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Clinical and genetic characteristics in patients with Huntington's Disease from Argentina

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ABSTRACT

Huntington's Disease (HD) is a neurodegenerative disease, caused by the expansion of an unstable $(CAG)_n$ in the *HTT* gene. There is scarce data about the disease in Argentina.
Objective: To describe the demographic, clinical and molecular data in patients with HD from Argentina.
Patients and methods: 59 HD patients were recruited at our department.
Comprehensive interviews, neurological examination and genetic analysis were performed in probands. Statistical analysis was conducted using G-Stat 2.0 and non-parametric tests (Wilcoxon).
Results: 32 women and 27 men were diagnosed with a mean age of 45.7 ± 16.2 years and a mean age at onset of 35.8 ± 14.8 years. We found no gender prevalence and an inverse correlation between size of mutant CAG repeat sequence and age at onset, $r = -0.58$, $r^2 = 33.6$, Pearson's correlation coefficient $p = 0.0008$.
Juvenile HD in this series of patients was higher than previously reported (16.6% vs. <10%). The mean CAG repeat in the expanded allele was 45.1. The number of CAG repeats in Argentinean controls was 17.8, which is similar to the literature of the European population.
Conclusions: This is the first series of Argentinean HD patients with demographic, clinical and molecular data. Our findings appear similar to the ones described in Western European populations.

1. Introduction

Huntington's Disease is an autosomal dominant and progressive neurodegenerative disease characterized by involuntary movements, cognitive impairment, and neuropsychiatric symptoms [1]. HD is caused by the expansion of an unstable CAG triplet in *HTT* gene located on the distal arm of chromosome 4 at 4p16.3. This gene encodes a large protein called huntingtin (HTT), which is involved in several cellular functions, including vesicle trafficking, energy production and transcription [2–4].

Recently, HD has been proposed as an interesting model of monogenic disease for the exploration of the mechanism of this disease and new therapeutic pathways [5].

In normal individuals, the number of CAG repeats averages between 17 and 20 units. Alleles with <26 CAG repeats are normal, whereas those with repeats between 27 and 35 are classified as intermediate or normal meiotically unstable alleles [1,5]. Alleles with 36–39 CAG repeats have a reduced penetrance and often a later onset; those with ≥40 CAGs are at risk for HD with 100% penetrance [4,5]. Approximately 99% show heterozygosis [5,6].

Classical studies have shown an inverse correlation between the length of the CAG repeat and the age at onset. However, the length of the CAG repeats accounts for only 50–70% of the variance in age of onset and does not provide information about the initial symptoms or the course or duration of the disease [7]. These findings suggest that the residual variance could be represented by other modifying genes or environmental factors [7–11].

Population studies have suggested that the mutation originated in Europe and then spread around the world through migration [12,13]. In consequence, prevalence of the disease varies in different populations. In individuals with European ancestry the prevalence of HD has been estimated to be 10-fold higher (3–10 affected

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persons per 100,000) than non-European descents (0.11–0.45 per 100,000 in Japan and <0.01 per 100,000 in South African blacks) [5,14–17].

In Latin American countries, HD shows a wide range of prevalence, with 4/100,000 in Mexico City [18] and 1/200,000 in the general population of Venezuela [7]. However, two widely known clusters of HD have a striking geographic prevalence: one of them in Indian-White inhabitants from the Valley of Cañete in Peru [19,20] and the other on the western coast of Lake Maracaibo, Zulia State, Venezuela (1/143 and 1/23,000, respectively) [7,8].

In Argentina, to date there is scarce information on HD [21]. The aim of the present study was to carry out a comprehensive analysis of demographic, clinical and genetic characteristics of HD patients from Argentina. We also compared our findings with those reported in different world populations.

2. Methods

A total of 59 consecutive outpatients, originating from various regions of Argentina were enrolled at the movement disorder department of the Institute of Neuroscience Buenos Aires (INEBA) between June 2003 and December 2010. Our department is one of 5 Argentinean centers with neurological training in HD mentioned by the International Huntington Disease Association.

Patients were included in the order they attended our institution. Initially they were evaluated at the general neurology department and then referred to the movement disorders department. The sample was composed by individuals with a positive family history of HD and with motor, cognitive or psychiatric signs or symptoms suggestive of HD or molecular diagnosis of HD. For those individuals without a molecular diagnosis, inclusion criteria were: a positive family history with at least one relative with positive genetic testing for HD; motor, cognitive or psychiatric signs or symptoms suggestive of HD; and an extensive negative battery of tests excluding other similar conditions. Written informed consent was given in all cases. The study was approved by the Institutional Review Board.

