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SHORT COMMUNICATION

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Post-zygotic de novo trinucleotide repeat expansion at spinocerebellar ataxia type 7 locus: evidence from an Indian family

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Abstract Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant cerebellar ataxia caused by CAG repeat expansion. We found expansion at *SCA7* locus in only two out of 235 Indian families clinically diagnosed for ataxia. In one of the families, a de novo mutation was observed wherein a paternal allele in intermediate range of 31 CAG repeats expanded to 59 in the offspring leading to the disease. No expanded alleles were observed in the sperm of the transmitting parent by small pool PCR. This suggests that de novo expansion by a pre-zygotic event is unlikely and could be post-zygotic. SCA7 expanded alleles from the two families were present on different genetic backgrounds, indicating multiple origins of the mutation.

Keywords Spinocerebellar ataxia 7 (SCA7) · Post-zygotic · De novo mutation · CAG repeat · Repeat instability

Introduction

Spinocerebellar ataxia type 7 (SCA7) is a progressive autosomal dominant neurodegenerative disorder characterised by cerebellar ataxia associated with progressive macular degeneration. The disease is caused by CAG repeat expansion in the coding region of the *SCA7* gene (David et al. 1997). Normal SCA7 alleles contain 4–35 CAG repeats, whereas pathological alleles contain 36–306 CAG repeats (Lebre and Brice 2003). SCA7 repeat is highly unstable and large jumps are observed in particular when transmitted paternally, though majority of the transmissions are maternal at this locus. The intermediate alleles (IAs) in the range of 28–35 CAG repeats can be meiotically unstable when transmitted paternally and de novo expansions occur in these large, but still normal repeat sequences (Stevanin et al. 1998; Giunti et al. 1999). These IAs represent a reservoir of chromosomes, at-risk for expansion and, therefore, explain the persistence of the disorder in spite of marked anticipation characteristic of SCA7. Other than SCA7, de novo expansions among the disorders caused by polyglutamine repeat expansion have been demonstrated in Huntington's disease, SCA6 and SCA17 (Myers et al. 1993; Shizuka et al. 1998; Koide et al. 1999).

SCA7 is a rare form of autosomal dominant cerebellar ataxia (ADCA), varying from 1.0 to 11.7% of genetically diagnosed ADCAs in different populations (Michalik et al. 2004), except in Sweden and Finland where SCA7 is the most prevalent (Jonasson et al. 2000). Different haplotypes have been reported to be associated with the diseased alleles, indicating multiple origins of the SCA7 mutation (Stevanin et al. 1999).

Subjects and methods

A total of 235 ataxia families of Indian origin were studied. Patients were diagnosed at the Neuroscience Centre, All India Institute of Medical Sciences, New Delhi. Ethical clearance was obtained and blood samples were collected after obtaining informed consent and ensuring confidentiality.

Genomic DNA was isolated from peripheral blood leukocytes using modified salting-out procedure (Miller et al. 1988). Genomic DNA was also isolated from sperm samples of AT238 and AT248. Small pool PCR (SP-PCR) was used to detect all the repeat variants

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present in the sperm DNA of the father (AT248) and in the peripheral leukocytes of the offspring (AT246). Genomic DNA from leukocytes (AT246) and sperm sample (AT248) were serially diluted to 600, 60 and 6 pg/μl equivalent to 100, 10 and 1 haploid genome/μl, respectively for SP-PCR. Reconstruction experiment was carried out as reported (Crawford et al. 2000) by pooling genomic DNA from AT246 (10, 59 CAG repeats) and AT248 (10, 31 CAG repeats) in a way so that the final concentrations of the expanded allele (59 CAG repeats) were 600, 60 and 6 pg/μl and those of smaller alleles were 100, 10 and 1 ng/μl in three different pools, respectively. Assuming equal concentrations of both the alleles (10 and 59 repeats of AT246) in the genomic DNA, the required concentrations of the expanded allele in the pools were achieved by pooling genomic DNA to the final concentrations of 1, 200, 120 and 12 pg/μl. These pools had expanded and smaller alleles in the proportion of 1:170 molecules.

CAG repeat at *SCA7* locus and microsatellite markers (D3S3566, D3S3698, D3S1600 and D3S3635) were amplified by PCR using published primers (David et al. 1996). Seven microsatellite markers from different chromosomes were also amplified by PCR for confirming the paternity. One of the primers in each case was fluorescently labelled and size estimation was carried out on an ABI Prism 377 automated DNA sequencer using the GeneScan software (Perkin Elmer, Foster City, USA). Sequences of the expanded repeats were confirmed by direct sequencing of the amplicons using dideoxy chain terminator chemistry on an ABI Prism 377 automated DNA sequencer.

Results and discussion

We found only two SCA7 families out of 235 families of Indian origin, clinically diagnosed for ataxia, indicating a very low prevalence (< 0.85%). There were three affected individuals, AT238, AT236 and AT246 with ages at onset 50, 27 and 14 years, respectively (Fig. 1). All of them had gait ataxia, pyramidal signs, slow saccades and reduced vision. Extrapyramidal features were found in two patients. Nerve conduction studies were normal. Imaging of brain showed cerebellar as well as pontine atrophy in all the three cases.

