Published Ahead of Print on July 8, 2016 as 10.1212/WNL.0000000000002944

Clinical manifestations of intermediate allele carriers in Huntington disease

Esther Cubo, MD, PhD
María A. Ramos-Arroyo, MD, PhD
Saul Martinez-Horta, PhD
Asunción Martínez-Descalls, PhD
Sara Calvo, PhD
Cecilia Gil-Polo, MD, PhD
On behalf of the European HD Network

Correspondence to Dr. Cubo: mcubo@saludcastillayleon.es

ABSTRACT

Objective: There is controversy about the clinical consequences of intermediate alleles (IAs) in Huntington disease (HD). The main objective of this study was to establish the clinical manifestations of IA carriers for a prospective, international, European HD registry.

Methods: We assessed a cohort of participants at risk with <36 CAG repeats of the huntingtin (*HTT*) gene. Outcome measures were the Unified Huntington's Disease Rating Scale (UHDRS) motor, cognitive, and behavior domains, Total Functional Capacity (TFC), and quality of life (Short Form–36 [SF-36]). This cohort was subdivided into IA carriers (27–35 CAG) and controls (<27 CAG) and younger vs older participants. IA carriers and controls were compared for sociodemographic, environmental, and outcome measures. We used regression analysis to estimate the association of age and CAG repeats on the UHDRS scores.

Results: Of 12,190 participants, 657 (5.38%) with <36 CAG repeats were identified: 76 IA carriers (11.56%) and 581 controls (88.44%). After correcting for multiple comparisons, at baseline, we found no significant differences between IA carriers and controls for total UHDRS motor, SF-36, behavioral, cognitive, or TFC scores. However, older participants with IAs had higher chorea scores compared to controls ($p = 0.001$). Linear regression analysis showed that aging was the most contributing factor to increased UHDRS motor scores ($p = 0.002$). On the other hand, 1-year follow-up data analysis showed IA carriers had greater cognitive decline compared to controls ($p = 0.002$).

Conclusions: Although aging worsened the UHDRS scores independently of the genetic status, IAs might confer a late-onset abnormal motor and cognitive phenotype. These results might have important implications for genetic counseling.

ClinicalTrials.gov identifier: NCT01590589. *Neurology*® 2016;87:1–8

GLOSSARY

COHORT = Cooperative Huntington's Observational Research Trial; **DCL** = diagnostic confidence level; **EHDN** = European Huntington's Disease Network; **HD** = Huntington disease; **HRQoL** = health-related quality of life; **IA** = intermediate allele; **PBA** = Problem Behavior Assessment–short form; **PHAROS** = Prospective Huntington At-Risk Observational Study; **SF-36** = Short Form–36 Health Survey; **TFC** = total functional capacity; **UHDRS** = Unified Huntington's Disease Rating Scale.

Huntington disease (HD) is an incurable, autosomal dominant, neurodegenerative disease caused by the expansion of the CAG trinucleotide repeat of the huntingtin (*HTT*) gene on the short arm of chromosome 4.[1] Patients with manifest HD have a CAG 36 repeats or more, although alleles sized 36–39 CAG repeats might display reduced penetrance, resulting in a later age at onset and slower disease progression.[2,3] After the characterization of the gene mutation, a distinct category of HD genes, named intermediate alleles (IAs), were recognized.[3,4] IAs have been consensually defined as those with a CAG repeat size between 27 and 35, a range just below the disease threshold of 36 repeats.[4–6] It has been shown that IAs confer genetic instability and might broaden into the disease range within one generation through the paternal line and, exceptionally, the maternal line.[5,7] The prevalence of IAs varies from 1.5% to 5.8% in both the

Editorial, page XXX

Supplemental data at Neurology.org

From the Neurology Department (E.C., C.G.-P.) and Research Unit (S.C.), Hospital Universitario Burgos; Genetic Department (M.A.R.-A.), Complejo Hospitalario de Navarra, Pamplona; Movement Disorders Unit, Neurology Department (S.M.-H.), Hospital de la Santa Creu i Sant Pau, Barcelona; and Neurology Department (A.M.-D.), Fundación Jimenez Diez, Madrid, Spain.

