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Intergenerational De Novo Spinocerebellar Ataxia 7 CAG Enlargement in a Moroccan Family Detected by Triplet Primed PCR

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Abstract

The autosomal dominant cerebellar ataxias (ADCA) are a genetically and clinically heterogeneous group of neurodegenerative. Spinocerebellar ataxia type 7 (SCA7) is a progressive neurodegenerative disorder with retinal degeneration caused by a CAG expansion in the ATXN7 gene located in chromosome 3p. The normal range of CAG triplets is 4-34 considering diseased over 37 to 306. In order to study a possible *de novo* large expansion, a triplet primed SCA7 PCR assay was made in a family with characteristic clinical symptoms. This assay confirmed the disease number of triplets for the father and detects a second allele in his son (patient) with a large expansion (>200 triplets). In conclusion, the specific SCA triplet primed PCR is a robust, reliable, and can be used in this case to describe for the first time an intergenerational *de novo* SCA7 CAG large expansion.

Keywords

Spinocerebellar ataxia type 7; Multiplex; Triplet primed PCR

Introduction

Autosomal dominant cerebellar ataxias (ADCA), also known as spinocerebellar ataxias (SCA), are a clinically and genetically heterogeneous group of disorders characterized by a slowly progressive cerebellar syndrome presenting as ataxia of gait, stance and limbs, dysarthria and/or oculomotor disorder due to cerebellar degeneration in the absence of coexisting diseases. The degenerative process can be limited to the cerebellum or can spread further to retina, optic nerve, ponto-medullary systems, basal ganglia, cerebral cortex, spinal tracts or peripheral nerves.

Currently, more than 30 types of SCA are described, from them SCA1, SCA2, Machado-Joseph or SCA3, SCA6, SCA7, SCA12, SCA17, and dentatorubral-pallidoluysian atrophy (DRPLA) are caused by (CAG)n repeat expansions in the ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, PPP2R2B, TBP, and ATN1 genes, respectively, and all lead to the expansion of a polyglutamine tract in the corresponding proteins, for this reason these diseases are also known as polyglutamine diseases (SCApolyQ) [1]. All so-called polyglutamine ataxias share many common features, including a negative relationship between age at onset and the number of repeats in the expansion, and a more severe disease with larger expansions.

The mean age at onset of symptoms for the most common ataxias SCA1, SCA2, SCA3, and SCA7 is generally in the third or fourth decade of life, but an average of 20 years later for SCA6 [2]. The threshold of diseased CAG expansions (SCA1: 39-82; SCA2: 34-400; SCA3: 55-84; SCA6: 21-33; SCA7: 37-306; [1,3]), or the number of expansions that determines disease carrier status, varies between the different forms of SCA as do the boundaries between what is considered an expanded and normal size (overlapping in SCA1) [1] .

Gait ataxia is the first symptom identified in the majority of cases of these diseases [4] have shown that only 12% of SCA1, 13% of SCA2, 15% of SCA3, and 24% of SCA6 patients have other symptoms before the onset of gait ataxia.

Epidemiological data on SCAs are limited. Most of them had been performed in isolated populations in remote regions. In these studies, the prevalence of SCAs had been estimated between 0.8 and 3.5: 100,000 [5]. However, in specific populations frequencies can be much higher due to founder effects, e.g. 1:750 for SCA2 in Holuguin on Cuba [6] or 1:140 for SCA3 in Flores on the Azores [7].

The frequency of genetic subtypes of SCA varies substantially in different ethnic groups. In general SCA1, SCA2, SCA3, SCA6 and SCA7 are the most frequent forms and account for 50 - 80% of ADCA families in most studies.

SCA 7 is a rare disorder [8] where the ataxia is associated with retinal degeneration [9]. The disease is caused by an unstable CAG repeat expansion in the 5´-translated region of ataxin 7 gene located at 13p12-13 chromosome [9]. The number of CAG repeats in the pathological protein varies from 37 to >200 [9,10] and from 55 repeats can give a severe disease with onset in childhood and a rapid fatal course [9,11,12].

It has a prevalence of less than 1:100,000 and accounts for around 2% of all SCAs/autosomal dominant ataxias [8]. As with all genetic conditions, there are regions of increased prevalence. Within the western hemisphere, two regions of increased prevalence of SCA 7, Brazil and Mexico, have been assessed and felt to be due to a founder effect, with a potential/probable European origin of the SCA 7 gene in both [13].

