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Genetic features of Huntington disease in Cuban population: Implications for phenotype, epidemiology and predictive testing

Yaimeé Vázquez-Mojena [a,*], Leonides Laguna-Salvia [b], José M. Laffita-Mesa [a], Yanetza González-Zaldívar [a], Luis E. Almaguer-Mederos [a], Roberto Rodríguez-Labrada [a], Dennis Almaguer-Gotay [a], Pedro Zayas-Feria [a], Luis Velázquez-Pérez [a,**]

[a] Centre for Research and Rehabilitation of Hereditary Ataxias (CIRAH), Holguín, Cuba
[b] Department of Neurology, Clinical & Surgical Hospital, Holguín, Cuba

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ABSTRACT

Huntington disease is the most frequent polyglutamine disorder with variable worldwide prevalence. Although some Latin American populations have been studied, HD prevalence in Cuban population remains unknown. In order to characterize the disease in Cuba, the relative frequency of HD was determined by studying 130 patients with chorea and 63 unrelated healthy controls, emphasizing in the molecular epidemiology of the disease. Sixty-two patients with chorea belonging to 16 unrelated families carried a pathological CAG expansion in the HTT gene, ranging from 39 to 67 repeats. Eighty-three percent of them come from the eastern region of the country. A significant inverse correlation between age at onset and expanded CAG repeats was seen. Intermediate alleles in affected individuals and controls represented 4.8% and 3.97% respectively, which have been a putative source of de novo mutation. This study represents the largest molecular characterization of Huntington disease in the Cuban population. These results may have significant implications for an understanding of the disease, its diagnosis and prognosis in Cuban patients, giving health professionals the tools to implement confirmatory genetic testing, pre-symptomatic testing and clinical trials in this population.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the expansion of the CAG trinucleotide repeats in the first exon of the HTT gene producing a long polyglutamine tract in the huntingtin protein [1]. The disease is characterized by motor dysfunction, involuntary choreic movements, cognitive impairment and psychiatric manifestations [2]. The size of the HTT CAG repeat contributes significantly to the onset and disease phenotype [3]. In the general population, the CAG repeat length varies from 6 to 35 trinucleotides [4]. Alleles carrying <26 CAG repeats are considered normal, whereas those with 27–35 triplets are intermediate. Interestingly, some individuals carrying intermediate alleles have developed the HD phenotype [5,6]. Pathological expansions exceed 35 CAG repeats [7,8], alleles carrying 36–39 CAG repeats exhibit reduced penetrance while ≥40 CAGs are fully penetrant HD alleles [9].

HD is the most frequent polyglutamine disorder with a variable worldwide prevalence according to the ethnic background [10–12] and to the geographic differences in HTT haplotypes [12]. The prevalence in European descendants, including the United States and Canada [13], is about 5–10 per 100000 inhabitants [14]. The disease reaches higher prevalence rates in specific regions, such as Maracaibo Lake (Zulia, Venezuela), Tasmania Island (Australia) and Moray Firth (Scotia) [15] whereas considerably reduced frequencies of HD have been reported in Japan, China, Finland, South African black populations and North American black populations [13,16].

Although some Latin American populations have been studied, little is known about the prevalence rate of HD in these countries, with the exception of Venezuela [13,17]. HD prevalence in the Cuban population is unknown. In order to characterize the disease in Cuban patients, we determined the relative frequency of HD in a large group of patients with chorea, emphasizing the molecular epidemiology of the disease.

2. Methods

2.1. Subjects

A total of 130 patients exhibiting chorea (54 m; 76 f), were tested for the HTT mutation in the Centre for Research and Rehabilitation of Hereditary Ataxias (CIRAH). A control group was recruited, composed of

* Correspondence to: Y. Vázquez-Mojena, Carretera Central, km. 5½ vía Habana, CP 80100, Holguín, Cuba. Tel.: +53 24 424090.
** Correspondence to: L. Velázquez-Pérez, Reparto Edecio, Pérez, Carretera Vía Habana, CP 80100, Holguín, Cuba. Tel.: +53 24 462296; fax: +53 24 424090.
E-mail addresses: yaimeevazquez@gmail.com (Y. Vázquez-Mojena), velazq63@yahoo.com (L. Velázquez-Pérez).

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63 healthy subjects (25 m; 38 f. who had no family history or clinical signs suggestive of HD or other neurological disorders. The clinical diagnosis of patients was made by the referring neurologists. Informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee of the Center.

2.2. Molecular genetics measures

Genomic DNA was extracted from peripheral blood leucocytes using standard procedures [18]. The CAG size was estimated through PCR amplification, using primers that flank the CAG repeat tract [19,20], and polyacrylamide gel electrophoresis.

2.3. Statistical analysis

Statistical analysis was conducted using the commercially available Statistica software package (StatSoft, Inc., 2003 STATISTICA data analysis software system, version 6. www.statsoft.com). The normality of all variables was assessed through the Kolgomorov–Smirnov test. Mean comparisons were performed using the Mann–Whitney U test. Statistical significance was considered when $p < 0.05$. Correlation was assessed by the Spearman test.

