

Family history and DNA analysis in patients with suspected Huntington's disease

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Abstract

Objectives—Until recently a definite diagnosis of Huntington's disease could be made by a combination of clinical findings, a positive family history, and pathological confirmation. Prevalence data are based on these criteria. After finding the gene and its pathogenic mutation direct diagnostic confirmation became available. The aim of this study was to determine to what extent the direct assessment of CAG repeat length has allowed the diagnoses of additional patients, with atypical psychiatric or neurological disease, or those without a family history, that could otherwise not be diagnosed using traditional criteria.

Patients and methods—From all 191 referred patients suspected of having Huntington's disease between July 1993 and January 1996 CAG repeat length was determined and the family history was reviewed in the Leiden roster. After a retrospective search the patients were subdivided in positive, negative, suspect, and unknown family histories. Patients with an expanded repeat (>35) were finally diagnosed as having Huntington's disease. The family history was compared with the repeat length and the clinical features.

Results—Clinical information was obtained for 172 patients. Of these, 126 patients had an expanded repeat, 77 had a positive, eight a negative, 40 a suspect, and one an unknown family history. Of the 44 patients with a normal repeat length four had a positive family history. Of the two patients with an intermediate repeat (between 30–36 repeats), one with a negative family history received a clinical diagnosis of Gilles de la Tourette's syndrome. The other had an unknown family history. **Conclusion**—Despite verification of the family history through the Leiden roster, many more patients and families could be diagnosed with the new approach than would have been possible with the traditional criteria. Because prevalence studies have been based on this type of information, the data suggest an underestimation of the prevalence of Huntington's disease in the community of 14%.

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Keywords: Huntington's disease; prevalence; sporadic; CAG repeat

Until recently, the diagnosis of Huntington's disease could only be made in the presence of

progressive choreic movements, behavioural disturbances, dementia, and a positive family history. The confirmation of the family diagnosis was based on the neuropathological findings in affected family members. Using these criteria various investigators generated prevalence data of ethnic populations world wide that yielded prevalence rates between 3/100 000 and 7/100 000 people of western European descent.¹ Crucial for the diagnosis was the elucidation of a family history consistent with an autosomal dominant inheritance.

After the discovery of the gene and its pathogenic mutation in 1993² a definitive diagnosis could be made in people with specific neurological signs, and reliable presymptomatic testing could be offered to those at risk. Moreover, the assessment of CAG repeat length allowed the identification of patients with atypical psychiatric or neurological disease, or those without a family history, as having Huntington's disease.

In The Netherlands, DNA testing for Huntington's disease as a diagnostic and presymptomatic tool for clinical use was centralised at the Department of Clinical Genetics in Leiden until 1996. Also, at this department a centralised roster of Huntington's disease families was kept, which was started in the mid-1930s. Before CAG assessment the roster had allowed clinicians all over the country to acquire information, that was otherwise unavailable, about the status of family members of patients suspected of Huntington's disease. It was considered that the use of such a roster had allowed near complete identification of Huntington's disease families in The Netherlands.

The aim of this retrospective study was to determine to what extent the direct assessment of CAG repeat length has allowed us to diagnose additional patients and families that could otherwise not be diagnosed with traditional criteria.

Patients and methods

Between July 1993 and January 1996, 191 requests were submitted from all over the country by neurologists (157 patients), psychiatrists (16 patients), and clinical geneticists (18 patients) to determine the presence or absence of an expanded CAG repeat in the IT15 gene because of a clinical syndrome of involuntary movements, or psychiatric disorders. The number of requests in 1993, 1994, and 1995 was 31, 99, and 61 respectively. To prevent presymptomatic testing, which requires specific counselling, the referring physicians had to describe the main symptoms on the request form.

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Because the information about the clinical features and the family history was not always described in sufficient detail on the form, further information was obtained retrospectively from the referring physicians, who obtained informed consent from the patients (or relatives).

For all patients the family history was checked with the Leiden roster. This roster contains pedigree information on over 3000 persons, extending back to the 18th century, from Huntington's disease kindreds in the Netherlands.³ Since the mid-1930s information on the pedigrees is obtained by families and is extended using parish records, municipal registers, and national archives. The roster is in total compliance with recent Dutch legislation for individual privacy and protection of sensitive medical data. A family history was considered to be positive when at least one other pathologically or genetically established patient with Huntington's disease was known in the family. A family history was considered to be negative when no other siblings had any signs or symptoms of Huntington's disease, and both parents were alive and healthy or lived without neurological or psychiatric disorders over the age of 65 years. A family history was considered to be suspect when one parent had an ambiguous history or had died before the age of 65 years, or when the family history disclosed a neurological (parkinsonism), or psychiatric disorder, or suicide. If no information could be obtained the family history was considered to be unknown. Age at onset was defined as the age at which the first appearance of involuntary movements, behavioural disorders, or character changes was seen by the patient, the family, or the physician.

