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HUNTINGTON DISEASE

DNA ANALYSIS IN BRAZILIAN POPULATION

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ABSTRACT - Huntington disease (HD) is associated with expansions of a CAG trinucleotide repeat in the HD gene. Accurate measurement of a specific CAG repeat sequence in the HD gene in 92 Brazilian controls without HD, 44 Brazilian subjects with clinical findings suggestive of HD and 40 individuals from 6 putative HD families, showed a range from 7 to 33 repeats in normal subjects and 39 to 88 repeats in affected subjects. A trend between early age at onset of first symptoms and increasing number of repeats was seen. Major increase of repeat size through paternal inheritance than through maternal inheritance was observed. Data generated from this study may have significant implications for the etiology, knowledge of the incidence, diagnosis, prognosis, genetic counseling and treatment of HD Brazilian patients.

KEY WORDS: Huntington disease, CAG repeats, Brazil, DNA, PCR.

Doença de Huntington: análise de DNA na população brasileira

RESUMO - A doença de Huntington (DH) está associada a expansões da seqüência repetitiva de trinucleotídeos CAG no gene HD. Através de análise do número de repetições CAG em indivíduos brasileiros, amostras de 92 indivíduos-controle não afetados pela DH, 44 pacientes com DH e 40 indivíduos de 6 famílias com a DH, demonstrou-se a presença de repetições de 7 até 33 trinucleotídeos CAG nos indivíduos-controle e de 39 até 88 nos alelos mutados dos indivíduos afetados. Foi constatada relação inversa entre a idade de manifestação dos primeiros sintomas da doença e o tamanho do fragmento encontrado. Também foi observado que o número de casos em que ocorrem expansões do trinucleotídeo foi maior quando o pai transmite o alelo mutado do que quando a mãe o transmite. Os dados gerados com este estudo têm importância significativa para a compreensão da etiologia, incidência, diagnóstico, prognóstico e tratamento dos pacientes com DH na população brasileira. O conhecimento da base genética desta doença na população brasileira permitirá um aconselhamento genético mais eficiente às famílias em risco.

PALAVRAS-CHAVE: doença de Huntington, repetições CAG, Brasil, DNA, PCR.

Huntington disease (HD) is a progressive, neurodegenerative disorder that presents with motor disturbances, psychiatric symptoms, and cognitive decline[1,2]. The mutation associated with clinical manifestations of the disease is an unstable CAG trinucleotide expansion (runs of glutamine in the gene product) in the first exon of a novel gene (called HD gene) located on 4p16.3[3]. The HD gene, which spans about 200 kilobases[3], encodes two distinct transcripts, the protein huntingtin of 10,3 and 13,6 kilobases[4], but its function is still unknown. Such mechanism of mutation has been shown to cause a number of other genetic disease such as myotonic dystrophy[5], dentatorubral-pallidoluysian atrophy[6], spinocerebellar ataxias[7], spinal and bulbar muscular atrophy[8], and fragile X syndrome[9].

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The American College of Medical Genetics/American Society of Human Genetics/Huntington Disease Genetic Testing Working Group[10] divided the genotype/phenotype correlation in 4 categories for CAG-repeat lengths in the exon 1 of the HD gene: normal allele (less than 27 CAG repeats, generating a normal phenotype), intermediate allele (27-35 CAG repeats, mutable normal allele generating a normal phenotype), HD allele with reduced penetrance (36-39 CAG repeats, generating a normal or HD phenotype), and HD allele (more than 39 CAG repeats, generating a HD phenotype). Alleles sized up to 26 CAG repeats have never been associated with a HD phenotype and have not been demonstrated to show mutability. Alleles of 27-35 CAG repeats have demonstrated meiotic instability when transmitted by a male. Allele sizes of 36-39 repeats have been associated with HD phenotype in some cases documented clinically and pathologically. However, these alleles have been reported in some clinically unaffected subjects aged > 70 years[11].

