Clinical and genetic investigation of a Brazilian family with Huntington's disease

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Summary

The aim of this study was to investigate a Brazilian family carrying full penetrance alleles for Huntington's disease (HD) in order to correlate each member's genetic and clinical features. To this end, the following scales were administered in each patient: the Beck Depression Inventory, the Mini-Mental State Examination (MMSE) and the Unified Huntington's Disease Rating Scale (UHDRS). The patterns of CAG and CCG polymorphic regions in the *HTT* gene were determined, the disease burden score was calculated, and genotypes were correlated with phenotypes within this family. We suggest that HD duration, the number of years of formal education, and UHDRS status variables can explain 96.6% of the MMSE variability in HD patients. A strong significant correlation was found between the disease burden score and the UHDRS ($r = 0.76$; $p\text{-value} = 0.049$) and the MMSE ($r = -0.90$; $p\text{-value} = 0.006$). The correlations between CAG allele size and the three clinical evaluations performed in the HD patients were not statistically significant.

KEY WORDS: CAG CCG repeats, clinical features, Huntington's disease, UHDRS

Introduction

Huntington's disease (HD) is a rare, neurodegenerative, progressive and fatal disease characterized by motor impairment, cognitive decline and psychiatric disturbances (The Huntington's Disease Collaborative Research Group, 1993).

It results from mutations in the *HTT* gene, which is located on the short arm of chromosome 4 (4p16.3). The mutation occurs as a variation in the number of CAG repeats, located in the first exon of *HTT*, which encodes a polyglutamine tract next to the amino-terminal region of the huntingtin protein (Margolis and Ross, 2003). Normal alleles have fewer than 27 copies of the CAG trinucleotide. Intermediate alleles have from 27 to 35 CAG repeats. Alleles with 36 to 39 CAG repeats have reduced penetrance, and alleles with more than 39 copies of CAG show full penetrance (The Huntington's Disease Collaborative Research Group, 1993).

Huntington's disease usually manifests itself in individuals aged 35 to 55 years. The juvenile form of HD accounts for 20% of all HD patients; 10% of the 20% with the juvenile form begin to show signs of a decline in motor function before the age of 20 years, and 5% before they are 14 years old (Andrew et al., 1993; Nahhas et al., 2005). In more than 8% of cases HD is a sporadic event resulting from *de novo* trinucleotide expansions. This kind of HD mutation is inherited from asymptomatic parents bearing intermediate alleles (Nahhas et al., 2005).

Huntington's disease is an autosomal dominant disorder (Andrew et al., 1993; Margolis and Ross, 2003; Nahhas et al., 2005; The Huntington's Disease Collaborative Research Group, 1993; Zuccato et al., 2001). Instability of CAG alleles is rare in individuals bearing normal chromosomes between the generations (<1%), moreover, it occurs at a slow rate. Expanded CAG alleles, on the other hand, have the highest frequency of instability (73%), with 70% of this 73% attributable to expansion events between the generations (Kremer et al., 1995; Trottier et al., 1994; Maat-Kievit et al., 2001). Intermediate and expanded alleles are unstable during meiosis and mitosis, mainly in the spermatogenesis process. For this reason, there is a higher probability of expansions in paternal than in maternal transmissions of CAG alleles (Wheeler et al., 2007).

Although there is another polymorphic region, with CCG repeats in tandem, immediately adjacent to the downstream CAG repeat in the *HTT* gene, it does not affect the age at onset of HD (Agostinho et al., 2012). The size of the CCG polymorphic region may indicate the ethnic origin of the HD mutation in chromosome 4 (Rubinsztein et al., 1993; Andrew et al., 1994).

A definite diagnosis of HD is based on detection of an expanded *HTT* allele associated with HD clinical manifestations, regardless of whether or not there is a family history of HD (Maat-Kievit et al., 2001). The first clinical symptoms of HD are chorea, involuntary clumsy movements and motor incoordination, mainly of the upper and lower limbs (Gil and Rego, 2008). The lack of reliable biomarkers to track disease progression is a major problem in clinical research of chronic neurological disorders (Kuan et al., 2015).

