A deletion in the prion protein gene in a Japanese family

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ABSTRACT

A 24-bp deletion between codons 54-76 in the prion protein gene was found in a Japanese family having a variant of Gerstmann-Sträussler-Scheinker disease (GSS) associated with a missense mutation at the codon 105. Our results have indicated that the deletion is a rare non-pathogenic polymorphism that is present in Japanese individuals beyond ethnic background. In a family member, the deletion coexisted with the pathogenic codon 105 mutation. The coexistence of the deletion may possibly influence the disease phenotype of the GSS.

Mutations in human prion protein (PrP) gene are associated with prion diseases including Gerstmann-Sträussler-Scheinker disease (GSS) and Creutzfeldt-Jakob disease (CJD) (5, 6). We reported Leu¹⁰⁵ mutation with Val¹²⁹ polymorphism in the PrP gene in a Japanese family with a new GSS variant (GSS¹⁰⁵) (9). The GSS¹⁰⁵ was characterized by (1) slowly progressive motor disturbance (spastic paraparesis and ataxia) and dementia in middle age, and (2) numerous PrP amyloid plaques and atrophy in the cerebrum (9). In further studies of the PrP gene in the patient's family, we found a deletion in the PrP gene in addition to the codon 105 and 129 changes. A case in the family had both the deletion and the codon changes.

The patient was a 53-year-old Japanese man at the time of death who presented progressive spastic paraparesis, ataxia, and dementia. The pathological and molecular genetic studies revealed that the patient suffered from a new variant of GSS associated with Leu¹⁰⁵ mutation with Val¹²⁹ polymorphism in the PrP gene (9). The family tree of the patient is shown in Fig. 1. The PrP gene in the patient's family was further investigated in this study.

The PrP open reading frame of genomic DNA isolated from the brain tissue of the patient or white blood cells of other family members was amplified by the polymerase chain reactions (PCR) with four sets of primers, [1,(5'-CAGAGCAGTCATTATGGCGA-3',5'-TACTCGGCTTGGTCCACTGA-3') for the amplification of nucleotides 37-359; 2,(5'-ATGTTGCGTGGGTGCTGCAA-3',5'-ATCCA- TGGCCTGTAGTACA-3') for nucleotides 303-550; 3,(5'-ATGAGCAGGCCCATCATACA-3',5'- CTGATCTGGGTGATACACACA-3') for nucleotides 449-706; and 4,(5'-CAAGCAGACAGGTCACCCA-3',5'-GTGATACCCGCTCCTCAA-3') for nucleotides 601-875], with 30 cycles (denaturation, 94°C, 1 min; annealing, 60°C, 2 min; extension, 72°C, 3 min). PCR products were analyzed by size fractionation with agarose gel electrophoresis and by direct sequencing using the ABI 373A DNA sequencer (Applied Biosystems, Foster City, CA).

The codon 105 and 129 changes (Pro to Leu at codon 105, and Met to Val at codon 129) were shown in the PCR products with the primer set 2 from the patient (II-2) and his two asymptomatic children (III-1 and III-2) as previously described (9) (Fig. 1).

Further, the patient's wife (II-3) and one of his children (III-2), who were asymptomatic, had a one-allele deletion of about 20 base pairs (bp) in the PCR products with the primer set 1 (Fig. 1). The two DNA fragments (normal and deletion products) were gel-purified after electrophoresis in 4% NuSieve agarose gel (FMC Bioproducts, Rockland, ME), and were subjected to direct sequencing. The direct sequencing revealed a 24-bp nucleotide deletion between nucleotides 209 and 276 (codons 54-
Fig. 1  The family tree showing a deletion in the PrP gene. Square, male; circle, female; slashed symbol, deceased; closed symbol, affected individual; arrow, the patient; left superscript, subject number; right subscript, age at death; and right superscript, presence (+) or absence (−) of the Leu105 and Val129 changes in the PrP gene. Some family members are shown in diamonds, and the birth order of some individuals has been changed for confidentiality. Electrophoretic patterns of the PCR products with the primer set 1 indicating a deletion are shown under the symbols of family members (M=marker). Two DNA fragments, one of the expected size (323 bp) and the other of smaller size with a deletion of about 20 bp, are demonstrated in the patient's wife (II-3) and one of his children (III-2). Note that III-2 has both the codon 105 and 129 changes and the deletion.

