to 36 repeats, with a single reduced-penetrance allele). After restricting the analysis to individuals self-reported as Caucasian, there was still a nominally significant difference between the allele frequency of normal versus high–normal and normal versus ≥ 27 CAG repeat alleles (Fisher's exact test, p = 0.04 and p = 0.03, respectively) when comparing subjects with bipolar disorder with control individuals from Cohort 2, again suggesting fewer normal HD counts among controls. However, the Mann–Whitney U-test revealed no significant difference in the distributions of the *HTT* CAG allele sizes between the control and bipolar disorder Caucasian chromosomes (Mann–Whitney U-test, p = 0.216) (Fig. 2).

Discussion

The genetic architecture of major psychiatric disorders is complex. One rare risk variant, suggested by psychiatric symptoms observed in a proportion of HD patients, may be the *HTT* CAG repeat, at least in the range of allele sizes reported to be associated with a clinical diagnosis of HD. We hypothesized that these *HTT* CAG repeat alleles, which encode a functionally important run of polyglutamines in the Huntingtin protein (12),

may sensitize certain individuals in the general population to genetic and environmental factors that increase the risk of developing particular psychiatric symptoms. In a prior study, overrepresentation of HD-associated CAG alleles appeared to further increase the risk of developing depression (6). Our current study aimed to determine the prevalence of HD-associated *HTT* CAG repeat alleles in a clinical population of individuals diagnosed with bipolar disorder, in light of the observation that some HD patients may exhibit manic/hypomanic symptoms as well as depressive symptoms.

We find that one in 2,229 individuals, or about 0.45 in every 1,000 individuals, diagnosed with bipolar disorder carry an expanded *HTT* CAG allele in the range that has been associated with development of clinical symptoms of HD, whereas approximately 1.64 in every 1,000 individuals from the general population carries an expanded *HTT* CAG allele. Thus, in contrast to our observations in major depressive disorder, HD seems unlikely to contribute significantly to bipolar disorder risk.

One caveat to this interpretation, relevant to all case–control studies, reflects the composition of the control comparison group, which should be representative of the general population and provide a precise estimate of the expected disease-allele prevalence. In previous studies, we have used as a comparison group the non-HD chromosomes from a large collection of individuals diagnosed with HD. This strategy provides a good representation of the *HTT* CAG allele distribution in the general population because HD is a truly dominant disorder (9) that is relatively rare (~1–7 in 100,000); therefore, we would expect the non-HD chromosome to be drawn from and reflective of the general population. Nevertheless, this selection criterion excludes reduced-penetrant as well as fully penetrant HD alleles, which do occur at some level in the general population. To eliminate this potential bias, we utilized a second comparison group that included individuals screened to exclude bipolar disorder and schizophrenia, but not necessarily other psychiatric disorders.

A further advantage of this second cohort is restriction to Caucasian origin, reducing the impact of population stratification in the analysis. There are known to be significant geographic differences in the prevalence and distribution of the *HTT* CAG repeat, related to different ancestral *HTT* haplotypes (13, 14). The modal *HTT* CAG repeat length in European and East Asian populations is 17 while African populations have a broader *HTT* CAG distribution, with most of the alleles having 15 CAG repeats (15, 16). However, despite the apparent advantages, the criteria used to select this second cohort may themselves introduce different biases into the analysis. None of the cord blood samples comprising one-third of the control group can be characterized in terms of psychiatric illness, while even the screened non-psychiatric control group excluded schizophrenia and bipolar disorder only. As the lifetime prevalence of major depression is roughly 17% (17), we cannot exclude the possibility that the reduced penetrance HD alleles observed in the control cohort could be associated with individuals with major depression, previously reported to have an enrichment of these alleles (6)—that is, the screened control cohort may include some misclassified individuals at greater risk to carry expanded alleles (though we note that, based upon prior analyses, even including subjects with major depressive disorder should only modestly increase risk to carry expanded alleles). In addition, we note that the lack of a formal neurologic examination and collection of family neurological history may be

insufficient to exclude a diagnosis of HD; although given the extremely low prevalence in unselected populations the impact of misclassification should be extremely modest.

