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Huntington's disease-like phenotype due to trinucleotide repeat expansions in the *TBP* and *JPH3* genes

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Summary

We report a group of 252 patients with a Huntington's disease-like (HDL) phenotype, including 60 with typical Huntington's disease, who had tested negative for pathological expansions in the *IT15* gene, the major mutation in Huntington's disease. They were screened for repeat expansions in two other genes involved in HDL phenotypes: those encoding the junctophilin-3 (*JPH3/HDL2*) and prion (*PRNP/HDL1*) proteins. In addition, because of the clinical overlap between patients with HDL disease and autosomal dominant cerebellar ataxia or dentatorubral and pallidoluysian atrophy (DRPLA), we investigated trinucleotide repeat expansions in genes encoding the TATA-binding protein (*TBP/SCA17*) and atrophin-1 (*DRPLA*). Two patients carried 43 and 50 uninterrupted CTG repeats in the *JPH3* gene. Two other patients had 44 and 46 CAA/CAG repeats in the *TBP* gene. Patients with expansions in the *TBP* or *JPH3* genes had HDL phenotypes indistinguishable from Huntington's disease. Taking into account patients with typical Huntington's disease, their frequencies were evaluated as 3% each in our series of typical HDL patients. Interestingly, incomplete penetrance of the 46 CAA/CAG repeat in the *TBP* gene was observed in a 59-year-old transmitting, but healthy, parent. Furthermore, we report a new configuration of the expanded *TBP* allele, with 11 repeats on the first polymorphic stretch of CAGs. Expansions in the *DRPLA* gene and insertions in the *PRNP* gene were not found in our group of patients. Further genetic heterogeneity of the HDL phenotype therefore exists.

Keywords: Huntington's disease; junctophilin-3; spinocerebellar ataxia 17; TBP; Huntington's disease-like phenotype

Abbreviations: ADCA = autosomal dominant cerebellar ataxia; DRPLA = dentatorubral and pallidoluysian atrophy; HDL = Huntington's disease-like; IT15 = important transcript 15; JPH3 = junctophilin-3 gene; PRNP = prion protein; TBP = TATA-binding protein

Introduction

Huntington's disease is an autosomal dominant disorder characterized by progressive movement abnormalities and impaired cognition (MIM 143100). The majority of Huntington's disease cases are caused by a CAG repeat expansion in the important transcript 15 gene (*IT15*) on chromosome 4 (Huntington's Disease Collaborative Research Group, 1993). Huntington's disease-like (HDL) phenotypes have, however, been described in which no expansions in the IT15 gene were detected (Xiang *et al*., 1998; Margolis *et al*., 2001). A 192-nucleotide insertion in the region of the prion protein gene (*PRNP*) encoding an octapeptide repeat in the prion protein is responsible for *HDL1* on chromosome 20 in a single family (Moore *et al*., 2001). In addition, a CTG repeat expansion has been identified in a few cases in the junctophilin-3 gene (*JPH3*) (*HDL2* locus) (Holmes *et al*., 2001).

Cerebellar signs, parkinsonism, dystonia and epilepsy are part of the clinical spectrum of Huntington's disease, mostly in young-onset patients, causing clinical overlap in some cases with dentatorubral and pallidoluysian atrophy (DRPLA) (Koide *et al*., 1994; Nagafuchi *et al*., 1994) and autosomal dominant cerebellar ataxias (ADCA) (Stevanin *et al*., 2000; Filla *et al*., 2002). Interestingly, several families presenting with ataxia, dementia and movement disorders, including chorea in some cases, were shown to carry abnormally expanded CAG/CAA trinucleotide repeats in one of the genes responsible for ADCA at the spinocerebellar ataxia type 17 (*SCA17*) locus, *TBP*, which encodes TATA-binding protein (TBP) (Koide *et al*., 1999; Fujigasaki *et al*., 2001; Zuhlke *et al*., 2001; Silveira *et al*., 2002).

We identified 252 index patients with an HDL phenotype who do not carry CAG repeat expansions in the *IT15* gene, 60 of whom were clinically assessed by movement disorder specialists as having 'typical' Huntington's disease; 192 other cases had been referred for genetic testing but with limited clinical data. Patients were tested for mutations in four candidate genes: *PRNP*, *JPH3*, *TBP* and *DRPLA*.

Patients and methods

Subjects

Group 1

Sixty index cases clinically diagnosed as having Huntington's disease by neurologists specializing in movement disorders and who had tested negative for the Huntington's disease mutation on chromosome 4 were selected for screening. Most of them (n = 49) presented with dementia associated with chorea (n = 46) and/or other movement disorders, such as dystonia (n = 13), parkinsonism (n = 7), myoclonus (n = 4) and cerebellar ataxia (n = 15). CAG repeat expansions at the *SCA1-3*, *-6* and *-7* loci were excluded in all patients with cerebellar ataxia. Seven patients had isolated chorea. The present series of 60 patients includes a group of 39 patients presenting with typical Huntington's disease (chorea and dementia) from a larger series of miscellaneous cases for which CTG repeat expansions in the *JPH3* gene have been analysed previously by our group (Stevanin *et al*., 2002).