It is difficult to define the smallest size of the CAG repeat expansion, which leads to the disorder. In most studies 37 repeats where reported as the lowest CAG repeat associated with HD on European descendant. The absence of HD pathology has not been documented in any individual with a HD allele size larger than 40 repeats[10]. The CAG length has a significant impact on age of onset[12], defined as the first time at which a patient had either neurological or psychiatric symptoms that represented a permanent change from the normal state. The mean age of onset is 35 to 44 years, the median survival time is 15 to 18 years and the average age at death is 54-55 years[1,12]. About 10% of patients with HD have juvenile onset before age 20 years[1] and 5% before 14 years old[13], whereas late onset (at >50 years of age) occurs in ~20%[14]. The CAG trinucleotide expansion is unstable during transmission from parents to offspring and it has been shown that the repeat length can expand during spermatogenesis. HD anticipation (offspring having symptoms at earlier ages of onset that their affected parents) is more intense in paternal transmission, that is, offspring of affected fathers on average have both longer repeat lengths and earlier ages of onset that offspring of affected mothers[15].

HD occurs with variable prevalence rates in different parts of the world with most countries having rates between 5-10 affected persons per 100,000[1,2]. In Japan and Africa however, there is remarkably, a 10 times reduced prevalence rate[16,17]. The lowest frequencies have been found in South African Blacks, with 0.01 affected person per 100,000, that is, 500-1,000 times lower than the worldwide prevalence, but this is probably an underestimate, because the authors used only 11 documented African cases to calculate it[17]. A study in blacks from North America showed a prevalence of 0.97 per 100,000 persons, about one-fifth the prevalence for Caucasoid patients with HD in the same population[18]. It has been showed that there is a relation between incidence of HD and the median size of normal CAG repeat in different populations. The larger the median size of normal CAG repeats, the higher is the incidence of HD[19,20].

The incidence as well as the genetic basis of HD in Brazil is unknown. Therefore we decided to use direct Polimerase Chain Reaction (PCR) from whole blood to genotype three groups of individuals: a) 92 healthy subjects older than 50 years old from the normal Brazilian population with no family history of HD, b) 44 unrelated HD subjects and c) 40 persons of 6 HD families.

METHOD

Ten microliters of whole blood in EDTA were collected from 92 Brazilian normal healthy controls (being 50 Caucasoids and 42 African-Brazilians), from 44 Brazilian subjects with clinical findings suggestive of HD, and 40 individuals from 6 HD families (Fig 1).

Neurologists, psychiatrists or both have examined the 40 individuals from the 6 HD families and the 44 subjects with HD. Information of sex and age at onset of first symptoms were recorded for each patient.

The age at onset of first symptoms for the 44 individuals with HD was: 1 patient under 10 years, 1 patient between 11 and 20 years, 10 patients between 21 and 30 years, 17 patients between 31 and 40 years and 15 patients older than 41 years.

