Analysis of spinocerebellar ataxia type 2 gene and haplotype analysis: (CCG)$_{1-2}$ polymorphism and contribution to founder effect

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Abstract
Spinocerebellar ataxia type 2 is a familial spinocerebellar ataxia with autosomal dominant inheritance. The gene responsible was recently cloned and this disorder was found to be the result of a CAG expansion in its open reading frame. We analysed 13 SCA2 patients in seven unrelated families in Gunma Prefecture, Japan. In four of the seven families, we detected CCG or CGGCCG interruptions in only the expanded alleles. Co-segregation of these polymorphisms with SCA2 patients was established within each family. Together with the results of haplotype analyses, we considered that at least two founders were present in our area and that these (CCG)$_{1-2}$ polymorphisms may make analysis of founder effects easier. By sequencing analysis we found that although the number of the long CAG repeat varied in each subclone of expanded alleles, these polymorphisms did not change their configuration. This finding suggests that CCG or CGGCCG sequences are stable when surrounded by the long CAG repeat and a single CAG. Moreover, the presence of these polymorphisms may lead to miscounting the repeat size by conventional estimation using a size marker such as an M13 sequencing ladder. Therefore we should consider these polymorphisms and accurately determine the repeat size by sequencing.

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Keywords: spinocerebellar ataxia type 2; CCG repeat polymorphism; founder effect

However, there have been no reports concerning the interruptions observed in the expanded CAG repeat of the SCA2 gene. In this study, we document two types of previously unreported polymorphisms present only in the expanded alleles and their contribution to easier analysis of the founder effect in association with data from haplotyping.

Materials and methods
Thirteen SCA2 patients (five males and eight females) from seven unrelated Japanese families (families A-G) and 60 unrelated normal controls who live in Gunma Prefecture (located about 100 km north of Tokyo with a population of approximately two million) were genetically examined. All patients had a family history of autosomal dominant inheritance. Blood samples were obtained from these cases with informed consent. Genomic DNA was extracted from blood by a standard phenol/chloroform method. Using a primer set of SCA2-A whose 5' end was Cy5 labelled and SCA2-B from Puls's group, the region containing the CAG repeat in the SCA2 gene was amplified by polymerase chain reaction (PCR) according to previously reported methods.

After the PCR products were analysed by 4% agarose gel electrophoresis with ethidium bromide staining, an aliquot of the PCR product was electrophoresed on 6% denaturing polyacrylamide gel with an automated DNA sequencer (ALF Express, Pharmacia LKB, Upsala, Sweden). The data were processed with fragment analysis software (Fragment Manager, Pharmacia) according to our previous methods. After confirming the presence of both normal and expanded alleles on agarose and sequencing gels, both alleles were excised from the agarose gel and purified by spin columns (GeneClean agarose spin columns, Supelco, Bellefonte, USA). Thereafter these purified fragments were subcloned into a plasmid vector (pBluescript II KS (+), Stratagene, La Jolla, CA, USA) and the sequence was determined with the above sequencer. All fragments were analysed by plasmid sequencing, while downstream of the reverse primer (SCA2-B) was not examined in this study. Similarly, analyses of six microsatellite markers (D12S84, D12S105, D12S1672, D12S333, D12S354, and D12S179) flanking the SCA2 gene (cen-D12S84/D12S105-1 cm-D12S1672 (at the first intron in the SCA2 gene)-20 kb-the CAG repeat in the SCA2 gene-200 kb-D12S333-4 cm-D12S354-2 cm-D12S333) were performed.
Polymorphisms of the SCA2 gene

In this study, we sequenced the region containing the CAG repeat in the SCA2 gene from seven unrelated SCA2 families in Gunma Prefecture, Japan. In two of them (families D and E), we detected the CCG polymorphism after the long CAG repeat, and in another two (families F and G) the

Discussion

In this study, we sequenced the region containing the CAG repeat in the SCA2 gene from seven unrelated SCA2 families in Gunma Prefecture, Japan. In two of them (families D and E), we detected the CCG polymorphism after the long CAG repeat, and in another two (families F and G) the
CGGCCG configuration was found only in the expanded allele. So far a similar interruption has not been reported in the genes responsible for other CAG-related disorders. Moreover, cosegregation of SCA2 patients with the expanded SCA2 gene with or without these polymorphisms was established within each family (table 1). Together with the results of haplotype analyses (tables 1 and 2) it was determined that at least two founders (family A and families D-G) are present in our area. If the founder of families D-G is the same, the CCG repeat has been ancestrally lost or gained through transmissions. From the results of haplotype analyses, families D-G obviously have a different founder from that of family A. Therefore, without analysis of flanking microsatellite markers, we could study the presence of a distinct founder in SCA2 families with the (CCG)1-2 polymorphisms. Thus the (CCG)1-2 polymorphisms may make analysis of founder effects easier. The fact that two founders are present in a geographically confined region suggests that the CAG expansion is prone to occur in the SCA2 gene in normal populations, which is compatible with a recent report.1 This may be associated with the relatively smaller size of the CAG expansion in the SCA2 gene.

Although the single or double CCG repeat was surrounded by the long CAG repeat and a single CAG, the long CAG repeat which skipped the CCG or CCGCCG repeat was never observed in any of the expanded alleles examined (table 1). Moreover, although the number of the long CAG repeat varied in each subclone of the expanded alleles, these polymorphisms did not change their configuration. The present results clearly show that the (CCG)1-2 sequence is stable when surrounded by the long CAG repeat and a single CAG. The data presented here may provide clues for elucidating the mechanisms of the expansion of trinucleotide repeats.

Because the multiple band pattern of the expanded alleles on polyacrylamide gel electrophoresis suggested that cell mosaicism occurs in the expanded SCA2 gene as well, several subclones were sequenced in each expanded allele. As described above, in contrast with the size variation of the long CAG repeat in each subclone, the CCG and CGCCCG polymorphisms and the single CAG after these polymorphisms never changed their configurations, suggesting that cell mosaicism in the expanded alleles is the result of the variation in the size of the long CAG repeat. In addition, in HD it has been noted that the CCG region immediately 3′ to the unstable CAG repeats in the IT15 cDNA is polymorphic, but only the CAG repeat is associated with its expansions and the disease.13-30 These findings suggest that the size of the long CAG repeat, not the size of the following (CCG)1-2 CAG, is probably responsible for the occurrence of the disease in our patients (families D-G). In CAG-related disorders, the size of the expanded allele is conventionally determined on polyacrylamide gel electrophoresis using a size marker such as an M13 subcloning ladder. Therefore, in analyses of the expanded alleles containing these polymorphisms, two or three repeats may be overestimated. Thus, we should examine in detail whether such polymorphisms are contained in the expanded alleles of the genes for other triplet repeat diseases as well as other group SCA2 families.

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1 Rosenberg RN. Autosomal dominant cerebellar phenotypes: the genotype has settled the issue. Neurology 1995; 45:1-5.
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