The family history was considered positive when at least one of the parents had an established diagnosis of HD. Onset was defined as the age at which behavioral, cognitive, psychiatric or motor abnormality were first reported by the patient, family, caregiver or identified at the medical records.

Comprehensive interviews and neurological examination were performed by movement disorder neurologists (EMG, JLE). Premanifest individuals were defined as carriers of the gene in absence of unequivocal HD signs, determined by a movement disorders specialist (EMG, JLE).

The $(CAG)_n$ length was determined after PCR amplification of genomic DNA isolated from peripheral white blood cells. Genetic analysis for HD was carried out in 43/59 probands and in 90 healthy controls.

The control series was recruited through volunteer donors at a local blood bank; it was composed by 90 healthy subjects who had no family history or clinical signs suggestive of HD or other neurological disorders.

Statistic analysis was conducted using G-Stat 2.0 and non-parametric tests (Wilcoxon). Correlation was calculated using Pearson-Chi-square correlation test. A $p \leq 0.05$ was considered statistically significant.

3. Results

This sample included 59 Caucasian individuals with diagnosis of HD. Nineteen patients did not have other family members included in this series, and forty patients belonged to 15 unrelated families (of these, 11 families had 2 affected members each, 3 had 3 affected individuals, and 1 had 9 affected individuals). The overall mean age was 45 ± 16 years (range 11–72 years) with 35 ± 14 ys mean age of onset (range 4–63); 10 patients had infantile or juvenile HD (Table 1). A paternal inheritance was identified in 25 subjects, 6 of them had juvenile HD (45.4% and 3.5%, respectively), maternal transmission in 30 individuals (50.8%), and parental transmission was unknown in 4 individuals (6.8%).

Eighty-three percent of HD patients were of Western European ancestry (45.6% of them from Spain); other ancestry included: Mestizo (mixed white and Amerindian ancestry) 1.7%, Arab or East Asian heritage 1.7%, Amerindian 0% and non available in 13.6%.

Motor symptoms of onset were identified in 19 cases (32.2%), including: chorea, dystonia, tics and parkinsonism. Psychiatric or behavioral symptoms were identified as initial symptoms in 38 patients (64.4%), and behavioral disorders included: apathy, depression, irritability and anxiety. Two patients were classified as "premanifest"; suicidal ideation was detected or reported in 2 patients, while 3 patients reported alcoholism.

Table 1
Demographic data of HD patients enrolled in the study.

	HD	Controls
N° of patients	59	90
Range age	11–72	18–60
European ancestry	83%	80%
Mean age at onset	35.82 ± 14.78 ys	N/A

Abbreviations: HD: patients with Huntington's Disease. N/A: not applied. ys: years.

Genetic analysis for HD was carried out in 43 probands (16 from Buenos Aires City and the remainder from at least 7 different states of Argentina,). In 16 patients genetic test was not available due to social (economic) reasons, were under the age of 18 years, or refused to undergo molecular genetic testing at the moment of the present study. Nevertheless, in all these patients HD diagnosis was supported by family history, by motor, psychiatric or cognitive symptoms, or by signs suggestive of HD, and all reported a molecular positive study in one relative (nine cases showed a positive genetic test in one parent, 4 in a grandmother, 2 cases in a son, and 1 in a sister).

Although molecular analysis was performed in 43 HD participants, in 6 of them molecular data concerning unexpanded allele was not available. All HD patients were heterozygote.

The mean expanded CAG repeat was 45.1 (range 36–80 repeats), while the mean number in the unexpanded HD allele was 18.9 (range 15–24). There was a significant inverse correlation between age of onset and expanded CAG repeats $r = -0.58$, $r^2 = 33.6$, for Pearson's correlation coefficient $p = 0.0008$ (Fig. 1).

Only 18.5% of the pooled normal alleles in HD had ≥21 CAG repeats. We failed to demonstrate a correlation between normal allele in HD patients and age of onset.

The mean CAG length of the expanded allele in patients with paternal inheritance was longer than patients with maternal inheritance, but this difference was not statistically significant (Wilcoxon, $p = 0.34$, with median CAG repeat length of 45 vs. 43, respectively) (Fig. 2).

Intermediate alleles (range between 29 and 35) were not found in this series. Three cases evidenced 36-39 CAG repeats with an incomplete penetrance; one of these cases was still asymptomatic at the age of 72 years.