In one of the families (AT062), we identified a de novo mutation in a subject with no family history (Fig. 1). The unaffected father (AT248) had CAG repeats in the intermediate range (31 repeats), which underwent pathological expansion to 59 repeats in the son (AT246), resulting in the disease. Paternity of the transmitting parent was confirmed by following the inheritance of seven microsatellite markers (data not shown). Haplotype analysis using microsatellite markers (D3S3566, D3S3698, D3S1600 and D3S3635) revealed that the expansion has occurred on the paternal allele (Fig. 1) as reported in other de novo cases (Stevanin et al. 1998; Giunti et al. 1999). Analysis of CAG repeat

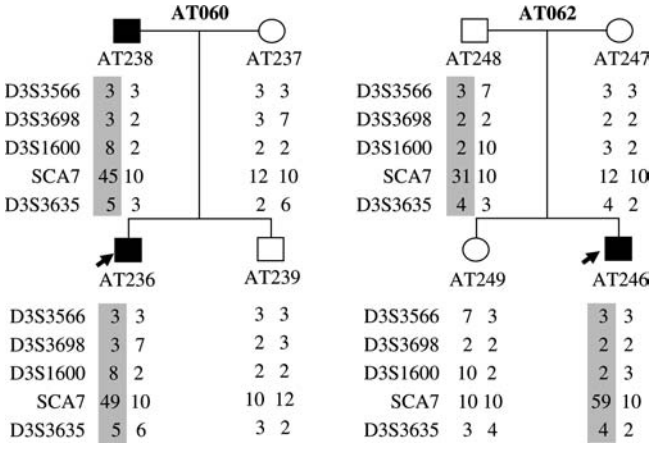

Fig. 1 Pedigrees of families with probands, AT236 and AT246, affected with SCA7. The CAG repeat sizes and alleles for the microsatellite markers used for haplotype analysis are given below the pedigree symbols

at *SCA7* locus in sperm sample of the transmitting parent (AT248) revealed no germinal mosaicism (Fig. 2). Additionally, SP-PCR analysis using as low as 60 pg of DNA suggested that expanded alleles were not present even in a small number of the father's sperms. We had tested the sensitivity of our PCR protocol and fluorescent based detection method by mimicking the situation in which the expanded alleles were present in small numbers in the father's sperms. Firstly, we were able to amplify 59 repeats, the maximum size of expanded allele we would expect—if present—in the father's sperms, even at very low DNA concentrations (60 pg). Secondly, we carried out reconstruction experiments to assess the impact that allele competition during PCR could have on the amplification of expanded allele. We could co-amplify as low as 60 pg of the expanded allele (59 CAG from AT246) with 10 ng of smaller alleles (10, 31 CAG from AT248) (data not shown). As no expanded alleles were observed in SP-PCR analysis of sperms, it is unlikely that a rare sperm

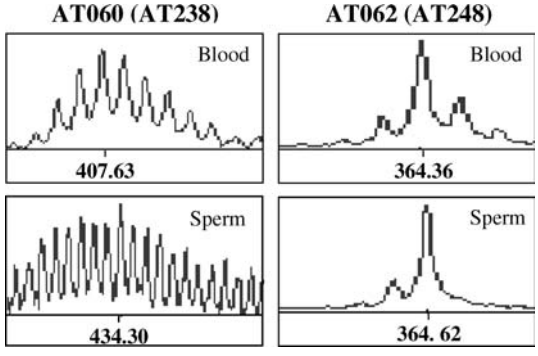

Fig. 2 Electropherogram showing the size of the larger CAG repeat allele in blood and sperm samples of the transmitting parents, AT238 and AT248. Numbers below the peaks show the amplicon size corresponding to respective CAG repeat alleles at *SCA7* locus given in pedigrees

cell with an expanded repeat, undetectable in the father's sperms, contributed to the zygote formation. Though we cannot rule out a pre-zygotic event completely, our findings suggest that the de novo expansion could be post-zygotic, which might have occurred during an early stage of embryogenesis as no IA was observed in somatic cells of the offspring using SP-PCR with ~10 haploid genomes. Moreover, it has been reported that repeat instability is greatest during early post-zygotic cell divisions (Wohrle et al. 1993).

In the other family (AT060), a pre-existing mutation with an increase of only four repeat units (45 to 49 repeats) was observed during paternal transmission. Unlike the de novo mutation, DNA analysis of the transmitting parent (AT238) revealed increased CAG repeat mosaicism in sperm samples compared to blood (Fig. 2). This is in corroboration with the earlier report, which showed that the marked anticipation in SCA7 is due to strikingly greater gonadal mosaicism than that observed in blood (David et al. 1998). Haplotype analysis using microsatellite markers showed that the two affected pedigrees had different genetic backgrounds (Fig. 1), indicating that different ancestral mutations were involved in SCA7 in the Indian population, as reported previously for other populations (Stevanin et al. 1998).

In summary, this is the first report of post-zygotic origin of de novo repeat expansion in SCA7 from the IA. As reported, we also provide evidence that a reservoir of IAs exists in a population that has a tendency to undergo de novo expansion, explaining the persistence of the disease. This observation further raises questions about the mechanism of repeat instability, which is usually attributed to germ line expansions. This study, therefore, emphasises the necessity of identification of modifier loci that govern the onset of repeat expansion.

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