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Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course

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Summary

Huntington disease is caused by a dominantly transmitted CAG repeat expansion mutation that is believed to confer a toxic gain of function on the mutant protein. Huntington disease patients with two mutant alleles are very rare. In other poly(CAG) diseases such as the dominant ataxias, inheritance of two mutant alleles causes a phenotype more severe than in heterozygotes. In this multicentre study, we sought differences in the disease features between eight homozygotes and 75 heterozygotes for the Huntington disease mutation. We identified subjects homozygous for the Huntington disease mutation by DNA testing and compared their clinical features (age at onset, symptom presentation, disease severity and disease progression) with those of a group of heterozygotes, who were assessed longitudinally. The age at onset of symptoms in the homozygote cases was within the range expected for heterozygotes with the same CAG repeat lengths, whereas homozygotes had a more severe clinical course. The observation of a more rapid decline in motor, cognitive and behavioural symptoms in homozygotes was consistent with the extent of neurodegeneration as available at imaging in three patients, and at the post-mortem neuropathological report in one case. Our analysis suggests that although homozygosity for the Huntington disease mutation does not lower the age at onset of symptoms, it affects the phenotype and the rate of disease progression. These data, once confirmed in a larger series of patients, point to the possibility that the mechanisms underlying age at onset and disease progression in Huntington disease may differ.

Keywords: CAG expansion; CAG mutation homozygosity; disease progression; age at onset; brain atrophy; UHDRS

Abbreviations: UHDRS = Unified Huntington's Disease Rating Scale; TFC = total functional capacity

Introduction

Huntington disease is an autosomal dominantly transmitted disorder characterized by motor, mood and cognitive signs caused by an expansion mutation beyond 36 CAG repeats in the *IT15* gene (Huntington's Disease Collaborative Research Group, 1993; Kremer *et al*., 1994). Chorea or other atypical disorders of movement may open the clinical picture (Di Maio *et al*., 1993*a*; Quinn and Schrag, 1998; Squitieri *et al*., 2000*a*), but neuropsychiatric symptoms and mood abnormalities can precede the motor signs (Di Maio *et al*., 1993*b*), being relatively independent of cognitive and motor aspects of the disease (Paulsen *et al*., 2001). The age at onset of symptoms inversely correlates with CAG repeat number (Huntinton's Disease Collaborative Research Group, 1993). Genetic (Farrer and Conneally, 1985; Gusella and MacDonald, 2002) and familial factors (Kremer *et al*., 1993; Squitieri *et al*., 2000*b*; Rosenblatt *et al*., 2001), other than the triplet expansion (Kieburtz *et al*., 1994), may also modulate the onset and severity of disease. Disease progression is probably caused by increasing neuronal loss, with time, in the cerebral cortex and striatum (Antonini *et al*., 1996; Penney *et al*., 1997; Andrews *et al*., 1999)—the brain structures most frequently involved in Huntington disease (Vonsattel *et al*., 1998).

The CAG repeat mutation in the *IT15* gene is translated into an abnormally long polyglutamine tract, which is believed to confer a deleterious gain of function on the mutant protein (Nucifora *et al*., 2001). Because patients homozygous for Huntington disease (i.e with two mutant alleles) receive the gain-of-function mutation in a double dose, one would expect a more toxic effect in homozygotes than in heterozygotes, similarly to other poly(CAG) diseases (Gusella and MacDonald, 2000). Huntington disease is widely believed, however, to be one of the rare genetic diseases that manifests 'complete dominance', and hence is indistinguishable in homozygotes and heterozygotes. In fact, earlier studies reported similar age at onset and clinical presentation in the two groups (Wexler *et al*., 1987; Myers *et al*., 1989; Kremer *et al*., 1994; Durr *et al*., 1999; Laccone *et al*., 1999). The studies reporting genetic analysis included only a small number of patients (one or two) (Kremer *et al*., 1994; Durr *et al*., 1999; Laccone *et al*., 1999), whereas those including more patients (Wexler *et al*., 1987; Myers *et al*., 1989) gave no data on CAG repeat number because they were conducted before the Huntington disease mutation was identified. The dogma of 'complete dominance' in Huntington disease, therefore, has to be investigated further and established.

We conducted a multicentre study and were able to collect clinical and genetic data on eight subjects homozygous for the expanded CAG repeat, and 75 heterozygous subjects. We investigated possible phenotypic differences between the two genetic groups, including age at onset of symptoms, phenotypic presentation and disease progression.

