Background: We previously discovered spinocerebellar ataxia type 14 (SCA14) in a single Japanese family with an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and intermittent axial myoclonus. The latter manifestation is selectively observed in patients with early onset. We mapped the locus to chromosome 19q13.4-qter, but the etiologic gene was not known. Recently, a mutation in the protein kinase Cγ gene (PRKCG) was identified in a US family of English and Dutch ancestry with autosomal dominant SCA whose disease mapped to a region overlapping that of the SCA14 locus. Different PRKCG mutations were found in another family with SCA and in a sporadic case from the United States. Axial myoclonus was not observed in any of these US families.

Objectives: To determine whether a mutation in the PRKCG gene is responsible for SCA14 and to investigate the prevalence of PRKCG mutations in Japanese patients with autosomal dominant SCA.

Patients and Methods: Direct nucleotide sequencing analysis of the 18 coding exons of the PRKCG gene was performed in the 19 members of the original Japanese family with SCA14 and in 24 Japanese probands with SCA. After identifying a PRKCG mutation, DNA samples from 72 patients with multiple system atrophy and 50 healthy individuals were examined for the mutation as controls.

Results: Sequence analysis revealed a novel missense mutation, Gln127Arg, in all affected members of the family with SCA14. This mutation was not found in 122 control individuals. No mutations in the PRKCG gene were detected in the group of 24 probands with SCA of unknown type.

Conclusions: These findings document that SCA14 is caused by mutations in the PRKCG gene. The observation that all 4 PRKCG mutations identified in patients with SCA to date are located in exon 4 suggests a critical role for this region of the gene in cerebellar function. Mutations in the same region of the gene can result in myoclonus in some families but not in others.
The family we studied (previously). In that study, 19 individuals were examined and investigations, disease course, and haplotype analysis was published previously. We examined 24 probands with ataxia and family histories consistent with autosomal dominant SCA who were residents of Hokkaido. The mean±SD age at onset was 43.4±11.2 years with a range of 19 to 69 years. Clinically, all of these patients had progressive limb and gait ataxia, dysarthria, and nystagmus. Limb reflexes were mostly normal, but a few affected individuals showed slight hyporeflexia or hyperreflexia with spasticity. None had myoclonus, extrapyramidal signs, ophthalmoplegia, retinal degeneration, skeletal muscle atrophy, or intellectual disturbance. Superficial sensations were intact, and some individuals had a slight reduction in vibratory perception. On brain magnetic resonance imaging studies in all patients, atrophic changes in the cerebellum were restricted to the vermis cerebelli and hemispheres; in 2 patients, the brainstem was also affected.

Seven-two patients with multiple system atrophy (MSA) and 50 healthy individuals were also studied as controls. The diagnosis of MSA was based on the criteria of Gilman et al. The mean±SD age at onset of MSA was 53.4±11.2 years with a range of 38 to 77 years.

After obtaining informed consent, blood samples were obtained from these individuals, and DNA studies were performed using protocols approved by the ethics committees of Hokkaido University Graduate School of Medicine (Sapporo, Japan). The DNA genotyping excluded SCA1, SCA2, Machado-Joseph disease, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, and dentatorubral-pallidoluysian atrophy in all 24 probands with SCA. In samples from 19 members of the family with SCA14 and the 24 probands with SCA, all 18 coding exons of the PRKCG gene were amplified with polymerase chain reaction using the primers and method that had been reported previously. Amplified polymerase chain reaction products were sequenced directly using a sequencing kit (Big Dye Terminator Cycle Sequencing Kit; PE Applied Biosystems, Tokyo, Japan) with a DNA sequencing system (ABI PRISM 377; PE Applied Biosystems). After identifying a PRKCG mutation, DNA samples from 122 control individuals were examined for the mutation by sequencing exon 4 as described.

Direct nucleotide sequencing analysis of the 18 coding exons revealed a heterozygous mutation within exon 4 of the PRKCG gene in all 11 affected family members, the 76-year-old asymptomatic obligate carrier, and 1 other asymptomatic person (aged 44 years) in the family with SCA14. This A-to-G missense mutation at nucleotide position 380 predicts an amino acid change at codon 127 from uncharged glutamine to positively charged arginine (Gln127Arg; Q127R) (Figure 2). This glutamine residue is completely conserved in protein kinase Cγ in all species studied and in all other isoenzymes of protein kinase C. The mutation was not found in the 3 unaffected members or 3 spouses in the family with SCA14, 50 healthy individuals, or 72 patients with MSA. No mutations in the PRKCG gene were detected in the group of 24 probands with SCA of unknown type.