The family history was compared with the length of the CAG repeat. This was determined in DNA isolated from venous blood with a

polymerase chain reaction assay as described previously.⁴ A repeat over 35 was considered as expanded and diagnostic of Huntington's disease.^{5,6} Of the patients in whom a discrepancy between family history and CAG repeat length was found, a detailed retrospective clinical description was extracted from the records.

STATISTICAL ANALYSIS

To assess differences in the length of the CAG repeats, the age at onset, and the durations of illness of the patients in data from the four different family history groups were analysed by ANOVA with Student's *t* tests for post hoc comparisons. The relation between the repeat length and the age at onset was assessed with the Pearson correlation coefficient. Data were analysed using the statistical package for social sciences (SPSS). *p* Values < 0.05 were considered to be statistically significant.

Results

Of the original 191 referred requests three could not be contacted by their referring physician to obtain informed consent and three patients refused to participate. For 13 patients, not enough clinical details could be obtained. Therefore, 172 patients were included in the further analysis (table 1).

POSITIVE FAMILY HISTORY

Of the 81 patients with a positive family history in the roster, 77 (95%) received a final diagnosis of Huntington's disease (mean repeat length 45.7, range 40–59). The remaining four patients had a normal repeat length (table 1). The positive family history was well known by the referring physicians before submission. Of these four with a normal repeat length, patient 1 (male) had an affected father and sister. Clinically, he had chorea combined with a behavioural disorder and dementia as presenting signs. During the course of the disease the patient developed rigidity soon after onset. No neuroleptic drugs were used. Dystonia, mainly seen in the upper limbs, was progressive. On CT cortical atrophy and atrophy of the caudate nucleus was present. No final diagnosis could be made (table 2). The other three patients had an atypical presentation of psychiatric symptoms without choreic movements (table 2, patients 2–4). Patient 3 was addicted to

Table 1 Family history of the 172 patients and the CAG repeat

Family history	CAG repeat length			Total
	>35	<30	Intermediate	
Positive	77	4		81
Negative	8	21	1	30
Suspect	40	17		57
Unknown	1	2	1	4
Total	126	44	2	172

Table 2 Characteristics of seven patients with a positive family history and discrepancy in prior clinical diagnosis and final diagnosis. Four patients had Huntington's disease (HD) as prior clinical diagnosis and a normal repeat. Three patients had a prior clinical diagnosis different from HD and an expanded repeat, leading to HD as final diagnosis

Patient	Age at onset (y)	First symptom	Prior clinical diagnosis	Duration of illness (y)	Chorea	Psychiatric disorders	Dementia	CT	CAG repeat length	Diagnosis
1	38	Chorea/behavioural disorders/dementia	HD suspect	7	–	–	+	a	18/19	Progressive degeneration, no HD
2	45	"Hot standing on firm ground"/depression/motor impersistence	HD suspect	5	–	+	–	n	19/20	No HD
3	32	Behavioural disorders	HD suspect	1	–	+	–	–	16/18	No HD
4	41	Behavioural disorders	HD suspect	9	–	+	–	–	15/17	Depressive regressive syndrome
5	59	Chorea/behavioural disorders	Chorea of unknown cause	11	+	+	–	a	16/42	HD
6	66	Chorea	Choreoathetosis	7	+	–	–	a	18/43	HD
7	32	Chorea/dystonia	Chorea of unknown cause	7	+	–	–	n	18/52	HD

+ = Observed; – = not observed; a = abnormalities on CT; n = no CT.

