

SHORT REPORT

Huntington's disease: confirmation of diagnosis and presymptomatic testing in Spanish families by genetic analysis

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Abstract

Huntington's disease is a neuropsychiatric disorder with late age of onset, caused by an elongation of a (CAG)_n repeat in the *IT15* gene. This trinucleotide repeat has been studied by polymerase chain reaction amplification in 86 members of 43 Spanish families with Huntington's disease and in 60 unrelated subjects from the general population. The number of (CAG)_n repeats in Huntington's disease chromosomes varied from 40 to 85, with 49 and 52 repeats being the most common, whereas in normal chromosomes it ranged from 12 to 32 with 20 (CAG)_n repeats being the most frequent allele. In four patients with juvenile onset the number of (CAG)_n repeats was greater than 50 and only one was of maternal transmission. There was a clear inverse correlation between the number of repeats and the age of onset of the disease. The study contributed to the diagnosis of 10 patients in whom the clinical diagnosis was uncertain, and identified 41 "at risk" patients after a previous psychological-psychiatric evaluation.

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Huntington's disease is a progressive neurodegenerative disorder characterised by involuntary movements, cognitive disturbances, and emotional problems, which inexorably leads to dementia and death 15 to 20 years from the onset of the disease. Histological changes are widespread in the CNS, but neuronal death and gliosis is mainly found in the caudate nucleus and putamen. The age of onset of clinical manifestations of Huntington's disease varies between 2 and 80 years, but the average age is within the fourth decade of life.¹

Huntington's disease has an approximate frequency of 1 in 10 000 in the European population and is inherited as an autosomal dominant trait with full penetrance and an extremely low mutation rate.² The genetic defect causing this disease was assigned to linkage to chromosome 4p16.3.³ Recently, the

defective gene (*IT15*) has been isolated⁴ and it has been shown to contain a (CAG)_n trinucleotide expansion at the 5' end of the *IT15* open reading frame in patients with Huntington's disease.

The *IT15* (CAG)_n repeat ranges from 11 to 34 (CAG)_n repeats on normal chromosomes, whereas it is expanded beyond 37 repeats in Huntington's disease chromosomes.^{5,6} The 34-38 range is considered to be an intermediate expansion or premutation, which, although it is not associated with clinical manifestations, can expand to the disease range due to meiotic instability and be transmitted to the next generation.⁷⁻¹⁰ The number of repeats undergoes variation depending on the parental Huntington's disease chromosome, with larger expansions when the Huntington's disease gene is transmitted through the male.^{5,6,11,12} The (CAG)_n expansion is inversely correlated with the age of onset of the disorder.¹³ Male sex bias in transmission of Huntington's disease and the inverse correlation of expansion and age of onset are clearly shown in patients with juvenile onset. Thus 80% of juvenile patients are due to paternal transmission, resulting in dramatic increases of the (CAG)_n repeat length.^{11,14}

We present here the molecular analysis of 43 Spanish families with Huntington's disease using the polymerase chain reaction (PCR) of the (CAG)_n repeat sequence of the *IT15* gene. The study has confirmed the clinical diagnosis of Huntington's disease in 36 patients and has allowed us to characterise 41 "at risk" subjects, 14 of whom present the (CAG)_n expansion. In all patients we provided genetic counselling supported by psychologists, psychiatrists, neurologists, and clinical geneticists.

Patients and methods

PATIENTS

A total of 146 DNA samples were studied for the (CAG)_n expansion at the *IT15* gene. Thirty four samples were from eight families with a history of Huntington's disease and clinical, pathological, and radiological features of the disease in at least one affected person (12 samples from affected and 22 from "at risk" subjects). Nineteen samples were of "at risk" persons from five families with a positive history of Huntington's disease, but without

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DNA from the affected members. Fifteen samples were from individual patients with Huntington's disease with family history, radiological signs, and clinical features of the disease. Eighteen samples were from subjects with uncertain family history and negative clinical or radiological data of Huntington's disease. Finally, 60 samples were from unrelated clinically normal subjects of over 60 years of age.