Coinvestigators are listed on the *Neurology*® Web site at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

general population and HD families, showing no significant differences between them, and with similar haplotype distributions.[6]

At present, IAs are not considered associated with the HD phenotype. However, there has been emerging evidence that some individuals with IAs might develop HD-like clinical and neuropathologic manifestations.[8,9] In this regard, mild clinical signs in patients with IAs have also been found in other diseases such as spinocerebellar ataxia type 2 and fragile X syndrome.[10,11] Therefore, there is still controversy about the clinical consequences of IAs in HD and subsequent genetic counseling implications. Because the majority of the clinical reports focusing on to the clinical phenotypes of IAs in HD are cross-sectional studies, there is a need to include prospective data looking at the progression of symptoms over time. Our aim was to characterize the clinical manifestations of IAs, their association with sociodemographic and environmental factors, and the clinical progression in a large cohort as compared to participants carrying <27 CAG repeats.

METHODS

Design. This was an international, retrospective-longitudinal, case-control study.

Standard protocol approvals, registrations, and patient consents. Clinical and sociodemographic data were obtained from patients enrolled in the European Huntington's Disease Registry Database (European Huntington's Disease Network [EHDN]). This registry is a large, prospective study observing the natural course, clinical spectrum, and management of HD in 140 centers from 17 European countries and 3 other countries.[12,13] For this observational study, participants provided written informed consent following the International Conference on Harmonization–Good Clinical Practice guidelines.[14] For participants who lacked capacity to consent, study sites followed country-specific guidelines for signing consent forms. Minors agreed with both parents authorizing for them. Ethical approval was collected from the local ethics committee for each study site contributing to the EHDN Registry (e-Methods on the *Neurology*® Web site at Neurology.org).[12,13]

Participants and clinical assessments. Data from the European Huntington's Disease Registry database from March 1998 to January 2014 with a larger allele of <36 CAG repeats within the huntingtin gene were included in this study. For this particular study, participants with significant comorbidity, including Parkinson disease, cranial trauma, tic disorders, stroke, or any neurologic condition with subsequent motor impairment, were excluded. Data collection adhered to a standard protocol including electronic case report forms and used identical study protocols of assessment and sampling of biomaterials. At each center, participants were evaluated by neurologists with longstanding experience in HD, and a detailed family and personal history was taken. The diagnostic confidence level (DCL) for HD was classified as normal (DCL = 0), nonspecific motor impairment (DCL = 1), motor impairment that may be sign of HD (DCL = 2), motor impairment that is likely sign of HD (DCL = 3), and motor impairment that is unequivocal sign of HD (DCL = 4).[15]

Motor and psychiatric signs were scored using the Unified Huntington's Disease Rating Scale (UHDRS).[16] Additionally, behavior was assessed by the Problem Behavior Assessment–short form (PBA).[17] For motor and psychiatric UHDRS scores and PBA, higher scores indicated worse motor and psychiatric impairment. For cognition, we used the cognitive UHDRS composite score (UHDRS total correct for letter fluency, symbol digit modalities test, and Stroop subscores for word reading, color, naming, and interference), with lower scores indicating lower performance.[13] Disease stage was obtained from total functional capacity (TFC) scores, with higher scores indicating better functional status.[18] Health-related quality of life (HRQoL) was assessed using the Short Form–36 Health Survey (SF-36), with higher scores indicating higher quality of life.[19]

Patients were followed up on a yearly basis according to the EHDN Registry protocol.[12,13] Study site raters were annually trained, evaluated, and certified to lessen interrater and intrarater variability. Data entry was reviewed online and on-site by monitors fluent in the language of the study site.[12,13] Data monitoring adhered to the principles laid out in the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use–Good Clinical Practice. The *HTT* CAG genotyping was performed at each local genetic laboratory (see e-Methods for further detail).

Data management. At-risk participants with 27–35 and <27 CAG repeats were defined as IA carriers and controls, respectively, as counseled by the American College of Medical Genetics.[4] Based on previous studies, IAs were further classified as high IAs (34–35 CAG repeats) and moderate IAs (27–33 CAG repeats).[20] Individuals younger than 60 years were classified as younger participants, and those aged ≥60 years as older participants. Demographics, CAG repeat numbers, and clinical information including motor, cognitive, and behavior UHDRS subscale scores, PBA, SF-36, TFC at baseline and follow-up, and pharmacologic treatments were collected from the EHDN database. With the UHDRS motor subscale, different domain subscores were calculated: the oculomotor domain (sum of the ocular pursuit, saccade initiation, and saccade velocity), chorea (sum of the chorea items [face, buccolingual, upper and lower extremities]), dystonia (sum of the trunk and upper and lower extremities scores), bradykinesia (sum of the finger taps, pronate/supinate, rigidity of each extremity, and body bradykinesia scores), and gait impairment (sum of gait, tandem, and retropulsion scores). To deal with missing values, case-wise deletions were adopted.