Table 3 Characteristics of 30 patients (8–37) with a negative family history. Eight patients had an expanded repeat and had HD as final diagnosis. One patient had an intermediate repeat

Patient	Age at onset (y)	First symptom	Prior clinical diagnosis	Duration of illness (y)	Chorea	Psychiatric disorders	Dementia	CT	CAG repeat length	Final diagnosis
8	40	Chorea/behavioural disorders	Chorea of unknown cause	2	+	+	+	n	24/44	HD
9	39	Chorea	Chorea of unknown cause	14	+	+	+	–	22/44	HD
10	23	Psychotic/insecure walking	Chorea of unknown cause	15	–	+	–	–	18/47	HD
11	52	Chorea	Chorea of unknown cause	10	+	+	–	–	17/44	HD
12	41	Chorea/behavioural/character disorders	Chorea of unknown cause	7	–	+	–	a	19/45	HD
13	50	Chorea/behavioural disorders	Chorea of unknown cause	5	+	+	–	–	17/44	HD
14	54	Chorea	HD suspect	14	+	+	–	a	22/44	HD
15	53	Chorea/behavioural disorders	HD suspect	5	+	+	–	–	18/42	HD
16	31	Behavioural disorders/psychotic/tics	HD suspect	6	+	+	–	n	22/32	Gilles de la Tourette
17	72	Chorea	Chorea of unknown cause	2	+	–	–	–	20/28	Steele-Richardson-Olsewski
18	39	Chorea	Chorea of unknown cause	7	+	–	–	–	16/16	Benign chorea
19	1	Chorea	Chorea of unknown cause	7	+	–	–	–	16/17	Benign chorea
20	62	Chorea/insecure walking	Chorea of unknown cause	4	–	+	+	n	20/21	Chorea of unknown cause
21	65	Mandibular dystonia	chorea of unknown cause	5	+	–	–	–	19/23	Meige/tardive dyskinesia
22	20	Hemichorea	Chorea of unknown cause	14	+	–	–	n	17/17	No symptoms
23	65	Chorea/strange thoughts	Chorea of unknown cause	8	+	–	+	n	17/19	Vascular
24	49	Chorea	Chorea of unknown cause	3	+	+	–	–	17/17	Neuroleptic induced chorea
25	26	Chorea/disturbance of coordination	Chorea of unknown cause	15	+	–	–	n	17/18	Chorea ataxia/higher function loss
26	66	Chorea	HD suspect	12	+	+	+	a	18/20	–
27	30	No balance	HD suspect	14	–	+	+	a	17/20	ADL
28	40	Chorea/no balance/vomiting	HD suspect	5	+	–	–	n	18/19	–
29	52	Chorea/behavioural disorders	HD suspect	3	+	+	+	–	12/15	Progressive dementia
30	28	Dysarthria/nervous face/CVA	Organic psychosyndrome	7	–	–	–	–	19/21	CADASIL/subcortical infarct
31	5	Chorea/low intellect	Non-progressive (non-)hereditary chorea	42	+	–	–	n	15/24	–
32	4	Chorea/tics/swearing	Gilles de la Tourette	16	+	+	–	–	15/17	Gilles de la Tourette
33	75	Chorea	Morbus Bechterew	1	+	–	–	–	18/21	Chorea during illness
34	64	Chorea/insecure walking	Torsion dystonia/chorea of unknown cause	8	+	–	–	n	20/22	–
35	–	Behavioural/character disorders	Not clear/oligofrenia	–	+	+	–	a	17/20	Chorea of unknown cause with oligophrenia+dementia
36	45	Chorea	StVitus dance/induced by neuroleptica	20	+	–	–	n	17/22	Remainder of StVitus dance
37	34	Strength loss	Faciohumeroscapular dystrophy (Landouzy-Dejerine)	27	–	+	+	–	20/24	Landouzy-Dejerine

+ = Observed; – = not observed; a = abnormalities on CT; n = no CT.

cocaine. The other two patients did not use medication.

Three patients with an expanded repeat length had a positive family history in the roster, but were submitted by the referring physicians as having negative (two patients) or unknown (one patient) family history. Clinically, these patients all had clear symptoms of Huntington's disease (table 2).

NEGATIVE FAMILY HISTORY

The clinical details of the 30 patients with a negative family history are described in table 3 (patients 8–37). Eight (27%) had Huntington's disease as the final diagnosis (mean repeat length 44.5, range 42–47) (table 3; patients 8–15). This contributed 6.3% of the 126 patients with an expanded repeat. Of the eight patients with an expanded repeat, six (patients 8–13) had chorea of unknown cause, and two (patients 14 and 15) had Huntington's disease suspected as a prior clinical diagnosis. Presenting signs were chorea (n=3), a combination of chorea and behavioural disorder (n=4), and psychosis with gait problems (n=1). During the

disease all eight patients had severe psychiatric disorders as their main clinical sign.

One patient (male, patient 16) had an intermediate repeat of 22/32 CAG repeats. Clinically, this patient presented with a behavioural disorder, mainly aggressive outbursts, psychosis, and tics as first symptoms. In the course of the disease he developed chorea. After CAG repeat determination his clinician considered Gilles de la Tourette's syndrome as the most likely diagnosis (table 3).