Methods

The family we studied (Figure 1) lives on Hokkaido, the northernmost island of Japan. Detailed information on the clinical evaluations, disease course, and haplotype analysis was published previously. In that study, 19 individuals were examined and interviewed to assess symptoms. Among 11 affected individuals, 5 patients with an early onset (at age <27 years) first showed intermittent axial myoclonus followed by ataxia. Patient II-3 was examined at age 76 years and was found to have no symptoms. However, 1 of the 2 children and all 3 grandchildren of this subject were clinically affected, indicating that patient II-5 is an obligate carrier. Neuroimaging studies were not performed for asymptomatic subjects, including the obligate carrier.

In addition, we examined 24 probands with ataxia and family histories consistent with autosomal dominant SCA who were residents of Hokkaido. The mean±SD age at onset was 43.4±11.2 years.
high expression in the Purkinje cells of the cerebellar cortex during dendritic development. Because a significant (≥20%) increase in all parameters of dendritic expansion of the Purkinje cells is found in slice cultures of PRKCG gene–deficient mice, this gene is thought to be a negative regulator of dendritic growth and branching in Purkinje cells. In addition, protein kinase C is known to be a key molecule for the expression of long-term depression at the parallel fiber–Purkinje cell synapse in the cerebellum. However, the exact effects of PRKCG mutations on cerebellar ataxia are difficult to predict. Haploinsufficiency at the PRKCG gene and/or gain of toxic function may contribute to disease progression. The lack of a neurologic phenotype in mice and rats that are heterozygous for null mutations argues against the former mechanism. A slowly developing gain of toxic function would be compatible with a delayed age at onset in this disease.

Protein kinase Cγ is a calcium-activated, phospholipid-dependent enzyme that comprises 2 domains. The catalytic domain contains adenosine triphosphate–binding and substrate recognition sites. The regulatory domain contains a calcium-binding region and 2 cysteine-rich regions, Cys1 and Cys2, that each bind zinc and diacylglycerol. The 4 PRKCG mutations associated with SCA14 to date cluster in exon 4 and affect highly conserved residues in the Cys2 region. This observation suggests that the Cys2 region may be critical for proper Purkinje cell function. Although our study suggests that the occurrence of SCA14 is rare in the Japanese population, the study of additional patients with SCA14 will be necessary to determine whether there is a restricted spectrum of PRKCG mutations that produce an SCA phenotype.

In 1998, Al-Maghtheh et al described 2 families with RP11-linked dominant retinitis pigmentosa in which a missense change (R659S) in the PRKCG gene cosegregated with the disease. Whereas mutations associated with SCA14 were localized to the regulatory domain, the mutations in patients with RP11 were located in the catalytic domain. None of our patients or those in the other families with SCA14 were reported to have retinitis pigmentosa. The difference in phenotypes might be dependent on the difference in PRKCG mutation sites.

In a previous investigation, we found that 42 (27%) of 155 unrelated families with late-onset familial SCA in Hokkaido did not have mutations in one of the known SCA genes. In the current study, with the exception of the index family, no PRKCG mutation was identified in 24 patients with SCA. Therefore, additional genes responsible for SCA remain to be identified.

Although no nucleotide repeat expansion was detected in the PRKCG gene, the pattern of age at onset in both the Japanese and US families with SCA14 is consistent with anticipation. In addition, the existence of 2 unaffected adult mutation carriers in the Japanese family is evidence of decreased penetrance. These observations imply that other genes or factors are involved in the clinical expression of this disease.

Our study suggests that axial myoclonus may be an early sign that distinguishes SCA14 from other ataxias. The exact cause of this involuntary movement is unknown. The lack of epileptic seizures and normal electroencephalogram results in this family suggest that the myoclonus may be of brainstem or spinal origin. It is unknown why this phenomenon has not occurred in the US families with slightly different mutations in the PRKCG gene. Careful clinical assessment of young family members at risk may allow early diagnosis.

Finally, identification of the molecular etiology of the PRKCG gene in SCA14 will aid in the classification of patients with SCA and benefit the development of clinical and genetic diagnostic strategies.

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Drs Yabe and Sasaki contributed equally to this work.

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