Statistical analysis. Analysis was performed using IBM SPSS 19 (Chicago, IL) software. Normal distribution of the variables was analyzed using the Kolmogorov-Smirnov test. Descriptive analysis of the participants' characteristics was performed in terms of frequencies (percentage), mean/median values with the corresponding SD or interquartile range, as appropriate, and 95% confidence intervals. Differences were compared using nonparametric tests (Mann-Whitney and Kruskal-Wallis tests) and parametric tests (Student *t* test). The χ^2 and Phi and Cramer tests were used to analyze categorical variables. A significance level of $\alpha = 0.002$, 2-sided tests was applied after post hoc Bonferroni multiple comparisons adjustments. Correlations were calculated using the Pearson (parametric) and Spearman (nonparametric) correlation coefficients. Linear regression analysis was used to estimate the association of age and CAG repeats with UHDRS scores. Longitudinal motor, cognitive, and behavioral scores of the UHDRS were compared between IA carriers and age- and sex-paired controls.

Table 1 Clinical characteristics comparison

	27–35 CAG repeats (n = 76)	<27 CAG repeats (n = 581)	p Value
Age, y	45.6 (15.6)	40.8 (13.3)	0.004
No.	76	581	
Female	44 (57.9)	358 (61.6)	0.53
No.	76	581	
Education, y	12.0 (10.7–17.2)	14.0 (11.0–17.0)	0.24
No.	38	363	
Body mass index	24.7 (22.2–27.6)	24.7 (21.9–27.9)	0.78
No.	73	565	
Currently working	20 (52.6)	242 (66.7)	0.08
No.	38	363	
Married and partnership	50 (68.4)	362 (63.2)	0.65
No.	73	572	
Toxics			
Nonsmokers	28 (37.3)	240 (41.5)	0.83
Non-alcohol users	51 (68.0)	431 (74.6)	0.43
No.	75	578	
Rural residence	15 (20.5)	70 (12.2)	0.04
No.	73	573	
TFC score <11	6 (9.1)	21 (3.9)	0.10
No.	66	535	
UHDRS, motor scores			
Total motor	0 (0–5)	0 (0–1)	0.01
No.	70	561	
UHDRS motor domains score ≥1			
Gait	10 (14.2)	50 (8.9)	0.13
Chorea	6 (8.6)	9 (1.6)	0.003
Dystonia	2 (2.9)	9 (1.6)	0.34
Bradykinesia	22 (31.4)	124 (22.1)	0.07
Ocular movements	15 (21.4)	52 (9.2)	0.003
No.	70	561	
UHDRS, behavior	6.0 (0–15)	4.0 (0–11.0)	0.28
No.	43	338	
PBA total	4.5 (0–17.2)	2.0 (0–12.0)	0.53
Depression	1.5 (0–10.2)	1.0 (0–6.0)	0.66
Irritability/aggression	0 (0–1.2)	0 (0–2.0)	0.36
Psychosis	0 (0–0)	0 (0–0)	0.43
Apathy	0 (0–1.2)	0 (0–0)	0.07
Executive function	0 (0–1.2)	0 (0–0)	0.42
No.	26	181	
UHDRS, cognition	313.5 (242.7–347.0)	314.0 (275.0–346.7)	0.64
No.	54	440	
SF-36	129.9 (111.3–143.8)	137.4 (125.6–145.4)	0.04
No.	28	207	

Continued

RESULTS Of 12,190 participants (as of January 2014) included in the EHDN registry, a total of 691 (5.6%) participants with <36 CAG repeats were identified in this study. Thirty-nine participants (0.03%) were excluded due to significant comorbidity likely associated with motor or cognitive impairment according to the investigators' criteria, and 657 (5.3%) were finally included, 402 women (61.2%) and 255 men (38.8%), with a mean age of 41.3 ± 13.7 years (range 18–91 years). Of these, 76 (11.6%) had 27–35 CAG repeats, and 581 (88.4%) had <27 CAG repeats in the larger allele. Instead, CAG repeats in the smaller allele ranged from 9 to 25, except for 1 participant who carried 2 intermediate alleles of 29 and 31 CAG repeats. Among IA carriers, 13 (17.1%) had high IAs, and 63 (82.9%) moderate to high IAs. In terms of data quality, UHDRS motor information was available in 631 (96.0%) participants, UHDRS composite cognitive scores in 494 (75.1%), UHDRS behavior in 381 (60.0%), SF-36 in 235 (35.7%), and PBA in 207 (31.5%). A total of 405 participants (61.6%) had baseline data only, 129 participants (19.6%) had a follow-up of 1 year, and 123 (18.7%) had a follow-up of ≥2 years. There were no differences in terms of parental HD inheritance between IA carriers and controls (affected father with HD, IA carriers: 27 [35.5%] vs controls: 215 [37.0%], $p = 1.00$; affected mother with HD, IA carriers: 30 [39.4%] vs controls: 272 [46.8%], $p = 0.26$; and unknown: IA carriers 19 [25.0%] vs controls: 94 [16.7%], $p = 0.10$).

