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Huntington's disease phenocopies are clinically and genetically heterogeneous

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Abstract
Purpose
Huntington's disease (HD) classically presents with movement disorder, cognitive dysfunction and behavioural problems but is phenotypically variable. 1% of patients with HD-like symptoms lack the causative mutation and are considered HD phenocopies. Genetic diseases known to cause HD phenocopies include HD-like syndromes HDL1, HDL2 and HDL4 (SCA17). HD has phenotypic overlap with dentatorubral-pallidoluysian atrophy, the spinocerebellar ataxias and neuroferritinopathy. Identifying the genetic basis of HD phenocopies is important for diagnosis and may inform the search for HD genetic modifiers. We sought to identify neurogenetic diagnoses in the largest reported cohort of HD phenocopy patients.
Methods
285 patients with syndromes consistent with HD, who were HD expansion-negative, were screened for mutations in *PRNP, JPH3, TBP, DRPLA, SCA1, SCA2, SCA3, FTL* and *FRDA*.
Results
Genetic diagnoses were made in 8 subjects: we identified 5 cases of HDL4, 1 of HDL1 and 1 of HDL2. One patient had Friedreich's ataxia. There were no cases of DRPLA, SCA1, SCA2, SCA3 or neuroferritinopathy.
Conclusions
HD phenocopies are clinically and genetically diverse and a definitive genetic diagnosis is currently possible in only a minority of cases. When undertaken, it should be clinically directed and patients and clinicians should be prepared for the low probability of reaching a genetic diagnosis in this group of patients.
Key words
Huntington's disease (HD), phenocopies, SCA17, familial prion disease, HDL1, HDL2

Introduction

Huntington's disease (HD) is a fatal neurodegenerative disorder with autosomal dominant inheritance that typically presents with a triad of movement disorder, cognitive dysfunction and behavioural problems, but the disease can arise in the absence of a family history and its clinical presentation and course are highly variable.[1] HD is caused by a CAG triplet expansion in the IT-15 gene encoding huntingtin.[2]

Approximately 1% of subjects with symptoms and signs suggestive of HD do not have the HD expansion.[3, 4] Such patients, referred to as HD phenocopies, present a diagnostic challenge. HD phenocopies are known to be produced by several neurogenetic syndromes[5]. These include the HD-like syndromes HDL1 (caused by an octapeptide repeat insertion in *PRNP*)[6] and HDL2 (caused by mutations in *JPH3*).[7] There is also clinical overlap between HD and dentatorubral-pallidoluysian atrophy (DRPLA)[8] and the autosomal dominant spinocerebellar ataxias including SCA1, SCA2, SCA3, SCA12 and SCA17 (HDL4).[9] Friedreich's ataxia has not previously been described as an HD phenocopy but is known to cause chorea.[10] Neuroferritinopathy, caused by mutations in the ferritin light chain gene (*FTL*), is an autosomal dominant basal ganglia disease with features similar to those of HD.[11]

Identifying the genetic basis of HD phenocopies is clinically important in the differential diagnosis of such patients, and has the potential to inform the search for genetic modifiers and intervention strategies for HD.

From samples referred for HD genetic testing, we identified 285 patients with syndromes consistent with a diagnosis of HD, in whom the HD expansion was not detected. DNA samples were tested for the mutations causing HDL1, HDL2, DRPLA, SCA1, SCA2, SCA3, SCA17, neuroferritinopathy and FA. We present the outcome of these genetic tests and descriptions of the patients in whom genetic diagnoses were reached, as well as a brief review of the literature in HD phenocopies.

Methods

DNA was analysed from 285 consecutive patients with HD phenocopy syndromes whose samples were referred for genetic testing to the Clinical Neurogenetics service of the National Hospital for Neurology and Neurosurgery, London. The service receives referrals from neurology and neurogenetics clinics across the United Kingdom, with a particular focus in London and south-east UK. The majority of referrals (206 of 285) were from experienced consultant neurologists and neurogeneticists within the National Hospital for Neurology and Neurosurgery, which also has a nationwide catchment with a focus on London and the south-east UK.

Subjects were defined as HD phenocopies on the basis of a clinical presentation consistent with HD when assessed by a consultant neurologist or neurogeneticist and a negative test for the pathogenic CAG repeat expansion in the gene encoding huntingtin. Clinical information was obtained from the abstract accompanying the referral, from hospital records and by direct communication with referring clinicians.

An autosomal dominant family history was not an absolute requirement because HD may arise *de novo* because of CAG repeat expansion, or appear to do so where the family history is unavailable, incomplete or inaccurate.