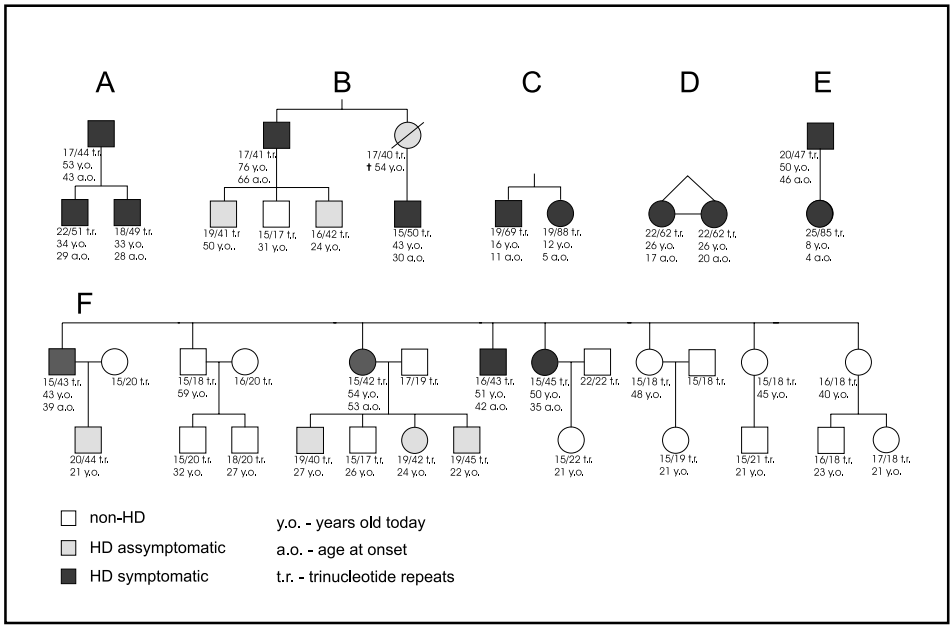

Fig 1. Families with HD.

CAG expansion was also assessed in 92 Brazilian control individuals older than 50 years old, without any signs, symptoms or family history of any neuropsychiatric disorder. They were 50 Caucasoids and 42 African-Brazilians, 64% male and 36% female. All of them were living in the Brazilian state of Paraná, but 60% were born in Paraná, 14% in the state of Minas Gerais, 8% in the states of Rio de Janeiro and Bahia, 4% in the state of São Paulo, and 2% in the states of Espírito Santo, Santa Catarina and Rio Grande do Sul.

Genomic DNA was extracted from leukocytes by standard procedures[21]. The primers used for these assay were: Hu1 5'-ATGAAGGCCTTCGAGTCCCTCAAGTCCTCC-3' and HD3 5'-GGCGGTGGCGGCTGTTGCTGCTGCTGCTGC-3'[22]. These sequences immediately flank the CAG repeat sequence and amplify specifically the CAG repeats, avoiding the downstream CCG polymorphism, and therefore avoiding errors in HD diagnostic test[12]. PCR was performed as described previously[23]. Sequencing standards were selected to allow exact determination of the size of the PCR product, including an M13 sequencing ladder and appropriate normal and abnormal controls whose CAG repeats have been sequenced independently.

Because of ethical implications of the results, all tests were done after a genetic counseling session and informed consent. The normal control group was tested without knowledge of any information that could be used to further identify the individuals, in accordance to the suggestions of the International Huntington Consortium Regulations[10].

RESULTS

A) Control subjects

The normal CAG repeat spectrum in the Brazilian population sample studied, as determined by the study of 184 chromosomes from 50 Caucasoids and 42 African-Brazilians normal control subjects, ranges from 7 to 33 trinucleotides in the Caucasoid sample and from 13 to 30 in the African-Brazilian sample (Table 1). Repeats with 17 triplets (37%) and 15 triplets (28.6%) were found at a peak frequency, while the mean was 17,7 and 17,9 CAG repeats in the Caucasoid and African-Brazilian samples respectively, (Fig 2).

A total of 8 persons with intermediate alleles (range 27-35 CAG repeats) were identified, being 4 Caucasoids and 4 African-Brazilians.

Table 1. Distribution of the number of CAG repeats in control subjects and in patients with HD, according to national or ethnic group.

National or ethnic group	Nº of subjects			References
		Non-HD hromosomes Nº of repeats		
Control subjects		median	range	
Brazilian Caucasoids	50	17	7-33	This study
Great Britain	32	18.5	12-35	25
Italy	29	19	15-29	25
Norway	10	19.5	17-27	25
Scotland	20	19	14-25	25
Sweden	103	19	11-29	25
South African	112	17	11-29	25
African-Brazilians	42	17	13-30	This study
		HD chromosomes Nº of repeats		
Patients with HD		median	range	
Brazil	44	45	39-88	This study
Netherlands	28	43	37-59	25
England	118	44	38-63	25
France	45	44	36-100	25
Germany	46	44	40-65	25
Great Britain	74	44	39-121	25
Ireland	43	42	39-52	25
Italy	52	44	39-54	25
Norway	23	46	39-71	25
Russia	22	41	37-47	25
Scotland	91	43	38-71	25
Sweden	103	43	38-88	25
Canada	35	45	36-75	25