Methods

Study sample

We studied eight subjects with CAG mutations in the *IT15* gene on both alleles, for whom complete data were collected in study centres from five countries (Canada, Australia, Scotland, France and Italy). As Huntington disease homozygosity is very rare, a study such as this needs an accurate retrospective analysis. As part of the clinical assessment, neurological examinations including motor, psychiatric, cognitive and functional assessments, conducted in each centre by a senior neurologist with expertise in Huntington disease, were available for all subjects. The onset of the disease was defined as the time when motor clinical manifestations (i.e. choreic movements), or severe psychiatric symptoms changing the normal life state (Brinkman *et al*., 1997), first became noticeable. Clinical and genetic data for heterozygous subjects came from the Italian Huntington disease database (Squitieri *et al*., 2001). To compare clinical data in homozygotes and heterozygotes, we selected from the database a cohort of 75 heterozygous patients (35 males and 40 females), for all of whom full data were available on clinical course and decline since the onset (mean number of disease years 9.7 $\pm$ 6.1, range 1–26), age at onset of the disease (41.3 $\pm$ 13.9 years, range 18–73) and expanded CAG repeat number (46.3 $\pm$ 10.5, range 39–70).

Clinical assessment

Neurological and psychiatric assessment

Motor symptoms and behavioural changes were assessed clinically with the Unified Huntington's Disease Rating Scale (UHDRS) (Huntington Study Group, 1996). As mood and behavioural changes may present years before the motor symptoms (Lovestone *et al*., 1996), details on patients' initial psychiatric disturbances, movement disorders and time in years between these two sets of clinical manifestations have been collected and included in a computed databank. Because rapid cognitive deterioration towards severe intellectual decline in some homozygotes precluded neuropsychological testing at the last assessment, detailed records were not always available. Homozygote 1 and her affected heterozygous brother underwent neuropsychological testing with the Wechsler Adult Intelligence Scale. Homozygotes 3, 4 and 5 underwent the Mini Mental State Examination. Homozygote 8 underwent the cognitive assessment according to the UHDRS. Notably, due to the necessary retrospective nature of this study, we had to rely on the availability of clinical records. For this reason, cognitive data were limited and psychiatric measures were not quantifiable. However, due to the rarity of the homozygotic Huntington disease condition, we have included these data to fill out the clinical picture.

Disability score

Disease severity was rated with standard scales for disability, independence and functional capacity. Disability was rated with the Independence Scale and also with the Physical Disability Scale. Both scales have high inter-rater reliability (Myers *et al*., 1991). The mean of these two scales was used as the summary 'disability score' (range 100 to 10, with lower scores indicating greater loss of independence) (Myers *et al*., 1991). The disease stage was calculated according to the Total Functional Capacity (TFC) score, on a scale ranging from 13 to 0, with lower scores indicating more severe dysfunction (Huntington Study Group, 1996).

Disease progression

The rate of functional decline and symptom progression was measured in units per year by the disability scores and TFC. The loss of units on the disability and TFC score per year was determined by the equation: patient's score at the onset minus patient's score at the last clinical evaluation, divided by the number of disease years (Penney *et al*., 1990). To calculate the time from the age at onset to the lowest score (i.e. most severe impairment), we considered the number of years from onset to the lowest summary disability and TFC scores. To ascertain the difference in disease duration between the two cohorts of patients, we examined the mean number of disease years from onset to the time when the disability score ranged between 10 and 55, a range identical to the most advanced stages in homozygotes.

Neuroimaging and neuropathological findings

CT and MRI brain scans, available in three homozygotes (homozygotes 1, 3 and 4) and in a heterozygous sibling, were re-analysed retrospectively at various disease stages in homozygote 1 and her brother, and in homozygote 3. One neuropathological report was available (homozygote 2) for a post-mortem examination performed at the Ottawa General Hospital, Ottawa, Canada.

Genetic assessment

DNA was extracted from blood lymphocytes obtained from the homozygous and heterozygous subjects after informed consent. The CAG trinucleotide repeats and the close CCG polymorphisms were analysed according to published techniques (Warner *et al*., 1993; Gellera *et al*., 1996). Reference samples and standardized sequence clones for sizing were shared among the collaborating centres and examined in each laboratory.