The evolution of HD is slow and the development of chorea is observed in 90% of adult cases (Wild and Tabrizi, 2007). As the disease progresses, the patient loses locomotion and communication abilities (Penney et al., 1990). Bradykinesia and rigidity are common symptoms in advanced stages of HD (Sánchez-Pernaute et al., 2000). The cause of death in HD patients is usually aspiration pneumonia or heart failure (Gil and Rego, 2008).

Because of the clinical variability of HD, and the fact that its symptoms may mimic those of other neurodegenerative disorders that have a better prognosis, a reliable genetic investigation with molecular tools is needed in order to arrive at an unequivocal diagnosis. We investigated a Brazilian family carrying full penetrance alleles in order to determine the variability of clinical symptoms between the HD patients and to correlate each subject's genetic and clinical features. To this end, the following scales were administered to each patient: the Beck Depression Inventory (BDI), the Mini-Mental State Examination (MMSE), and the Unified Huntington's Disease Rating Scale (UHDRS). The patterns of CAG and CCG polymorphic regions in the *HTT* gene were determined and genotypes were correlated with phenotypes within this family. We thus investigated whether the individuals who had the same MMSE score had a distinct phenotype, different from what we would have expected had we considered only HD duration.

Methods

Subjects

This was a cross-sectional study. Participants were recruited from the outpatient Genetics Clinic at the Gaffrée and Guinle University Hospital and from the outpatient Movement Disorders Clinic at the Pedro Ernesto Hospital (State University of Rio de Janeiro), Rio de Janeiro, Brazil. The pedigree was built using the 2007 version of the GenoPro program. Patients and their relatives were interviewed in order to obtain the family history. We calculated the median value of HD duration to find the number that best split our sample into two equal

parts. Half of the cases had values smaller than the median (11 years) and this sample was named Group 1, and the other half, Group 2, had values greater than the median.

Clinical evaluation

The clinical evaluation and the assessment of the degree of mental and motor impairments were carried out in accordance with the UHDRS, a research tool specifically developed by the Huntington Study Group (1996) to provide a uniform assessment of the clinical features and course of HD. It has several components including cognitive, behavioral, and functional sections. However, in the present study we assessed only motor impairment. In the UHDRS motor section, features like chorea, rigidity and dysarthria are evaluated and assigned a score, per item, of up to 4, giving a possible maximum score of 120. A higher UHDRS score indicates more severe disease progression (Klempir et al., 2006).

We also used the BDI, an instrument widely employed to assess the intensity of depression in different populations. It is a questionnaire with a score range from 0 to 63. The scores are interpreted as follows: 0–13: minimal depression, 14–19: mild depression, 20–28: moderate depression, and 29–63: severe depression (Smarr and Keefer, 2012).

Folstein's Mini-Mental State Examination (MMSE) is a simplified scored form of the cognitive mental status examination. It is widely used in both clinical and research settings to screen for dementia in patients with cognitive complaints. It is also used to estimate the severity and progression of cognitive impairment and to follow the course of cognitive changes in individuals over time. It is a 30-point questionnaire which evaluates, among other things, registration, attention and calculation, recall, language, the ability to follow simple commands, and orientation. In general, a score below 24 is associated with cognitive impairment, but the results depend largely on educational attainment. The CAG expansion of all the symptomatic subjects was known, and the disease burden score (DBS) of each gene carrier was calculated for correlation with genotype (DBS = [CAG repeat – 35.5] x age) (Penney et al., 1997).

Ethics

This study was approved by the Ethics in Research Committees of both the Gaffrée and Guinle University Hospital/UNIRIO (number 03/2009) and Pedro Ernesto Hospital/UERJ (number 20468). All participants had to fill in and sign an informed consent form.

Statistical analysis

Univariate analysis of the data was performed: mean ($\pm$ standard deviation), median and proportions were calculated using the IBM SPSS Statistics software (version 22.0). The t-test for independent samples was used to compare, between Group 1 and Group 2,

the results obtained. The correlation (two-tailed t-test) between MMSE and all the other variables listed in table I was done to select candidate variables for predicting the variation of the MMSE results. We performed a multiple regression analysis, considering the MMSE score as the dependent variable and UHDRS, years of formal education, and HD duration as independent variables.

