De novo expansion of intermediate alleles in spinocerebellar ataxia 7

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Spinocerebellar ataxia 7 (SCA7) is the eighth neurodegenerative disorder caused by a translated CAG repeat expansion. Normal SCA7 alleles carry from four to 35 CAG repeats, whereas pathological alleles carry from 37 to ∼200. Intermediate alleles (IAs), with 28–35 repeats in the SCA7 gene are exceedingly rare in the general population and are not associated with the SCA7 phenotype, although they have been found among relatives of four SCA7 families. In two of these families, IAs bearing 35 and 28 CAG repeats gave rise, during paternal transmission, to SCA7 expansions of 57 and 47 repeats, respectively, that were confirmed by haplotype reconstructions in one case and by inference in the other. Furthermore, the four haplotypes segregating with IAs were identical to the expanded alleles in each kindred, but differed among the families, indicating multiple origins of the SCA7 mutation in these families with different geographical origins. Our results provide the first evidence of de novo SCA7 expansions from IAs that are not associated with the phenotype but can expand to the pathological range during some paternal transmissions. IAs that segregate in unaffected branches of the pedigrees might, therefore, constitute a reservoir of chromosomes at-risk for expansion. This would help to explain the persistence of the disease in these kindreds in spite of marked anticipation (11–14) that should otherwise result in its extinction.

RESULTS

A de novo expansion of an intermediate allele and additional evidence for meiotic instability

In four out of 43 SCA7 families studied, there was one definite and three inferred de novo IA expansions (Fig. 1). In the Italian family VN, the de novo expansion was clearly demonstrated by haplotype reconstruction that excluded false paternity. It occurred on an allele with 35 repeats that was transmitted stably from the unaffected grandmother (VN-14902) to the father (VN-14901), then to the granddaughter (VN-14899), who were also unaffected. During transmission to the grandson (VN-14898), however, a pathogenic SCA7 expansion resulted in 47 CAG repeats and expression of the SCA7 phenotype.

In the three other families, evidence was more indirect. In Italian family PT (Fig. 1), an IA with 28 repeats was transmitted from a father, with no known history of disease and whose DNA was not available, to unaffected children PT-1561 and PT-1562. The same 28 repeat allele, or another allele with the same haplotype at seven markers spanning 10 cM in the SCA7 region (13,15), was transmitted to the affected daughter (VN-14899), who were also unaffected. During transmission to the grandson (VN-14898), however, a pathogenic SCA7 expansion resulted in 57 repeats and expression of the SCA7 phenotype.

IN INTRODUCTION

Spinocerebellar ataxia 7 (SCA7) is one of the eight neurodegenerative disorders caused by a translated CAG repeat expansion (1). The SCA7 alleles may carry from 37 to >200 CAG repeats, while the normal alleles have only 4–35 (2–5). The vast majority of the normal alleles (75%) contain 10 CAG repeats. Those in the range 20–35 are extremely rare (<0.5%).

In other diseases caused by trinucleotide repeat expansions, it has been proposed that expansions arise on a class of at-risk chromosomes bearing the largest normal number of repeats, also referred to as intermediate alleles (IAs) (6). De novo cases of disorders caused by translated CAG repeats, resulting from the expansion of an IA, have only been described in Huntington’s disease (HD) (7–10). We report here that SCA7 IAs can be meiotically unstable during paternal transmission and that de novo SCA7 expansions can occur on these large, but still normal, repeat sequences. In our series, IAs that are not implicated in an SCA7 phenotype are exclusively found among relatives of SCA7 families and might therefore represent a reservoir of chromosomes at-risk for expansion. This would help to explain the persistence of the disease in these kindreds in spite of marked anticipation (11–14) that should otherwise result in its extinction.

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transmitted, with 48 repeats, to the affected grandson (PT-1565). It could not be determined whether it was the 28 repeat allele that expanded during transmission or whether the father had a second IA or an expansion. The former hypothesis is greatly favoured by the low frequency of the haplotype (<0.4%; data not shown) and the rarity of normal alleles with >20 repeats (<0.5%).

Alleles with 35 CAG repeats have already been described in a Tunisian SCA7 family (TUN) (3) that segregates with the same 10 cM haplotype that is linked to the expansion in this family (Fig. 1). A 35 repeat IA was stably transmitted from the unaffected mother (TUN-23) to three (TUN-49, 50 and 55) of her five children. An allele with the same haplotype was transmitted from the father (TUN-22) to four affected children, with expansions of 51, 47, 60 and 47 repeats, respectively. The same chromosome haplotype carrying 35 CAG repeats was detected on another branch of the family in individual TUN-31.