Statistical analysis

Student's *t* test was used to compare differences in the median age at onset in the two groups. To compare the disease duration and changes in the TFC score per stage in heterozygotes versus homozygotes, we selected from the 75 heterozygotes a subset of 13 patients with a range of onset ages (40–60 years), CAG repeats (40–46), disability scores (10–55) and advanced disease stages (III–V) identical to homozygotes (homozygotes 1–5 and 8). A non-parametric test (Mann–Whitney *U*) was used to compare the mean decline in scores and the mean number of disease years in heterozygotes versus homozygotes. Because homozygotes 1 and 3 showed the most rapid disease course and might be outliers compared with the other homozygotes (Table 1), we analysed the data with and without this group. Because only part of the quantitative UHDRS assessment was available for homozygotes 6 and 7 (no accurate TFC score per year available), only some of their data were used for statistical comparison with the heterozygotes. Data, expressed as means $\pm$ SD, were considered statistically significant at $P \leqslant 0.05$.

Results

The characteristics of all the homozygous subjects (four males and four females), including genetic and clinical data, are summarized in Table 1. A 'quantitative' clinical assessment was available in the homozygous patients (Fig. 1).

Neurological and psychiatric presentation in homozygous and heterozygous patients

In four of the eight (50%) homozygotes (homozygotes 1, 4, 5 and 8), the first symptoms were characterized by movement disorders other than chorea, such as parkinsonism (homozygotes 4, 5 and 8) and limb and gait ataxia (homozygote 1). These motor manifestations were either associated with choreic movements or occurred alone, but they always predominated (Table 1). The analysis of the previously characterized at onset motor symptoms in heterozygotes showed that atypical motor signs other than chorea occurred in 7.5% patients.

Homozygotes 1, 2, 4, 5 and 8 had severe dementia at the last clinical assessment after 6, 7, 10, 10 and 12 years of disease, respectively. In homozygote 1, a moderate cognitive impairment, estimated by the Wechsler Adult Intelligence Scale as ~25% of the normal performance, developed 1 year after the onset of disease (age 58 years), followed 1 year later by a severe cognitive deterioration. The Mini Mental State Examination scores, available only for homozygotes 3 and 4, and rated at 3 and 6 years after the onset of disease, showed appreciable cognitive impairment (Table 1). Homozygote 8 showed severe intellectual deterioration at the UHDRS cognitive assessment, after 8 years of disease (Table 1).

Severe behavioural changes occurred in homozygotes 1, 3–5 and 8 (Table 1). In most cases (homozygotes 1 and 3–5), these symptoms were nearly concomitant with the initial motor manifestations (Table 1). In all the 75 heterozygotes,

Table 1 *Characteristics of the subjects homozygous for the Huntington disease mutation*

Subject	Databank	Sex and current age	CAG repeats in homozygotes	No. of heterozygotes with the same highest CAG repeat, median motor/psychiatric age at onset (corresponding range of motor/psychiatric onsets in years)*	Huntington disease years[+]	Current Huntington disease stage	TFC[†] (mean decline per year)[§]	Disability score[‡] (mean decline per year)[¶]	Age (years) of first motor symptoms	Age (years) of main behavioural changes	Neurological status at last clinical evaluation	Cognitive status at last clinical evaluation	Affected parent
1	Italian	F 69	40–46	57, 45/41 (20–58/20–58)	6	V	0 (2.2)	10 (14.2)	57 Limb and gait ataxia, chorea	56 Obsessions/compulsions, depression, irritability	Cachexia, severe rigidity and dystonic postures	Dementia	Mother and father
2	Canadian	F 62	36–44	71, 46/45 (17–82/15–78)	7	IV	2 (1.6)	35 (9.3)	51 Chorea	50 Slight personality changes	Severe chorea and dystonia	Dementia	Suspected mother
3	Italian	M 60	42–44	71, 46/45 (17–82/15–78)	3	III	3 (3.3)	50 (16.7)	57 Chorea	57 Delusions, aggressive behaviour; 59 Severe apathy	Severe chorea and dystonia, incontinent	MMSE 20	Mother
4	Italian	F 49	39–43	57, 47/46 (20–77/15–82)	9	IV	2 (1.2)	45 (6.1)	40 Parkinsonism (bradykinesia)	40 Depression, suicide attempts, obsessions/ compulsions, aggressive behaviour	Choreathetosis, bradykinesia	MMSE 13	Mother
5	French	M 71	37–40	16, 52/52 (38–80/36–80)	10	III	3 (1.0)	45 (5.5)	60 Parkinsonism (rigidity)	57 Irritability; 60 Obsessions/ compulsions; 71 Hallucinations, depression, delusions, apathy	Severe rigidity, bradykinesia	Dementia	Suspected mother and father
6	Australian	M 70	36–37	1, 51/50 (51 and 50)	10	IV/V	–	35 (6.5)	45 Chorea	45 'Nervous', slight personality changes	Severe chorea and dysarthria	Dementia	–
7	Australian	F 76	40–41	46, 56/55 (20–72/20–67)	3[†]	III/IV	–	55 (8.3)	– Chorea	58 Delusions, obsessions/ compulsions	Severe chorea	Intellectual decline, memory problems	–
8	Scottish	M 61	40–44	71, 46/45(17–82/15–78)	12	V	0 (1.1)	30 (5.8)	49 Parkinsonism (bradykinesia)	54 Depression, 60 Sleeping problems	Severe chorea, facial dystonia, severe gait ataxia	Dementia	Mother

