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Clinical and genetic analysis of 29 Brazilian patients with Huntington's disease-like phenotype

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder characterized by chorea, behavioral disturbances and dementia, caused by a pathological expansion of the CAG trinucleotide in the *HTT* gene. Several patients have been recognized with the typical HD phenotype without the expected mutation. The objective of this study was to assess the occurrence of diseases such as Huntington's disease-like 2 (HDL2), spinocerebellar ataxia (SCA) 1, SCA2, SCA3, SCA7, dentatorubral-pallidoluysian atrophy (DRPLA) and chorea-acanthocytosis (ChAc) among 29 Brazilian patients with a HD-like phenotype. In the group analyzed, we found 3 patients with HDL2 and 2 patients with ChAc. The diagnosis was not reached in 79.3% of the patients. HDL2 was the main cause of the HD-like phenotype in the group analyzed, and is attributable to the African ancestry of this population. However, the etiology of the disease remains undetermined in the majority of the HD negative patients with HD-like phenotype.

Key words: Huntington's disease, Huntington's disease-like, chorea-acanthocytosis, Huntington's disease-like 2.

Análise clínica e genética em 29 pacientes brasileiros com fenótipo doença de Huntington-símile

RESUMO

A doença de Huntington (DH) é uma doença neurodegenerativa caracterizada por coréia, alterações comportamentais e demência, causada por uma expansão patológica do trinucleotídeo CAG no gene *HTT*. Vários pacientes têm sido descritos com o fenótipo típico para a DH porém sem a mutação esperada. O objetivo deste estudo foi avaliar a ocorrência de doenças como doença de Huntington-símile 2 (DHS-2), ataxias espinocerebelares tipo 1, 2, 3 e 17, atrofia dentatorubral-palidoluisiana e coreo-acantocitose (CAc) entre 29 pacientes brasileiros com fenótipo doença de Huntington-símile. No grupo analisado, encontramos 3 pacientes com DHS-2 e 2 pacientes com CAc. O diagnóstico permaneceu obscuro em 79,3% dos pacientes. DHS-2 foi a principal causa do fenótipo DH-símile no grupo analisado, provavelmente devido a ancestralidade africana na população brasileira. Entretanto, a etiologia permaneceu indeterminada na maioria dos pacientes avaliados.

Palavras-chave: doença de Huntington, doença de Huntington-símile, coreo-acantocitose, doença de Huntington-símile 2.

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Huntington's disease (HD) is a progressive, neurodegenerative disorder characterized by chorea, dystonia or parkinsonism, cognitive impairment and behavioral abnormalities, with typical onset in young adulthood. HD is caused by a pathological expansion of CAG trinucleotide repeats in the *HTT* gene.

Since the identification of the causative mutation, it has been recognized that a number of patients with the classical HD phenotype have disease due to another etiology. These include prion diseases such as HDL1; autosomal dominant disorders due to inheritance of an expanded trinucleotide repeat sequence, such as HDL2, and DRPLA (dentatorubral-pallidoluysian atrophy); disorders affecting primarily the cerebellum, in which movements disorders can also be seen - FRDA (Friedreich ataxia), SCA (spinocerebellar atrophy) 1, SCA 2, SCA 3 and SCA 17; the neuroacanthocytosis syndromes, chorea-acanthocytosis (ChAc) and McLeod syndrome; and the neurodegeneration with brain iron accumulation (NBIA) syndromes, neuroferritinopathy, aceruloplasminemia and Pantothenate kinase-associated neurodegeneration (PKAN).

There have been several published studies addressing the etiological diagnosis of patients with an HD-like phenotype[1-14]; however, these have been mainly drawn from European and North American populations. We performed a transversal study of the genetic and clinical findings from a group of non-HD HD-like patients who attended a Brazilian movement disorders clinic.

METHOD

The medical records of 108 patients who were tested for HD between 1998 and 2006, were reviewed. Patients with an HD-like phenotype, defined as a progressive disorder with chorea, dystonia, parkinsonism, ataxia or myoclonus associated with a cognitive, psychiatric or behavioral impairment who did not have a pathological expansion on *HTT* gene underwent further clinical examination and testing for DRPLA, HDL2, SCA 1, 2, 3, 17 and ChAc. Although a true HD-like phenotype requires autosomal dominant inheritance, we did not use this criterion to select our patients, as an absent family history is non-informative.

DNA analysis of *ATN1* (DRPLA), *JPH3* (HDL2), *ATX1* (SCA1), *ATX2* (SCA2), *ATX3* (SCA3) and *TBP* (SCA17) were performed by sizing of fluorescent PCR products encompassing the CTG/CAG expansion site. The PCR products were loaded on 3730 DNA Analyzer or Megabace 1000 (GE) and analyzed using GeneMapper software [AppliedBiosystems] or Fragment Profiler (GE). As the standard PCR protocol does not allow detection of alleles carrying very large expansions as described for the juvenile, severe form of HD, triplet repeat primed PCR[15], which permits detection of large expansions, was performed for all patients who were not heterozygous at the *HTT* locus and/or the *JPH3* locus.