The MMSE was used as the dependent variable because our aim was to investigate whether other variables (namely UHDRS, number of years of formal education, and HD duration) could interfere with the cognitive mental status of HD patients. Parametric statistics were used when Shapiro-Wilks test indicated normality of the data. Pearson's correlation was used to correlate the DBS and the MMSE, UHDRS and BDI scores. A p-value <0.05 was considered statistically significant.

DNA genotyping

Analysis of polymorphic (CAG)*n* and (CCG)*n* repeats in the *HTT* gene was conducted in 10 patients from

one Brazilian pedigree. The genotyping of CAG and CCG repeats in the *HTT* gene was performed using the primers in accordance with Agostinho et al. (2012) and the CAG and CCG alleles were resized by Chris Kay in the Hayden Lab – Huntington Disease Research Group, at the Centre for Molecular Medicine and Therapeutics (University of British Columbia, Vancouver, Canada).

Results

Patients

Of the 10 patients investigated in this study, six were males. One patient (numbered III.8) did not carry the HD expanded allele. The age at onset of symptoms ranged from 29 to 57 years (mean 49.5±6.4) and the disease duration ranged from 3 to 28 years (mean 12.5 years±8.4). These mean values were calculated without taking into account individuals III.7 and III.8 (Fig. 1): motor symptom onset in individual III.7 occurred at the age of 30 — the data obtained from this individual

Table I - Scales used in the clinical evaluation of HD patients.

Individual	<i>HTT</i> upper CAG allele	Beck Depression Scale	MMSE	UHDRS	Years of formal education	Age at onset	HD duration (Jan 2015)
II.2	41	*	9	62	3	50	28
II.3	41	NA	NA	NA	NA	50	22**
III.1	42	5	30	1	12	42	3
III.2	43	6	25	15	16	39	14
III.3	43	15	28	31	16	44	11
III.4	42	21	25	17	9	57	6
III.5	42	5	22	31	6	49	16
III.6	42	11	21	43	5	56	8
III.7	49	19	19	44	6	30	8
III.8	19	NA	NA	NA	NA	NA	NA

Abbreviations and symbols: MMSE=Mini-Mental State Examination; UHDRS=Unified Huntington's Disease Rating Scale; HD=Huntington's disease; NA=not applied; * Evaluation difficult due to the cognitive disorder; ** This HD patient died in 2013.

Figure 1 - HD pedigree.

Individuals represented with totally filled symbols have a positive genetic test for HD. The upper CAG allele, as well as its phased CCG allele, carried by HD patients, are shown under the symbols. Symptomatic individuals related to HD affected patients without confirmed HD genetic test are represented by partially filled symbols. X: deceased

were excluded from the statistical analyses because the age at onset was an outlier —, while individual III.8 was unaffected. All patients bore full penetrance alleles.

Pedigree analysis

A four-generation HD family was investigated and the HD history was confirmed. The subjects were all from the city of Rio de Janeiro. In this study 10 individuals were genetically investigated for the *HTT* gene (CAG and CCG polymorphic regions) (Fig. 1). As regards the size of CAG expanded alleles ($n=9$) determined in affected individuals, the smallest expansion had 41 repeats of CAG trinucleotides and the largest had 49 units (overall mean 42.8 ± 2.4).

In the first generation (I), the bearer (I.1) transmitted HD expanded alleles to his four children, three males and one female; the CAG allele size of I.1 was unknown. In the second generation, the individuals II.2 and II.3 were genetically tested and they showed 41 CAG units. Individual II.2 carried an expanded allele with 41 repeats and transmitted this mutation to his son (III.7) with an extra expansion of eight CAG units (for a total of 49); his other child (III.8) was unaffected. All the children of II.1 inherited expanded alleles, four alleles with 42 CAG repeats and two with 43 repeats. Individuals clinically reported to have HD but in whom there was no genetic testing are represented in the pedigree with partially filled in symbols. After excluding the unaffected individuals of the second generation, 18 individuals from the pedigree were included as a risk group for HD.

With regard to the CCG polymorphic region, also investigated in this study, all tested individuals carried seven CCG repeats phased to the CAG expanded allele.