Age at onset was 44 ± 22 years (mean ± SD), ranging from onset in childhood up to the age of 83 years. Seventeen had similarly affected first-degree relatives; no other cases were reported in the families of the remaining index patients (n = 43). Other causes of chorea, such as acanthocytosis or the presence of anti-nuclear, anti-DNA and anti-thyroperoxidase antibodies, were excluded in the apparently sporadic cases.

Most of the patients were Caucasians of French ancestry (n = 46). Five came from North Africa, five had Hispanic ancestry and one originated from Colombia. Three were black, from the French West Indies (n = 2) and Africa (n = 1). Blood samples were obtained with informed consent according to French and European law, and genomic DNA was extracted from leucocytes using standard methods.

Group 2

We selected 192 patients with less restrictive criteria, i.e. chorea and/or cognitive or psychiatric impairment, referred to the Laboratoire de Biochimie et Génétique Moléculaire (Hôpital Cochin, Paris) for diagnosis of the CAG repeat expansion in the *IT15* gene, but who were non-carriers and for whom details of clinical features were not fully available.

PCR analysis for the PRNP, JPH3, TBP and DRPLA genes

CAG/CTG repeats in the *TBP*, *DRPLA* and *JPH3* genes and the octapeptide-coding region of the *PRNP* gene were amplified by PCR (polymerase chain reaction) under conditions described previously (Nagafuchi *et al*., 1994; Koide *et al*., 1994, 1999; Moore *et al*., 2001; Stevanin *et al*., 2002; Leber *et al*., 2003). The numbers of CAG and octapeptide-coding repeats were determined by electrophoresis on an ABI-3100 automated sequencer (Applied Biosystems, Foster City, CA, USA) followed by analysis using GeneScan and Genotyper software (Applied Biosystems). The expanded repeats were sequenced using the Big-Dye Terminator kit v2™ (Applied Biosystems) and analysed with Sequencing Analysis software (Applied Biosystems).

Results

Screening for the PRNP, JPH3, TBP and DRPLA mutations

We identified two patients from Group 1 carrying expanded alleles in the *JPH3* gene and two others with expansions in the *TBP* gene. No patients had pathological alleles in the *DRPLA* gene. No insertions were found in the *PRNP* gene, but a deletion of one octapeptide, described as a polymorphism, was detected in two cases (Vnencak-Jones and Phillips,

Table 1 *Clinical characteristics of index patients AAR-028 and SAL-2309, with identified mutations in the TBP gene, and index patients FDF-571 and SAL-2289, with identified mutations in the JPH3 genes*

	Mutated gene			
	TBP		*JPH3 (HDL2)*	
	AAR-028	SAL-2309	FDF-571	SAL-2289
Expansion size (trinucleotide repeats)	46	44	43	49
Origin	France	France	French West Indies	Morocco
Family history of Huntington's disease	No. Father unaffected but carries the mutation	No. Mother died at 60 (cancer); father alive but not sampled	No. Mother demented but non-carrier; father died aged <60 (cardiac arrest)	No. Father died at 54; mother alive but not sampled
Sex	F	F	M	F
Age at onset (years)	23	29	51	42
Age at examination (years)	30	45	59	44
Signs at onset	Behavioural changes, chorea	Gait instability, behavioural changes	Behavioural changes, memory loss	Chorea
Chorea	Upper limbs	Diffuse	Diffuse	Diffuse
Dementia	Yes	Frontal behaviour, dementia	Dementia, apraxia	Frontal behaviour
Cerebellar gait	Moderate	Severe	Moderate	No
Dysarthria	Moderate	Moderate	Mild	No
Reflexes	Increased (lower limbs)	Increased, plantar extensor	Normal	Increased
Parkinsonism	Rigidity	No	No	No
Ophthalmoplegia	No	No, nystagmus	No	No
Cerebral MRI	Olivo-pontocerebellar atrophy	Severe cerebellar and pontine atrophy	Subcortical atrophy	Caudate nucleus and cortical atrophy

1992). No mutations in the four analysed genes were found in patients of Group 2.

Molecular and clinical features of patients with CAG/CAA repeat expansions in the TBP gene

Patients SAL-2309 and AAR-028 carried 44 and 46 CAG/CAA repeats, respectively, in the *TBP* gene (Table 1). Direct sequence analysis in both cases revealed the following repeat structure: $(CAG)_3$ $(CAA)_3$ $(CAG)_{9/11}$ CAA CAG CAA $(CAG)_{24}$ CAA CAG. Neither patient had any affected relatives. The mother of case SAL-2309 died at age 60 years and the father was not available for testing. The father of case AAR-028 was normal on examination at age 59 years, but, interestingly, carried 38 repeats on one allele and the same 46-unit expanded repeat as his daughter on the other allele. The mother carried 38- and 39-repeat alleles.

The clinical features are summarized in Table 1. Patients SAL-2309 and AAR-028 were both of French origin. Both patients presented with a disease starting before 30 years of age, with behavioural changes and gait instability; one of the two patients also presented mild chorea at onset. Both had brisk reflexes and parkinsonism was observed in the patient with earlier onset. Interestingly, and consistent with ADCA, both had marked pontocerebellar atrophy.