At baseline, IA participants were similar to controls in terms of age, HRQoL, PBA, TFC, UHDRS motor, behavior, and cognitive scores, use of antidopaminergic drugs, body mass index, education background, tobacco and alcohol exposure, residence, and marital and working status (table 1).

In the group of older participants, the UHDRS motor score was correlated with age ($r_s = 0.83$, $p = 0.003$) in the IA group only. In both groups, IA carriers and controls, the UHDRS composite cognitive scores were moderately correlated with the number of years of education ($r_s = 0.49$, $p = 0.005$ [IA group], $r_s = 0.36$, $p = 0.001$ [controls]), and inversely with age ($r_s = -0.35$, $p = 0.008$ [IA group], $r_s = -0.16$, $p = 0.001$ [controls]). On the contrary, UHDRS behavior and total PBA scores were not significantly correlated with age in either group.

In terms of motor phenotype, the UHDRS motor scores were not correlated with the CAG repeat size in the smaller allele ($p = 0.45$) in either group. Compared to younger participants, in both groups, older participants had higher UHDRS motor scores ($p < 0.0001$) and higher frequency of participants with a UHDRS gait, chorea, and bradykinesia score ≥1 (table 2, figure). However, within the group of older

Table 1 Continued

	27–35 CAG repeats (n = 76)	<27 CAG repeats (n = 581)	p Value
Treatments			
Antidepressants and anxiolytics	16 (37.2)	88 (31.2)	0.18
Antidopaminergics	5 (11.6)	12 (4.2)	0.05
No.	43	282	

Abbreviations: PBA = Problem Behavior Assessment–short form; SF-36 = Short Form–36 Health Survey; TFC = total functional capacity; UHDRS = Unified Huntington's Disease Rating Scale.
Data are mean (SD), median (interquartile range), or n (%) unless otherwise indicated.

participants, compared to controls, IA carriers had higher scores in the chorea item of the motor UHDRS, and similar motor, cognitive, and behavior UHDRS scores. The same pattern was seen in the group of high IAs compared to moderate IAs and controls (table 3). IA carriers were more frequently diagnosed with DCL for HD ≥2 compared to controls (10 [14.3%] vs 9 [1.6%], $p < 0.0001$).

In the linear regression analysis performed on the group of older participants, using the logarithm of the UHDRS motor score as the dependent variable and aging and CAG repeats as the dependent variables, aging was the most contributing factor to increased UHDRS motor scores (a 5.8% increase in the motor UHDRS score per 1 additional year of life) (table 4). This model was insufficient for precise information of the younger participants' group. There were no linear associations with the total UHDRS composite cognitive or behavior scores with age or CAG repeats.

One-year follow-up data analysis was available in 25 IA participants and 25 paired sex-age controls for UHDRS motor, and 18 IA participants and 39 paired age-sex controls for UHDRS cognitive domain. IA carriers and control participants were similar in terms of motor UHDRS score change ($p = 0.63$), but individuals with IAs had greater cognitive decline compared to controls (median follow-up minus baseline UHDRS composite scores of −10.7 [−19.8 to 8.3] in the IAs group vs 16.0 [3.0–29.0] in the control group [$p = 0.002$]). Fewer than 10 IA participants had UHDRS behavior and HRQoL follow-up data, and longitudinal analyses were not performed.

DISCUSSION In this longitudinal analysis of European HD, older participants, either IA carriers or controls, were frequently found to have more subtle motor and cognitive abnormalities using the UHDRS compared to younger participants. This finding suggests that aging might worsen the UHDRS scores independently of the genetic status. However, within the group of older participants, mild chorea was more frequently found in the IA group compared to controls, especially in the high IA participants. Supporting this finding, based on the raters' perspective, IA participants were receiving at least a diagnosis of motor abnormalities that may be signs of HD more frequently compared to controls, suggesting that these motor abnormalities were clinically meaningful. Environmental factors were not significantly associated with IA carriers' clinical manifestations. Interestingly, IA participants had faster cognitive decline compared to controls, in contrast to motor manifestations, which remained stable at least for 1 year. However, the small number of IA participants, especially in the group of older participants, limits the interpretation of these findings and further studies are needed.