F = female; M = male; MMSE = Mini Mental State Examination. *Data on patients, heterozygous for the Huntington disease mutation, whose CAG repeat and age at onset were available, are generated from the Italian Huntington disease databank (n = 500) (Squitieri *et al*., 2001). [+] No. of disease years from the onset to the patient's lowest disability score (⩽55). [†]Disease years available from a disability score of 80–55 (Huntington disease stage III–IV). [‡]Total functional capacity and disability score at the patient's last clinical evaluation. [§]Loss of points per year at TFC obtained by the equation (13 – patient's score)/no. of disease years (see Methods). [¶]Loss of points per year at the disability score obtained by the equation (100 – patient's score)/no. of disease years (see Methods).

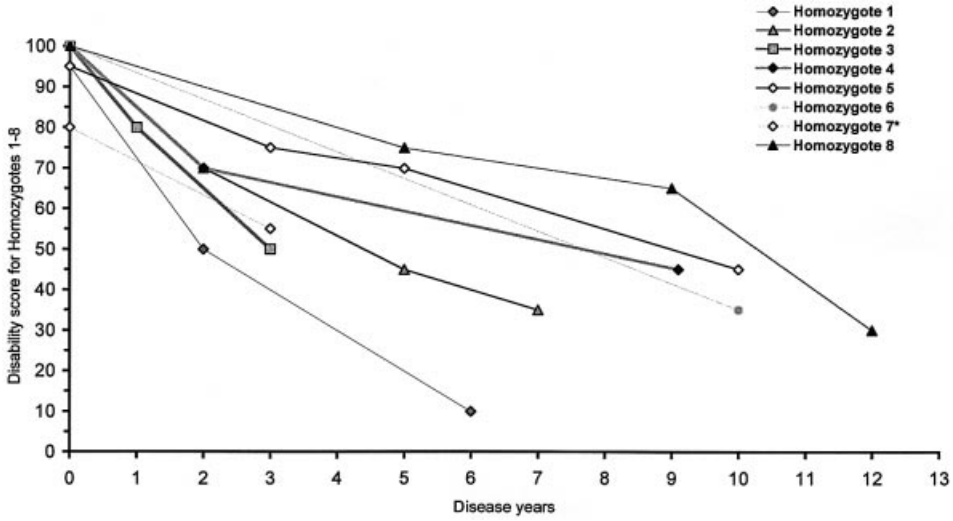

Fig. 1 Disability scores for the Huntington disease homozygotes in whom a quantitative assessment was available, and number of disease years. *Indicates homozygote 7, whose disability score was known but whose last assessment yielded a dubious Huntington disease stage and unreliable TFC scores.

Table 2 *Difference in the TFC score change and in the number of disease years, in each disease stage, between homozygotes and the heterozygotes selected per range of disability score, onset ages and CAG expansion identical to homozygotes*

Stage	Patients	Mean TFC score per year (no. of patients)	*P* value	Mean number of years per stage (no. of patients)	*P* value
I	Homozygotes	2.7 ± 1.8 (6)*	0.036	2.2 ± 1.6 (6)*	0.014
	Heterozygotes	1.1 ± 0.3 (13)		4.7 ± 1.6 (13)	
II	Homozygotes	1.8 ± 1.0 (6)*	0.020	2.7 ± 1.6 (6)*	0.002
	Heterozygotes	0.9 ± 0.3 (13)		8.7 ± 3.4 (13)	
III	Homozygotes	1.4 ± 0.7 (3)[+]	0.0024	4.8 ± 3.2 (3)[+]	0.005
	Heterozygotes	0.5 ± 0.2 (7)		12.5 ± 4.9 (7)	
IV	Homozygotes	1.2 ± 0.7 (2)[†]	0.016	5.2 ± 3.5 (2)[†]	0.014
	Heterozygotes	0.2 ± 0.2 (3)		14.7 ± 4.3 (3)	
V	Homozygotes	–(2)[‡]	–	–(2)[‡]	–
	Heterozygotes	0.1 ± 0.9 (2)		18.5 ± 6.3 (2)	

*Homozygotes 1–5 and 8; [+]homozygotes 1, 2 and 8; [†]homozygotes 1 and 8 (homozygote 2 died before reaching stage V); [‡]homozygotes 1 and 8 (these patients currently are in stage V of Huntington disease).

with the exception of two, mild to severe behavioural changes were concomitant with (n = 58) or pre-dated by one or more years (n = 5, mean 7.0 ± 5.0, range 1–15 years) the onset of motor symptoms.

