

Methods

Alcohol consumption and TPQ test

Mean values of alcohol consumption (ethanol g / month / person) of patients with PD were estimated from those of their premorbid ages (40~50 years old) by direct interview, and those of controls were obtained by questionnaire.

TPQ of PD patients were tested by direct interview, and control subjects were given the test in Japanese conventional questionnaire form (Takeuchi et al., 1993).

Genotypes

A 135 base pair fragment of exon 12 involving the mutation site of the ALDH2 gene (1510 G→A) was amplified using the primers (Table 2) labeled with indodicarbocyanine fluorescent dye (Cy5, Pharmacia Biotech, Uppsala) (Gong et al., 1998). The primer sets labeled with Cy5 were prepared for amplification of the CCK gene.

Polymerase chain reaction (PCR) was performed using a 25 μ l volume of Tris-HCl buffer (pH 8.0) containing 100ng genomic DNA, 8.25p mol of each primer, 50mM KCl, 1.5mM MgCl₂, 200 (M of dNTP, and 1U Taq DNA polymerase (TaKaRa Biomedicals, Tokyo).

The cycling conditions of the PCR were as follows: an initial 3 min denaturation phase at 97°C, 30 cycles of the denaturing phase at 97°C for 30 sec, using an annealing temperature step (Table 2) with a duration of 30 sec. Followed an extension step at 72°C for 30 sec, and a final extension step at 72°C for 10 min (Gene Amp 9600, Perkin Elmer, Norwalk, CT). The PCR products were visualized under UV light after staining with ethidium bromide.

Fluorescence-based Single Strand Conformational Change

Polymorphism (SSCP) was carried out by using DNA sequencer (ALF express, Pharmacia Biotech, Uppsala) equipped with a short gel plate. One μ l of the each PCR product was diluted with 14 μ l of a loading solution containing 99.5% deionized formamide and 0.5% blue dextran. After denaturation at 96°C, 5 min, 2 μ l of the mixture was applied to a 10% polyacrylamide gel (acrylamide and bisacrylamide ratio; 99:1 for the ALDH2 genotypes and 49:1 for the CCK genotypes). Electrophoresis was done at 700V, 20mA and 20W for 4 hr at 18°C.

DNA sequences of the PCR products showing altered banding patterns based on the SSCP analysis were determined directly using a cycle sequencing system (Dye Terminator Cycle Sequencing Ready Reaction, ABI PRISM™ 310 genetic Analyzer; Perkin-Elmer, Japan).

Statistical analysis

The comparison between PD patients and control subjects were analyzed using a chi-square test, or alternatively, by a one-way analysis of variance (ANOVA) test (P values less than 0.05 were considered statistically significant). Bonferroni correction for multiple comparisons was carried out for the positive result to exclude type I error.

Analysis of linkage disequilibrium between two given loci were performed by using the ASSOCIAT (version 2.32) software in conjugation with the LINKAGE UTILITY programs (Terwillinger JD et al., 1994). D' values for linkage disequilibrium were also calculated according to the previous report (Chen et al., 1997).