Taken together, our results do not support an obvious association between *HTT* and bipolar disorder. This finding stands in contrast to the overrepresentation suggested in individuals diagnosed with major depressive disorder. This apparent difference may suggest an area of contrast between the two disorders which otherwise share some heritable risk, with only the latter being sensitive to the effects of the *HTT* CAG repeat. Further large studies examining the prevalence of the *HTT* CAG repeat in other psychiatric disorders, such as schizophrenia, among others, may further clarify the subset of disorders that is sensitive to the effects of the CAG repeat that is the root cause of HD.

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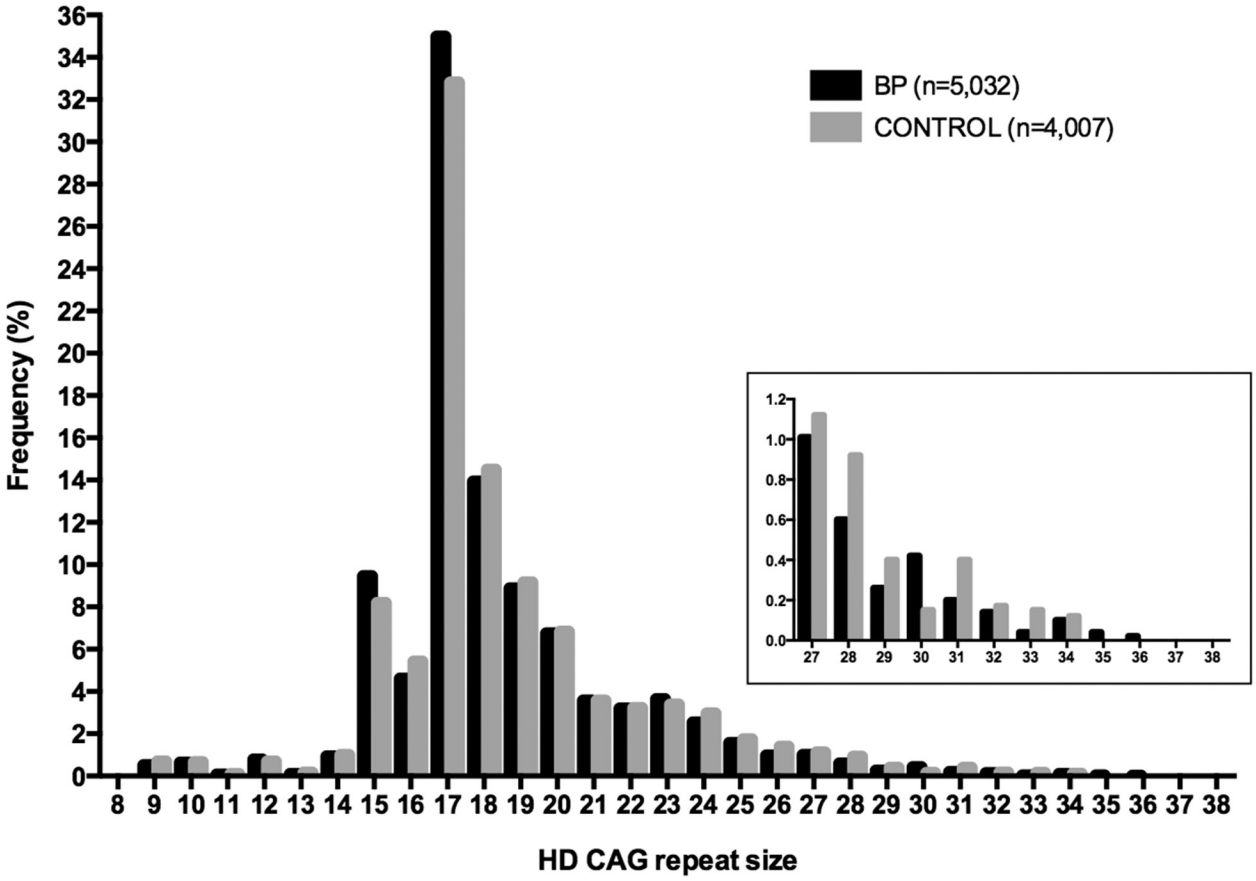

Fig. 1.