Unaffected individuals ($n=12$) in the second generation (II) are not shown in table I for practical reasons.

Clinical evaluation

Eight family members were evaluated by neurologists who are movement disorders specialists. The mean age at disease onset in these subjects was 49.5 ± 6.4 years and the mean HD duration was 12.5 ± 8.4 years at the time of this study (January 2015). Formal schooling amounted to a mean of 9.1 ± 5 years. The DBS was calculated in eight HD symptomatic patients and gave a mean value of 391.2 ± 66.5 .

Two individuals were not clinically evaluated: one had died (II.3) before this study and the other was not affected (III.8).

Chorea was the initial symptom in all the patients but one (87.5%); cognitive decline was the initial complaint in this one patient. The main current symptom was chorea (in six patients, 75%), irritability in one, and dementia in the other (the oldest, who was 77). All the patients were administered the MMSE, BDI and UHDRS. The results are reported in table I. MMSE scores ranged from 9 to 30. The lowest score was

recorded by the oldest individual, who had a disease duration of 22 years and fewer years of formal education. The other individuals were cognitively functional. BDI scores ranged from 5 to 21. Applying pre-established parameters, depression symptoms were considered minimal in four patients, mild in three and moderate in one.

The UHDRS is a research tool for assessing the clinical features of HD. We applied its motor section and the results were variable, ranging from a score of 1 in a patient with minimal disease manifestations, to 62, scored by the most affected and elderly family member.

The patients scored an average of 22.4 ± 6.5 points on the MMSE and 30.5 ± 19.4 on the UHDRS. The individuals carrying the same size of CAG allele (III.1, III.4, III.5 and III.6) had a distinct phenotype as shown in table I. We compared our results with those of other studies that used the same scales (Table II).

Genetic and clinical correlation

Three variables were selected as candidates to predict the variation of MMSE results because they showed statistical significance when correlated with MMSE score: UHDRS score ($p=0.03$), years of formal education ($p=0.02$), and HD duration ($p=0.02$). On multiple regression analysis, in which the MMSE score was taken as the dependent variable, and UHDRS score, years of formal education, and HD duration as the independent variables, we observed a statistically significant association, with $R^2 = 0.93$ ($p=0.008$). On the basis of the results here reported we suggest that HD duration, years of formal education, and UHDRS score could explain 96.6% of the variability of the MMSE results in HD patients. No statistically significant differences in the BDI, UHDRS and MMSE scores were found between Group 1 and Group 2 ($p>0.05$) (Table III). A strong significant correlation was found between the DBS and the UHDRS ($r = 0.76$; $p\text{-value} = 0.049$), and between the DBS and the MMSE ($r = -0.90$; $p\text{-value} = 0.006$).

The size of CCG allele in *HTT* was a constant value (CCG7) in all the HD patients here tested.

Discussion

Before the discovery of the *HTT* gene, HD was diagnosed on the basis of clinical features, family history, and pathological information (Siesling et al., 2000). After the *HTT* gene had been localized (Gusella, 2001), HD diagnosis became easier. HD classically presents with movement disorders, cognitive dysfunction and behavioral disturbances, although phenotypes are variable (Wild et al., 2008).

We investigated a Brazilian family carrying full penetrance alleles in order to determine the variability in clinical symptoms of each HD patient and to correlate his/her genetic and clinical features. To this end, the following scales were administered to each one: BDI,

MMSE and UHDRS. The patterns of CAG and CCG polymorphic regions in the *HTT* gene were determined and genotypes were correlated with phenotypes within this family. We thus investigated whether the indi-

viduals who had the same MMSE score had a distinct phenotype different from what we would have expected had we considered only HD duration. As regards the CCG alleles, (CCG)7 was the only

Table II - Different studies that assessed the clinical features of HD patients using the MMSE, UHDRS and BDI.