Age at onset and disease progression in homozygotes and heterozygotes for CAG expansion

The ages at onset calculated as motor symptoms in homozygotes were similar to the median ages at onset of heterozygotes matched for CAG repeat number (homozygotes 51.3 ± 2.7 versus heterozygotes 48.6 ± 3.9 years, $P > 0.05$; paired t test) (Table 1); ages at onset also did not differ between homozygotes and heterozygotes when we compared the ages at which the first behavioural changes occurred (homozygotes 52.1 ± 6.6 versus heterozygotes 48.0 ± 11.6, $P > 0.05$; paired t test) (Table 1). The most notable finding was the accelerated rate of disease progression in Huntington disease homozygotes. The six homozygotes who underwent the TFC assessment (homozygotes 1–5 and 8) had a significantly higher mean loss of units per year than the 75 heterozygotes (1.75 ± 0.9 versus 0.64 ± 0.5 units; P = 0.0005): notably, heterozygotes in our series showed a progression rate similar to that previously reported in a larger cohort of patients (loss of 0.72 TFC units per year) (Marder *et al*., 2000).

The mean loss per year in the disability score was significantly higher in the eight homozygotes than in the 75 heterozygotes assessed (9.07 ± 4.2 units versus 3.44 ± 2.8 units, P = 0.0002) (Myers *et al*., 1991). The difference also