For presymptomatic analysis the subjects were informed of the details of the disease, prognosis, and genetic risk. Psychological evaluation was undertaken. Presymptomatic diagnosis was only performed in patients older than 18 years. If they were younger we refused to perform the analysis, as recommended by the International Huntington Association and the World Federation of Neurology Research Group on Huntington's chorea.¹⁵ The analysis was only carried out in young people when clinical manifestations of the disease were present and the neurologist requested confirmation of the diagnosis.

IT15 (CAG)_n ANALYSIS

Genomic DNA isolation was performed as described.¹⁶ Chemiluminescent blotting polymerase chain reaction (CB-PCR) was as previously described.¹⁷

Results

The range of normal (CAG)_n alleles of the *IT15* gene, calculated from 120 chromosomes of 60 unrelated clinically normal subjects of over 60 years of age, was 12 to 32 repeats, the 20 repeats allele being the most common, present in 60% of the chromosomes.

Twenty seven clinically affected patients with Huntington's disease were analysed. All but one (96.3%) presented expansion of the *IT15* (CAG)_n repeat, ranging between 40 and 63 repeats. One patient with Huntington's disease was homozygous for expanded (CAG)_n repeats (40 and 53 repeats). Among 41 "at risk" subjects analysed, 14 (34.1%) had (CAG)_n repeats above normal, ranging between 46 and 60 repeats.

Eighteen patients either without family history of Huntington's disease or with uncertain diagnosis were referred for confirmation of the clinical diagnosis. Ten patients (55.5%) had expansion of the (CAG)_n repeat (between 48 and 85 repeats), and eight patients were within the normal range.

In the 53 chromosomes studied in 52 patients with Huntington's disease (one homozygous), the (CAG)_n alleles at the *IT15* gene ranged from 40 to 85 repeats, with the alleles of 49 and 52 repeats respectively being the most common, with a frequency of 14% each.

The age of onset of the manifestations of Huntington's disease was known for 31 of the 52 patients, with the average being 41 (13.9), ranging between 11 and 65 years.

Four juvenile patients were detected, with 51, 71, 63, and 85 CAG repeats. The mean number of repeats in the juvenile patients was

67.0 (14.2), and in the non-juvenile patients it was 51.8 (4.2), showing no significant difference between groups.

The transmission of the (CAG)_n expansion through two generations was followed in five patients with paternal and five with maternal transmission, including a juvenile patient for each of the two groups. The mean expansion through males was of 6 (10.9) repeats, and in females it was 1.4 (3.6) repeats.

Discussion

Our results indicate that an expansion of the (CAG)_n triplet in the *IT15* gene is present in 96.3% of Spanish patients affected by Huntington's disease. These results are similar to those reported by authors from other countries,¹⁸ as well as from Spain.¹⁹ Furthermore, we found no overlap between affected and normal alleles.

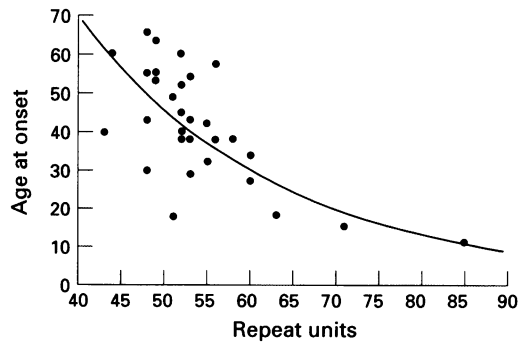
The only clinically affected patient without the (CAG)_n expansion was a 40 year old woman with a family history of psychiatric illness (father and two siblings). The first symptoms of the patient (behavioural problems) appeared when she was 36. Clinically she had chorea, dysarthria, behavioural problems, and epilepsy. Brain MRI showed cerebral atrophy, without any other alterations. On the basis of the data, we cannot exclude the possibility of a mutation in the *IT15* gene, distinct from the (CAG)_n expansion. Other neurodegenerative disorders cannot be disregarded and other genes containing (CAG)_n expansions should be analysed in the patient, as previously reported.¹⁸

Four of the patients with unclear clinical diagnosis that had a (CAG)_n repeat expansion had parents older than 60 without clinical manifestations of Huntington's disease. Unfortunately, DNA was not available for molecular studies and the confirmation of these patients as sporadic was not possible. In 55.5% of these sporadic or uncertain cases, we contributed substantially to an unequivocal diagnosis, which could not have been accomplished solely by clinical evaluation.

