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Polymorphism Report

C306A single nucleotide polymorphism in the human CEBPD gene that maps at 8p11.1–p11.2

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INTRODUCTION

The CCAAT/enhancer binding protein delta (CEBPD, GenBank accession number NM_005195, OMIM entry number 116898) is a member of the CEBP family of the basic-leucine zipper class of transcriptional regulators. While low levels of CEBPD mRNA are detectable in several organs of adult mice and humans, expression is dramatically induced by bacterial lipopolysaccharide and inflammatory cytokines, suggesting a role in the acute phase and inflammatory responses.[1] In the lung, however, CEBPD is constitutively expressed. In rabbit fetal lung, expression increases to adult levels by late gestation. CEBPD expression is also activated during differentiation of human fetal lung tissue in culture.[2] A number of differentiation specific genes are implicated as targets for CEBP regulation, e.g. Clara cell secretory protein, surfactant protein and annexin I.[3,4,5] By immunostaining, the CEBPD protein was localized to rat alveolar type II cells[6] and bronchiolar Clara cells.[7] These cell types are the primary origin of pulmonary adenocarcinoma.[8] Therefore, to investigate the possible role of the CEBPD gene in causing lung cancer, we have done mutation analysis of the gene, with the single-strand conformational polymorphism (SSCP) method, on a collection of normal/tumour-paired DNA samples obtained from non-small-cell lung cancer patients.[9,10]

During this study, a C306A single nucleotide polymorphism (SNP) was found in 1/34 patients (frequency: 2·9%). It is a same sense mutation that does not change the corresponding amino acid residue (Pro 102). This base change is classified as a polymorphism as it was found in 1/42 healthy CEPH[11] Caucasian individuals (frequency: 2·3%) and 2/36 healthy Asian individuals (frequency: 5·4%). It was not found in any of 33 healthy African–American individuals.

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Table 1. Changes in the restriction pattern introduced by the C306A SNP

Change	Position in the PCR product (bp)	Pattern
Abolished sites		
*Bcn*I	56	Three bands (10 bp, 61 bp, 82 bp)
*Nci*I	56	As above
*Bsi*SI	57	Three bands (9 bp, 61 bp, 83 bp)
*Hap*II	57	As above
*Hin*2I	57	As above
*Hpa*II	57	As above
*Msp*I	57	As above
Created sites		
*Bst*2UI	56	Three bands (13 bp, 43 bp, 97 bp)
*Bst*NI	56	As above
*Bst*OI	56	As above
*Eco*RII	56	As above

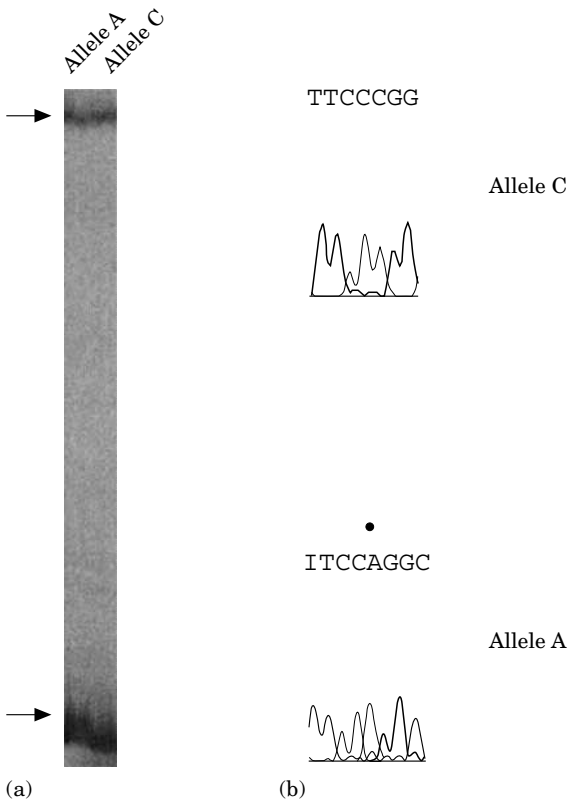

Fig. 1. SSCP mutation analysis of the CEBPD gene in lung cancer patients with the 2BFw–2Rv primers revealed the presence of the C306A SNP, present also in normal control individuals. (a) SSCP profile of allele 'A' and allele 'C'. (b) Corresponding sequence.

MATERIALS AND METHODS

Primer sequences

Two primers were used, with the following sequences: CEBP-2BFw, 5′ TGG AGC TGT GCC ACG ACG AG3′; CEBP-2Rv, 5′ AGT CGG GCT CGC GCT TGA GC3′.

Polymerase chain reaction–single-strand conformational polymorphism (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 α^{35}S-dATP, 0·5 M GC-RICH resolution buffer (GC-RICH PCR System, Roche Molecular Biochemicals, Indianapolis, IN, USA). Primers amplify a 153-bp product under the following cycling conditions: 3 min at 95°C (1 min at 95°C, 30 s at 64°C, 1 min at 72°C) for 35 cycles; 7 min extension at 72°C. After heat denaturation (8 min at 90°C) in formamide buffer (Stop Solution, Amersham, Arlington Heights, IL, USA), PCR products (154 bp) were run overnight in a 0·5 × Mutation Detection Enhancement (MDE) gel (FMC Bioproducts, Rockland, ME, USA), 0·6 × Tris Borate EDTA buffer (TBE), at room temperature, 8 W constant power; transferred on 3MM 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) or automatically (ABI 373 Stretch Automated DNA Sequencer, Applied Biosystems, Foster City, CA, USA).

Restriction analysis

The C306A SNP introduces the restriction pattern changes summarized in Table 1. They can be analysed on high-resolution gel, such as 4·5–5% NuSieve GTG Agarose (FMC, Rockland, ME, USA).

RESULTS AND DISCUSSION

Frequency

Forty-two Caucasian CEPH[11] control individuals were analysed. One (1334-02) was found heterozygous for the allele 'A' (Fig. 1). The carrier family 1334 has been analysed for this polymorphism to prove Mendelian co-dominant inheritance of the genotypic trait (data not shown).

Two out of 36 Asian normal individuals were found heterozygous for the same SNP. None out of the 33 normal African–American individuals tested carries the polymorphism.

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