Our results are in agreement with another observational study, the Cooperative Huntington's Observational Research Trial (COHORT), which also found statistically significant dissimilarities in some UHDRS motor and cognitive scores between the normal and the IA group.[21] The Prospective Huntington At-Risk Observational Study (PHAROS), however, found that IA carriers did not deviate from the controls in their motor and cognitive evaluations.[22] In contrast to a previous study,[23] we observed that the smaller allele CAG repeats were not associated with the UHDRS motor scores. Although not statistically significant, lower quality of life and higher apathy were found in the IA population compared to controls, in agreement with the COHORT and PHAROS studies.[21,22] Whether these findings reflect a characteristic abnormality in participants with IAs, or a tendency that is potentiated by aging, is confusing.[21]

If the prevalence of IAs is relatively high in the general population, why are the clinical manifestations of IAs reported so infrequently? There are several possibilities: first, because older individuals and IA carriers are frequently underrepresented in HD cohort studies; second, because, besides mild chorea, other clinical features such as cognitive impairment are not specific for HD, and therefore attributed to other causes, especially aging; third, because the differences between the control and the IA group may have occurred as a result of an error in measurement.[21] In this regard, the UHDRS was originally developed to assess patients with manifest HD, and it is considered the reference standard in HD clinical trials due to high degree of internal consistency and sensitivity for tracking HD changes over time.[16] However, use of the UHDRS in normal, IA, and aged populations is not well-delimited[21]; fourth, because of the presence of HD phenocopies.[21] In this regard, the likelihood of ascertaining IAs with a HD phenocopy or HD-like disorder within the context of family members with HD may be controversial[3]; finally, because of the possibility of a laboratory error, particularly when interpreting alleles bordering on different penetrance classes.[21] In this regard, the European HD Registry has found that clinically significant differences occurred in 4.0% of

Table 2 Baseline motor, cognitive, and behavior UHDRS scores stratified by age

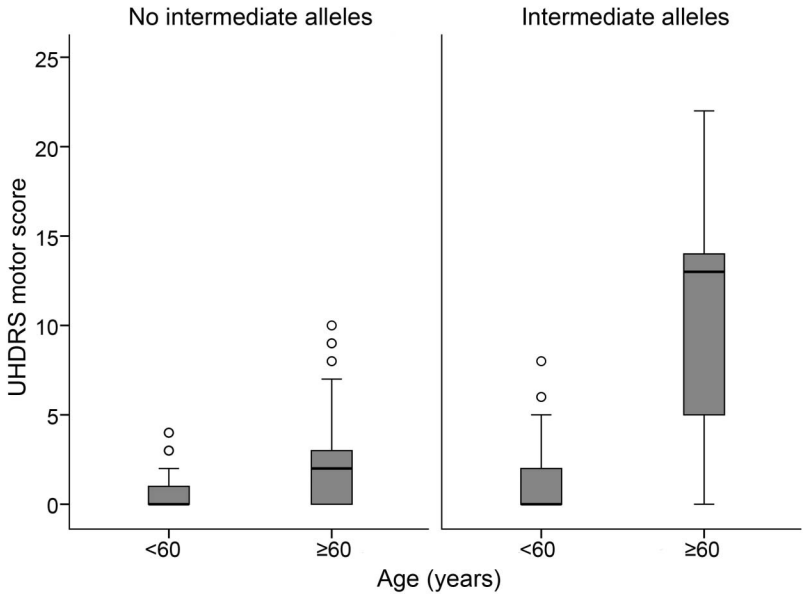
	27–35 CAG repeats	<27 CAG repeats	*p* Value
Older participants (≥60 y)			
Total UHDRS motor			
Median (IQR)	13.0 (3.7–15.0)	2.0 (0–3.0)	0.004
Sample	10	46	
UHDRS-chorea			
Participants with score ≥1 (%)	4 (40)	0	0.001
UHDRS-bradykinesia			
Participants with score ≥1 (%)	5 (50)	20 (43.5)	0.73
UHDRS-dystonia			
Participants with score ≥1 (%)	1 (10)	1 (2.2)	0.32
UHDRS-gait			
Participants with score ≥1 (%)	4 (40)	8 (19)	0.21
UHDRS-ocular movements			
Participants with score ≥1 (%)	6 (50)	9 (17.6)	0.01
UHDRS-cognition			
Median (IQR)	198.0 (87.0–252.5)	256.5 (164.7–312.5)	0.05
Sample	9	36	
UHDRS-behavior			
Median (IQR)	16.0 (12.0)	1.0 (0–5.0)	0.01
Sample	3	25	
Younger participants (<60 y)			
Total UHDRS motor			
Median (IQR)	0 (0–2.0)	0 (0–1.0)	0.17
Sample	60	515	
UHDRS-chorea			
Participants with score ≥1 (%)	2 (3.2)	9 (1.7)	0.31
UHDRS-bradykinesia			
Participants with score ≥1 (%)	17 (28.3)	104 (20.0)	0.13
UHDRS-dystonia			
Participants with score ≥1 (%)	1 (1.7)	8 (1.5)	1.00
UHDRS-gait			
Participants with score ≥1 (%)	6 (10.5)	42 (8.4)	0.61
UHDRS-ocular movements			
Participants with score ≥1 (%)	9 (14.3)	43 (8.1)	0.12
UHDRS-cognition			
Median (IQR)	325.0 (281.0–354.5)	317.0 (279.0–348.0)	0.42
Sample	45	404	
UHDRS-behavior			
Median (IQR)	4.5 (0–15.0)	4.0 (0–11.0)	0.66
Sample	40	313	