In family WI, from Jamaica, an affected mother transmitted an expanded SCA7 allele with 48 repeats stably to her affected son (WI-1861) and with 55 repeats to her affected daughter (WI-1608). This affected daughter and the unaffected WI-1859

![Figure 1](image-url)
also received, from the unaffected father, IAs with the same haplotype and 29 and 30 CAGs, respectively, verified by sequencing. The CAG repeat increased in size by one repeat unit in the germline of the father during transmission of the 29 repeat allele to individual 1859 (Fig. 1).

The number of IAs in these four families is remarkable, in particular because they are found not only in the SCA7 lineage or in other branches of the pedigrees (TUN-31), but, curiously, also among the married-in relatives: individual 23 in TUN and the spouse in WI. In a sample, however, of 196 normal chromosomes from unaffected unrelated subjects of North African (n = 62), French (n = 94) or other origins (n = 40), as well as on >500 normal chromosomes of various origins previously reported (2,4,5), no alleles had >18 repeats. In two other SCA7 kindreds (not shown), two spouses carried alleles with 17 and 19 repeats, but, unlike the families shown here, the haplotypes differed from the one that segregated with the disease in each family. The fact that the ‘married-in’ with IAs had the same haplotype as the familial SCA7 chromosomes in families TUN and WI, which originate from small and highly inbred rural areas, suggests that they may be relatives of these families.

IAs are not associated with the SCA7 phenotype

Onset and severity of the disease were not enhanced in the three patients carrying both the expansion and an IA in the Tunisian (3) and in the West Indian kindreds. Furthermore, there was no parental history of ataxia or maculopathy in any of those carrying IAs only, except for the 84-year-old grandmother of family VN (VN-14902), whose slight visual loss due to non-progressive maculopathy appeared by the end of her sixties and can be attributed to age. Maculopathy is frequent beyond the age of 75, in particular in women (16). Furthermore, the first sign in later-onset SCA7 patients is always ataxia (3) and this subject does not present signs of ataxia or dysarthria after >10 years with maculopathy. In family TUN, a 35 repeat IA was detected in an individual (TUN-31) who had suffered from an autosomal dominant pigmentary retinopathy since his early childhood, but with no neurological symptoms after 49 years of disease duration. There were no records of either retinopathy or cerebellar ataxia in his parents, who died after 70 years of age. The pigmentary retinopathy was transmitted to his two children, who received an allele with 12 CAG repeats from their father and carried neither an IA nor an SCA7 expansion. The retinopathy was therefore considered to be unrelated to SCA7.

The presence of IAs with 28–35 repeats in unaffected family members was verified in 10 subjects and inferred in another, eight of whom were at least examined at age 50–84.

Mechanism of IA expansion

Meiotic instability. In families VN and PT, the pathological expansion occurred during paternal transmission. In family WI, the father transmitted an IA with 30 repeats, one more than his own IA repeat, to one of his offspring, indicating the occurrence of slight instability of the 29 repeat allele. Stable paternal transmissions of 35 repeats were observed in families VN and TUN to one and three children, respectively, whereas only one stable transmission of a paternal IA of 35 repeats was confirmed in family VN. These results are in accordance with the much greater instability observed in paternal transmission of the SCA7 mutation (3).

Mitotic instability. The visual aspect of PCR amplification products, illustrated in Figure 2, is clearly different between alleles with 30 CAG repeats or less and the IA carrying 35 repeats. In alleles with 35 repeats, at least one additional band of higher molecular weight, thus not a PCR artifact, is observed. This is reminiscent of the somatic mosaicism observed, to a much greater degree, with pathological expanded alleles, whereas no sign of somatic instability is detected with 30 repeats or less.

Sequence analysis of the 28–35 repeat IAs revealed no polymorphisms or interruptions, compared with the published SCA7 sequence (EMBL accession no. AJ000517), that would explain their tendency to expand. It was observed, however, that the 29 and 30 CAG IAs from family WI contained three CCG repeats downstream from the CAG repeat sequence. Note that mitotic instability of the 35 CAG repeat IA allele is less than that of expanded alleles (37–55 repeats), where instability increases as a function of CAG repeat numbers.