Authors	Sample	MMSE	UHDRS	BDI	Expanded CAG allele	Current age	Disease burden score
This study	8 symptomatic HD patients from Brazil	22.4±6.5	30.5±19.4	11.7±6.7	42.8±2.4	54.6±12 (38–77)	391.2±66.5
Dorsey et al., 2013	333 from Australia, Canada and the United States	26.2±3.6	NA	NA	43.7±3.2	52±10.8 (in 2013)	NA
Vaccarino et al., 2011	327 from United States, Canada, Europe and Australia	NA	43.06±0.55	NA	44.78±0.15	52.07±0.30	442.25±2.73 (91.5–1165.5)
Oliva et al., 1993	26 from Italy	22.6±5.6	NA	NA	NA	46.1±15.5	NA
Jurgens et al., 2008	16 from New York and Netherlands	28.4±1.2	3.5±3.5	NA	42 (40–49)	41.9±10.0	NA
De Souza et al., 2009	12 from Birmingham, UK	NA	NA	26.08±13.97	NA	51.2±10.35	NA
Reedeker et al., 2010	150 from New York	26 (22–28)	NA	NA	45±3	51±11	NA
Reilmann et al., 2011	19 symptomatic HD patients from Muenster (Germany)	NA	26±12.9	NA	46±2.9	43.5±8.5 (27–61)	456±107.2

Abbreviations: MMSE=Mini-Mental State Examination; UHDRS=Unified Huntington's Disease Rating Scale; BDI=Beck Depression Inventory; NA=not applied

Table III - Clinical and genetic details of HD patients split into two groups on the basis of HD duration (Group 1: less than 11 years of HD duration and Group 2: more than 11 years of HD duration).

Patients	Group	CAG allele	CCG allele	Age at onset	HD duration	Years of formal education	MMSE	BDI	UHDRS	DBS
1	1	42	7	42	3	12	30	5	1	279.5
2	1	43	7	44	11	16	28	15	31	352.5
3	1	42	7	57	6	9	25	21	17	364
4	1	42	7	56	8	5	21	11	43	390
5	1	49	7	30	8	6	19	19	44	513
6	2	41	7	50	22	*	*	*	*	423.5
7	2	41	7	50	28	3	9	*	62	*
8	2	43	7	39	14	16	25	6	15	397.5
9	2	42	7	49	16	6	22	5	31	409.5

Abbreviations: HD=Huntington's disease; MMSE=Mini-Mental State Examination; BDI=Beck Depression Inventory; UHDRS=Unified Huntington's Disease Rating Scale; DBS=disease burden score; *not applied

allele encountered in the family reported here. The most common CCG alleles have seven or 10 repeats. In Western Europe, (CCG)7 is the most frequent allele in the population with high HD prevalence, and (CCG)10 is the most frequent in populations with low HD prevalence, as in Japan (Garcia-Planells et al., 2005; Xu and Wu, 2015). The Brazilian patients previously studied by Agostinho et al. (2012) showed seven units of CCG with a higher frequency, comparable to what is found in Western Europe, known to be the place of the HD founding event (Andresen et al., 2007).

With regard to the correlation between CAG allele size and the three clinical evaluations performed, no statistically significant results were found: the p-value was 0.97 for the MMSE score, $p=0.74$ for the UHDRS, and $p=0.29$ for the BDI. Some studies suggest that the interference of modifier genes plays a role in the variability of age at onset in individuals that bear the same size of expanded CAG alleles. Also, genetic and environmental factors have both been suggested to be causes of variation in age at onset and in the clinical features observed in HD (Andrew et al., 1993). In a case report by Raskin et al. (2000), identical twin sisters, both bearing the same CAG repeat numbers (22/62) on the *HTT* gene, had slightly different disease onset ages (17 and 20 years). They were monozygotic twins, as proved by their having the same DNA genotypes in nine different short tandem repeats loci. These results show that there are also non-genetic factors influencing HD variability in symptoms, and that other genes may be involved in the final phenotype, too.

Huntington's disease is characterized by progressive loss of medium spiny neurons in the striatum (Raymond et al., 2011). This occurs during the third to fifth decade of life, showing slow progression. In general, affected individuals die within 15 to 20 years of onset of symptoms (Yu et al., 2014). The longer sizes are typical of juvenile HD and can be a result of paternal or maternal expansion. Large maternal expansions are rare events as reported by Nahhas et al. (2005), who found a CAG allele expansion of 60 repeats inherited from the mother. Maternal expansions rarely expand beyond 20 units (Sánchez et al., 1997).