HTT CAG repeat length in 4,007 non-Huntington's disease (HD) control chromosomes (Cohort 1) and 5,032 bipolar disorder (BP) chromosomes. Insert shows a zoomed view of high–normal and reduced penetrance HD CAG alleles (27 to 38 CAG repeats).

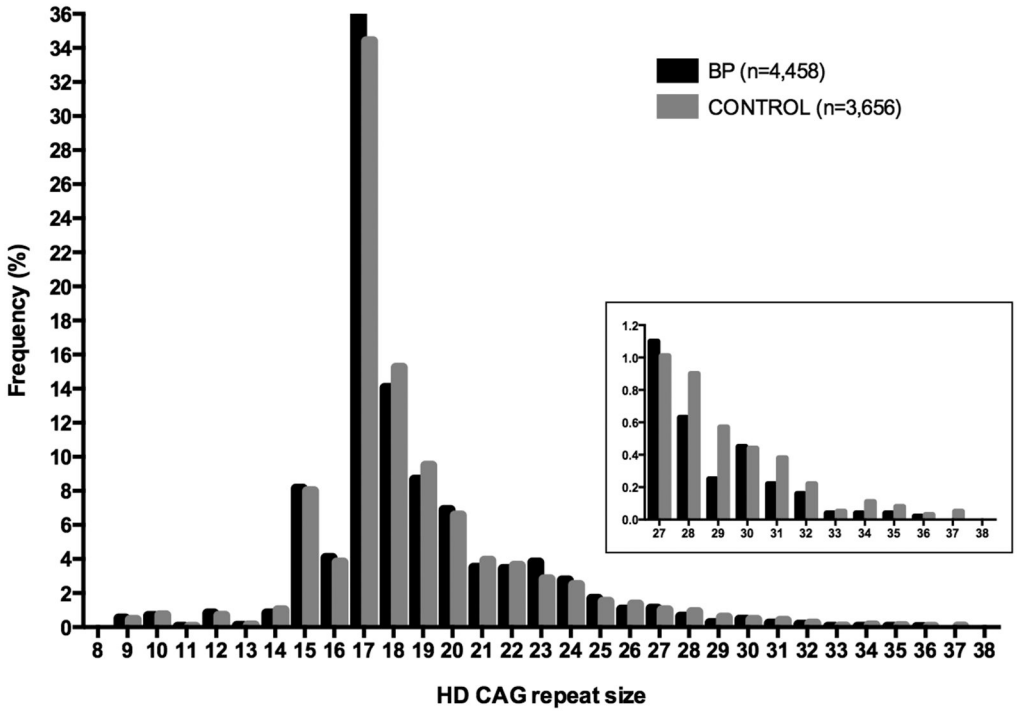

Fig. 2.

HTT CAG repeat length in 3,656 control chromosomes (Cohort 2) (from 1,288 psychiatrically screened and 547 cord blood control samples) and 4,458 bipolar disorder (BP) chromosomes, where all individuals have Caucasian origin. Insert shows a zoomed view of high–normal and reduced penetrance Huntington's disease (HD) CAG alleles (27 to 38 CAG repeats).

Table 1.

HTT CAG repeat alleles in bipolar disorder

	Normal HD allele (< 27 CAGs)	High–normal HD allele (27–35 CAGs)	At risk HD allele (> 35 CAGs)
Bipolar disorder chromosomes	4890 (97.18%)	141 (2.80%)	1 (0.02%)
Caucasian	4326 (97.04%)	131 (2.94%)	1 (0.02%)
Control chromosomes			
Cohort 1	3869 (96.56%)	138 (3.44%)	0 (0%)[a]
Cohort 2	3515 (96.14%)	138 (3.77%)	3 (0.08%)

The allele frequencies in the individuals with bipolar disorder were not different from control Cohort 1 (Fisher's exact test: normal versus high–normal alleles, p = 0.086 and normal versus ≥ 27 CAGs, p = 0.099), but there was a significant difference with control Cohort 2 chromosomes (p = 0.011 and p = 0.008, respectively). When considering only Caucasian individuals, there was still a nominally significant difference (p = 0.040 and p = 0.030, respectively) when comparing subjects with bipolar disorder with control individuals from Cohort 2.

[a]Cohort 1 has 0 alleles with > 35 CAG repeats by design not observation.