Abbreviations: IQR = interquartile range; UHDRS = Unified Huntington's Disease Rating Scale.

cases with a potential unapparent misdiagnosis rate of 0.3% out of 1,326 duplicate result analysis, within acceptable measurement errors proposed by the American College of Medical Genetics.[4,24,25]

The results of this study support that IAs could produce a mild phenotype in some participants. In this regard, higher chorea were more frequently observed in the group of high IAs, suggesting a continuum in terms

Figure Unified Huntington's Disease Rating Scale (UHDRS) motor comparison



of clinical manifestations associated with CAG repeats in HD. Our study also highlights the possibility of modifiers that affect the penetrance of these alleles.[3,26] Aging could then be considered as a modifying factor, since an inverse correlation between the age at onset and the number of CAG repeats has been observed,[27] and motor signs were more frequently found in the group of older individuals with IAs. A potential mechanism for the relationship between aging and IA clinical manifestations would be the loss of neuronal scaffold associated with aging, leading to clinical manifestations of the disease.[26]

Clinical manifestations of HD in patients with IAs might also be potentially accelerated by associated medical conditions, treatments, and environmental factors.[27] After correcting for multiple comparisons, the living status, exposure to antidopaminergic drugs tobacco and alcohol, marital status, and education background were similar in the IAs and controls, and did not have any association with UHDRS scores. Opposite results were found in another study, where tobacco and lower education background were related to clinical manifestations in those individuals with CAG repeat lengths of 36–39.[28] Likewise, weight loss, which has been correlated with CAG repeat length,[29] was similar between IA carriers and controls. These findings suggest that environmental factors are more likely to influence when the HD mutation is within the pathogenic level.

In humans, intermediate repeats on some specific HD haplotypes might contribute to CAG repeat instability and expansion.[6,7,30] Interestingly, recent studies show that a striking somatic mosaicism of CAG repeats is present in the brain, with noticeable cell-specific expansion in the striatum.[7,31] In this regard, a CAG repeat number of 35 or less extracted from peripheral blood samples does not necessarily duplicate the length and toxicity of the CAG repeats in the striatum.[7] Therefore, neuropathologic assessment of the symptomatic carriers of IAs will be useful, since an autopsy of a patient with 29 CAG repeats has been criticized because no huntingtin inclusions were found in this brain.[7,8] Recently, Tomé et al.[32] have reported that CAG instability is also associated with polymorphisms of DNA repair genes, which may have prognostic implications for various repeat-associated diseases. These authors observed that *MSH3* polymorphisms and protein levels were associated with CAG stability in animal models.[32] In this regard, we agree with Killoran et al.[22] that "given the wide range of estimated risk of disease associated

Table 3 High, moderate, and intermediate CAG repeats: UHDRS characteristics

	34–35 CAG repeats (n = 13)	27–33 CAG repeats (n = 63)	<27 CAG repeats (n = 581)	*p* Value
UHDRS-motor[a]	0 (0–8)	0 (0–5)	0 (0–1)	0.05
Sample	11	59	561	
No. of participants with UHDRS-chorea score ≥1 (%)	2 (15.4)	4 (6.3)	9 (1.5)	<0.0001
No. of participants with UHDRS-bradykinesia score ≥1 (%)	5 (38.5)	17 (27.0)	124 (21.3)	0.54
No. of participants with UHDRS-dystonia score ≥1 (%)	0	2 (3.2)	9 (1.5)	0.09
No. of participants with UHDRS-gait score ≥1 (%)	0	10 (15.9)	50 (8.6)	0.06
No. of participants with UHDRS-ocular movements score ≥1 (%)	2 (15.4)	13 (20.6)	52 (9.0)	0.01
UHDRS, behavior[a]	0 (0–8.2)	6.0 (0.5–16)	4.0 (0–11)	0.12
Sample	6	37	338	
UHDRS, cognition[a]	298.0 (277.0–326.0)	318.5 (240.7–347.2)	314.0 (275.0–346.7)	0.70
Sample	8	46	440	

Abbreviation: UHDRS = Unified Huntington's Disease Rating Scale.
[a] Data are median (interquartile range).

