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NO EVIDENCE FOR A POINT MUTATION AT CODON 713 AND 717 OF AMYLOID PRECURSOR PROTEIN GENE IN JAPANESE SCHIZOPHRENICS

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Summary A point mutation at codon 717 of amyloid precursor protein (APP) gene has been demonstrated to play an important pathogenic role in some cases of familial Alzheimer's disease (FAD). Recently, a single case of chronic schizophrenia with a point mutation at codon 713 of APP gene which sits very close to the mutation in FAD was reported. We screened for these two kinds of mutations in 39 schizophrenic patients using polymerase chain reaction (PCR) and restriction enzyme technique. A mutation of codon 713 creates a *Mae*III restriction site and that of codon 717 creates a *Bcl*I site. Enzyme digestion with amplified PCR product revealed no restriction site in all subjects. None of our subjects had either of these two kinds of mutations. Our findings support the hypothesis that the case of a mutation at codon 713 of APP gene is a natural non-pathogenic variant and, as well as a mutation at codon 717, has no relation with the genetic predisposition to schizophrenia.

Key Words schizophrenia, amyloid precursor protein gene, point mutation

INTRODUCTION

Mutations at codon 717 in exon 17 of the amyloid precursor protein (APP) gene which is located on chromosome 21 have been previously shown to segregate with early-onset Alzheimer's disease in several families (Goate *et al.*, 1991; Naruse

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et al., 1991; Murrel *et al.*, 1992; Chartier-Harlin *et al.*, 1992). Following reports showed the evidence for other kinds of mutations in exon 16 or 17 of APP gene segregating with some types of familial Alzheimer's disease (FAD) (Hendriks *et al.*, 1992; Mullan *et al.*, 1992; Balbin *et al.*, 1992). Thus implications of mutation in APP gene and presumable unusual metabolic pathway of APP are now in the spotlight for the pathogenesis of a proportion of cases with FAD.

Jones *et al.* (1992) reported a single case of chronic schizophrenia with a C to T nucleotide substitution which yields an alanine to valine change at codon 713, just four codons removed from the cluster of the FAD mutations. It is interesting because some subtypes of schizophrenia progressively lead to intellectual deterioration, formerly called as "dementia praecox." Mant *et al.* (1992) recently reported that they failed to detect any abnormality in APP gene among schizophrenic families including a total of 191 individuals by the use of linkage analysis and single strand conformation polymorphism (SSCP) analysis.

To re-evaluate the correlation between a point mutation on APP gene and schizophrenia, we investigated whether a C to T nucleotide substitution which results in an alanine to valine change at codon 713 reported by Jones *et al.* (1992) or a G to A substitution which results in a valine to isoleucine change at codon 717 reported by Goate *et al.* (1991) might be present in 39 unrelated Japanese schizophrenics using PCR and restriction enzyme technique.

SUBJECTS AND METHODS

Thirty-nine unrelated schizophrenics diagnosed according to DSM-III-R were recruited from inpatients and outpatients of Department of Psychiatry, Teikyo University Hospital. Subjects consists of 20 males aged 33.6 ± 11.9 years (mean $\pm$ S.D.) and 19 females aged 40.0 ± 11.7 years (mean $\pm$ S.D.). Oral and written informed consents were obtained from all subjects.

Genomic DNA was isolated from peripheral white blood cells using standard phenol/chloroform methods. We obtained 319 bp product of exon 17 of the APP gene after the PCR amplification of genomic DNA using the oligonucleotide primer set which is identical with that of Jones *et al.* (1992): 5′-CCTCATCCAAATGT-CCCCGTCATT-3′ and 5′-GCCTAATTCTCTCATAGTCTTAACCCAC-3′.

Mutation at codon 713 creates a *Mae*III restriction site (/GTnAC) yielding fragments of 189 bp and 130 bp, and mutation at codon 717 creates a *Bcl*I restriction site (T/GATCA) yielding fragments of 199 bp and 120 bp, while wild-type DNA remains uncut.

We digested PCR product of exon 17 of the APP gene with *Mae*III or *Bcl*I according to manufacturer's recommendations. Following exposure to the restriction enzyme, the DNA was electrophoresed in 10% polyacrylamide gels. ϕX174/*Hae*III digest was used as a size marker. The polyacrylamide gels were stained with ethidium bromide and DNA fragments were visualized using a UV-transil-

luminator.