In the present work, the novo expansion of the SCA7 CAG repeat region in one generation from a Moroccan family obtained by triplet primed PCR and the effect of the repeat length on the clinical manifestation is described using a multiplex PCR screening technology and specific primed triplet PCR.

Materials and Methods

Samples and clinics

10-days male neonate was referred to a pediatric neurologist because of parental history of SCA7. Parents were from Moroccan

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origin and not consanguineous. Mother was healthy and a father presented an affectation of spinocerebellar ataxia type 7 diagnosed 2 years ago.

Family history was compatible with autosomal dominant inheritance consisting in one brother of the father probably affected of SCA7 (no genetic study was done) and the grandmother of the patient who died at age 62 with symptoms compatible with the same disease. Other SCA genes (1, 2, 3, 6, and 8) were studied besides of SCA7.

From a clinical point of view the patient properly acquires the developmental milestones and remains asymptomatic until 6 months of age. When he was seven months old the examination revealed a discrete truncal ataxia and an intentional tremor in handling.

Does not return to review until being 10-11 months old, when we appreciated a significant psychomotor regression with generalized hypotonia and hyperreflexia, poor visual fixation and erratic eye movements, lack of comprehensive and expressive language, truncal ataxia, intentional tremor and dysphagia.

Ophthalmologic examination showed a retinal atrophy with macular pigment migrations. MRI of the brain showed a vermian and hemispheric cerebellar atrophy, all compatible with SCA7.

The patient died at 12 months in the context of a hemodynamic shock secondary to an infectious disease.

DNA extraction and fragment analyses

Genomic DNA was isolated from peripheral blood using Mini Kit (Qiagen, Crawley, UK) according to the manufacturer's instructions.

Multiplex SCA1, SCA3 and SCA7 amplification and capillary electrophoresis

Two standard multiplex PCR reactions of the SCA genes (SCA1, 3, 7; present paper and SCA2, 6, and 8; Martinez-Laso, unpublished results) were made in order to detect the presence of the different alleles of the SCA genes mentioned.

The multiplex SCA1, 3, and 7 reaction of 50ul consisted of SCA 1 primers (SCA1-F-HEX: HEX-5´-CCAACATGGGCAGTCTGAG-3´; SCA1-R: 5´-GAACTGGAAATGTGGACGTACTG; 2,2 pmol each), SCA 3 primers (SCA3-F-ROX: ROX-5´-GTGACTACTTTGATTCGTGAAACAATG-3´; SCA3-R: 5´-AGG-TAGCGAACATGATGAATG-3´, 2.0 pmol each), and SCA7 primers (SCA7-F-FAM: 6´-FAM- 5´- TAGGAGCGGAAAGAATGTCG-3´; SCA7-R: 5´- CCAGCATCACTTCAGGACTG-3´, 1,8 pmol each) The PCR reaction contained 25 UL GoTaq long mastermix (Promega, WI), Betaine (20% V/V) (Sigma, Germany), DMSO (4% v/v) (Sigma, Germany), and 100 ng of genomic DNA purified from peripheral blood as described above. Reactions were performed in a "Veriti" thermal cycler for 1 cycle at 98º for 5 minutes, 30 cycles at 97º for 45 seconds, 60º for 45 seconds, 68º for 6 min, and a final extension at 72ºC for 30 minutes. One ul of the reaction product was added to a 10 ul of formamide (HiDi formamide, Applied Biosystems, CA) and 0,5 ul of GS500-LIZ internal molecular weight standard (Applied Biosystem, CA).

Analysis of Fluorescent PCR Fragments and Interpretation of Data Analysis of the fluorescent PCR products was performed using an ABI-Prism 3730xl automatic sequencer (Applied Biosystems, CA, USA) on a 50-cm capillary array with the POP7 polymer and a LIZ-GS500 as internal standard marker (Applied Biosystems, CA, USA). Data were examined using the Peakscan 1.0 software (Applied Biosystems, CA, USA).

The mixture was denatured at 95º C for 2 minutes and placed on ice for a 3 minutes and then was injected into a 3730xl DNA analyzer on a 50-cm capillary array with the POP7 polymer (Applied, Biosystems, CA). Analysis of Fluorescent PCR Fragments and Interpretation of Data Analysis of the fluorescent PCR products was performed using the Peakscan 1.0 software (Applied Biosystems, CA, USA).