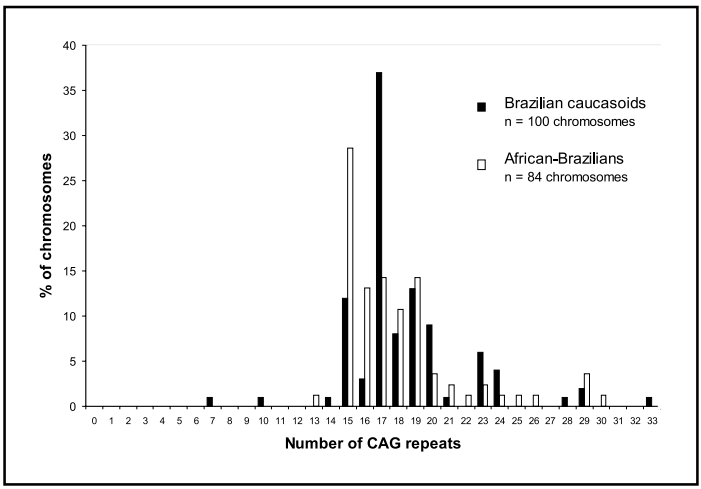

Fig 2. Number of CAG repeats and % of chromosomes in Caucasoids and African-Brazilians control subjects.

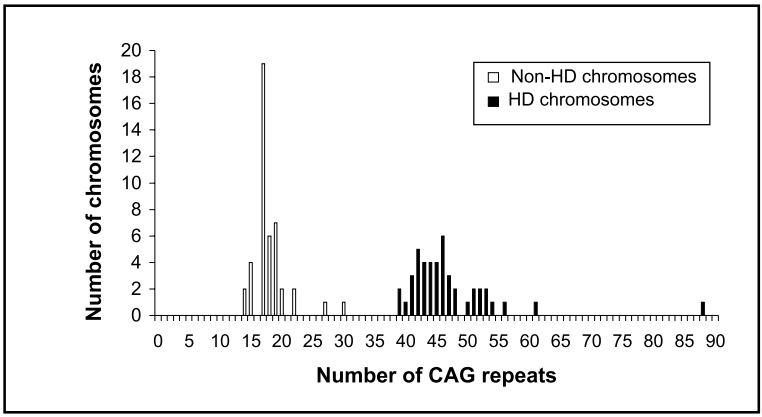

Fig 3. Number of CAG repeats x number of chromosomes in HD patients.

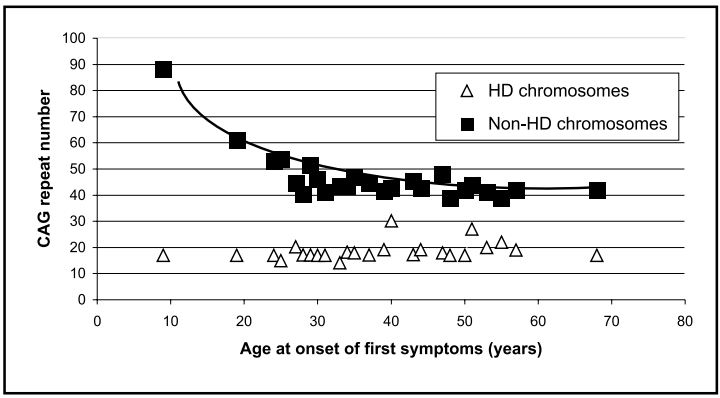

Fig 4. CAG repeat number x age at onset of first symptoms in years, from affected subjects, showing inverse correlation in HD chromosomes. Some of the colored squares represent more than 2 patients.