The CAG profile and the smallest expanded CAG repeat size responsible for the HD phenotype are difficult to determine given the many differences between individuals and populations (Agostinho et al., 2012). In a study in India, the shortest allele identified had 36 repeats and the longest had 56 (Pramanik et al., 2000). In a Brazilian study, the CAG size ranged from 39 to 88 repeats, and in another from 43 to 73 repeats (Raskin et al., 2000; Lima et al., 2000). It is important to mention that intermediate CAG alleles in asymptomatic individuals with a family history of HD are more unstable than intermediate alleles from members of the general population without a family history, and can lead, in offspring, to sporadic HD, resulting from *de novo* trinucleotide expansions (Maat-Kievit et al., 2001).

Chorea is the prototypical movement disorder in HD and is usually present in subjects with onset in middle age or old age; the full spectrum of motor impairment in HD includes eye movement abnormalities, parkinsonian features and dystonia (particularly in juvenile HD), myoclonus, tics, dysarthria and dysphagia, and spasticity with hyperreflexia and extensor plantar responses. Unsurprisingly, a longer disease duration has been found to be associated with higher scores on the UHDRS motor assessment in most patients (Kirkwood et al., 2001). A longer disease duration is also related to worse MMSE scores (Jurgens et al., 2008; Reedeker et al., 2010).

A high degree of internal consistency between the motor, behavioral, cognitive and functional components of the UHDRS is observed. The scores on the motor, cognitive and functional sections of the scale have been shown to be highly intercorrelated, although the total behavioral score did not correlate well with the other sections (Huntington Study Group, 1996). There is evidence that schooling can influence performance in cognitive assessment testing. In developing countries, formal education is limited; therefore, the evaluation of illiterate and low educated individuals can distort the results of the MMSE (Brito-Marques and Cabral-Filho, 2004).

In our sample, after six to 16 years of HD progression, chorea remained the main symptom in 75% of the patients. All but one of our patients reported chorea as the initial symptom. Kirkwood et al. (2001) evaluated the progression of symptoms in HD and found involuntary movements to be the earliest reported symptom. On the other hand, Wild et al. (2008) suggested that some individuals at the preclinical stage of HD may have psychiatric manifestations without movement disorders. Depression, anxiety, irritability and apathy have all been described in the early progression of HD (Kirkwood et al., 2002). However, the same study described other symptoms with progression of the disease, including cognitive and behavioral ones.

We suggest, on the basis of the data reported in this article, that HD duration, years of formal education, and UHDRS score could explain 96.6% of the variability of MMSE scores in HD patients. When we ran the multiple regression analysis using the BDI ($p=0.57$) or the UHDRS ($p=0.052$) score as the dependent variable, each with the same independent variables (HD duration, years of formal education, and UHDRS score), we observed that these independent variables cannot be identified as predictors of the variation of results recorded on the UHDRS or BDI. It should be pointed out that although the MMSE is widely used by investigators, its heavy emphasis on language and memory may not efficiently capture the cognitive domains most affected in HD.

Pillai et al. (2012) reported a mean score of 23.7 (15–28) for cognitive impairments assessed using the MMSE in the moderate stages of HD and 24 (20–30) in the severe stages. However, our MMSE results were very similar to those of other studies for the range of 42–46 CAG expanded alleles (Reedeker et al., 2010; Jurgens et al., 2008; Oliva et al., 1993;

Dorsey et al., 2013). The results obtained assessing the UHDRS varied more when individuals bearing 42–46 CAG repeats were compared (Jurgens et al., 2008; Vaccarino et al., 2011).

There are few studies on the application of these scales in Brazilian patients with HD. We therefore issue an invitation to Brazilian Health Centers with registered HD patients to try this clinical investigation. It is, however, important to stress that, because the HD clinical picture is variable, a definite clinical diagnosis requires caution. To improve the accuracy and specificity of HD detection, analysis of clinical symptoms and genotyping should always be combined. Greater understanding the natural history of HD symptoms will better inform patients, caregivers and clinicians on the disease course and assist researchers in the design of clinical studies. Further studies should be conducted to investigate the relationship between genetic, epigenetic, environmental and clinical features of HD.