RESULTS

As shown in Fig. 1 and Fig. 2, none of the PCR products of subjects' DNA was cut after the *Mae*III or *Bcl*I digestion. No evidence for a C to T nucleotide substitution at codon 713 or a G to A at 717 was detected.

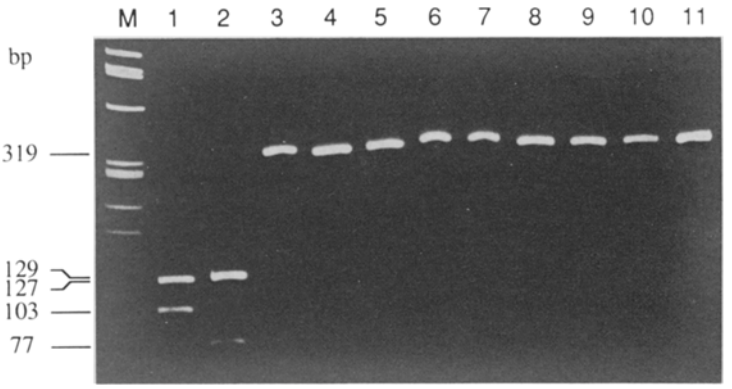

Fig. 1. Polyacrylamide gel analysis of amplified exon 17 after digestion with *Mae*III. Lane 1 and lane 2, PCR products of exon 2 of human leukocyte antigen DRB1 gene which contain a *Mae*III site as a positive control for the restriction enzyme reaction; from lane 3 to lane 11, samples from schizophrenic subjects. DNA from 39 unrelated schizophrenics remains uncut. ϕX174/*Mae*III digest was used as a marker to estimate fragment size (lane M).

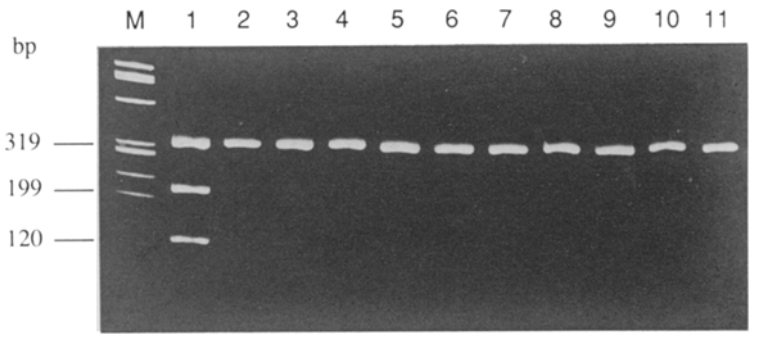

Fig. 2. Polyacrylamide gel analysis of amplified exon 17 after digestion with *Bcl*I. Lane 1, a sample from a case of early-onset FAD with a Val→Ile mutation at codon 717 (generous gift of Dr. S. Naruse and Prof. S. Tsuji, Department of Neurology, Brain Research Institute, Niigata University) as a positive control for the restriction enzyme reaction; from lane 2 to lane 11, samples from schizophrenic subjects. DNA from 39 unrelated schizophrenics remains uncut. ϕX174/*Hae*III digest was used as a marker to estimate fragment size (lane M).

DISCUSSION

The detection of a point mutation on APP gene in a case of chronic schizophrenia described by Jones *et al.* (1992) is interesting because there have never been a solid molecular biological evidence to explain the genetic basis for schizophrenia. To re-evaluate the significance of this mutation, further screenings were promptly conducted. Mant *et al.* (1992) found no evidence for a mutation on exon 17 of APP gene using SSCP analysis. Carter *et al.* (1993) and Coon *et al.* (1993) reported absence of a C to T nucleotide substitution at codon 713 by the use of PCR in 104 and 86 unrelated schizophrenics, respectively.

In this study, none of our Japanese subjects had either of these two kinds of mutations. Putting the findings by other investigators cited above together with our findings, the frequency of having these kinds of mutations among schizophrenics is estimated to be very low.

Our findings support the hypothesis that the case of a mutation at codon 713 of APP gene is a rare non-pathogenic variant of no medical importance as Jones *et al.* (1992) initially implied. It is unlikely that the sequence variants such as at codon 713 or 717 of APP gene have any relation with the genetic predisposition to schizophrenia.

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