Table 4 Linear regression model (log Unified Huntington's Disease Rating Scale-motor as the dependent variable) (n = 56)

	B	Standard error	*p* Value	95% CI
Age (≥60 y)	0.05	0.01	0.002	0.02 to 0.09
CAG repeats (larger allele)	0.04	0.02	0.06	−0.002 to 0.09

Abbreviation: CI = confidence interval.
R^2 = 0.223.

with these partially penetrant alleles, it might be more informative to replace the use of diagnostic cut-off values in favor of a diagnostic probability spectrum based on expansion length and possibly other relevant genetic or environmental factors as they become known."

The main limitations of this study are the small sample of IAs and the lack of robust follow-up data for IAs, which do not allow for meaningful interpretation for UHDRS changes. In addition, adjustments for multiple comparisons were applied. A Bonferroni correction on all 27 hypothesis tests performed established a *p* value of less than 0.002 for statistical significance. Although controversial, classical adjustments for multiple comparisons have also been argued, because of the danger of erroneous dismissal of meaningful results.[33,34] Despite the above limitations, this study documents the clinical profile and progression of HD signs of a representative sample of participants with IAs. We would like to highlight that these observational data were obtained from a database without any prespecified hypotheses at the time of data collection, which preclude sample selection bias. Therefore, the results of this study provide additional information that might be of critical relevance in genetic counseling and medical care of HD families.

AUTHOR CONTRIBUTIONS

Dr. Cubo: study concept, design, and writing the manuscript. Dr. Ramos-Arroyo: interpretation and critical revision of the manuscript. S. Martinez-Horta: interpretation and critical revision of the manuscript. Dr. Martinez-Descalls: critical revision of the manuscript. S. Calvo: data analysis. Dr. Gil-Polo: interpretation and critical revision of the manuscript.

ACKNOWLEDGMENT

The authors thank Michael Orth and the EHDN REGISTRY Study Group investigators for collecting the data and the participating REGISTRY patients for participation.

STUDY FUNDING

European Huntington Disease Registry (data mining project: #530), SACYL (GRS 1021A14).

DISCLOSURE

E. Cubo has consulting fees for UCB, Allergan, and Abbvie. M. Ramos-Arroyo, S. Martinez-Horta, A. Martinez-Descalls, S. Calvo, and C. Gil-Polo report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received November 17, 2015. Accepted in final form March 28, 2016.

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72:971–983.
2. Rubinsztein DC, Leggo J, Coles R, et al. Phenotypic characterization of individuals with 30–40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36–39 repeats. Am J Hum Genet 1996;59:16–22.
3. Semaka A, Hayden MR. Evidence-based genetic counselling implications for Huntington disease intermediate allele predictive test results. Clin Genet 2014;85:303–311.
4. Bean L, Bayrak-Toydemir P. American College of Medical Genetics and Genomics Standards and Guidelines for Clinical Genetics Laboratories, 2014 edition: technical standards and guidelines for Huntington disease. Genet Med 2014;16:e2.
5. Semaka A, Kay C, Belfroid RD, et al. A new mutation for Huntington disease following maternal transmission of an intermediate allele. Eur J Med Genet 2015;58:28–30.
6. Semaka A, Kay C, Doty CN, Collins JA, Tam N, Hayden MR. High frequency of intermediate alleles on Huntington disease-associated haplotypes in British Columbia's general population. Am J Med Genet B Neuropsychiatr Genet 2013;162B:864–871.
7. Groen JL, de Bie RM, Foncke EM, Roos RA, Leenders KL, Tijssen MA. Late-onset Huntington disease with intermediate CAG repeats: true or false? J Neurol Neurosurg Psychiatry 2010;81:228–230.
8. Kenney C, Powell S, Jankovic J. Autopsy-proven Huntington's disease with 29 trinucleotide repeats. Mov Disord 2007;22:127–130.
9. Ha AD, Jankovic J. Exploring the correlates of intermediate CAG repeats in Huntington disease. Postgrad Med 2011;123:116–121.
10. Elden AC, Kim HJ, Hart MP, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010;466:1069–1075.
11. Hagerman RJ, Hagerman PJ. The fragile X premutation: into the phenotypic fold. Curr Opin Genet Dev 2002;12:278–283.
12. Orth M, European Huntington's Disease N, Handley OJ, et al. Observing Huntington's disease: the European Huntington's Disease Network's Registry. J Neurol Neurosurg Psychiatry 2011;82:1409–1412.
13. Orth M, Handley OJ, Schwenke C, et al. Observing Huntington's disease: the European Huntington's Disease Network's Registry: version 2. PLoS Curr 2010;2:RRN1184.
14. ICH Harmonised Tripartite Guideline Guideline for Good Clinical Practice E6(R1): Current Step 4 version. 1996. Available at: http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/good-clinical-practice.html.
15. Rupp J, Blekher T, Jackson J, et al. Progression in prediagnostic Huntington disease. J Neurol Neurosurg Psychiatry 2010;81:379–384.
16. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. Mov Disord 1996;11:136–142.
17. Kingma EM, van Duijn E, Timman R, van der Mast RC, Roos RA. Behavioural problems in Huntington's disease using the Problem Behaviours Assessment. Gen Hosp Psychiatry 2008;30:155–161.
18. Shoulson I. Huntington disease: functional capacities in patients treated with neuroleptic and antidepressant drugs. Neurology 1981;31:1333–1335.