3. Results

Sixty-two out of 130 (47.7%) patients with chorea belonging to 16 unrelated families carried a pathological CAG repeat expansion in the HTT gene. Among them, 83% came from the eastern region of the country, specifically from the Municipality of Banes. The HD patients' mean age of onset was 38 ± 12.56 years (range 9–65 years). All HD patients were heterozygotes for a pathological mutation.

Sixteen different alleles were identified, in the range of expanded alleles from 39 to 67 trinucleotides (mean 45.3) (Fig. 1). The distribution of expanded alleles was asymmetrical (skewness: 2.48 ± 0.38) and did not fit a normal distribution ($D = 0.24$; $p < 0.05$). Chromosomes carrying 45 repeats were the most frequent in HD patients (16.7%). Fully-penetrant alleles (>40 CAG) were observed in 98.3% of the HD mutation carriers. The longest expanded chromosomes carried 55, 67 and 59 units, corresponding to cases of infantile and juvenile HD respectively. There was a significant inverse correlation between age at onset and expanded CAG repeats ($r = -0.82$; $p = 0.000001$) (Fig. 2).

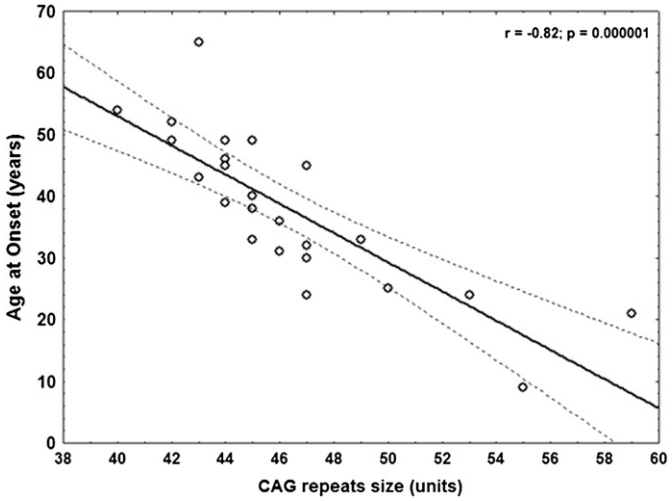

Fig. 2. Age of onset and CAG correlation: Significant inverse correlation between age of onset and expanded CAG repeats $r = -0.82$ for Spearman's correlation coefficient $p = 0.000001$.

Thirteen different normal alleles were identified in HD patients, ranging from 13 to 29 units (mean 18.4). In this series, 17.7% of the chromosomes had ≥21 CAG units and intermediate alleles represented 4.8% of the total. No correlation was demonstrated between the normal CAG repeats and the age of onset in HD patients ($r = 0.0012$; $p = 0.9955$).

No statistically significant differences were detected between the expanded allele CAG size in patients with paternal versus maternal inheritance ($p = 0.53$, with the median CAG repeat length of 46.2 ± 5.89 vs. 45.3 ± 3.41 respectively) (Fig. 3). CAG repeat instability in HD chromosomes was analyzed in seven sib-ship transmissions, four maternally and three paternally inherited. CAG size was stable in four transmissions; the remaining showed an increase in 6, 13 and 20 CAG units respectively; the last two corresponding to paternal transmission.

In order to gain insights into the CAG allele frequency at the HTT locus for the Cuban population, a series of 126 chromosomes from unaffected individuals was analyzed. All controls had unexpanded CAG repeats. We found 17 different alleles ranging from 12 to 31 CAG repeats

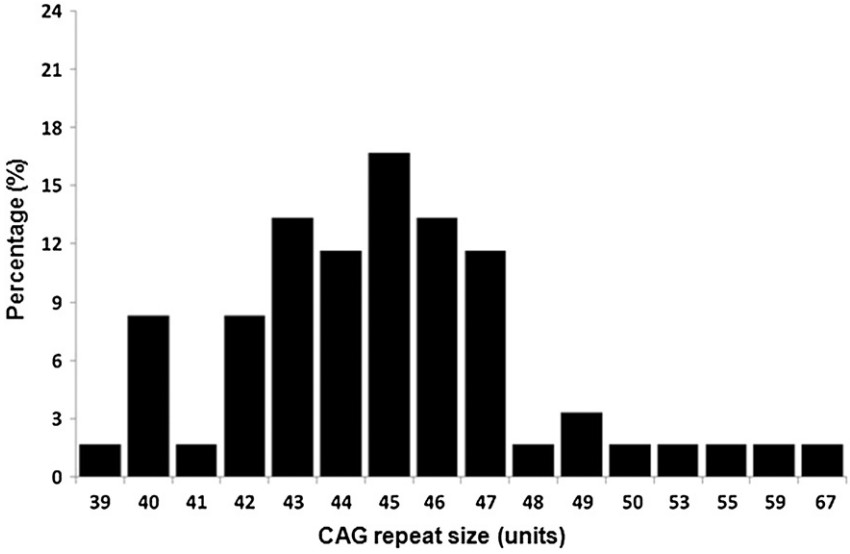

Fig. 1. Distribution of (CAG)n size of the Huntington disease gene in a Cuban population.