Patients who were found to have acanthocytes on peripheral blood smear, elevation of serum CK levels, or evidence of myopathy or peripheral neuropathy, had

Table 1. Clinical and molecular findings in HD and HDL patients.

	HD	HDL	p
Number of cases	37	29	
Age of onset	35.1±12.8	30.7±17.6	0.25†
Male:Female ratio	16:21	14:15	0.68‡
Chorea	37 (100%)	22 (75%)	0.002#
Ataxia	7 (18.9%)	8 (27.5%)	0.40‡
Myoclonus	0	2 (6.8%)	0.19#
Dystonia*	0	2 (6.8%)	0.19#
Tremor	0	1 (3.4%)	0.43#
Parkinsonism*	0	0	–
Psychiatric disturbance	17 (45.9%)	15 (51.7%)	0.64‡
Dementia	25 (65.7%)	20 (68.9%)	0.90‡
Epilepsy	4 (10.8%)	4 (13.7%)	0.72#
Familiar history	34 (91.8%) AD:34	19 (65.5%) AD: 14 (48.2%) non-AD: 5 (17.2%)	0.007‡
htt CAG range	37-87	15-27	

HD: Huntington's disease; HDL: Huntington's disease-like; AD: autosomal dominant; *Dystonia or parkinsonism as a predominant manifestation; †Student's t test; ‡χ^2 test; #Fisher's exact test.

Table 2. Review of HD-like screening studies.

Year	Reference	Population	Nº HDL	Evaluation	Results
1998	1	US and UK	15	SCA 1,2,3,6 and DRPLA	0
2000	2	European (France)	32	SCA 1,2,3,6,7, DRPLA RED test	1 patient with an unknown CAG expansion
2001	3	American (African ancestry)	330	HDL2	4 HDL2
2002	4	European (Germany and Austria)	1600	HDL2	0
2003	5	European (France)	252	HDL 1, HDL2, SCA 17 and DRPLA	HDL2 = 2 (3.3% of typical cases) SCA 17 = 2 (3.3% of typical cases)
2004	6	European (Germany and Austria)	1712	SCA 17	9 (0.005%)
2004	7	(North America Japan, Mexico)	538 (NA) and 44 (Japan)	HDL2	North America 6/538 (1.1%) Japan 0/44
2004	8	European (Italy)	98	SCA 17. HDL2 previously excluded	1 possible case with 43 repetitions
2005	9	European (Yugoslav)	48	HDL1, HDL2, SCA 1,2,3,17, DRPLA, NFP	0
2005	10	African (South Africa)	50	HDL2	15 (30%)
2006	11	European (Portugal)	107	HDL1, HDL2, SCA 17, DRPLA, NFP	0
2008	12	European (Spain)	95	FXTAS	1 (1.6%)
2008	13	European (UK)	285	HDL1, HDL2, SCA 1,2,3,17, DRPLA, NFP, FRDA	SCA 17 = 5 (1.8%) HDL1 = 1 (0.4%) FRDA = 1 (0.4%) HDL2 = 1 (0.4%)
2008	14	European (Polish)	224	HDL2, SCA17, DRPLA	SCA 17= 1 (0.44%)
2011		Brazilian	29	HDL2, SCA 1,2,3,17, DRPLA, ChAc	HDL2 = 3 (10.3%) ChAc = 2 (6.8%)

HDL: Huntington's disease-like; DRPLA: dentatorubral-pallidoluysian atrophy; FRDA: Friedreich ataxia; SCA: spinocerebellar atrophy; ChAc: chorea-acanthocytosis; NFP: neuroferritinopathy; FXTAS: Fragile X–associated tremor/ataxia syndrome; RED: repeat expansion detection technique.

chorein levels in peripheral blood qualitatively analyzed by Western blot[16] (n=2).

Statistical analysis was performed with SPSS version 10.0 (SPSS, Inc., Chicago, IL, USA). This study was approved by the ethical board of the Hospital das Clínicas de Ribeirão Preto and all patients have signed an informed consent.

RESULTS

Of the 108 patients tested for HD, seven were excluded due to insufficient data in their medical records. From the remaining subjects, 37 were diagnosed with HD, 35 were not classified as having an HD-like phenotype, and 29 were classified as a HD-like phenotype. As a result, considering the patients with a typical or compatible HD phenotype, we found 29/66 patients (43%) without pathological CAG expansion on *HTT* gene.