![Diagram](image)

Fig. 2  The deletion site in five octameric peptide repeats encoded by codons 51–91 of the PrP gene. A deletion of 24-bp nucleotides is located at any site between nucleotides 209 and 276 (shown as the nucleotide sequence between two arrows) (codons 54–76) as demonstrated in direct sequencing of the deletion products. The deletion results in a loss of one of five octameric peptide repeats encoded by codons 51–91.

CCT CAG GCC GGT GGT GGC TGC GGC GAG CAG 226
Pro His Gly Gly Gly Gly Gly Trp Gly Glu
51 52 53 54 55 56 57 58 59

CCT CAT GGT GGT GGC TGC GGC GAG CAG 250
Pro His Gly Gly Gly Gly Gly Trp Gly Glu
60 61 62 63 64 65 66 67

CCT CAT GGT GGT GGC TGC GGC GAG CAG 274
Pro His Gly Gly Gly Gly Gly Trp Gly Glu
68 69 70 71 72 73 74 75

CCT CAT GGT GGT GGC TGC GGC GAG CAG 298
Pro His Gly Gly Gly Gly Gly Trp Gly Glu
76 77 78 79 80 81 82 83

CCT CAG GCC GGT GGT GGC TGC GGC GGA CAG 322
Pro His Gly Gly Gly Gly Gly Trp Gly Glu
84 85 86 87 88 89 90 91

76), which would result in the deletion of one of five octameric peptide repeats (Pro His Gly Gly Gly Trp Gly Glu) encoded by codons 51–91 (Fig. 2). Analyses with restriction enzymes indicated that the deletion was located between Dde I site at nucleotide 201 (codon 51) and the Nco I site at nucleotide 277 (codon 76) (data not shown).

DNA samples obtained from 100 unrelated Japanese individuals were included in this study to test for the presence of deletion in the PrP gene. We were unable to demonstrate this deletion in 100 unrelated Japanese individuals (data not shown).

In one of the patient’s children (III-2), who was asymptomatic in the twenties, had both the deletion and the codon 105 and 129 changes (Fig. 1). The deletion and the base changes were located on the different alleles, as demonstrated in the analyses of the longer PCR products (nucleotides 37–550) that encompassed both the deletion and codon changes (data not shown).

A 24-bp deletion between codons 54–76, which would result in the loss of one of five octameric peptide repeats encoded by codons 51–91, was demonstrated in family members of the GSS patient associated with the pathogenic codon 105 mutation with codon 129 polymorphism in the PrP gene (9). The deletion was not linked with the disease ex-
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pression, because the deletion was found in the patient's wife (II-3), but not in the patient (II-2).

Similar 24-bp deletions between codons 51–91 that result in a loss of one of the octapeptide repeats have been reported in Moroccan, Tunisian, and American Caucasian and Black individuals with or without prion disease, and in both the genomic Hela and human brain cDNA library (1, 3, 4, 7, 8). These reports have indicated that the deletions are not associated with prion disease, but represent polymorphisms in the PrP gene. Our results demonstrated that such a non-pathogenic 24-bp deletion is present also in Japanese individuals beyond ethnic background, and that the frequency of the deletion would be rare in the Japanese since the deletion could not be detected in 100 unrelated Japanese individuals.

The octamer peptide repeats encoded by codons 51–91, a site of the deletion, are important, because the presence of an insertion resulting in an increased number of the repeats is associated with familial CJD (5, 6), and the repeating octapeptides are predicted to contribute to a β-pleated sheet conformation of the PrP (7). Importantly, one of the patient's children (III-2) have both the 24-bp deletion and Leu105 mutation with Val129 polymorphism (Fig. 1) that are located on the different alleles. The pathogenic codon changes came from the farther (the patient) (II-2), and the non-pathogenic deletion from the mother (II-3) and the child (III-2) is devoid of normal PrP. Pathomechanism by which changes in the PrP gene cause or influence the development of prion diseases is unknown. Mice homozygous or heterozygous for disrupted PrP genes are protected against prion disease completely or partially, respectively (2). The coexistence of this deletion may influence the disease phenotype expressed by the Leu105 mutation with modification of Val129 polymorphism.

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REFERENCES