Molecular diagnosis of a sample of the Cuban population with spinocerebellar ataxia type 2

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INVESTIGACIÓN

ABSTRACT

CAG repeat unstable expansions causing spinocerebellar ataxia 2 (SCA2) disease were localized into a CpG island on the exon 1 of the SCA2 gene, previously linked to the chromosomic region 12q23-24.1 of human chromosome 12. At molecular level, 392 patients with dominant ataxia from Holguín, Cuba, were analyzed, showing an inverse correlation between CAG repeat length and the age of disease onset. The smallest CAG repeat expansion causing neurodegeneration among the SCA2 patients was also found. For SCA2 patients with $(CAG)_n$ stretches of 32 to 40 repeats, there is a high variability with respect to the age of disease manifestation. These findings suggest that for each patient other specific genetic and/or environmental factors are very significant for the begining and evolution of the disease for small sizes of CAG repeat expansions.

Keywords: CAG repeat expansions, disease, spinocerebellar ataxia 2

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RESUMEN

Diagnóstico molecular de una muestra de la población cubana con ataxia espinocerebelosa tipo 2. Las expansiones inestables de las repeticiones de CAG que causan la ataxia espinocerebelosa 2 (SCA2), fueron localizadas en un islote de CpG en el exón 1 del gen *SCA2*, previamente ligado a la región cromosómica del cromosoma 12q23-24.1. Se analizaron 392 pacientes de Holguín, Cuba, con ataxia dominante, y se observó una correlación inversa entre la longitud de las repeticiones de CAG y la edad de inicio de la enfermedad. Se encontró la expansión más pequeña que causa neurodegeración entre los pacientes con SCA2. Para los pacientes enfermos de SCA2 con un número de unidades entre 32 y 40 (CAG), existe una alta variabilidad en relación con la edad de inicio de la enfermedad. Estos hallazgos sugieren que, para cada paciente, otros factores genéticos y/o ambientales específicos son muy significativos en las tallas pequeñas de las expansiones de las repeticiones de CAG para el inicio y la evolución de la enfermedad.

Palabras claves: ataxia espinocerebelosa 2, enfermedad, expansiones de repeticiones de CAG

Introduction

Spinocerebellar ataxias (SCA) constitute a heterogeneous group of neurodegenerative disorders characterized by progressive neuronal loss in the cerebellum, brain stem nuclei and spinocerebellar tract [1]. Spinocerebellar ataxia 2 (SCA2) is an autosomal dominant disease mainly characterized by gait ataxia, cerebellar dysarthria, dysmetria, adiadochosinesia, cramps, tremor, hypotonia, abnormal reflexes and slowed-limited eye movements [2].

SCA2 was linked to the human chromosomic region 12q23-24.1 [3] and over the past few years large SCA2 pedigres were well identified in Italy [4], Canada [5], USA [6], Tunisia [7], Martinique [8], Japan [9], Germany [10], and China [11].

The positional cloning of the SCA2 gene was developed by three independent work groups in 1996, and the mutation responsible for the clinical features of SCA2 disease was defined as the intergenerational expansion of a CAG tract on exon 1 of the gene [1, 12, 13] contained into a region very rich in G/C [12]. This G/C rich region was defined as a typical CpG island, like it occurs for exon 1 of the Huntington's disease (HD) gene [14, 15].

Clinical diagnosis of SCAs is very difficult because their principal phenotypes overlap very much [2]. In this study, 392 patients from Holguín, Cuba, have been diagnosed for the first time at molecular level for dominant ataxia. In this province, the prevalence of the SCA2 disease was reported to be 133:100 000 [16].

Materials and Methods

Patients

A total of 392 Cuban patients with familial history of dominant ataxia were clinically examined by a specialized neurologist at Lenin Hospital in Holguín. Since 1994, a computerized data base of the phenotypes and a computerized genetic register were created at ataxia laboratories in Holguín, with the aid of a collaborative group from Düsseldorf University, Germany.

Methods

DNA was extracted according to standard phenol-chloroform protocols [17]. Polymerase chain reaction (PCR) for the detection of the SCA2 mutation was carried out using the following pairs of primers previously published: SCA2a (5'-gggcccctcaccatgtcg-3', with Tet fluorescence) and SCA2b (5'-cgggcttgcg gacattgg-3') [12], or DAN1 (5'-cgtgcgagccggtgtatggg-3') and UH10 (5'-ggcgacgctagaaggccgct-3') [1]. PCR reactions were

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developed on a Perkin Elmer 9600 thermal cycler (USA) according to the following profile: 1 cycle at 95°C for 2min, 35 cycles at 94°C for 1 min, 65°C for 1min and 72°C for 1min, and a final cycle at 72°C for 5min, using a SuperTaq-Pol kit (Perkin Elmer, USA). PCR product sizes were determined on an ABI automated sequencer 373A (Perkin Elmer, USA) using TAMRA 350 as internal size standard and the Genescan 672 software (Perkin Elmer, USA). To determine the composition of the CAG repeat, PCR products were directly sequenced on the ABI automated sequencer 373A at the laboratory of the German collaborative group at Düeseldorf University. Sequence reactions were perfomed on a Perkin Elmer 9600 thermal cycler according to the profile: 25 cycles at 96°C for 30s, 50°C for 15s and 60°C for 4min, using a Prism ready reaction Dye Deoxy Terminator kit (Applied Biosystems, USA). Data were collected and analyzed using the ABI 373A Analysis and Data collection software.