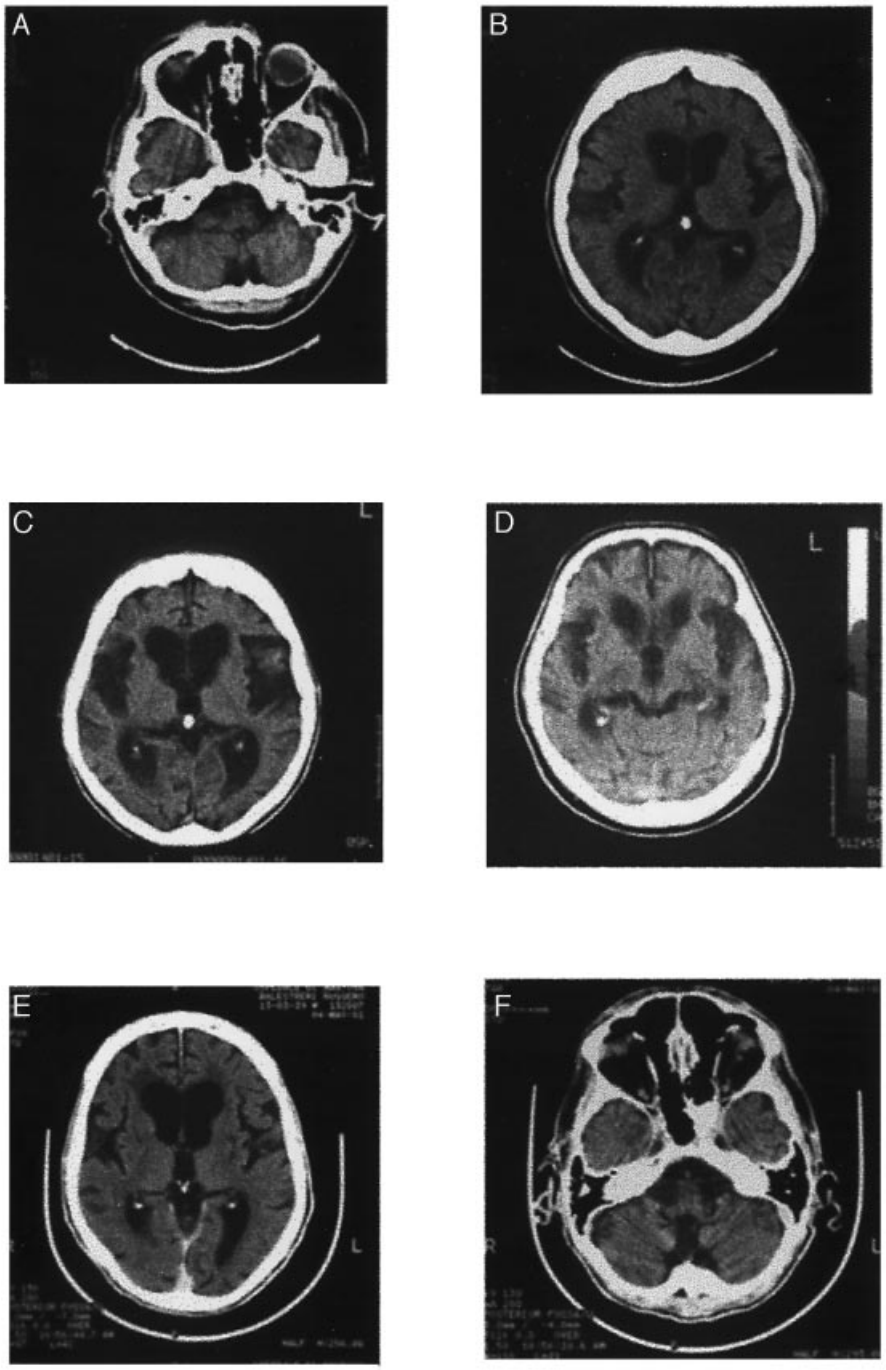

Fig. 2 The CT scan, obtained 1 year after the patient's disease onset (age 59), shows brain atrophy. (**A**) CT scan showing cerebellar, (**B**) cortical and subcortical atrophy in homozygote 1. (**C**) Cerebral CT scans of homozygote 1, performed at age 66, and (**D**) of her affected heterozygote sibling, obtained at advanced disease stages. Note the more severe brain damage, cortical and basal ganglia atrophy in the homozygote compared with the heterozygote, despite the greater number of disease years in the heterozygote. (**E**) CT scan showing cortical and (**F**) cerebellar atrophy in homozygote 3. The CT scan, obtained 4 years after the patient's disease onset, shows a severe degree of brain atrophy.

remained significant when we excluded the data for homozygotes 1 and 3 (1.25 ± 0.2 versus 0.64 ± 0.5 units per year at the TFC, $P = 0.0069$; and 6.94 ± 1.5 versus 3.44 ± 2.8 units per year, $P = 0.002$ at the disability score). The analysis of the mean TFC score change and of the mean number of disease years, in each disease stage, also showed a significant difference between the homozygotes and a cohort of 13 heterozygotes at advanced disease stages (III–V) and selected for range of disability score, onset ages and CAG repeats identical to homozygotes 1–5 and 8 (range of P values 0.036–0.0024, per TFC score changes; range of P values 0.014–0.002, per number of disease years; Table 2; see

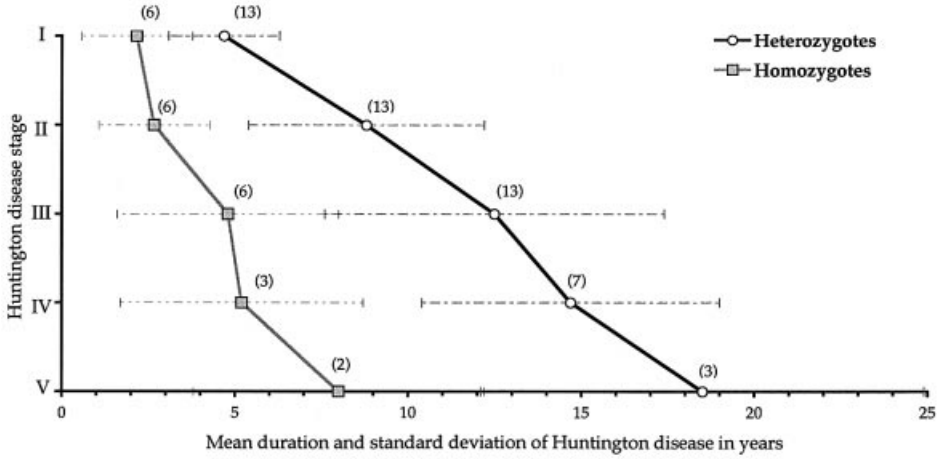

Fig. 3 Duration of Huntington disease (in years) in the homozygotes and heterozygotes. *x*-axis: mean duration and SD of Huntington disease in years. *y*-axis: Huntington disease stage (the number of patients per stage is given in parentheses: stages I and II completed by homozygotes 1–5 and 8; stage III by homozygotes 1, 2 and 8; stage IV by homozygotes 1 and 8). The more rapid disease progression in homozygotes (grey line) compared with heterozygotes (black line) is evident.

