Polymorphism of trinucleotide repeats in non-translated regions of SCA8 and SCA12 genes: allele distribution in a Polish control group

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Abstract. Spinocerebellar ataxias (SCAs) are a group of neurodegenerative disorders caused by dynamic mutations of microsatellite repeats. Two novel forms of SCAs have been described recently: SCA8, with expansions of CTA/CTG repeats in 3'UTR of the SCA8 gene, and SCA12, caused by expansion of the CAG tract in 5'UTR of the SCA12/PP2R2B gene. Analysis of CTA/CTG and CAG polymorphism in those two genes was performed in a Polish control group consisting of 100 individuals without any neurological signs. The distribution and ranges of the number of non-pathogenic repeats were similar to those observed in other populations described previously. Expansion of CTA/CTG repeats in the SCA8 locus was found in 2 of 100 controls and in 5 probands among 150 pedigrees affected with unidentified ataxias. As such expanded alleles were also observed in their healthy relatives, the pathogenic role of expansions in the SCA8 gene remains uncertain.

Key words: SCA – spinocerebellar ataxia, dynamic mutation, trinucleotide repeats.

Spinocerebellar ataxias (SCAs) are a heterogenous group of disorders characterized by progressive cerebellar ataxia of gait and limbs, dysarthria, dysphagia, and other neurological signs. The genetic classification of the autosomal dominant types of SCA comprises 20 loci for different SCAs. Eight of those have been identified, revealing a dynamic mutation in the trinucleotide repeat regions as a molecular defect leading to microsatellite expansion. Six of the SCAs (SCA1,
2, 3, 6, 7, 17) are due to CAG repeat expansions in the coding regions of the corresponding genes translated into abnormally long polyglutamine stretches (Stevanin et al. 2000, Gilman 2000). These types of SCA – together with Huntington’s disease (HD), dentatorubropallidoluysian atrophy (DRPLA) and spinobulbar muscular atrophy (SBMA) – are classified as polyglutamine disorders. CAG repeats are highly polymorphic with regard to the number of trinucleotide repeats, and normal as well as pathological ranges of repeat numbers for particular genes have been established.

Besides dynamic mutations involving microsatellite repeats that are translated into proteins, a novel type of the mutation in SCA was described during the last four years – consisting of trinucleotide repeats expansions in the non-coding regions of DNA. Two such repeat expansions – CTA/CTG and CAG – appear to be responsible for two dominantly inherited spinocerebellar ataxias: SCA8 (OMIM 603680) and SCA12 (OMIM 604326) (Koob et al. 1999, Holmes et al. 1999).

The combined CTA/CTG repeat expansion in 3’ UTR of the SCA8 gene [13q21] (a gene of unknown function) is controversial with regard to its pathologic significance. Alleles with large expansions of CTA/CTG repeats occur in both affected and unaffected individuals (Nemes et al. 2000). Tetramodal distribution of the alleles is used in determining the pathogenicity of the CTA/CTG tract observed among tested subjects: 15-21, 22-37, 40-91 and > 100 CTA/CTG repeats (Vincent et al. 2000).

Alleles with 100 to 155 CTG repeats have usually been detected in SCA 8 patients, although expansions ranging from 80 to 300 repeats have also been reported in 1.25% of psychiatric patients and 0.7% of healthy subjects (Stevanin et al. 2000).

In the SCA12 locus a CAG tract is located in the 5’ UTR of the SCA12/PPP2R2B gene [5q31-q33] encoding the brain-specific subunit of the serine-threonine protein phosphatase PP2A. The normal, non-pathogenic number of CAG repeats has been established in different ethnic populations in the ranges 9-18 (Fujigasaki et al. 2001) and 7-28 (Holmes et al. 1999). The pathogenic CAG repeat expansions in the range of 66-78 were observed in patients of German origin (Holmes et al. 1999) and of 55-69 in Indian SCA12 families (Fujigasaki et al. 2001, Srivastava et al. 2001).

The aim of this study was to investigate the range and distribution of trinucleotide repeats in SCA8 and SCA12 genes and the frequency of the respective alleles in a Polish control group. We analysed 100 unrelated individuals (200 alleles) unaffected with any neurological conditions. DNA was obtained from peripheral blood by using standard methods. PCR reactions were performed with specific primers flanking CTA/CTG and CAG regions of the SCA8 and SCA12 genes, respectively. To establish the repeat numbers, PCR products were separated in denaturing polyacrylamide gels with M13mp18 as a size marker.
The distribution of the alleles according to the repeat numbers and their frequencies are presented in Figure 1A for the SCA8 gene and in Figure 1B for the SCA12 gene.

The number of CTA/CTG repeats in SCA8 alleles ranged from 14 to 100 repeats, with the frequency of particular alleles similar to the distribution described elsewhere (STEVANIN et al. 2000).
Most alleles were grouped in two ranges according to the postulated tetramodal distribution: 15-21 (17%) with the most frequent allele of 18 repeats (11.7%) and 22-37 (81.6%) with the most frequent alleles of 23 (19%), 25 (14%), and 26 repeats (14.6%). Large alleles of 91 and 100 repeats were observed in 2 individuals (1% of analysed alleles).

The number of CAG repeats in the SCA12 gene ranged from 9 to 23 trinucleotides, and it did not differ markedly from the ranges presented previously (OMIM 2003). The most frequent allele (65%) contained 10 CAG repeats.

Establishing non-pathological ranges of the trinucleotide repeats in SCA8 and SCA12 genes characteristic of the Polish ethnical background, allowed us to introduce in our patients with spinocerebellar ataxias two new tests – for SCA8 and SCA12. The number of repeats in SCA8 and SCA12 loci was further analysed in a group of 150 Polish SCA patients in whom SCA1, 2, 3, 6, 7, 17 and DRPLA were previously excluded. In that group we did not observe any SCA12 pathogenic expansion, but in 5 of 150 probands we found SCA8 expanded alleles ranging from 80 to 150 CTA/CTG repeats. However, the same expanded alleles were also present in their healthy relatives. Thus, the significance of the expanded CTA/CTG repeat expansion in the SCA8 gene remains uncertain and the frequencies of the expanded alleles in the control (1%) and in the SCA group (1.7%) do not differ significantly (IZUMI et al. 2003, SULEK et al. 2003).

It is assumed now that the normal range of the repeats in the SCA8 gene varies between 15 to even >100 trinucleotides and in the expanded alleles the number of repeats ranges from 71 to over 800. The most common alleles associated with the disease have 90 to 250 repeats, but the alleles with fewer repeats than 100 show reduced penetrance (DALTON et al. 2003). Thus, in the case of SCA8 the significance of trinucleotide expansions remains unexplained.

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REFERENCES


1 In the entire group of 520 patients suspected of SCA we diagnosed 95 cases of SCA1 (in 60 families), 27 cases of SCA2 (in 14 families) and 2 cases of SCA17 (1 family).