Figure 2. Analysis of PCR products in four SCA7 kindreds. Note that the 29 (WI-1608) and 30 (WI-1859) CAG repeat alleles appear as 30 and 31 units due to an additional CCG repeat downstream from the CAG repeat sequence. Note that mitotic instability of the 35 CAG repeat IA allele is less than that of expanded alleles (37–55 repeats), where instability increases as a function of CAG repeat numbers.
DISCUSSION

In summary, we have observed large normal alleles or IAs with 28–35 CAG repeats in four out of 43 SCA7 families that are absent in the normal population. They have the same haplotype as the pathological allele in all cases and, in one case, clearly gave rise to an SCA7 expansion. The IA were not associated with the SCA7 phenotype. The lack of phenotypic expression cannot be explained by age-dependent penetrance, since eight of the IA carriers were 50–84 years of age. This is in contrast to HD, where reduced penetrance has been reported with small expansions of 36–40 CAG (17,18). Furthermore, onset did not decrease and disease severity did not increase in the patients with both the IA and the SCA7 expanded allele. Koob et al. (5) reported a 35 repeat units allele in an asymptomatic at-risk individual with ‘symptomatic consistent with the early stages of ataxia’. It is not clear, however, whether the phenotype and the haplotype are those associated with the expanded allele segregating in the family.

IAs are prone to slight instability (±1 repeat), as in HD (19,20), and can expand to the pathological range on some paternal transmissions, giving rise to de novo mutations, also described in HD (7–10). The threshold for meiotic instability appears to be >27 repeat units. A moderate degree of mitotic instability of IAs with 35 CAG units is detectable on PCR products obtained from blood DNA. IAs giving rise to de novo mutation in HD are reported to be in the same size range. Such events are infrequent, however. In the present study, direct and indirect evidence of de novo mutation from an IA represents 10% of the families. A figure of 3% is reported in HD (9). In HD, as in our study, pathological expansions were only observed during paternal transmission. Expansions of IAs into the pathological range have also been detected in Friedreich’s ataxia (21–23), myotonic dystrophy (24) and fragile X syndrome (25), all associated with large trinucleotide repeat expansions. In French SCA3/MJD families (26) and Japanese DRPLA (27), large normal alleles share the same intragenic haplotype as expanded alleles. As a consequence, the relative frequencies of the different spinocerebellar ataxias are related to the frequency of IAs in each population (28).

The range (9–15 CAG) and distribution of normal alleles in our control population was similar to previous reports (2–5). The explanation for the large number of IAs in unaffected and married-in individuals in the Tunisian and West Indian families, where consanguinity could not be demonstrated, can only be explained by the isolated rural origins of these populations, where a common ancestral gene is disseminated locally and constitutes a reservoir for future de novo mutations. This would explain why, despite the extreme anticipation observed in SCA7 (20 years/generation; 11–14), the disease has not disappeared.

MATERIALS AND METHODS

Forty three SCA7 families from various geographical origins were analysed, including 38 married-in individuals. Normal allele distribution was assessed in 98 unrelated and unaffected subjects from the general population.

PCR reaction was performed using 25 pmol each primers 4U716 (5’-CAGCAGCTGTCCACGACCTACATT-3’) and 4U1024 (5’-TGTTCACTTGTAGGAGGCGGAA-3’) in a final volume of 25 µL containing 200 ng genomic DNA, 240 µM each dNTP and 10% DMSO. One unit of Taq polymerase (Perkin Elmer-Cetus) was added during the first denaturation step of 94°C for 10 min. Then, 35 cycles of denaturation at 94°C for 1 min, 57°C for 1 min and 72°C for 1 min were performed on a Gene Amp PCR system (Perkin Elmer-Cetus), followed by a final extension step of 2 min. Aliquots of the PCR products were diluted 1:1 in formamide loading buffer, denatured and electrophoresed on a 6% acryla–mide–7 M urea gel. DNA was then transferred to a nylon membrane (Hybond N*; Amersham) and hybridized at 42°C for 3 h with a 32P-end-labeled (CAG)7 oligonucleotide in Aminosin buffer (29). Membranes were washed at room temperature for 15 min in 2x SSC, 0.1% SDS and exposed to X-ray film for autoradiography. The number of CAG repeat in IAs was verified by sequencing, after separation of the PCR products on 2% agarose gels, using an ABI dye terminator kit on an ABI-Prism 377 automatic sequencer according to the manufacturer’s conditions (Perkin Elmer-Cetus).

Genotypes at seven microsatellite markers (Fig. 1) were determined using standard conditions (13,15).

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