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No evidence of association between *BDNF* gene variants and age-at-onset of Huntington's disease

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Huntington's disease (HD) is a late-onset, autosomal dominant neurodegenerative disease caused by a CAG trinucleotide expansion. The number of repeats on the HD chromosome explains most of the variability in age of onset, but genetic factors other than the HD gene are responsible for part of the residual variance. Based on the role played by the brain derived neurotrophic factor (BDNF) in neurodysfunction and neurodegeneration in HD, we searched for novel polymorphisms in the neuron restrictive silencer element located in the *BDNF* promoter. Then, the effect of the Val66Met variant in determining age of onset was tested in a large sample of HD carriers by using a multivariate regression approach. The CAG repeat number accounted for 62% of the variance. After correction for the predominant effect of the CAG expansion, no multiple regression model provided evidence of association between the Val66Met genotype and variation in age-at-onset. Additional studies are warranted to further investigate BDNF as genetic modifier of the HD phenotype.

Keywords: Huntington's disease; Age-at-onset; Genetic modifiers; BDNF; Val66Met polymorphism; Regression analysis

Introduction

Huntington's disease (HD) is a fatal, dominantly inherited neurodegenerative disease characterized by involuntary movements (chorea), psychiatric disturbances and cognitive decline. The illness usually starts in adult life, but age of onset ranges from infancy to the elderly. The disease is caused by the expansion of a polymorphic CAG trinucleotide repeat sequence in the IT15 gene (chromosome 4p16), which encodes a polyglutamine tract. The smallest HD chromosomes have 36 CAG repeats, but the number of repeats may raise up to >200 in very young affected individuals (Bates et al., 2002).

The length of the pathogenic expansion correlates inversely with age-at-onset, accounting for approximately 50–60% of its variance (Bates et al., 2002). Soon after the discovery of the gene (HDCRG, 1993), it was hypothesized that familial factors independent of CAG repeats account for a proportion of the age-at-onset variance (Andrew et al., 1993; Duyao et al., 1993; Snell et al., 1993). Moreover, the residual variance is at least in part heritable, though the influence of genetic and environmental factors has yet to be elucidated. The U.S.–Venezuela Collaborative Research Project (Wexler and The U.S.–Venezuela Collaborative Research Project, 2004) estimated that the heritability of the variance of age of onset attributable to genetic determinants other than the CAG expansion is about 40%. Also a multicenter study on Italian families with HD confirmed the role for familial factors in influencing age-at-onset (Squitieri et al., 2000).

Multiple candidate genes were investigated as HD modifiers, based upon their role in neurodegeneration, neurotransmission and/or brain development. To date, a few genes were demonstrated to account for a small proportion of the age-at-onset variance each, namely the genes encoding the GluR6 kainate receptor (*GRIK2*) (Rubinsztein et al., 1997), the NMDA receptor subunit 2B (*GRIN2B*) (Arning et al., 2005), the ubiquitin carboxy-terminal hydrolase L1 (*UCHL1*) (Metzger et al., 2006). Using a genome-wide approach, the HD-MAPS study identified positive linkage signals at chromosomes 4p16, 6p21–23 and 6q24–26 (Li et al., 2003). Djoussé and co-workers (2003) further investigated the 4p16 locus, and provided additional clues that also the normal HD allele may explain part of the variability of the phenotype.

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Brain derived neurotrophic factor (BDNF), a neurotrophin that is particularly important for the survival of striatal neurons and for the activity of cortico-striatal synapses (Widmer and Hefti, 1994; Nakao et al., 1995; Alcantara et al., 1997; Ivkovic and Ehrlich, 1999; Jovanovic et al., 2000), may be implicated in the development of the HD phenotype. *In vitro* and *in vivo* data showed that wild-type huntingtin, but not the mutant protein, stimulates cortical BDNF production by acting at the level of BDNF gene transcription. BDNF is then anterogradely transported to the striatal neurons where it exerts its pro-survival and modulatory function. Earlier studies showed that brain cells and mice overexpressing wild-type huntingtin produce increased BDNF mRNA and protein, an effect that is lost in the presence of overexpressed mutant huntingtin (Zuccato et al., 2001) or in cells and mice depleted of wild-type huntingtin (Zuccato et al., 2003; Zuccato, under submission). In vitro, full-length wild-type huntingtin also stimulates (but mutant huntingtin represses) BDNF vesicular trafficking in neuronal cells, and BDNF transport is attenuated by reducing the levels of wild-type huntingtin using RNAi (Gauthier et al., 2004).