The choice of genetic tests to which samples were subjected was determined by those conditions previously screened for in HD phenocopy cohort studies[12-15] and newly-described genetic causes of HD phenocopies (i.e. neuroferritinopathy). Because the cohort was drawn from patients whose DNA had been sent for genetic analysis following clinical assessment by experienced neurologists, we did not test for conditions that can readily be detected by clinical tests likely to have been performed already, such as neuroacanthocytosis (usually

excluded by examination of the blood film for acanthocytes[16]), Wilson's disease (which causes biochemical and MRI abnormalities[17]) or PANK-2 (which causes characteristic MRI scan abnormalities[18]). Acanthocyte-negative cases of neuroacanthocytosis have been reported,[19, 20] and it is therefore theoretically possible, though unlikely, that a *VPS13A* mutation may have been missed by our genetic tests. Benign hereditary chorea was not tested for because, though it is an hereditary cause of chorea, it is not known to cause cognitive impairment or psychiatric features.[21, 22]

DNA was extracted using standard techniques using an NA3000 automated DNA extractor (AutoGen, MA). *JPH3* (HDL2), *TBP* (SCA17/HDL4), *DRPLA*, *ATXN1* (SCA1), *ATXN2* (SCA2) and *ATXN3* (SCA3) were analysed by fluorescent PCR amplifying the triplet repeat region followed by size fractionation and fragment sizing using an Applied Biosystems 3730XL genetic analyser and GeneMapper software (Applied Biosystems, CA). *FTL* exons 3 and 4 (neuroferritinopathy) was analysed by sequence analysis and SeqScape software (Applied Biosystems). The *PRNP* octapeptide region was amplified by PCR, followed by electrophoresis on ethidium bromide stained agarose gels and viewed using a Gel Doc 1000 transilluminator using Quantity One 4.5.1 software (both from Bio-Rad, CA). 1-OPRD and 6-OPRI controls were run on each gel. Following the independent diagnosis of one patient in the cohort with Friedreich's ataxia, the entire cohort was screened for *FRDA* expansions as described above.

Results
285 subjects met the criteria for HD phenocopy syndromes. Of these, 60 (21.1%) had a family history consistent with autosomal dominant inheritance. Positive genetic diagnoses were made in 8 subjects (2.8%). The clinical history and salient features of each diagnosed subject are described below and summarised in Table 2. Five subjects (1.8%) were found to have expansions in TBP causing SCA-17; one subject (0.4%) had a 6-octapeptide insertion in the *PRNP* gene consistent with familial prion disease; one subject was found to have HDL2 caused by a pathogenic expansion in *JPH3*; and one subject was subsequently diagnosed with Friedreich's ataxia with a homozygous expansion in *FXN*. No cases of DRPLA, SCA1, SCA2, SCA3 or neuroferritinopathy were identified in this cohort. Screening the entire cohort for *FRDA* mutations revealed 4 heterozygous expansion carriers (consistent with the population frequency of 1 in 70) and no further homozygotes. Clinical features of the 8 genetically-diagnosed patients are summarised in Table 1 and full clinical case descriptions are available as supplementary data.

Discussion
In this series of 285 patients with HD phenocopy syndromes—the largest investigated to date—8 patients (2.8%) were identified with genetic mutations. SCA17 was the most commonly identified disorder (5 cases) with HDL2, familial prion disease and Friedreich's ataxia also identified (1 case each).

The commonest neurogenetic diagnosis in this cohort was SCA17. This causes intellectual deterioration, cerebellar ataxia, epilepsy and chorea, in 100, 90, 50 and 20% of patients respectively.[12] All 5 SCA17 patients we report here presented with chorea and falls and developed ataxia and cognitive impairment, but cerebellar atrophy was not a universal feature on brain imaging. Thus, HD phenocopy patients represent an atypical subset of SCA17 cases. Within a given pedigree, SCA17 HD phenocopy syndromes are relatively phenotypically homogeneous.[23] SCA17 is caused by CAG-CAA expansions in the TATA-box binding protein which is an important transcription initiation factor.[24] This is of relevance to HD where transcriptional dysregulation occurs early in disease pathogenesis.[25]
As well as being the largest HD phenocopy cohort studied to date, this study is the first time Friedreich's ataxia (FA) has been described as an HD phenocopy. Unlike HD, FA has autosomal recessive inheritance, but a family history is not seen in all cases of HD because repeat length instability can cause HD to arise *de novo*, or a parent who would have become

symptomatic may die from other causes before onset.[1] HD can produce cerebellar ataxia and atrophy[26, 27] and chorea has been reported as a rare manifestation of FA[10]; but the unusual combination of chorea, eye movement restriction and cognitive impairment with the more classical findings of ataxia and sensory impairment has not previously been described in FA. Patient 7 had GAA repeat expansions in the *FRDA* gene of 850 and 76 repeats. Typically, FA patients have alleles of 600-1200 but the smallest reported disease-causing allele, which resulted in a typical FA phenotype, is 66 repeats.[28] It is not known why a subset of FA patients develop chorea.[10]

Familial prion disease is a rare cause of HD phenocopy syndromes. HDL1 is caused by an 8-octapeptide insertion in the *PRNP* gene. All cases reported to date have had an eight-octapeptide (192-nucleotide) insertion. Patient 6, who was felt to have presentation consistent with ataxic/rigid HD, was found to have a 6-octapeptide insertion (6-OPRI) in *PRNP* and was subsequently established to be a member of the largest known 6-OPRI pedigree.[29] Even within a single pedigree, 6-OPRI mutations produce a variety of phenotypes including behavioural change, dementia, myoclonus, seizures, chorea and pyramidal, cerebellar and extrapyramidal signs.[29]

HDL2, caused by CTG/CAG repeat expansions in *JPH3*, the gene encoding junctophilin-3, predominantly affects patients of African ancestry.[15] It has not been described in Caucasian or Japanese subjects and, to our knowledge, patient 8 is the first report of a person of middle-eastern ancestry.

