SHORT REPORT

Molecular diagnostic analysis for Huntington's disease: a prospective evaluation

J C MacMillan, P Davies, P S Harper

Abstract

The availability of mutation analysis for the CAG repeat expansion associated with Huntington's disease has prompted clinicians in various specialties to request testing of samples from patients displaying clinical features that might be attributable to Huntington's disease. A series of 38 cases presenting with clinical features thought possibly to be due to Huntington's disease were analysed prospectively. In 53% of such cases presenting initially with chorea and 62·5% with psychiatric symptoms an expansion was identified, a considerable lower proportion than found in previous series where the diagnosis was considered definite on clinical and genetic grounds. Mutation analysis is likely to be of considerable value in the diagnosis of Huntington's disease, especially where the family history in previous generations is inadequate or apparently negative.

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Huntington's disease is an inherited progressive neurodegenerative disease, typically with onset of choreiform movements and cognitive decline in midlife or later.[1] Over 99% of patients with a firm clinical diagnosis of Huntington's disease have been shown to have an expansion in an unstable CAG trinucleotide repeat in the IT15 gene.[2–4] These patients were mainly studied for research purposes and their DNA stored and subsequently analysed by units engaged in the cloning of the Huntington's disease gene. They are therefore biased in that the samples come from patients with "typical" clinical manifestations and, in the great majority, a positive family history for Huntington's disease or a disorder diagnosed as such. There have been some data published on the presence of the Huntington's disease CAG expansion in retrospective series of "sporadic" cases of Huntington's disease[5]—namely, those without a positive family history but with a suggestive clinical phenotype, those with "suspected" Huntington's disease,[6] in series of patients with schizophrenia,[7] and most recently in symptomatic patients reported along with results from those requesting presymptomatic prediction.[8]

We have found that the availability of a sensitive and specific molecular test for this genetic disorder now leads to requests for such an analysis in a wider range of clinical diagnostic situations than previously resulted in a request for DNA banking for future analysis. We here report the series of requests for Huntington's disease diagnostic mutation analysis that followed its establishment in the service laboratory of our unit.

Materials and methods

Requests for Huntington's disease mutation analysis were received from clinicians throughout England and Wales. Mutation analysis was only undertaken when the clinician had obtained informed consent from the patient (or their family where the patient was unable to give consent) and had completed a standardised data sheet detailing clinical manifestations, family history, and the results of neurological investigations such as CT and MRI. Diagnostic mutation analysis was only offered in the context of an affected patient with symptoms considered likely to be due to Huntington's disease, not in asymptomatic patients at risk who were assessed with previously validated counselling protocols,[9] and for whom data are not reported here.

Blood samples (20 ml) in EDTA were forwarded by first class post to our unit and DNA was extracted from peripheral blood leucocytes by standard techniques. Polymerase chain reaction amplification of the region of IT15 containing the CAG repeat polymorphism was carried out as described previously.[3] The normal range for our laboratory, based on 1160 normal chromosomes, is nine to 34 repeats with a mean of 18.

Results

Initial diagnostic enquiries concerning 43 cases were received over a 12 month period. Thirty nine blood samples were subsequently received for analysis from which 38 (97·4%) mutation results were successfully generated. Seventeen referrals (15 subsequent samples

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Clinical and molecular data for the cases in the series

Case	*Sex*	*Age at presentation*	*Presentation*	*Family history*	*Repeats*
1	F	75	C	A	39/17
2	M	30	C	A	43/16
3	M	70	C	O	20/17
4	M	Un	C	A	40/19
5	F	75	C	N	21/18
6	F	24	C	N	18/14
7	M	28	C	N	28/15
8	M	55	C	N	41/17
9	M	9	C	N	23/17
10	F	Un	C	N	40/17
11	F	76	C	N	23/21
12	M	59	C	N	19/17
13	F	55	C	N	39/18
14	M	58	C	N	40/20
15	F	45	C	U	41/16
16	M	35	P	A	42/16
17	M	57	P	A	40/17
18	F	65	P	N	28/18
19	M	40	P	N	21/15
20	M	41	P	N	54/14
21	M	25	P	N	42/14
22	M	54	P	N	41/16
23	M	18	P	N	17/17
24	M	56	CP	N	43/19
25	M	28	CP	N	16/15
26	F	30	CD	O	54/16
27	M	31	CD	O	49/16
28	M	Un	CD	N	44/20
29	F	59	CD	N	41/23
30	M	Un	CD	N	20/15
31	M	Un	CD	N	19/18
32	F	54	CD	N	21/16
33	M	60	CD	N	38/16
34	F	60	CAt	A	41/15
35	M	37	CR	A	46/16
36	M	48	CDP	U	44/15
37	F	40	CDP	U	41/16
38	M	65	CDPAt	N	38/15

Un = Unknown; C = chorea; P = psychiatric symptoms; D = dementia; R = rigidity; At = ataxia; A = affected parent; O = other affected relative; N = no family history; U = uncertain.

and analyses) were received from neurologists, two from psychiatrists (one sample and analysis), one from a psychogeriatrician, and 23 from clinical geneticists (22 subsequent samples and analyses). Seventeen enquiries related to female and 26 to male patients. The age range of the cases at the time of the request for analysis was 13 to 87 whereas the age at onset of symptoms ranged from 9 to 75 (mean 43 (SD 22)) years.