The finding that normal but not mutant huntingtin stimulates *BDNF* gene transcription/protein production and its axonal transport prompted analyses of BDNF levels in the cortex and striatum of transgenic mice and patients with HD (Cattaneo et al., 2005). With a few exceptions (Gauthier et al., 2004; Ferrer et al., 2000), a consistent reduction in cortical BDNF protein and its mRNA was reported in all studies. In fact, reduced BDNF mRNA and protein levels were found in cerebral cortex from: YAC mice expressing the full-length mutant protein (Zuccato et al., 2001; Hermel et al., 2004); R6/2 mice expressing a 63-amino-acid N-terminal portion of mutant huntingtin (Luthi-Carter et al., 2002; Zhang et al., 2003; Zuccato et al., 2005); mice expressing a human N-terminal truncated huntingtin with 82 polyQ repeats (Duan et al., 2003); mutant huntingtin knock-in mice (Gines et al., 2003); post-mortem cortical tissue from a small cohort of patients with HD (Zuccato et al., 2001).

The impact of reduced cortical BDNF in HD was further reinforced by the finding that Emx-BDNF conditional knock-out mice, in which cortical BDNF is almost completely eliminated, showed a hindlimb clasping phenotype similar to that observed in mouse models of HD (Baquet et al., 2004). In another experiment, earlier onset and enhanced severity of motor alterations were detected in mice expressing only one functional BDNF allele on a background of an overexpressed N-terminal mutant huntingtin fragment (the R6/1 line) (Canals et al., 2004). Together, these data suggest that reduced BDNF levels affect the development of HD in mice and possibly in patients.

Investigation of the mechanism by which wild-type huntingtin stimulates *BDNF* gene transcription showed that the normal but not the mutant protein sequesters the transcriptional regulator RE-1 silencing transcription factor/neuronal restrictive silencing factor (REST/NRSF) in the cytoplasm, therefore blocking its translocation to the nucleus and its binding to the neuron restrictive silencer element (NRSE/RE1) located in the BDNF promoter (and in the promoters of many other neuronal genes). In normal conditions, the NRSE is inactive and *BDNF* is properly transcribed. Mutant huntingtin causes the pathological entry of REST/NRSF into the nucleus, thus leading to repressor complex formation and reduced transcription (Zuccato et al., 2003).

In the light of these lines of evidence, the *BDNF* gene appeared an excellent candidate as modifier of the age-at-onset of HD. Interestingly, the effect of the *BDNF* gene variants was suggested to act as susceptibility factor in different psychiatric illnesses, namely bipolar disorder (Neves-Pereira et al., 2002), eating disorders (Ribasès et al., 2004), schizophrenia (Neves-Pereira et al., 2005), as well as in normal personality traits (Itoh et al., 2004). All studies included the valine–methionine substitution at codon 66 (Val66Met; dbSNP accession number: *rs6265*). The Val66Met single nucleotide polymorphism is highly conserved across species and was demonstrated to influence human memory and hippocampal activation by affecting intracellular processing and activity-dependent secretion of BDNF (Egan et al., 2003). A recent study examined 122 patients of Spanish ancestry with HD and 95 anonymous controls using a multiple linear regression approach, providing preliminary evidence that the Val66Met polymorphism exerts an influence on age-at-onset (Alberch et al., 2005). The limited size of the study indeed precludes a final indication of the role of Val66Met in HD.

We herein designed a study aimed at: (i) searching for polymorphisms located in the region of NRSE of *BDNF*; (ii) testing the hypothesis that genotypes of *BDNF* functional polymorphisms are associated with variations in age-at-onset of HD, by evaluating a large cohort of Italian patients.

Methods

Dataset

The inverse correlation between number of CAG repeats and age-at-onset was estimated on a cohort of 255 affected individuals, belonging to 213 families (mean CAG repeat number: 44.43, SD: 5.38, range: 38–85; mean age-at-onset: 46.91, SD: 13.00, range: 5–85). Age of onset was defined as the onset of motor impairment, or apparent cognitive or behavioural disturbances. The Val66Met genotype was available for 244 individuals (213 families).

Fifty-nine asymptomatic carriers of the CAG expansion (belonging to 50 families; mean CAG repeat number: 43.40, SD: 3.35, range: 38–54) were genotyped for the Val66Met polymorphism and included in the categorical analysis, using as variable the age at last negative neurological examination (mean age at examination: 32.54, SD: 7.62, range: 18–52).

Molecular analysis

The CAG repeat size was determined according to Warner et al. (1993). A 195 bp-long fragment encompassing the *BDNF* NRSE was amplified by PCR (primers pair: 5′-aagccttttcctcctgctgtg-3′; 5′-agccccgatctcagtgtgag-3′) and screened for nucleotide variants by Single Strand Conformation Polymorphism (SSCP) and direct sequencing (detailed protocols are available on request). The Val66Met single nucleotide polymorphism was genotyped through restriction fragments analysis using *Nla*III as described by Ribasés et al. (2004).

Statistical analysis

The dependence of age-at-onset on CAG repeat number on the HD chromosome was assessed by linear regression. The best fit estimated by the R^2 value was obtained after log transformation of age-at-onset (see below). Residuals from this model did not show deviations from normality and from expected outliers (data not shown). Multiple regression models were used to test the effect of