B) HD patients

In all 44 HD patients the clinical diagnosis was confirmed by PCR analysis. Expanded CAG repeats ranged from 39 to 88 (mean 46.7 and median 45) and the normal CAG repeats from these patients ranged from 14 to 30 (mean 18 and median 17) (Fig 3).

Figure 4 shows the relationship between age at onset of first symptoms and the CAG repeat size in HD alleles in Brazilian affected subjects. An increase correlation for the age at onset of first symptoms and the number of CAG repeats was detected (Table 4).

C) Families with HD

DNA analysis was carried out in 40 individuals who were members of 6 HD families (Fig 1 - Families A-F). Fifteen were HD patients (symptomatic), 13 were their relatives at 50% risk to develop this disease, 6 were their relatives at 25% risk to develop this disease, and 5 were married to a HD patient or to a person at 50% risk to develop HD. Results showed an expanded CAG repeat in all 15 HD patients tested and in 6 of their 13 first-degree relatives (46,1%), previously at a 50% risk (Fig 4). The age of these asymptomatic family members who inherited one HD chromosome range from 21 to 50 years (median = 23/24 years, n = 6).

CAG analysis showed that the expanded repeat in families with HD ranged between 40 and 88 (mean = 50.7 and median = 44/45), and normal chromosomes ranged between 15 and 25 (mean = 21.2 and median = 18).

The analysis of 4 of these 6 families resulted in molecular data from 6 HD father-child pairs and 4 HD mother-child pairs affected with HD (Tables 2 and 3).

Table 2. CAG repeat instability during 6 paternal and 4 maternal transmissions in 3 Brazilian HD families.

	Transmission	
	Father-child (n=6)	Mother-child (n=4)
Contract	0	1
Stable	1	1
Expansion	5	2

Table 3. Comparison of the CAG repeat instability during paternal transmission.

Transmission	Expansions/total
*HD families of Brazilian origin	5/6
**Late onset causes from HD families of varied ethnic origin	8/10
**HD families from various parts of Greece	7/9
**HD families of varied ethnic origin	8/10
**HD families of varied ethnic origin	32/37
**HD families of Dutch origin	10/16
**HD families of Scottish origin	21/31
**HD families of Italian origin	18/19

* This study; **In Tzagournissakis et al., 1995[27].

Table 4. Median age at onset of first symptoms in HD patients.

CAG repeat size	Median age at onset in years*	Age at onset in years**
	N=886[35]	N=35
39	66 (72-29)	55/48
40	59 (61-56)	28
41	54 (56-52)	53/39/31
42	49 (50-48)	68/57/50/44/40
43	44 (45-42)	44/34/33
44	42 (43-40)	51/40/37/27
45	37 (39-36)	43/40
46	36 (37-35)	44/40/39/34/33/30
47	33 (35-51)	37/35
48	32 (34-30)	47/40
49	28 (32-25)	—
50	27 (30-24)	34

*Age by which 50% of individuals will be affected[27]. **Due to our small sample size, we decided not to include the median ages, but the age of onset of each HD patient.

DISCUSSION

A) Control subjects

Table 1 shows the distribution of the CAG repeats in the normal Brazilian sample. The frequency of intermediate alleles (meiotic unstable alleles) in the Brazilian Caucasoid general population is 4% (4/100) of chromosomes, therefore one in each 12 persons in the general Brazilian Caucasoid population may have an intermediate allele. This result is higher than the literature reported, where the frequency of intermediate alleles on normal Caucasoids chromosomes is 0.93%, one in each 50 persons, as showed in the general Canadian population[24]. The frequency of intermediate alleles in the African-Brazilian general population is 4.7% (4/84) of chromosomes, therefore one in each 10 persons in the general African-Brazilian population may have an intermediate allele. This result is also higher than the ones reported in the literature, where no intermediate allele was found in normal chromosomes by Almqvist et al.[20] and Squitieri et al.[19], and 12/1595 were found by Kremer et al.[25] in the South-African population. In the African-Brazilian control population the CAG repeat number mean (17.9) is higher than in African populations (15.9[20] and 16.2[19]). Although this is in accordance to studies of African-Americans showing that they have a significant higher prevalence inherited alleles than Africans[18], one has to be very careful comparing this means, considering the small sample analyzed. Even though, these two findings together suggest that the incidence of HD in the African-Brazilian population may be higher than in other African-descendant populations. One explanation could be the known higher rates of admixture between African-Brazilians and Brazilian Caucasoids that could have brought larger and more intermediate alleles to the African-Brazilian gene pool. Further studies are in progress to enlarge the normal population sample to confirm this initial finding.