Molecular and clinical features of patients with CTG repeat expansions in the JPH3 gene

Patients FDF-571 and SAL-2289 carried 43 and 50 uninterrupted CTG repeats, respectively, in the *JPH3* gene. Both patients were of African descent: FDF-571 was black and was living in the French West Indies and SAL-2289 was from southern Morocco.

The mother of FDF-571, reported to have dementia of the Alzheimer's type, did not carry an expansion in the *JPH3* gene. The father died of cardiac arrest before age 60 years, with no known history of neurological disease. Patient SAL-2289 had no family history of neurological disease and has been reported elsewhere (Stevanin *et al*., 2002).

The phenotypes of these two patients differed (Table 1). The ages at onset may reflect the sizes of the repeats. The Moroccan patient presented with chorea and frontal behaviour, a clinical profile indistinguishable from that of classical Huntington's disease, after a disease duration of only 2 years. The 59-year-old patient from the French West Indies had clinical features compatible with Huntington's disease associated with prominent cerebellar ataxia.

Discussion

We have identified four patients initially referred for Huntington's disease testing who do not carry expansions in

the *IT15* gene but have expansions in other genes: *TBP* and *JPH3*. Interestingly, these patients had no family history of the disease. Genetic heterogeneity in Huntington's disease was thought to be anecdotal since >90% of Huntington's disease patients have *IT15* CAG expansions. We showed previously, however, that *JPH3* expansions account for <3% of HDL patients, indicating further genetic heterogeneity. Interestingly, the absence of this mutation in Caucasians (Holmes *et al*., 2001; Bauer *et al*., 2002; Stevanin *et al*., 2002) was confirmed in this group of patients. The phenotype was indistinguishable from Huntington's disease, as reported previously (Holmes *et al*., 2001).

Expansions in the *TBP* gene were initially found in ADCA patients. Detailed clinical descriptions of 17 of these patients have been published (Koide *et al*., 1999; Fujigasaki *et al*., 2001; Nakamura *et al*., 2001; Zuhlke *et al*., 2001; Silveira *et al*., 2002). All these patients had intellectual deterioration. Cerebellar ataxia, epilepsy and chorea were present in 90, 50 and 20% of the patients, respectively. The TBP cases presented here had prominent behavioural changes and abnormal movements at onset, including typical chorea in one case, which led to a diagnosis of Huntington's disease. This study is the first report of HDL patients carrying *TBP* gene expansions, and it highlights the clinical overlap between Huntington's disease and ADCA. According to previous reports, there is overlap between the sizes of normal (25–44 repeats) and pathological (43–63 repeats) alleles in the *TBP* gene (Koide *et al*., 1999; Fujigasaki *et al*., 2001; Zuhlke *et al*., 2001; Silveira *et al*., 2002). Interestingly, the father of one of our patients was asymptomatic at age 59 years while carrying 46 repeats in the *TBP* gene. His daughter, severely affected at age 30 years (onset at 23 years), carried a repeat of the same size. This observation illustrates the incomplete penetrance associated with small expansions in the *TBP* gene. This accounts for the lack of family history and suggests the influence of modifying genetic or environmental factors. It also shows the absence of instability, even through paternal transmission of the disease-bearing allele, which can be explained by CAA interruptions in the CAG tract. Interestingly, the first stretch of polymorphic $(CAG)_n$ reported to date in expanded *TBP* alleles carried nine repeats (Koide *et al*., 1999; Fujigasaki *et al*., 2001; Zuhlke *et al*., 2001), probably because this is the most frequent number of repeats in normal alleles (Zuhlke *et al*., 2001). We report here the first case (Patient AAR-028) carrying 11 repeats on the first polymorphic stretch of CAGs in *TBP*.

Repeat expansions in the *DRPLA* gene and insertions in the octapeptide-coding region of the *PRNP* gene were not found in our series of patients. In the case of *PRNP*, this was not surprising since the phenotype of the single HDL1 family was atypical, with chorea present in only four out of seven patients but epileptic seizures with abnormal EEG in all patients (three out of three) (Xiang *et al*., 1998), a phenotype closer to DRPLA. The exclusion of CAG expansions in the *DRPLA* gene in our series of HDL patients can be explained by the rarity of this mutation in Europeans (Leber *et al*., 2003).

In conclusion, the TBP- and JPH3-associated phenotypes were variable but remained within the spectrum of features observed in Huntington's disease patients. It is therefore not surprising that no mutations were found in these genes in the series of patients with less restrictive criteria. In addition, mutations in the *JPH3* and *TBP* genes accounted for only 6% of typical HDL patients without *IT15*/CAG repeat expansions, suggesting further genetic heterogeneity. Given their low frequencies, these genes should be analysed in patients presenting with typical Huntington's disease who are either of African origin (*JPH3*) or present ataxia (*TBP*). Lastly, family histories may have been overlooked due to incomplete penetrance or to a censor effect, as in our four cases. It is therefore worthwhile analysing CAG/CTG repeats in both the *TBP* gene and the *JPH3* gene in typical HDL patients, regardless of their family history.

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