Eight (8) patients had a juvenile onset (age of onset 10–20 ys) and 2 had infantile onset (age of onset <10 ys). Six (6) patients had paternal transmission, 3 maternal transmission, and in 1 patient

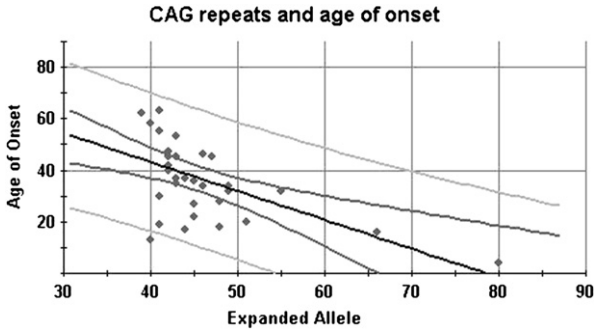

Fig. 1. Age at onset and CAG correlation: Significant inverse correlation between age of onset and expanded CAG repeats $r = -0.58$, $r^2 = 33.6$, for Pearson's correlation coefficient $p = 0.0008$.

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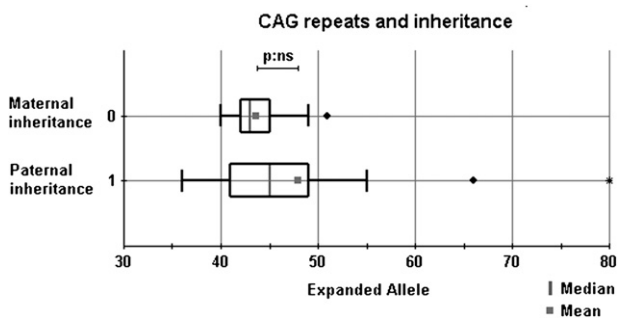

Fig. 2. Parental inheritance and CAG repeats: Paternal and maternal inheritance of CAG repeats expansion. | correspond to media and □ correspond to mean.

parental transmission could not be determined as he was adopted. Infantile or juvenile onset represented 16.9% of our HD series, with molecular studies performed in 7 of these cases. Mean CAG repeat length obtained was 53 ± 15 (range 41–80), with a mean age of onset of 15 ± 5 ys (range 4–20 ys old).

Two young patients were siblings and developed hypokinetic and bradykinetic motor syndrome (Westphal variant) associated with learning difficulties at school. The remaining patients showed epileptic syndrome (2 cases) or behavioral, psychiatric and obsessive compulsive disorders (9 cases).

All control individuals showed a non-elongated CAG repeat (number of CAG_n ≤26). The average CAG repeats in normal chromosomes from our 90 controls was 17.8 (range 12–26), 85% of them with <20 CAG repeats.

Finally, although treatment was not systematically assessed in this study, most patients were managed with Coenzyme Q10 (42 individuals) as a neuroprotective approach. Olanzapine and amantadine were employed for abnormal hyperkinetic movement disorders. Additional treatments included antidepressants, mainly serotonin reuptake inhibitors. Tetrabenazine is not commercially available in Argentina.

4. Discussion

In the present study we report a demographic, clinical and molecular comprehensive data analysis in a series of HD patients from Argentina.

In agreement with previous reports in the literature, the present study showed a similar prevalence in both genders as well as an inverse correlation between the number of CAG repeats in the expanded allele and age of onset [1,6]. This observation was supported by data showing that the average of expanded CAG repeat length was 45.1 in overall HD individuals, while the juvenile variant was 53.

We failed to demonstrate an interaction among normal and expanded alleles in HD age of onset.

There is extensive information concerning HD in some clusters from Latin America, such as in the Maracaibo or Cañete populations [7,8,19,20]; however, reports from other countries of the region are scarce. At present, in Argentina there are no studies determining the prevalence of HD patients.

The carrying out of epidemiological studies represents a major challenge in our region. The assessment of the average CAG tract size in general populations has been suggested as an indirect method to estimate the prevalence of HD. A larger average CAG tract size was associated with an increase of CAG instability and a higher risk of HD in terms of prevalence [14,22,23].

The worldwide average control CAG tract size in the general population is 17–20 repeats [22]. When our data were compared with different populations from around the world, we found that normal CAG repeat length in the Argentine control group was longer than those found in Africa (mean = 16.2) [17], Japan or China, but were smaller than in Mexican populations. Perhaps not surprisingly, controls in the Argentine population demonstrated an identical CAG repeat length as European controls (17.82 vs. 17.8) [14] (Fig. 3).