19. Ho AK, Robbins AO, Walters SJ, Kaptoge S, Sahakian BJ, Barker RA. Health-related quality of life in Huntington's disease: a comparison of two generic instruments, SF-36 and SIP. Mov Disord 2004;19:1341–1348.
20. Semaka A, Kay C, Doty C, et al. CAG size-specific risk estimates for intermediate allele repeat instability in Huntington disease. J Med Genet 2013;50:696–703.
21. Ha AD, Beck CA, Jankovic J. Intermediate CAG repeats in Huntington's disease: analysis of COHORT. Tremor Other Hyperkinet Mov Epub 2012 Feb 2.
22. Killoran A, Biglan KM, Jankovic J, et al. Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS. Neurology 2013; 80:2022–2027.
23. Djoussé L, Knowlton B, Hayden M, et al. Interaction of normal and expanded CAG repeat sizes influences age at onset of Huntington disease. Am J Med Genet A 2003; 119A:279–282.
24. Quarrell OW, Handley O, O'Donovan K, et al. Discrepancies in reporting the CAG repeat lengths for Huntington's disease. Eur J Hum Genet 2012;20:20–206.
25. The American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group. ACMG/ASHG statement: Laboratory guidelines for Huntington's disease genetic testing. Am J Hum Genet 1998;62:1243–1247.
26. Panegyres PK, Shu CC, Chen HY, Paulsen JS. Factors influencing the clinical expression of intermediate CAG repeat length mutations of the Huntington's disease gene. J Neurol 2015;262:277–284.
27. Rosenblatt A, Brinkman RR, Liang KY, et al. Familial influence on age of onset among siblings with Huntington disease. Am J Med Genet 2001;105:399–403.
28. Panegyres PK, Goh JG. The neurology and natural history of patients with indeterminate CAG repeat length mutations of the Huntington disease gene. J Neurol Sci 2011;301:14–20.
29. Aziz NA, van der Burg JM, Landwehrmeyer GB, et al. Weight loss in Huntington disease increases with higher CAG repeat number. Neurology 2008;71:1506–1513.
30. Goldberg YP, McMurray CT, Zeisler J, et al. Increased instability of intermediate alleles in families with sporadic Huntington disease compared to similar sized intermediate alleles in the general population. Hum Mol Genet 1995;4: 1911–1918.
31. Gonitel R, Moffitt H, Sathasivam K, et al. DNA instability in postmitotic neurons. Proc Natl Acad Sci USA 2008; 105:3467–3472.
32. Tomé S, Manley K, Simard JP, et al. MSH3 polymorphisms and protein levels affect CAG repeat instability in Huntington's disease mice. PLoS Genet 2013;9:e1003280.
33. Savitz DA, Olshan AF. Describing data requires no adjustment for multiple comparisons: a reply from Savitz and Olshan. Am J Epidemiol 1998;147:813–814.
34. Cubo E, Martín PM, Martin-Gonzalez JA. Motor laterality asymmetry and nonmotor symptoms in Parkinson's disease. Mov Disord 2010;25:70–75.