Table 3 *Clinical status comparison between homozygote 1 and her affected heterozygote sibling*

Items investigated	Homozygote 1	Heterozygote sibling
Age at onset of motor symptoms (years)	57	58
Age of first behavioural changes (years)	56	58
CAG repeats on upper allele	46	42
Disease duration in years until TFC = 0 (patient in bed)	6	12
Years after onset for autonomy impairment	3	–
TFC and disability score decline (units per year)	2.2 and 14.2	1.1 and 7.5
UHDRS chorea score in 1998 (years of disease)	24 (11)	17 (15)
Huntington disease stage at the last clinical evaluation	V	V
Disability score (years of disease)	10 (11)	10 (15)
Number of years to severe mental deterioration (WAIS)	2	10
Disease years at the CT scan (age of patients)	9 (66)	12 (70)

WAIS = Wechsler Adult Intelligence Scale.

Table 3 for comparison between homozygote 1 and her affected heterozygote sibling). We also found that the 13 heterozygotes took longer than the same homozygotes (homozygotes 1–5 and 8) to reach the same advanced stage of Huntington disease (15.5 ± 4.5 years versus 7.8 ± 3.2 years, $n = 6$; $P = 0.0037$) (Fig. 3). The difference remained significant when we excluded the data for homozygotes 1 and 3 (15.5 ± 4.5 years versus 8.5 ± 3.9 years, $P = 0.023$). When we evaluated the impact of psychiatric symptoms at onset, behavioural changes anticipated the motor onset in four out of 13 heterozygotes (mean 5.0 ± 2.9, range 1–8 years); therefore, also when the age of psychiatric symptom appearance was taken into account, the statistical difference in the Huntington disease progression rate between homozygotes and heterozygotes was not affected (data not shown, Table 1).

Neuroimaging and neuropathological findings in homozygotes

A CT scan, obtained in homozygote 1 at age 59 years (2 years after onset), showed a generalized brain atrophy also involving the cerebellum (Fig. 2 A and B). A subsequent CT scan, at 66 years of age, showed a marked progression of brain atrophy (Fig. 2C). In homozygote 3, a CT scan obtained at age 58, 1 year after onset, was nearly normal, showing only marginal atrophy of the cortex and basal ganglia. A second CT scan, 4 years after onset, showed marked brain atrophy (Fig. 2E), also involving the cerebellum (Fig. 2F). An MRI scan, obtained in homozygote 4, 9 years after Huntington disease onset, showed generalized brain atrophy.

An autopsy report was available for homozygote 2, who died 11 years after the onset of Huntington disease. It showed moderate to marked cerebral atrophy, mainly of the frontal and temporal lobes. The neostriatum showed marked atrophy, with severe neuron loss and gliosis of the caudate, putamen and globus pallidus. The cerebellum also showed neuron loss, with mild to moderate loss of Purkinje cells. Loss of neurons was noticed in the reticular zone of the substantia nigra, and marked atrophy was also noticed in the brainstem, especially the basis pontis and cerebral peduncles, and in the Clark's column. Finally, there was an axonal degeneration of corticospinal tracts and fasciculus gracilis. Outside the CNS, a mild atrophy of the spleen was reported.

Discussion

CAG repeat expansion on both alleles of the *IT15* gene is a rare occurrence (Wexler *et al*., 1987; Myers *et al*., 1989; Kremer *et al*., 1994; Durr *et al*., 1999; Laccone *et al*., 1999). The multicentre design of our study allowed, for the first time, a comparison between heterozygotes for the Huntington disease mutation and the largest homozygous patients' series to date. Our data suggest subtle but significant clinical differences between the two groups. First, the disease progresses more rapidly in homozygotes than in heterozygotes. More rapid deterioration was evident by measures of neurological function and functional independence. In fact, all homozygous patients reached a severe disability in a shorter time and displayed a significantly larger rate of functional capacity decline, in each disease stage, than heterozygotes (Table 2, Fig. 3). Secondly, four of the eight homozygotes (50%) had non-choreiform movements at presentation. While the numbers of cases are too small to allow us to draw definitive conclusions, and motor disorders can be difficult to categorize accurately, it is possible that homozygotes may present with a slightly different phenotype, including a wider spectrum of neurological symptoms other than chorea compared with heterozygotes (Squitieri *et al*., 2000*a*) (Table 1). Accordingly, we observed a more extensive and more severely progressive brain atrophy in homozygotes, though limited to three patients, documented by neuroimaging (homozygotes 1 and 3; Fig. 2) and neuropathology (homozygote 2). The unique autopsy report evidenced remarkable and widespread brain atrophy in the homozygous patient analysed, although, unfortunately, we cannot provide any quantitative neuropathological data as biological samples for studies on the neuronal distribution of huntingtin and inclusions were not available.