Table 1 summarizes the main clinical features of HD and HD-like patients. MRI exams were performed in 24 patients and brain CT in 3. The most common finding was brain atrophy in 23 (85%) patients. We found no evidence of iron deposition on those patients who underwent MRI exams. All 29 HDL patients had their DNA analyzed for trinucleotide repeat expansions of *HTT, JPH3, ATX1, ATX2, ATX3, TBP* and *ATN1*. Two patients met criteria for chorein testing and had absent or markedly reduced levels in peripheral blood. From these tests, we diagnosed 3 (10.3%) cases of HDL2 and 2 (6.8%)

cases of ChAc. The patients with HDL2 were clinically indistinguishable from those with typical HD phenotype. However, patients with ChAc presented clear clinical differences as absence of autosomal dominant history, presence of peripheral involvement and epilepsy in both cases. The patients with HDL2 and ChAc have been previously reported in detail elsewhere[17,18].

One patient of this study was a deceased sister of one ChAc patient, who presented with chorea and cognitive abnormalities, but whose diagnosis could not be confirmed by Western blot due to the non-availability of required biomaterial. The remaining 23 (79.3%) patients tested negative for HD, HDL2, SCA1, 2, 3, 17, and DRPLA.

DISCUSSION

This is the first study to address the diagnosis of diseases responsible for the HD-phenotype in Brazilian patients. Compared with previous studies (Table 2), we found a higher frequency of ChAc and HDL2 among our patients. Considering the 3 unrelated HDL2 cases reported here, together with the patients reported by Teive et al.[19] and Santos et al.[20], it is possible that HDL2 is the most common cause of HD-like phenotype in Brazil. A probable explanation is that 44% of Brazilian population is of African descent[21], even though this ancestry may be occult[19,20]. This regional difference contrasts with the article of Wild and colleagues[13], in which SCA 17 was considered the most important cause of the HD-like phenotype in patients from the UK.

Based upon previous studies, we initially decided to test for disorders whose frequencies were higher than 0.5% of all HD phenocopies. Consequently, we included SCA 17 and HDL2 and excluded HDL1 and FRDA[13]. We have not included neuroferritinopathy because it appears to be even rarer in HD-like patients, with a very small number of families being reported to date, and it is unlikely to occur in patients without evidence of iron deposition in the basal ganglia on MRI[22].

Screening for ChAc was performed as the clinical characteristics of our patients suggested the diagnosis. It was important to include the investigation for ChAc because Brazil represents the largest population of Japanese and descendants outside Japan[21], and ChAc appears to be particularly prevalent in Japanese subjects[23]. DRPLA was included for the same reason. In addition, studies have demonstrated that the number of DRPLA cases in non-Asian populations may be higher than previously considered[24,25]. Finally, chorea has been reported as an occasional non-ataxic symptom on SCAs, occurring in approximately 7% of SCA 1 and 2 and 10% of SCA 3 patients[26].

Our data showed that almost 43% of the patients who had a phenotype compatible with HD tested negative for CAG expansions at the *HTT* gene. This data diverges from reference studies in this field, which suggest that the frequency of HD-like phenocopies is about 1% of HD cases[27,28]. We believe that this discrepancy cannot be addressed only as an ascertainment bias, because our sample was selected based on restrictive criteria. In addition, other studies have found proportions of HD-like patients in 33%[10], 35.5%[11], and 36.3%[9] of their study populations. Krause and colleagues[10] provided an insight in this problem, showing that, in their population, only 16% of white patients were HD phenocopies, in contrast to 64% of the black patients. Therefore, the percentage of HD-like patients may differ according to the ethnicity of the study population.

Our clinical data shows that the absence of an autosomal-dominant history and signs of peripheral involvement or seizures may help differentiate between ChAc and HD. Although the presence of acanthocytes may improve the diagnostic accuracy, their absence does not rule out the diagnosis of ChAc[29]. In addition to screening for acanthocytes, we considered the diagnosis of ChAc in patients with elevated CK levels and clinical signs of myopathy or peripheral neuropathy.

The patients with HDL2 were clinically very similar to the classical HD phenotype, and this diagnosis should be suspected in the presence of typical HD phenotype with absent *HTT* mutation, especially if an autosomal dominant inheritance and African ancestry is present.

However, this survey has some limitations. First, the small number of patients identified as having an HD-like phenotype, what can be explained by the rarity of these disorders and because we have enrolled patients from only one clinical center. Second, referral bias is unavoidable when including patients from a referral movement disorders clinic, therefore, it is possible that the relative frequency of HD-like disorders was overestimated. Third, in spite of screening systematically for seven diseases, 79.3% of our patients remained undiagnosed, confirming a considerable etiologic heterogeneity among HD-like disorders and indicate the need for additional studies, using a broader panel of diagnostic tests. Considering the discrepancy between the high percentage of cryptic cases and the rareness of the remaining diseases to be tested for, it is likely that among the HD-like phenotypes some causalities have not been described yet.

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