Based upon the aforementioned hypothesis, we can speculate that the mean CAG length of repeats (17.8) in our control group may suggest, in terms of instability, a similar prevalence to European countries [14] and a higher prevalence than in Asian or Black African populations.

Interestingly, ≅80% of the Argentinean population is of European ancestry [24], most of them from Spain and Italy. Therefore, it is not surprising that 45.58% of HD patients in the present study reported Spanish ancestry, when considering the high prevalence of HD in Spain. Nevertheless, we can not exclude other additional genetic, geographical or environmental factors that may influence HD prevalence in this population.

On the other hand, 64.4% of patients in our series showed psychiatric and behavioral symptoms of onset. These non-motor symptoms were recently identified as a very early expression of the disease, with occurrence years before HD diagnosis. This observation provides a new venue for exploration in premanifest individuals [25–28]. Early detection of these symptoms could favor an early introduction and monitoring of potential disease-modifying therapies [29].

The occurrence of juvenile HD in this series of patients was higher than previously reported (16.6% vs ≅10%). Nevertheless, clinical presentation was similar to other international series that included Parkinsonism, epilepsy, behavior disturbances and learning difficulties at school. In this group paternal transmission was determined in >60% of cases, with a slightly lower percentage than previously detected in other populations. These findings, however, should be analyzed with caution, taking into consideration institutional bias and the small sample size.

This study constitutes the first approach to describe the epidemiological, clinical and molecular characteristics of HD in our population as well as the first to indirectly estimate the prevalence of the disease in Argentina.

Recently, it has been hypothesized that different HTT haplotypes (A, B and C) provide an explanation for different geographic

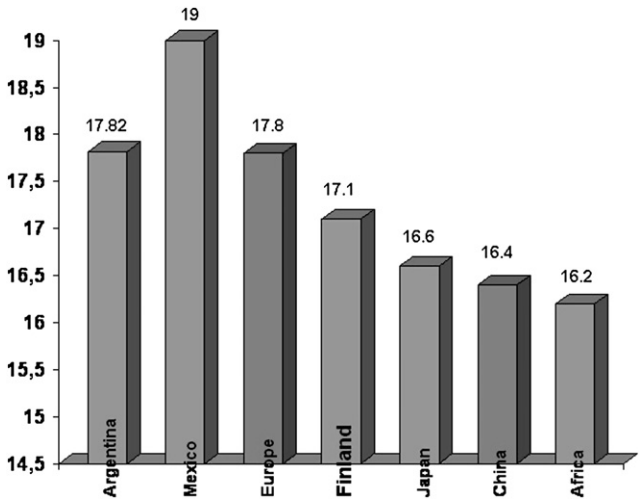

Fig. 3. CAG length in healthy populations: Distribution of normal CAG repeats length in the controls group around the world. The range observed is 16.2–19, Argentina present a CAG repeats mean of 17.8 similar to Europe.

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mutation rates and prevalence, with a majority of haplogroup A in HD patients from European countries [14]. In the near future, it may be worth looking at the three major haplotypes in our population as an additional assessment for the risk factor for HD.

More extensive studies of our largely European ancestry population, as well as comparative studies between Argentinean and European populations, are still needed to be able to identify potential modifying factors for age of onset and phenotype expression of HD.

In conclusion, in the advent of newer promising treatments, it will be helpful to gain access to larger study populations from multiple areas of the world including Argentina and other South American countries.