We have one homozygous patient with a (CAG)_n expansion in both chromosomes. She is a 58 year old woman with an age at onset of 54, displaying choreic movements in the limbs, tongue protrusion movements, mood changes, and progressive forgetfulness. Brain MRI did not show significant changes. Regarding the family history, the mother was affected whereas the father was clinically normal. The parents of the patient were not consanguineous, but were from the same village of 700 inhabitants. The affected patient has five children, who should all be affected, unless a regression of one of the repeats has occurred.⁵ DNA was only available from the patient.

We have four juvenile patients with ages at onset of 11, 17, 17, and 18 years. Three of these patients were of paternal and one of maternal transmission. We could only study one father in the three patients with paternal transmission. The father had 60 repeats and the son had 85. The other two patients with

Figure 1 Correlation between age at onset and (CAG)_n repeat length in the IT15 gene in 31 Spanish patients with Huntington's disease. Two patients had identical age of onset and number of repeats; therefore their points are in the same position.



paternal transmission had 51 and 71 repeats and the affected fathers were deceased. In the patient with maternal transmission, the mother had 60 (CAG)_n repeats and the affected 17 year old son had 63 repeats. The mean number of repeats in the juvenile patients was 67.5, slightly higher than previously reported.¹¹ In the juvenile patient with 51 (CAG)_n repeats, the expansion is well below the mean for early onset and it cannot be explained by the number of (CAG)_n repeats alone. In the juvenile patient with 63 (CAG)_n repeats, the expansion is also below the mean and was of maternal transmission, suggesting that factors other than repeat number and paternal transmission affect the clinical phenotype, as has been stated by other authors.¹¹

We have found a significant negative correlation between age of onset and the (CAG)_n repeat length as has been previously reported⁵ (figure). In our study, we can infer a greater increment in the number of repeats between generations when the father is the transmitter of the disease (6 (10.9)), than when it is the mother (1.4 (3.6)), although the small size of the sample and the fact that the increments in the juvenile patients (one with 25 repeats) were included have to be considered. Our data agree with the fact that paternal transmission implies an increase in the number of (CAG)_n repeats and, therefore, an earlier onset of Huntington's disease, as shown by other authors.^{11 12}

The analysis of the (CAG)_n repeat in Huntington's disease permits confirmation of clinical diagnosis, correlation of clinical features, and presymptomatic testing. In addition to molecular confirmation of Huntington's disease, our study allowed us to confirm the diagnosis in 60% of patients with uncertain clinical diagnosis. Furthermore, presymptomatic testing, performed in 41 patients, permitted the detection of the (CAG)_n mutation in 14 patients, before the development of the disease. There are other diseases phenotypically similar to Huntington's disease in which anticipation is involved. Patients with an uncertain diagnosis who are negative for the IT15 mutation are studied for (CAG)_n expansions at other possible loci (*SCA1*, *DRPLA*, Machado-Joseph).²⁰

Patients with presymptomatic diagnosis of Huntington's disease received the appropriate psychological and psychiatric support. In our

experience, the analysis of the (CAG)_n triplet in the IT15 gene helps in the clinical diagnosis of Huntington's disease, permitting the determination of the (CAG)_n repeat status in "at risk" subjects without involving the rest of their family. The information provided by this analysis is very important for these people and must be delivered with extreme care by an expert team including psychiatrists, psychologists, neurologists, and geneticists.

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