Triplet primed PCR for SCA7 expansions

In the case to obtain only one peak in the sample, a second SCA specific amplification based on triplet primed PCR technology, in this case, SCA7, was used in order to test the homocigozity or the presence of expanded allele (>200 CAG triplets). The SCA7 triplet primed amplification was made using the same primers than in the multiplex (2 pmol/each) and the primers SCA7-CAG-R: 5´-CAGGAAACAGCTATGACCTGCTGCTGCTGCTG-3´ and universal primer M13R: 5´-CAGGAAACAGCTATGAC-3´(3 pmol each). The PCR reaction contained 15 UL GoTaq long mastermix (Promega, WI), Betaina (20% V/V) (Sigma, Germany), DMSO (4% v/v) (Sigma, Germany) , and 45 ng of genomic DNA purified from peripheral blood as described above. Reactions were performed in a "Veriti" thermal cycler for 1 cycle at 98º for 5 minutes, 30 cycles at 97º for 35 seconds, 60º for 35 seconds, 68º for 4 min+10 seconds/cycle, and a final extension at 72ºC for 30 minutes. One ul of the reaction product was added to a 10 ul of formamide (HiDi formamide, Applied Biosystems, CA) and 0,5 ul of GS500-LIZ internal molecular weight standard (Applied Biosystem, CA). Fragment analyses were analyzed by the same software.