Statistical analysis

The number of CAG repeats for each patient and the age of disease onset were compiled in an Excel data base, and the Spearman correlation coefficient was calculated.

Results

CAG repeat and correlation with the age of disease onset

It was analyzed whether the size of the CAG repeat expansion might influence the age of disease onset in Cuban patients with SCA2. The age of disease onset was inversely correlated with the CAG repeat expansion ($r=-0.826, P < 0.0001$, Figure 1). Sixty seven percent of the patients with $(CAG)_{41}$ and 99% of the patients with $(CAG)_{42-79}$ presented clinical symptoms at 30 years old or younger. Two patients presented symptoms at 65 years old, harboring 35 and 37 CAG repeats, respectively. One of the longest alleles causing the SCA2 neurodegeneration was also found. A child carrying 79 trinucleotide units developed clinical manifestations at 2 years old.

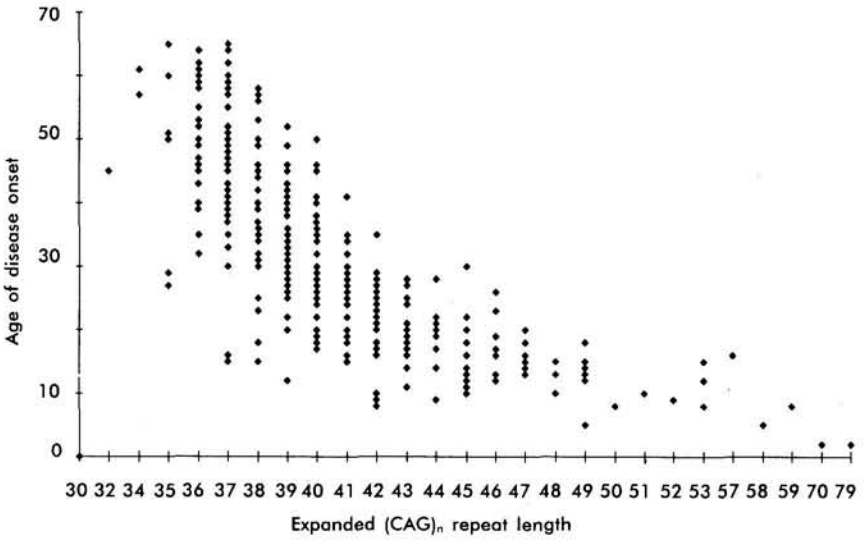

Figure 1. Relationship between the expanded $(CAG)_n$ repeat lengths in the SCA2 gene and the age of manifestation of the disease in 392 Cuban patients. The correlation coefficient was calculated: $r = -0.826$, $P < 0.0001$.

Distribution of CAG repeat expansions in the total number of affected chromosomes

Figure 2 represents the alleles causing SCA2 phenotypes in the sample of patients. The expanded alleles carried 32 to 79 codons and were found usually at single doses and without CAA interruptions. The normal alleles ranged from 13 to 30 CAG repeats, with one or two CAA interruptions. Two affected twin sisters were found with disease manifestation at 57 and 61 years old, respectively, being homozygotic for $(CAG)_{34}$ without CAA interruptions.

A 45-year-old heterozygotic female patient was found with $(CAG)_{23}$ as a normal allele and $(CAG)_{32}$ without CAA interruptions as disease allele, inherited through one contraction in $(CAG)_{40}$ allele from her affected father. This is the shortest CAG repeat reported for SCA2 that causes neurodegeneration.

The most common allele that produces neurodegeneration in SCA2 patients of this group was $(CAG)_{37}$, which represents a 14% of the total chromosomes.

