

*Short Communication*

DUPLICATION POLYMORPHISM WITHIN THE  
THIRD INTRON OF THE *p53* GENE IS A RARE  
EVENT IN JAPANESE POPULATION

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One of the tumor suppressor genes, human *p53* gene has been assigned to 17p13.1 (Isobe *et al.*, 1986; McBride *et al.*, 1986) and its molecular abnormalities were frequently found in various tumor cells. The sequence polymorphisms within tumor suppressor genes are useful for the detection of loss of allele, and also it is important to be aware of rare sequence polymorphisms in the screening for gene mutations. Lazar *et al.* have identified a new simple sequence repeat polymorphism from the 11951st to the 11966th of the *p53* gene (Chumakov *et al.*, 1991), and they found that 28% of individuals were heterozygotes for this polymorphism in the Caucasian population examined (Lazar *et al.*, 1993). We report here the allele frequency of this polymorphism in Japanese population, since it was significantly different from that found in Caucasian population.

*Materials and Methods*

We obtained genomic DNA from peripheral leucocytes or non-cancerous lung tissues from 183 unrelated Japanese individuals including 56 lung cancer patients. PCR primers were designed to amplify a 460 bp (A1) and 476 bp (A2) containing the polymorphic repeat on the third intron of the *p53* gene.

Genomic DNA (100 ng) was amplified in a 50  $\mu$ l reaction mixture containing 200  $\mu$ M each dNTP, 1.5 mM MgCl<sub>2</sub>, 0.25  $\mu$ M each primer (sense and antisense primers: 5'-GGACTGACTTTCTGCTCTTG-3', 5'-TGAAGTCTCATGGAAGCC-

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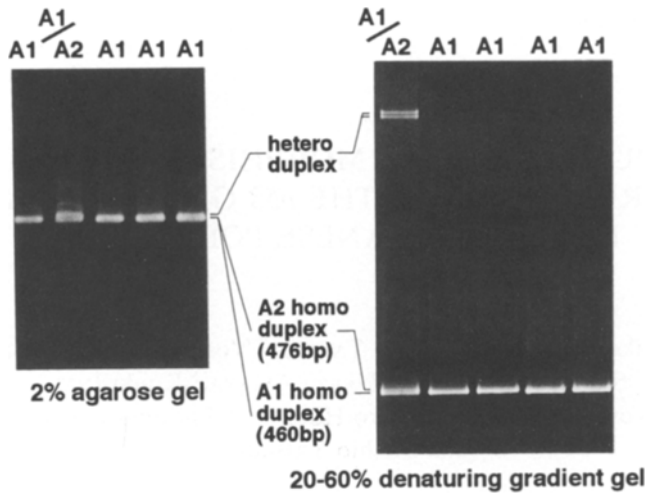


Fig. 1. Ethidium bromide stained agarose gel (left) and denaturing gradient gel (right), showing the heteroduplex bands of PCR products.

AG-3'), and 1 unit of Taq DNA polymerase (Wako, Osaka, Japan). A program temperature control system (Astec, Fukuoka, Japan) was used for 40 cycles of: 60 sec at 95°C, 60 sec at 60°C, and 90 sec at 74°C.

The heteroduplex bands consisting of one strand of A1 allele and another strand of A2 allele were detected on a 2% agarose gel and a 20–60% (100% = 7 M urea, 40% Formamide) denaturing gradient gel following the method reported previously (Takahashi *et al.*, 1990) (Fig. 1).

### Results and Discussion

Allele frequencies from 183 unrelated Japanese were calculated. Allele A1 corresponding to the *p53* wild-type sequence was found in 361 chromosomes (98.6%), while allele A2 with the 16-bp duplication was found in 5 chromosomes (1.4%). The observed heterozygosity was 0.027, and the frequency of allele A2 was significantly lower than that reported in Caucasian (20%, Lazar *et al.*, 1993) ( $P < 0.001$ ). The discrepancy between Caucasian and Japanese in allele frequency of this polymorphism may reflect the racial difference.

Lazar *et al.* reported that this polymorphism was not associated with a predisposition to breast cancer. In our study, among 56 patients with lung cancer, only one patient showed the heterozygosity of this polymorphism (0.018). This polymorphism seems not to be associated with a predisposition to lung cancer as well as breast cancer.

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