Eight of 15 cases presenting initially with chorea had one allele with more than 37 CAG repeats and the remaining seven had two alleles each with less than 30 repeats, whereas five of eight cases with initial psychiatric symptoms had expanded repeats. Eleven of the remaining 15 cases, who had combinations of symptoms at onset, also possessed expanded repeats. The table summarises, for each case, the major clinical features at presentation, the recorded family data, and the results of the mutation analyses. Neuroradiological data (CT or MRI) were available for 23 patients, 14 mutation positive and nine mutation negative. Of the 14 mutation positive cases, the scan was normal in five (all CT), showed specific caudate atrophy in five, and generalised atrophy in four. Of the mutation negative cases, the scan was normal in two, showed generalised atrophy in one, general and more pronounced caudate atrophy in one, and offered alternative diagnoses in five (one caudate infarct, two widespread infarcts, one demyelination, and one frontal atrophy). In all five cases with an alternative radiological diagnosis the family history was negative for Huntington's disease.

Discussion

In this series only 47% (seven of 15) of patients analysed who presented initially with chorea as their major manifestation had a normal length CAG repeat in the Huntington's disease gene. This is not surprising given that in six of these cases there was no evidence for Huntington's disease in preceding generations, but contrasts with the very high proportion (98·4% to 100%)[10 11] of clinically established cases with a known family history that show the presence of the mutation. Ashizawa *et al*[6] recently reported that five of 12 (42%) patients thought clinically to have Huntington's disease but for whom no positive family history could be documented failed to demonstrate CAG expansions. The single mutation negative case (case 3) in our study where choreiform movements were the presenting feature and the patient had a positive family history for Huntington's disease merits some discussion. The affected relatives in this family did not include the patient's appropriate parent who had died at the age of 71 without any documented neurological dysfunction. The patient himself had been on antiparkinsonian medication before the onset of his chorea at the age of 70. We did not have access to any DNA from other affected patients in this family that we could use to confirm the presence of the Huntington's disease mutation and therefore his clinical diagnosis of Huntington's disease remains insecure. We have previously reported a parent-child pair, clinically diagnosed as having Huntington's disease, both of whom were expansion negative (cases A and B, table 1, MacMillan *et al*[12]). Both have subsequently been found to have expansions in the CAG repeat sequence associated with dentatorubropallidoluysian atrophy (Ross *et al*, in preparation) emphasising that, wherever possible, even in the case of symptomatic patients said to have a "positive" family history, the mutation state of clinically affected family members should be ascertained.

Three of the six cases in this series presenting initially with psychiatric manifestations but with no family history of Huntington's disease were mutation positive. The first of these was a patient who presented with paranoid delusions at age 41 and developed a choreiform movement disorder 18 months later while taking phenothiazines. The involuntary movements were predominantly orofacial with lesser involvement of the upper limbs. There was no disturbance of eye movements, no evidence of cognitive decline, and his gait was normal. His mother was alive and well in her 70s and his father had died of a cardiac event in his 50s. There had been no history of a movement disorder in his three sibs although one had died at the age of 26. The second case was of a man who presented with a personality disorder at the age of 25 and subsequently developed progressive chorea (without a history of medication) from the age of 45. His mother had died aged 92 and his father at the age of 70, neither having

displayed any features of Huntington's disease. His only sib was reported well at the age of 63. The third case had presented with a psychiatric disturbance in her early 50s, which was shortly followed by chorea and dementia. Computed tomography showed only generalised cerebral atrophy. Her mother was alive and well at the age of 87 and her father had died aged 62 of malignancy. In all three cases the lack of a positive family history had made the referring clinicians suspicious of making a diagnosis of Huntington's disease although all developed a movement disorder (as had the three mutation negative patients) subsequent to the psychiatric presentation.

The results reported here are not directly comparable with those of Davis *et al*[5] who examined a series of banked DNA samples from "isolated individuals with Huntington's disease" in addition to patients with clinically doubtful Huntington's disease. They defined a negative family history as both parents having died over 65 years of age without neurological or psychiatric disease or both parents still being alive. In our study the age at which parents had died was often not available. In four of our cases with negative family history it had been documented that both parents died over the age of 65 and without a history of a neurological disorder. In two of these cases an expanded repeat sequence was identified. It was not possible, due to the nature of the study, to exclude non-paternity as a reason for the negative family history. We think that a more likely explanation, however, is that these expansions (42 and 43 repeats) arose from "intermediate" alleles.[13] Davis *et al*[5] found expanded repeat sequences in 89% of patients defined as clinically probable Huntington's disease and in 31% of those with clinically doubtful Huntington's disease. The referring clinicians in our study clearly thought that Huntington's disease was a possibility: we have not, however, attempted to subdivide the cases into "probable" or "doubtful" on the basis of the information provided. Benjamin *et al*[8] reported mutation analysis results on 171 symptomatic patients, of which 56 were without a positive family history. Thirty eight (70%) of these 56 had expansions of more than 36 repeats (Huntington's disease range >36 in their study). They found two symptomatic patients with repeat lengths in the normal range in families with mutation established Huntington's disease, a similar situation to that reported in our original series.[12] Davis *et al*[5] defined a repeat length of less than 40 identified using the original primers as a borderline result and it is noteworthy that in all such cases repeat analysis with primers excluding the CCG region resulted in an increase in the recorded length of the CAG repeat. In our series we considered a CAG repeat length of 38 or greater to be disease related and did not find any values in the range 30–37. It is also our experience (P Davies, unpublished data) that allele lengths in the intermediate zone analysed with the original primer pair tend to enlarge when analysed with primers that omit the polymorphic CCG sequence.

This study, like those of Davis *et al*[5] and Ashizawa *et al*,[6] shows how mutation analysis for the Huntington's disease CAG repeat expansion can be applied in clinical practice. It emphasises the need to be wary of a "negative" family history not only where there is limited knowledge of the status of "missing" parents but also where the parents have died at advanced age without neurological symptoms. The test undoubtedly is an important augmentation to the diagnosis of Huntington's disease in clinical practice. We would urge, as did Benjamin *et al*,[8] that it remains prudent to "proceed with care".

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