This is also the first large cohort tested for mutations in *FTL* causing neuroferritinopathy. Autosomal dominantly inherited and causing chorea and cognitive impairment, it shares several features of HD. The absence of causative mutations from this and previous cohorts may be explained by the condition's rarity and its association with relatively specific abnormal imaging findings.[30]

Prior series of HD phenocopy patients from France, Portugal and Yugoslavia, tested for multiple genetic phenocopy syndromes, have reported varying rates of positive genetic diagnosis, between nil and 1.6%.[12-14] In the largest previous series of 252 subjects, 2 cases each of HDL2 and SCA17 were identified. This cohort was not screened for mutations causing prion disease or neuroferritinopathy. Pooling the findings of our analysis with the three previous cohorts, the overall genetic discovery rate in 665 cases is 1.8%. Across cohorts, SCA17 represents 1.1% of HD phenocopy syndromes, and HDL1 and Friedreich's ataxia each 0.2%. The frequency of HDL2 in HD phenocopy cases was examined in a large (582-patient) and clinically heterogeneous cohort from the USA and Japan.[15] Pooling that series with our findings and the other European series, the frequency of HDL2 in the 1247 cases reported is 0.7%.

Our findings suggest principally that currently available genetic testing is unlikely to yield a diagnosis in HD-like patients where genetic testing for HD is negative. Detailed clinical assessment combined with directed clinical testing (e.g. blood films, biochemical tests and expertly interpreted MRI imaging) should therefore be the first resort in the assessment of such patients. Our findings do suggest a possible clinically-directed approach to genetic diagnosis in HD phenocopy syndromes but patients and clinicians should be prepared for the low probability that a diagnosis will be reached, and genetic testing should not be embarked upon blindly. SCA17 is a close clinical phenocopy and may be screened for in all HD phenocopy cases. Familial prion disease causes such a wide spectrum of motor abnormalities that mutations may be sought in HD phenocopies with cognitive decline. Friedreich's ataxia may be suspected when ataxia or pyramidal signs are present and the inheritance is not clearly autosomal dominant. Junctophilin mutations may be sought in non-caucasian patients with HD phenocopy syndromes. The possibility of neuroferritinopathy,

DRPLA and the other spinocerebellar ataxias should also be considered, although none of these have been found in any series of HD phenocopies described to date.

In our cohort, as with other HD phenocopy populations,[12-15] genetic diagnosis is possible in only a small minority of cases, suggesting that as-yet undescribed conditions account for the majority of HD phenocopies. We are currently assessing the patients from this cohort with autosomal dominant inheritance to try and identify novel HD phenocopy genes.

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Acknowledgements

Tables

Table 1 Clinical characteristics of subjects successfully diagnosed by genetic analysis in this 285-patient

HD phenocopy cohort.

	Patient							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sex	M	F	F	F	F	F	M	F
Diagnosis	SCA17	SCA17	SCA17	SCA17	SCA17	Prion	FA	HDL2
Age at onset	37	45	45	41	40	32	53	41
Family History								
Autosomal dominant	●	●	●			●		
None				●	●		●	●
History								
Irritability	●	●	●					●
Aggression	●	+	+					●
Depression		+						●
Cognitive decline	+	+	●	●	+	+	●	●
Falls		●		●	●	+	●	●
Gait disturbance		●	●	+	●	●	●	
Coordination problems				+	●	+	●	
Dysarthria		+	+	+	+	+	+	
Gait								
Postural instability		+			+			
Broad-based gait	+	+	+				●	
Tandem gait impairment	+	+	+	+	+	+	●	
Freezing		+	+					
Eye movements								
Saccadic pursuits	+				+	+		
Delayed saccade initiation	+	+	+	+				
Slowed saccades					+			
Nystagmus						+		
Gaze restriction					+		+	
Speech / swallow								
Dysarthria	+	●	+	+	+	+	●	
Dysphagia					+		+	
Limbs								
Limb ataxia	+		+	+		+	●	
Dystonia					+			+
Chorea	●	+	●	●	●		●	+
Myoclonus		+						
Bradykinesia	+	+	+		+	+		
Brain imaging								
Global cortical atrophy	+	+			+			+
Putaminal enhancement				+				
Cerebellar volume loss	+	+		+		+		

●, present at onset; +, present subsequently.