B) HD patients

Among the affected patients no differences were found in CAG alleles frequencies from Brazilian subjects when compared to different national and ethnic groups (Table 1).

In the Brazilian HD patients studied, the lowest size associated with disease was 39 repeats and the highest 88.

The patient which HD chromosome had 88 CAG repeats, manifested HD with 9 years old. The age of onset of first symptoms of this patient was not surprising when compared with other studies of juvenile onset forms of HD[26], and strength the current knowledge that the higher the number of CAG repeats, the earlier will be the age of onset.

C) Families with HD

Although we studied a small sample of parents/sibling pairs, the molecular data obtained from 6 HD father-child pairs and 4 mother-child pairs affected with HD suggest an increase of CAG repeat size on paternal but not always on maternal transmission (Table 2). The instability of the CAG repeat during paternal transmission in this sample is similar to that observed in most HD families studied worldwide (Table 3).

Two siblings, clinically followed by one of us (M.S.H.) (Family C in Figure 1), manifested HD with 5 and 11 years and their HD chromosomes have 88 and 69 CAG repeats respectively. The age of onset of first symptoms of these patients is now understandable, when compared with other studies showing correlation of large repeats with early age of onset and juvenile onset forms[26]. This two cases as well as the other 9 years old case described above with the HD patients, show that the general suggestion, to avoid testing asymptomatic minors for HD, should take in account that the disease may manifest in individuals with less than 21 years old.

It is also interesting to note the case of identical twin sisters, clinically followed by one of us (G.L.) (Fig 1 D) which have the same CAG repeat numbers (22/62) on the HD gene, but slight

different onset ages (17 and 20 years). They are monozygous twins, as proved by having both the same DNA genotypes in 9 different Short Tandem Repeats loci tested[28]. It seems by this case that there are also non-genetic factors influencing the age at onset of first symptoms of HD.

Due to absence of specific complementary tests to diagnose or to exclude HD PCR analysis of the CAG trinucleotide repeat number is the only definitive laboratorial diagnostic tool of HD, useful not only for research but also when clinical diagnosis is not quite clear.

HD occurs with different prevalence rates in different parts of the world. It has been suggested that European immigration and genetic admixture could account for the variation in prevalence rates[1]. The Brazilian population, with high rates of admixture between European descendants and African-Brazilians will be an interesting sample to learn more about the history, incidence and spread of HD.

Data generated from this type of studies may have significant implications for the knowledge of the incidence, diagnosis and prognosis in HD Brazilian patients. The aim of new therapies in HD is to slow or stop the progression of HD in affected persons and to delay or prevent onset in persons with CAG > 35 repeats. An appropriate design of clinical trials for increased-risk individuals will need to take into account the expected age at onset of first symptoms of HD for a particular person, in order to determine the potential efficacy of therapy[26]. Knowledge of the molecular basis of the disease in the Brazilian population should help families at risk and improve genetic counseling. As HD DNA test becomes routine in Brazil, all efforts must be provided so that psychiatric support is available for all persons that HD predictive testing is offered. Further studies are needed in the Brazilian population to asses the frequency of catastrophic events, such as suicide, suicide attempt and psychiatric hospitalization after receiving an HD DNA predictive test result, as it is now known that almost 1% of pre-symptomatic HD patients may have a catastrophic event after receiving the result of a predictive DNA-based test[29].

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