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Polymorphism Report

A G-to-A single nucleotide polymorphism in intron 2 of the human CACNA2D2 gene that maps at 3p21.3

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SOURCE/DESCRIPTION

A single nucleotide polymorphism (SNP) was found in intron 2 of the human Alpha 2 Delta calcium channel subunit 2 gene (CACNA2D2, GenBank acc. AF042792) by single strand conformational polymorphism (SSCP) method, during mutation analysis of normal/tumor paired DNA samples obtained from small-cell lung cancer patients.[1,2] The Alpha 2 Delta 2 gene maps at 3p21.3, in the region that most frequently undergoes loss of heterozygosity (LOH) in lung cancer. The polymorphism consists of a G-to-A transition (Fig. 1(b)) and is located on cosmid LUCA 9 (residue 2208, GenBank acc. Z75743). The allele 'A' has an index of heterozygosity of 33% among small-cell lung cancer patients. This SNP is informative with regard to LOH investigation in cancer patients (Fig. 1(a)). No biased loss of one allele over the other was observed. Since no data are available on the ethnic background of the patients tested, CEPH[3] individuals were used as control. Among them, the allele 'A' has an index of heterozygosity of 22%. The G-to-A transition eliminates a *Dde*I restriction site from the 115-bp PCR product obtained with the following primers.

PRIMERS SEQUENCE

Lu9 2Fw: 5′ GTC TCC TCT TTG GAC AGA TTC TG
Lu9 2Rv: 5′ TGG AGA TGA CTG TAA CAA GGG CAC

PCR-SSCP ANALYSIS

The radioactive reaction was performed in a total reaction volume of 12·5 µl, containing 100 ng of genomic DNA, 12·5 pmol of each primer, 200 µM dNTPs, 1·5 mM $MgCl_2$, 1·25 nCi α35S-dATP. Primers amplify a single product under the following cycling conditions: 3 min at 95°C; 35 × (1 min at 95°C, 30 s at 64°C, 1 min at 72°C); 7 min at 72°C. After heat

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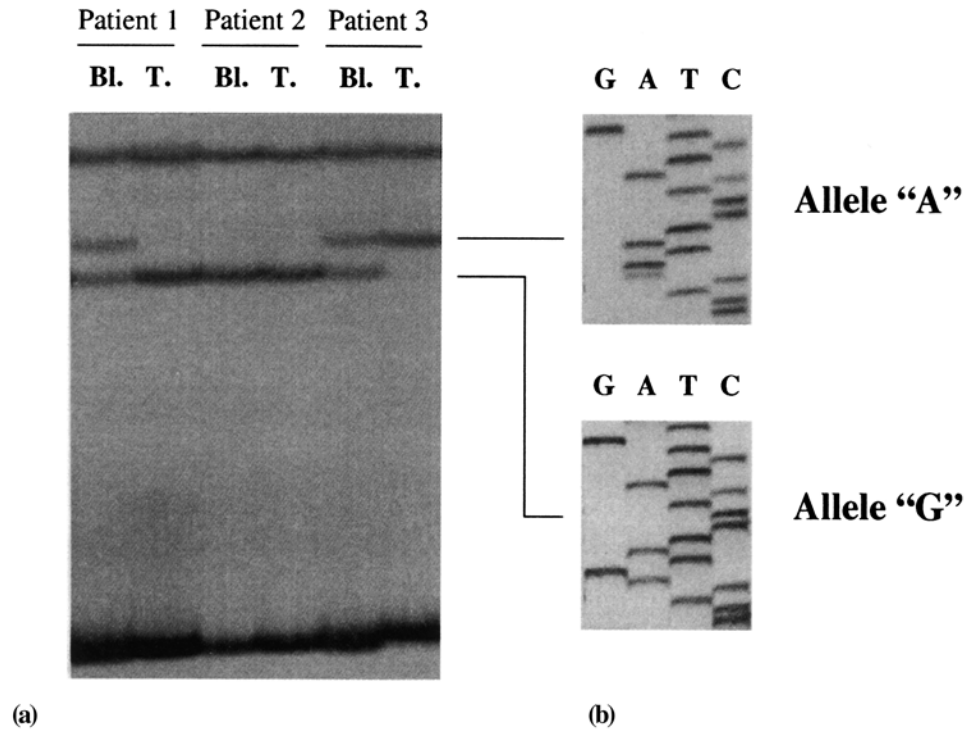

Fig. 1. Mutation analysis of the CACNA2D2 gene in small cell lung cancer patients, by means of Lu9 2Fw-2Rv primers, revealed the presence of a SNP located in intron 2. (a) SSCP profile of three different patients. Allele 'A' gives the upper band, allele 'G' gives the lower band (sequence in (b)). B1, DNA extracted from blood; T, DNA extracted from tumor. This heterozygous marker is a diagnostic tool of LOH in lung cancer patients.

denaturation (8 min at 90°C) in formamide buffer (Stop Solution, Amersham, Arlington Heights, IL, USA), PCR products (115 bp) were run overnight in a 0·5 × MDE gel (FMC Bioproducts, Rockland ME), 0·6 × TBE, at room temperature, 8 W constant power; transferred on 3 MM paper, dried and exposed to autoradiography film (X-OMAT AR, Kodak, Rochester, NY, USA).

SEQUENCING

Sequencing reactions were done either manually (T7 Sequenase Kit, Amersham, Arlington Heights, IL, USA) or automatically (ABI 373 Stretch Automated DNA Sequencer, Applied Biosystems, Foster City, CA, USA).

FREQUENCY

In total, 21 pairs of normal/tumor DNA, obtained from lung cancer patients, were analysed. Nine individuals (paired samples number: 8066/866, 5297/5301, 43/5322, 7161/6395, 5305/5310, 8362/8364, 7995/10213, 930/825, Bl1/H128, affected with small cell lung cancer), were found heterozygous for the allele 'A' (Fig. 1). The polymorphic allele was found in eight CEPH[3] control individuals (1349–01; 23–02; 35–02; 1345–01, homozygous; 1424–02; 28–01; 1355–01; 1447–01) out of 36 analysed. The allele 'A' index of heterozygosity corresponds to 33% among small cell lung cancer patients, and to 22% in the CEPH control population.

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