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References

- Agostinho Lde A, Rocha CF, Medina-Acosta E, et al (2012). Haplotype analysis of the CAG and CCG repeats in 21 Brazilian families with Huntington's disease. *J Hum Genet* 57:796-803.
- Andresen JM, Gayán J, Cherny SS, et al (2007). Replication of twelve association studies for Huntington's disease residual age of onset in large Venezuelan kindreds. *J Med Genet* 44:44-50.
- Andrew SE, Goldberg YP, Theilmann J, et al (1994). A CCG repeat polymorphism adjacent to the CAG repeat in the Huntington disease gene: implications for diagnostic accuracy and predictive testing. *Hum Mol Genet* 3:65-67.
- Andrew SE, Goldberg YP, Kremer B, et al. (1993). The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat Genet* 4:398-403.
- Brito-Marques PR, Cabral-Filho JE (2004). The role of education in mini-mental state examination: a study in Northeast Brazil. *Arq Neuropsiquiatr* 62:206-211.
- De Souza J, Jones LA, Rickards H (2009). Validation of self-report depression rating scales in huntington's disease. *Mov Disord* 25:91-96.
- Dorsey ER, Beck CA, Darwin K, et al (2013). Natural history of Huntington disease. *JAMA Neurol* 70 :1520-1530.
- García-Planells J, Burguera JA, Solís P, et al (2005). Ancient origin of the CAG expansion causing Huntington disease in a Spanish population. *Hum Mutat* 25:453-459.
- Gil JM, Rego AC (2008). Mechanisms of neurodegeneration in Huntington's disease. *Eur J Neurosci* 27:2803-2820.
- Huntington Study Group (1996). Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. *Mov Disord* 11:136-142.
- Gusella JF (2001). Huntington Disease. In: *Encyclopedia of Life Sciences*: John Wiley & Sons, Ltd. <http://www.els.net/WileyCDA/ElsArticle/refId-a0000147.html>. DOI: 10.1038/npg.els.0000147
- Jurgens CK, van de Wiel L, van Es AC, et al (2008). Basal ganglia volume and clinical correlates in 'preclinical' Huntington's disease. *J Neurol* 255:1785-1791.
- Kirkwood SC, Siemers E, Viken R, et al (2002). Longitudinal personality changes among presymptomatic Huntington disease gene carriers. *Neuropsychiatry Neuropsychol Behav Neurol* 15:192-197.
- Kirkwood SC, Su JL, Conneally P, et al (2001). Progression of symptoms in the early and middle stages of Huntington disease. *Arch Neurol* 58:273-278.
- Klempir J, Klempírova O, Spacková N, et al (2006). Unified Huntington's disease rating scale: clinical practice and a critical approach. *Funct Neurol* 21:217-221.
- Kremer B, Almquist E, Theilmann J, et al (1995). Sex-dependent mechanisms for expansions and contractions of the CAG repeat on affected Huntington disease chromosomes. *Am J Hum Genet* 57:343-350.
- Kuan WL, Kasis A, Yuan Y, et al (2015). Modelling the natural history of Huntington's disease progression. *J Neurol Neurosurg Psychiatry* 86:1143-1149.
- Lima E Silva TC, Serra HG, Bertuzzo CS, et al (2000). Molecular diagnosis of Huntington disease in Brazilian patients. *Arq Neuropsiquiatr* 58:11-17.
- Maat-Kievit A, Helderma-van den Enden P, Losekoot M, et al (2001). Using a roster and haplotyping is useful in risk assessment for persons with intermediate and reduced penetrance alleles in Huntington disease. *Am J Med Genet* 105:737-744.
- Margolis RL, Ross CA (2003). Diagnosis of Huntington disease. *Clin Chem* 49:1726-1732.
- Nahhas FA, Garbern J, Krajewski KM, et al (2005). Juvenile onset Huntington disease resulting from a very large maternal expansion. *Am J Med Genet A* 137A:328-331.
- Oliva D, Carella F, Savoirdo M, et al (1993). Clinical and magnetic resonance features of the classic and akinetic-rigid variants of Huntington's disease. *Arch Neurol* 50:17-19.
- Penney JB Jr, Vonsattel JP, MacDonald ME, et al (1997). CAG repeat number governs the development rate of pathology in Huntington's disease. *Ann Neurol* 41:689-692.
- Penney JB Jr, Young AB, Shoulson I, et al (1990). Huntington's disease in Venezuela: 7 years of follow-up on symptomatic and asymptomatic individuals. *Mov Disord* 5:93-99.
- Pillai JA, Hansen LA, Masliah E, et al (2012). Clinical severity of Huntington's disease does not always correlate with neuropathologic stage. *Mov Disord* 27:1099-1103.
- Pramanik S, Basu P, Gangopadhaya PK, et al (2000). Analysis of CAG and CCG repeats in Huntingtin gene among HD patients and normal populations of India. *Eur J Hum Genet* 8:678-682.
- Raskin S, Allan N, Teive HA, et al (2000). Huntington disease: DNA analysis in Brazilian population. *Arq Neuropsiquiatr* 58:977-985.
- Raymond LA, André VM, Cepeda C, et al (2011). Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. *Neuroscience* 198:252-273.
- Reedeker N, Van Der Mast RC, Giltay EJ, et al (2010). Hypokinesia in Huntington's disease co-occurs with cognitive and global dysfunctioning. *Mov Disord* 25:1612-1618.
- Reilmann R, Bohlen S, Kirsten F, et al (2011). Assessment of involuntary choreatic movements in huntington's disease—toward objective and quantitative measures. *Mov Disord* 26:2267-2273.
- Rubinsztein DC, Barton DE, Davison BC, et al (1993). Analysis of the huntingtin gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of onset of Huntington's disease and CAG repeat number.