SUSPECT FAMILY HISTORY

Of the 57 patients with a suspect family history, of whom the roster was unable to provide a positive family history, 40 (70%) had Huntington's disease as the final diagnosis (mean repeat length 43.7, range 39–66) (table 1). In 17 patients the parents had died before the age of 65 years and in two patients the biological father remained unknown. In 35 patients one parent was suspected of having Huntington's disease. The suspicion was based on restless movements (n=14), parkinsonism (n=4), psychiatric symptoms (n=10), and dementia

(n=7). A member in the families of three patients had committed suicide.

During the disease, all the 40 patients with an expanded repeat developed chorea. In 25 patients this was combined with dementia, in 30 with a psychiatric disorder, and in 15 with dysarthria. In the 17 patients with normal repeat length 15 developed chorea, which was combined with dementia in two patients. Eight patients had psychiatric disorder only.

UNKNOWN FAMILY HISTORY

Of the four patients with a family history unknown to the referring physician, and whose family history could not be identified in the roster, one had an expanded repeat (16/40; table 1). This patient had choreic movements and dysarthria and was diagnosed as suspected of having Huntington's disease. Of the remaining three patients with an unknown family history two patients were submitted to exclude Huntington's disease. In one patient the final diagnosis of tardive dyskinesia and in the other a diagnosis of probable multiple sclerosis was made. One patient (female) who was born outside The Netherlands, had an intermediate repeat length of 17/31 CAG repeats (table 1). She clinically showed chorea as an isolated sign and was submitted with chorea of unknown cause as prior clinical diagnosis. After the repeat determination no final diagnosis was established.

COMPARISON OF THE DEFINITE FAMILY HISTORY GROUPS

The unknown family history group was too small to take into account for the statistical analysis, thus lengths of the expanded repeats in the three other family history groups were compared and a significant longer repeat was disclosed in the patients with a positive family history than in patients with a suspect family history ($p < 0.05$). The mean age at onset in the patients with Huntington's disease with an expanded repeat and a positive family history was 40.1 years (n=74, range 4–67 years), which was significantly lower than in the patients with a suspect family history (n=34, mean age at onset 50.5 years, range 22–69 years, $p < 0.01$). The mean age at onset in the patients with a negative family history was 44.0 years (n=8, range 23–54 years), which did not differ from the other two CAG expanded groups. The duration of illness did not differ between the three groups.

A significant inverse relation was found between the repeat length and the age at onset in the three groups (positive family history -0.69 , negative family history -0.82 , and suspect family history -0.67 , all $p < 0.01$).

Discussion

Before the discovery of the Huntington's disease gene and its pathogenic mutation, a diagnosis of Huntington's disease was based on clinical features, family history, and pathological confirmation. This family history could be verified through a roster, such as the one in Leiden. Such a roster may provide the best information possible to assess family details for

clinical use, although from an epidemiological and research point of view, the information would be incomplete.

In the literature, over 99% of patients with a clinical diagnosis of Huntington's disease and a positive family history have been found to have an expanded CAG repeat.^{2 7-10} In our study this percentage was 95%, which is comparable with the findings of Sanchez *et al.*¹¹ and Zülke *et al.*¹² In retrospect, the 5% represent three atypical patients and one possible phenocopy. The three atypical patients were diagnosed as having Huntington's disease with a positive family history (table 2, patients 2–4), but the diagnoses made in these clinically atypical patients may have been prejudiced by the positive family history. The patient with clear signs of Huntington's disease could represent a phenocopy (table 2, patient 1). A few patients in a similar situation have been described previously. Andrew *et al.*¹⁰ found in a cohort of 1022 patients, seven patients (0.7%) with a positive family misdiagnosed, and 12 patients (1.2%) to be possible phenocopies. Eight of these 12 patients (0.8%) had a positive family history, which is comparable with our results.

Eight of 30 patients with a negative family history (27%) had an expanded repeat, which is somewhat lower than the 40% described by Mandich *et al.*¹³ who used the same definitions for a negative family history as we did.

The proportion of those with an expanded repeat and a suspect family history was higher than the proportion of those with an expanded repeat and a negative family history. Of those with a suspect family history (n=57), 40 persons (70%), had an expanded repeat. This proportion is similar to the 65% of Davis *et al.*¹⁴ and 85.7% of Mandich *et al.*¹³ All these patients had clinically obvious and typical Huntington's disease features.