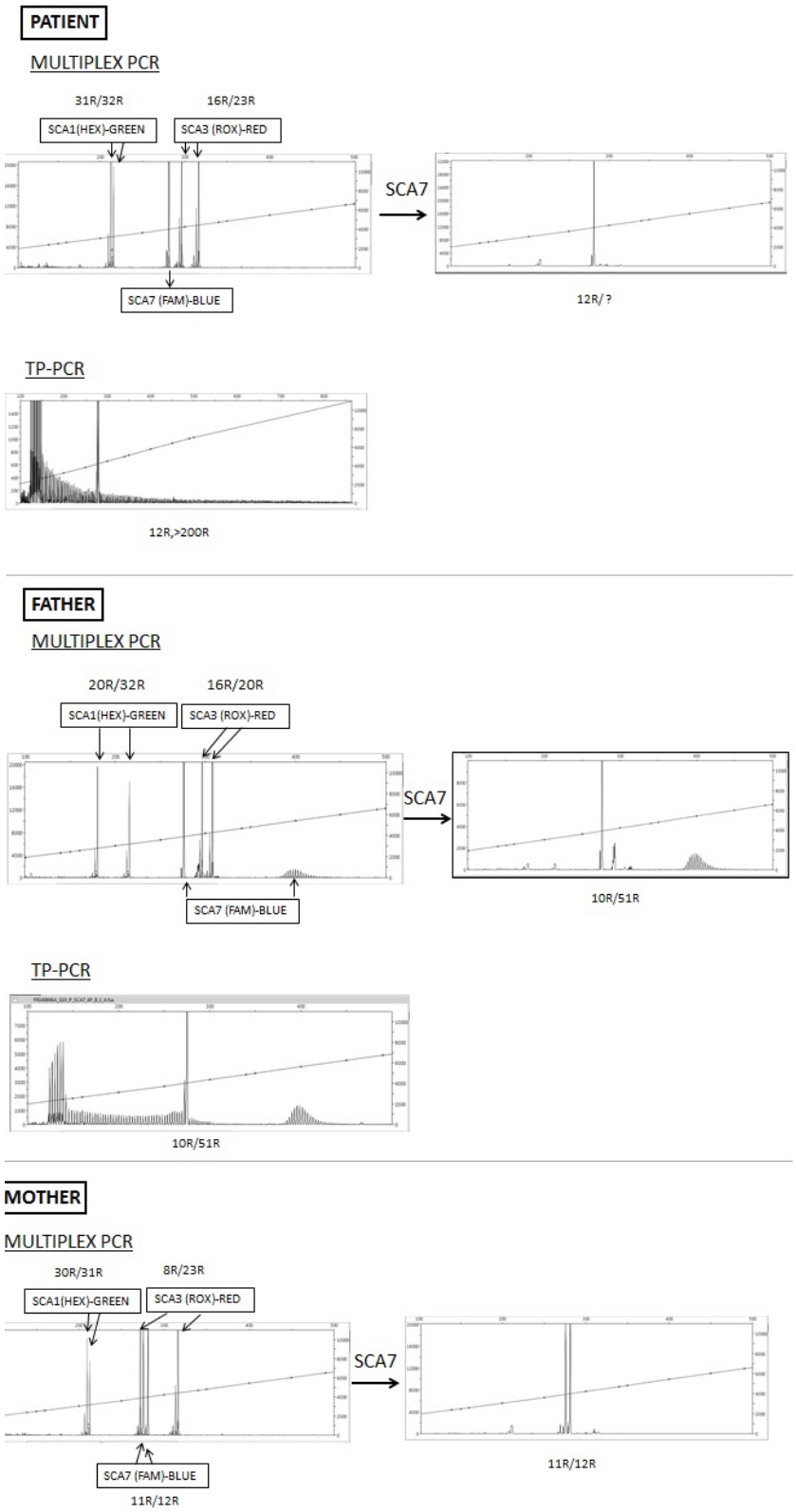
Results and Discussion

Autosomal dominant cerebellar ataxias share many common features (see introduction) and sometimes are necessary to test the different and most common regional SCA genes. A new multiplex single PCR test is described in other to minimize the number of PCRs (from the classical 6 reactions to only 2) to do a screening of the SCA1, 2, 3, 6, 7 and 8 and on the other hand, a specific SCA7 triplet primed PCR are described.

Fluorescent triplet-primed PCR is a robust technique used to detect large-repeat expansions that cannot be amplified using standard PCR in other neurodegenerative diseases with large number of triplet expansions as X-fragile, Myotonic dystrophy type 1 or Friederich ataxia [4-16] Classically, large expansions of SCA2, SCA7 and SCA 8 have been described and need Southern-based approaches to be detected.

Triplet-primed PCR showed a short series of discrete peaks with 3-bp periodicity when subjects within the normal range were tested; large expansions yielded clearly distinguishable profiles, with multiple peaks of progressively lower intensity down to fluorescence close to. The results presented in this paper suggest that this technology is reliable for large CAG expansions in the SCA7 gene, although their detection is proved only up to 306 triplets in SCA7. On the other hand, this technology has several advantages over the genomic- or PCR-Southern blot as the small amount of DNA used, does not need the use of radiochemicals the time required to perform the technique and follows the recommended procedures [17,18].

The family studied was tested in the first time with the multiplex single PCR for the SCA1, 3, and 7 (Figure 1) and for SCA2, 6, and 8



Multiplex PCR was made in all relates and Triplet Primed PCR was made in father and patient to confirm the data obtained in the single PCR

SCA1 peaks are in green color (HEX), SCA 3 in red color (ROX) and SCA7 in blue color (FAM)

The numbers with R are the corresponding repeats (or triplets) of the SCA genes tested

Figure 1: Fragment analysis of the family.

(data not shown) obtained two peaks with a normal number of SCA7 triplets for the mother (11, 12 triplets), two peaks with one healthy allele (10 triplets) and one disease allele (51 triplets) in the father, and only one peak with a healthy allele of 12 triplets in the patient with the lack of a second allele. In order to confirm the heterozygosis (one healthy allele and one disease – expanded allele) of the father and the patient a SCA 7 triplet PCR was made (Figure 1). Due to the structure of the kit the previous data of the multiplex single PCR were confirmed in the two individuals and the triplet primed PCR showed clearly a large expansion in the second allele of the patient not found with the single multiplex PCR establishing the final result as 12, >200 triplets. With these results is confirmed the intergeneration expansion from a 51 triplets to >200 in one generation as described previously for other neurodegenerative disease as X-fragile [19] establishing the instability of a mutated SCA7 gene with low number of triplets to generate an allele with a large expansion.

In conclusion, the use of the combined multiplex single PCR for the screening of the SCAs named and the specific triplet primed PCR for each of the SCAs as a confirmatory method for homozygosis or heterozygosis with one large expansion allele is an easy, operative and alternative method avoiding the single PCR for each of the SCAs and Southern blot.

Finally, it is also possible to apply the tests to routine screening for infantile- or juvenile-onset diseases that suggest the involvement of large expansions in these genes.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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