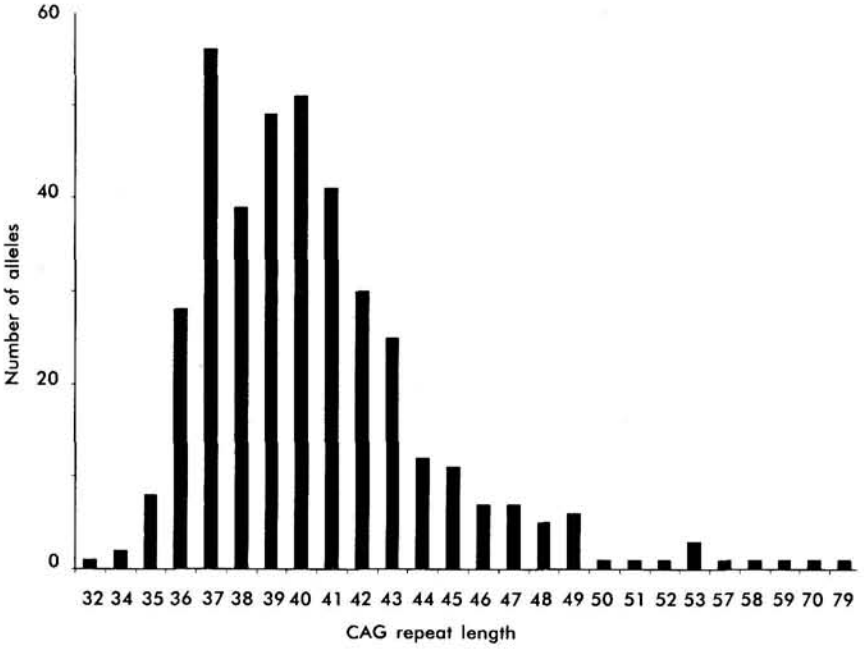

Figure 2. Distribution of $(CAG)_n$ sizes in 392 Cuban patients of a founder population with SCA2 disease.

Variability between the affected individuals at the age of disease onset

Figure 1 represents 100% of the total expanded alleles in the SCA2 gene that cause neurodegeneration in this group of patients. For alleles with the smallest sizes of CAG trinucleotide repeat ranging from 32 to 40, a high variability at the age of disease manifestation was observed among the affected individuals, in contrast with the observations for trinucleotide repeat sizes of 41 or longer.

Discussion

SCA1 [18], SCA3/Machado-Joseph disease (SCA3/MJD) [19], HD [20], dentatorubral pallidoluysian atrophy (DRPLA) [21,22], SCA6 [23], SCA7 [24], and

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spinobulbar muscular atrophy (SBMA) [25] constitute, like SCA2 [1, 12, 13], the seven nown neurodegenerative disorders caused by an expanded CAG tract in the coding region of the gene.

In the last few years, a strong inverse correlation has been demonstrated between CAG repeat length and the age of disease onset in some neurodegenerative diseases, for example, DRPLA [21, 22], SBMA [25], HD [26], SCA1 [27], SCA3/MJD [28], SCA2 [10], SCA6 [29], and SCA7 [24]. In our study, the SCA2 patients also presented an inverse correlation with values for correlation coefficients and significance similar to those of previous studies.

Alleles of 32 CAG repeats interrupted by one or two CAA were previously considered as normal alleles in the SCA2 gene [10], because no evidence of expansions were observed through this type of CAG/CAA repeat length structure through further generations. Moreover, the identification of a Japanese SCA2 patient, a 48-year-old man that developed cerebellar ataxia at the age of 39 transmitted from the allele of an uninterrupted 32-CAG repeat of his 75-year-old father—at present unaffected—[30], showed that for a 32-CAG repeat expansion the structure of the $(CAG)_n$ tract is very important for the transmission of the disease phenotype to the following generation. Compared to other known neurodegenerative diseases due to trinucleotide repeats, these results show that expanded alleles for SCA2 gene with a 32-CAG repeat without CAA interruptions can also produce the disease phenotype. This CAG repeat size constitutes the shortest expansion causing neurodegeneration in SCA2 patients. This finding favors the hypothesis that for ataxin 2 the flanking translated sequence is less protective against the "gain of function" produced by the polyglutamine expanded tract [10].

The high number of patients analyzed in this study produced important data to evaluate the characteristics of the SCA2 phenotype, and the transmission of the disease to the next generation. It has been noted that for each of the mutated alleles with $(CAG)_{32\text{-}40}$ repeats, the age of disease onset was very variable among individuals. Velázquez and Medina [2] reported in neurophysiological studies different degrees of alteration for patients with $(CAG)_{36\text{-}40}$ repeats, which was not observed in patients with 41 CAGs or more. A complete blockade of conduction was observed in some patients from the group of $(CAG)_{36\text{-}40}$ repeats two years after disease onset, and in others after 8 years. These differences in neuropathological responses for the smallest CAG repeat length suggest that other factors are involved in the begining and evolution of the disease.

As the polyglutamine diseases seem to be produced mainly by the action of the mutated protein in the cell nucleus [31-33], several genetic factors could explain the high variability at the age of disease onset for $(CAG)_{32\text{-}40}$ in the SCA2 disease; for example, the levels of ataxin 2 among individuals, the occurrence of alternative splicing, the presence of proteases, and the rate of protein transport to the nucleus. Other explanations like environmental factors could also be possible.

Further studies to determine the causes of this high variability at the age of disease onset could help to find therapies to retard disease manifestation.

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