This remarkably high number of patients with an expanded CAG repeat and an uninformative family history, which we found in this study, either because of a negative (n=8), a suspect (n=40), or an unknown family history (n=1) may be explained in several different ways. The sporadic patient could represent a de novo CAG expansion, which may occur in about 3% of the affected patients¹⁵ as a result of expansion of "intermediate alleles" usually through the male germline.¹⁶⁻¹⁹ Several investigators expect the appearance of patients with new mutations to be higher.²⁰⁻²³ In our study we could not obtain DNA from the parents of the eight patients with sporadic Huntington's disease because they were deceased (n=4) or no family contact existed (n=4). If all eight sporadic patients were regarded as new mutations this study would suggest an upper limit to the mutation rate of 6.3% (8/126).

Non-paternity, which could not be excluded in this study, could have been another explanation for a few "sporadic" patients. The incidence of non-paternity in the general population has been estimated to be about 5%.²⁴

A third explanation may be the late onset of clinical signs in the parents with anticipation in

the probands. The risk of being affected after the age of 50 is estimated to lie between 10% and 25%, which is probably an underestimate as death may occur from other causes before the onset of Huntington's disease.²⁵ Late onset disease is usually milder in presentation with milder chorea and less cognitive decline.²⁶⁻²⁷ It could easily have been overlooked or misdiagnosed, thus obscuring the family history. The longer repeat length would explain the earlier onset in the children. This could explain the shorter repeat length and higher age at onset in the patients with a suspect family history compared with the patients with a positive family history. Moreover, first signs in a patient with a positive family will be recognised earlier, resulting in a lower age at onset. Another explanation for the apparently healthy survival in old age of the parents could be due to an incomplete penetrance of the CAG mutation (range 36–39), which may rarely occur in those without any clinical or pathological manifestation of the disease.²³⁻²⁸⁻³⁰

Two patients with neurological abnormalities had repeat lengths in the intermediate range (17/31 and 22/32 respectively). Clinically, one had chorea and the other chorea combined with psychiatric disorders and tics as atypical features. The question arises whether these are patients with Huntington's disease with an extremely low repeat size. In the literature eight patients with CAG sizes within the range of 30 to 37 repeats have been described with clinical features.⁸⁻²⁹⁻³⁰⁻³¹ Hanning *et al*²³ found repeat lengths between 30–39 repeats in 41 out of 2592 patients (1.6%). Spector *et al*³⁰ found five out of 181 patients (2.8%) to have repeats in this range. In both studies the number of patients with repeat lengths between 30 and 36 repeats was not described.

The intermediate sized alleles could represent the tail of the normal range. Extrapolating the correlation of the age at onset and the CAG repeat length,³² repeat lengths in this range would be associated with an extremely late age at onset. However, Andrew *et al*³² determined at the lower end of the range of CAG repeat lengths very broad confidence limits for age at onset prediction. Thus, CAG repeat lengths between 30 and 36 repeats could give a disease phenotype given a long enough survival.

This study again illustrates the contribution of determination of CAG repeat length to the diagnosis of Huntington's disease in patients with a suspect or negative family history as well as in patients with a positive family history. Despite verification and extension of the family history through the Leiden roster more than one third of the patients could not be linked to a known family. Because prevalence studies traditionally have been based on these types of criteria, the number of patients with an expanded repeat and a negative, suspect, or unknown family history implies an underestimate of the prevalence of Huntington's disease in the community. Because not all patients with definite clinical Huntington's disease and a positive family history have applied for CAG repeat determination an estimate of the actual prevalence can only be made by extrapolation

of the percentage of patients with Huntington's disease with positive family history applied by the department of neurology of the Leiden University Medical Centre. Sixty four patients were applied for CAG repeat determination by the department of neurology of the Leiden University Medical Centre, of whom 35 patients had a positive family history and expanded CAG repeat.

During the period of this study the number of patients with Huntington's disease with a known family history in Leiden was 159. Thus 22% of these patients with a positive family history study have been sent for determination of the CAG repeat. Under the assumption that all referring physicians sent the same percentage of their patients with Huntington's disease with a known family history for CAG repeat determination the 77 patients with a positive family history and an expanded repeat of this study should be 22% of the total, which is 350 patients.

Assuming that all 49 patients with Huntington's disease (with an expanded CAG repeat) with a suspect, negative, or unknown family history have been applied for CAG repeat determination the prevalence increases with 14% (49/350). Subtle aspects of the clinical examination, knowledge about the family history, and the physician's experience with Huntington's disease all influence the need to obtain genetic analysis to confirm or exclude the disease.

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