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Polymorphism Report

A G-to-A single nucleotide polymorphism in the human Alpha 2 Delta 2 calcium channel subunit gene that maps at chromosome 3p21.3

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SOURCE/DESCRIPTION

A single nucleotide polymorphism (SNP) in the human Alpha 2 Delta subunit 2 calcium channel gene (CACNA2D2; GenBank accession #AF042792) was found by single-strand conformation polymorphism (SSCP) method, during mutation analysis of normal/tumor paired DNA samples obtained from 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), it is intronic and located on cosmid LUCA 11 (GenBank accession #Z84492). The allele 'A' has an index of heterozygosity of 23% among lung cancer patients and 8% in a group of CEPH[3] control individuals.

The G-to-A transition creates an *Alu*I restriction site that cleaves into two fragments of 172 and 63 bp, the 235 bp PCR product obtained with the primers indicated below.

PRIMER SEQUENCES

MJE7 Fw: 5′-GGA GGG CTG AGA GCT GCC TG
MJE7 Rv: 5′-TTG AGG TTA CTG CTG TGG CCA C

PCR-SSCP analysis

A total of 100 ng of DNA was used per each reaction. Normal DNA was extracted from peripheral white blood cells, tumor DNA from surgically resected tumor tissue. The radioactive PCR was performed in a total reaction volume of 12·5 µl, containing, 12·5 pmol of each primer, 200 µM dNTPs, 1·5 mM MgCl2, 1·25 nCi α35 S-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 62°C, 1 min at 72°C); 7 min at 72°C. After heat denaturation (8 min at 90°C) in formamide buffer (Stop Solution, Amersham, Arlington Heights IL, USA), PCR products (235 bp)

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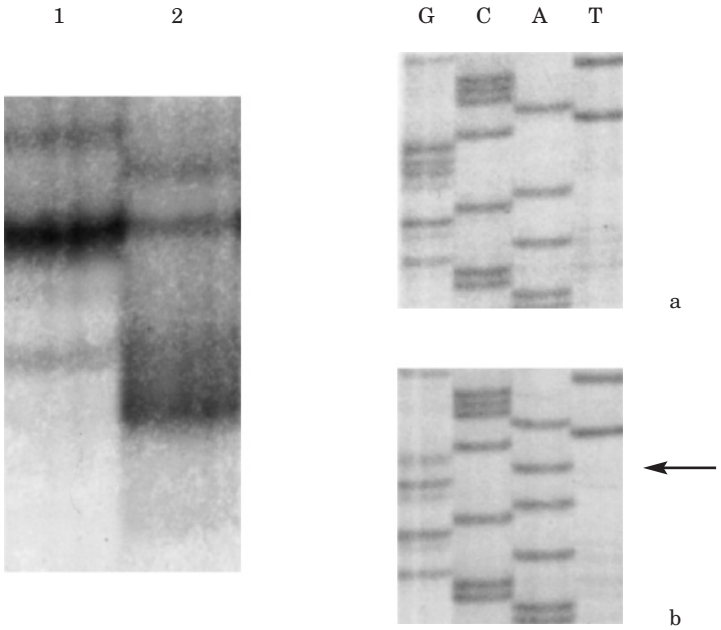

Fig. 1. SSCP analysis of the Alpha 2 Delta 2 calcium channel gene in lung cancer patients, by means of MJE7 primers, revealed the presence of an intronic SNP. Lane 1: migration profile given by the more frequent allele 'G' (sequence in inset a); Lane 2: profile of the allele 'A' (sequence in inset b), which was found in six out of 23 lung cancer patients and two out of 24 CEPH individuals.

were run overnight in a 0·5 × MDE gel (FMC Bioproducts, Rockland ME, USA), 0·6 × TBE, at RT, 8 W constant power; transferred on 3 MM paper, dried and exposed to autoradiography film (X-OMAT AR, USA, 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

Twenty-three pairs of normal/tumor DNA, obtained from lung cancer patients, were analysed. Six individuals (paired samples number: 34/856; 8906/8361; 848/1237 and 1245/963, affected with small cell lung cancer; 932/1192 and 5364/5360, affected with non-small-cell lung cancer), were found heterozygous for the allele 'A' (Fig. 1). The polymorphic allele was found in two CEPH[3] control individuals (45-01 and 1344-01) out of 24 analysed. The allele 'A' index of heterozygosity corresponds to 23% among lung cancer patients, and to 8% in the control population. Further studies are ongoing, on a larger sample of lung cancer patients and control individuals, to better estimate these values.

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REFERENCES

1. Johnson, B. E., Makuch, R. W., Simmons, A. D., Gazdar, A. F., Burch, D., Cashell, A. W. (1988). myc family DNA amplification in small cell lung cancer patients' tumors and corresponding cell lines. *Cancer Research* **48**, 5163–6.
2. Phelps, R. M., Johnson, B. E., Ihde, D. C., Gazdar, A. F., Carbone, D. P., McClintock, P. R. *et al.* (1996). NCI-Navy Medical Oncology Branch cell line data base. *Journal of Cellular Biochemistry* **24**, 32–91.
3. Dausset, J., Cann, H., Cohen, D., Lathrop, M., Lalouel, J. M., White, R. (1990). Centre d'etude du polymorphisme humain (CEPH): collaborative genetic mapping of the human genome. *Genomics* **6**, 575–7.