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References

[1] Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 2011;10(1):83–98. Jan.
[2] Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 2010;90:905–81.
[3] Renna M, Jimenez-Sanchez M, Sarkar S, Rubinsztein DC. Chemical inducers of autophagy that enhance the clearance of mutant proteins in neurodegenerative diseases. J Biol Chem 2010;285:11061–7.
[4] Roze E, Saudou F, Caboche J. Pathophysiology of Huntington's disease: from huntingtin functions to potential treatments. Curr Opin Neurol 2008;21:497–503.
[5] Johnson CD, Davidson BL. Huntington's disease: progress toward effective disease-modifying treatments and a cure. Hum Mol Genet 2010;19(Review Issue 1):R98–102.
[6] Ross CA, Shoulson I. Huntington disease: pathogenesis, biomarkers, and approaches to experimental therapeutics. Parkinsonism Relat Disord 2009;15(S3):S135–8.
[7] Paradisi I, Hernández A, Arias S. Huntington disease mutation in Venezuela: age of onset, haplotype analyses and geographic aggregation. J Hum Genet 2008;53:127–35.
[8] Wexler NS, Lorimer J, Porter J, Gomez F, Moskowitz C, Shackell E, et al. U.S.-Venezuela Collaborative Research Project (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci 2004;101:3498–503.
[9] Gusella JF, MacDonald ME. Huntington's disease: the case for genetic modifiers. Genome Med 2009;1:80.
[10] Aziz NA, Jurgens CK, Landwehrmeyer GB, van Roon-Mom WMC, van Ommen GJB, Stijnen T, et al. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. Neurology 2009;73:1280–5.
[11] Nørremølle A, Budtz-Jørgensen E, Fenger K, Nielsen JE, Sørensen SA, Hasholt L. 4p16.3 haplotype modifying age at onset of Huntington disease. Clin Genet 2009;75:244–50.
[12] Harper PS. The epidemiology of Huntington's disease. Hum Genet 1992;89:365–76.
[13] Andrew SE, Hayden MR. Origins and evolution of Huntington disease chromosomes. Neurodegeneration 1995;4:239–44.
[14] Warby SC, Visscher H, Collins JA, Doty CN, Carter C, Butland SL, et al. HTT haplotypes contribute to differences in Huntington disease prevalence between Europe and East Asia. Eur J Hum Genet 2011;19:561–6.
[15] Chang CM, Yu YL, Fong KY, Wong MT, Chan YW, Ng TH, et al. Huntington's disease in Hong Kong Chinese: epidemiology and clinical picture. Clin Exp Neurol 1994;31:43–51.
[16] Nakashima K, Watanabe Y, Kusumi M, Nanba E, Maeoka Y, Nakagawa M, et al. Epidemiological and genetic studies of Huntington's disease in the San-in area of Japan. Neuroepidemiology 1996;15:126–31.
[17] Hayden MR, MacGregor JM, Beighton PH. The prevalence of Huntington's chorea in South Africa. S Afr Med J 1980;58:193–6.
[18] Alonso ME, Ochoa A, Boll MC, Sosa AL, Yescas P, López M, et al. Clinical and genetic characteristics of Mexican Huntington's disease patients. Mov Disord 2009;24:2012–5.
[19] Cuba JM, Torres L. Genealogic trees of families with Huntington's chorea in Cañete (Peru). Rev Neurol 1989;145:482–4.
[20] Velez M, Cosentino C, Torres L. Huntington disease in Perú. Mov Disord 1998;13:221.
[21] Gatto E, Micheli F, Fernández M, Casas Parera I, Giannaula R. Análisis de una serie clínica: corea de Huntington. Evaluación Clínica. Rev Chil Neuro-Psiquiat 1994;32:127–8.
[22] Falush D. Haplotype background, repeat length evolution, and Huntington's disease. Am J Hum Genet 2009;85:939–42.
[23] Warby SC, Visscher H, Butland S, Pearson CE, Hayden MR. Response to Falush: a role for cis-element polymorphisms in HD. Am J Hum Genet 2009;85:942–5.
[24] Quiroga Micheo E, Vilaseca AB, Bonder MC, Quiroga Vergara ER. Frecuencia de los grupos sanguíneos y análisis de la progresiva disminución del factor Rh negativo en la población Argentina. Revista Medicina 1988;48:355–60.
[25] Tabrizi SJ, Langbehn DR, Leavitt BR, Durr A, Roos RA, Leavitt BR, et al. Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet Neurol 2009;89:791–801.
[26] Paulsen JS, Langbehn DR, Stout JC, Aylward E, Ross CA, Nance M, et al. Predict-HD investigators and coordinators of the Huntington Study Group. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. J Neurol Neurosurg Psychiatry 2008;79:874–80.
[27] Paulsen JS, Wang C, Duff K, Barker R, Nance M, Beglinger L, et al. PREDICT-HD investigators of the Huntington Study Group. Challenges assessing clinical endpoints in early Huntington disease. Mov Disord 2010;25:2595–603.
[28] Stout JC, Paulsen JS, Queller S, Solomon AC, Whitlock KB, Campbell JC, et al. Neurocognitive signs in prodromal Huntington disease. Neuropsychology 2011;25:1–14.
[29] Gagnon KT. HD therapeutics – CHDI fifth annual conference. Drugs 2010;13:219–23.

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