In previous studies on Huntington disease (the largest study preceding the discovery of the gene and referring to subjects with only a likelihood of having two mutated alleles), the most frequently evaluated marker of disease severity was the difference in the age at onset (Wexler *et al*., 1987; Myers *et al*., 1989; Kremer *et al*., 1994; Durr *et al*., 1999; Laccone *et al*., 1999). In agreement with these earlier reports, our study shows that homozygotes and heterozygotes had a similar age at onset. In addition to these studies, we found the patients' age at onset was within the range expected for that particular CAG repeat number. It is noteworthy that studies in other CAG triplet diseases, such as the dominant cerebellar ataxias, have reported a more severe phenotype in homozygotes than heterozygotes, including earlier onset of gait disturbances and rapid progression (Gusella and MacDonald, 2000). Unlike cerebellar ataxias, determining the precise age at onset of disease in Huntington disease (Penney *et al*., 1990) may be difficult due to the overlap of psychiatric, cognitive and motor manifestations in the early stages of the disease. In this study, to reduce the chances of bias from age at onset, we obtained detailed longitudinal data on our patients' clinical presentations and could evaluate separately the age of onset for motor and psychiatric manifestations. By both measurements, we observed that disease progression is significantly more rapid in homozygotes than in heterozygotes, suggesting that in this context the double mutant gene dosage is deleterious in patients.

More rapid progression in homozygotes can be the consequence of a greater toxic effect due to the presence of two, rather than one, mutated protein products which may cause more aggregate formation (Scherzinger *et al*., 1999; Gusella and MacDonald, 2002) and/or sequestration of critical cellular components in the targeted cells (Cha, 2000). However, an alternative explanation can be that the polyglutamine mutation in huntingtin not only causes a toxic gain of function, but also abolishes a putative protective function of the wild-type protein on neuronal survival. In this hypothesis, the CAG expansion homozygosity would mimic a 'recessive' loss of wild-type huntingtin function, in addition to the dominant toxic effect of the mutation. Although the animal model may not reproduce the comprehensive wide spectrum of the human disease phenotype (Gusella and MacDonald, 2002), recent experimental data generated in mice should be taken into account. For instance, a mouse model, where the wild-type homologue of the *IT15* gene was inactivated, showed progressive neurodegenaration, suggesting that a loss-of-function mechanism may contribute to Huntington disease pathogenesis (Dragatsis *et al*., 2000). In support of this, a knock-in mouse model of Huntington disease confirmed a more aggressive phenotype in animals homozygous for the expansion mutation, and pointed to a role for wild-type huntingtin in neuronal survival (Lin *et al*., 2001). Also recent work by Gervais *et al*. (2002) finely describes functional differences between wild-type and mutant huntingtin protein, demonstrating that the expansion mutation abolishes the ability of normal huntingtin to bind cellular proteins such as Hip1, chlatrin and AP2, thus inhibiting endocytosis and secretion of neurotransmitters, and possibly activating apoptotic processes. These data stress the possible double nature of the pathogenetic mechanism in Huntington disease, which results in the gain of toxic function (Nucifora *et al*., 2001) and in the loss of normal function (Cattaneo *et al*., 2001). Other data in human Huntington disease are in favour of such a scenario, as wild-type huntingtin has been shown to increase the production *in vivo* of cortical neurotrophins which act on the survival of striatal neurons (Zuccato *et al*., 2001), suggesting a neuroprotective function of normal huntingtin.

As Huntington disease homozygosity is very rare, a study such as this had to be undertaken retrospectively. This meant that we had to rely on the available clinical data from different study centres where Huntington disease patients are assessed with different protocols. While the combination of these factors means that we cannot be dogmatic about our interpretation, we believe that the data suggest that Huntington disease homozygotes do not have an earlier onset of disease than heterozygotes, but the available

evidence is consistent with the homozygote state having a more aggressive disease course.

In conclusion, despite the similar age at onset of symptoms, Huntington disease progresses more rapidly in homozygotes than in heterozygotes, and is probably associated with a wider neuronal degeneration. Our study would suggest that the mechanisms underlying onset and progression of Huntington disease may differ. Further elucidation of the molecular bases of onset and progression may offer clues for further therapeutic strategies in Huntington disease and, possibly, in other expanded CAG repeat diseases.

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