- Hum Mol Genet 2:1713-1715.
- Sánchez A, Milà M, Castellvi-Bel S, et al (1997). Maternal transmission in sporadic Huntington's disease. *J Neurol Neurosurg Psychiatry* 62:535-537.
- Sánchez-Pernaute R, Kunig G, del Barrio Alba A, et al (2000). Bradykinesia in early Huntington's disease. *Neurology* 54:119-125.
- Siesling S, Vegter-van de Vlis M, Losekoot M, et al (2000). Family history and DNA analysis in patients with suspected Huntington's disease. *J Neurol Neurosurg Psychiatry* 69:54-59.
- Smarr KL, Keefer AL (2012). Measures of depression and depressive symptoms: Beck Depression Inventory-II (BDI-II), Center for Epidemiologic Studies Depression Scale (CES-D), Geriatric Depression Scale (GDS), Hospital Anxiety and Depression Scale (HADS), and Patient Health Questionnaire-9 (PHQ-9). *Arthritis Care Res (Hoboken)* 63 Suppl 11:S454-S466.
- The Huntington's Disease Collaborative Research Group (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72:971-983.
- Trottier Y, Biancalana V, Mandel JL (1994). Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset. *J Med Genet* 31:377-382.
- Vaccarino AL, Anderson K, Borowsky B, et al (2011). An item response analysis of the motor and behavioral subscales of the unified Huntington's disease rating scale in huntington disease gene expansion carriers. *Mov Disord* 26:877-884.
- Xu M, Wu ZY (2015). Huntington Disease in Asia. *Chin Med J (Engl)* 128:1815-1819. d
- Yu M, Li X, Wu S, et al (2014). Examination of Huntington's disease in a Chinese family. *Neural Regen Res* 9:440-446.
- Wheeler VC, Persichetti F, McNeil SM, et al (2007). Factors associated with HD CAG repeat instability in Huntington disease. *J Med Genet* 44:695-701.
- Wild EJ, Mudanohwo EE, Sweeney MG, et al (2008). Huntington's disease phenocopies are clinically and genetically heterogeneous. *Mov Disord* 23:716-720.
- Wild EJ, Tabrizi SJ (2007). Huntington's disease phenocopy syndromes. *Curr Opin Neurol* 20:681-687.
- Zuccato C, Ciammola A, Rigamonti D, et al (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* 293:493-498