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Research article

Use of a PCR-based amplification analysis as a substitute for the Southern blot method to determine the *C4A* and *C4B* genes

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Abstract

The human C4 complement components of the *C4* gene are encoded by two genes, *C4A* and *C4B*, located on chromosome 6p21.3 of the major histocompatibility complex (MHC) of the human leukocyte antigen (HLA) class III region. Genetic determination of these two genes was by the Southern blot method: the 276- and 191-bp *Nla*IV fragments represent the *C4A* gene with the sequence, PCPVLP, at residues 1101–1106; the 467-bp *Nla*IV fragment represents the *C4B* gene with the sequence, LSPVIH, at residues 1101–1106. Here, we describe a PCR-based approach for differential amplification of the *C4* genes adjacent to the respective *CYP21A1P* and *CYP21A2*, followed by *Nla*IV restriction digestion in a secondary PCR product and direct analysis by electrophoresis on an agarose gel to determine the *C4A* and *C4B* genes. From the results of this study, we concluded that 87% and 85% of the *C4* genes adjacent to the *CYP21A1P* and *CYP21A2* genes carried the *C4A* and *C4B* genes, respectively. The frequencies of the *C4A* and *C4B* genes comprising the *C4* locus were 51.5 and 49%, respectively in this ethnic Chinese (Taiwanese) cohort. Since no radiolabelling application is involved, the protocol is reliable as a substitute for the Southern blot method for *C4A* and *C4B* determination.
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Keywords: C4 component complement; PCR-based amplification; Southern blot; RFLP; Rodgers and Chido; HLA class III

1. Introduction

The human C4 complement components of the *C4* gene are encoded by two genes, *C4A* and *C4B*, located on chromosome 6p21.3 of the major histocompatibility complex (MHC) of the human leukocyte antigen (HLA) class III region. In this region, the RCCX module is composed of part of the *RP* gene (serine/threonine nuclear protein kinase) (Shen et al., 1994), a full-complement *C4* gene, the entire *CYP21A2* (*CYP21A1P*) gene, and a portion of the *TNX* gene (Gitelman et al., 1992; Bristow et al., 1993). The *TNX* gene contains *XA* (*TNXA*) and *TNXB*. *TNXB*, located downstream of the *CYP21A2* gene, is partially duplicated in the downstream *CYP21A1P* gene, where a truncated gene is termed

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TNXA. *TNXA* and *TNXB* are transcribed on opposite strands. There are two *RP* genes, *RP1* and *RP2*. The *RP2* gene is truncated and corresponds to *RP1* adjacent to *TNXA*. These genes are arranged in an *RP1-C4A-CYP21A1P-XA-RP2-C4B-CYP21A2-TNXB* gene sequence and are designated the bimodule of RCCX (Koppens et al., 2002) (Fig. 1). The bimodule is composed of a long module including part of *RP1*, *C4A* (long), *CYP21A1P*, and *TNXA* and a short module containing *RP2*, *C4B* (short), *CYP21A2*, and part of the *TNXB* gene (Fig. 1). The RCCX module has three possible forms: monomodular, bimodular, and trimodular in Caucasians; the bimodular form occurs most frequently (Yu, 1998; Blanchong et al., 2001). Therefore, the copy number of *C4* genes in a diploid chromosome predominantly varies from two to six (Yu, 1998; Blanchong et al., 2001).

Both *C4A* and *C4B* contain 41 exons with 1744 amino acid residues sharing > 99% nucleotide sequence homology (Belt et al., 1984). The difference in the biochemical properties of these two proteins is caused by four amino acids at positions 1101, 1102, 1105, and 1106 in exon 26 of the C4d region (Dodds et al., 1996). The isotypic residues at position 1101–1106 of *C4A* contain the sequence PCPVLD and *C4B* contains the sequence LSPVHI (Dodds et al., 1996). However, D1106 is the critical residue affecting the favorable binding of *C4A* towards IgG immune aggregates (Carroll et al., 1990). Based on gross charge differences, there are 41 isoforms of allotypes of *C4A* and *C4B* (Mauff et al., 1998). In addition, the Rodgers (Rg) and Chido (Ch) blood group antigens were shown to be correlated with the C4d region of *C4A* and *C4B*, respectively (Yu et al., 1986). Usually, *C4A* expresses Rg and *C4B* expresses Ch (Blanchong et al., 2000). The Rg residue of PCPVLD has a higher affinity for binding antigens containing amino groups, whereas the Ch residue, LSPVIH, has a higher affinity for antigens containing hydroxyl groups (Blanchong et al., 2000). Excess production of component C4 could possibly

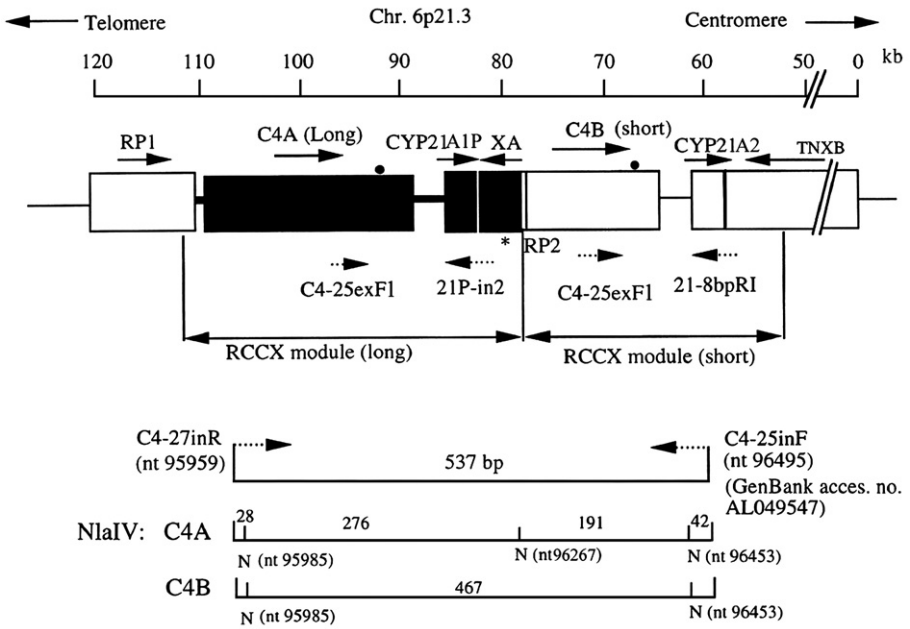

Fig. 1. Bimodular form (*RP1-C4A-CYP21A1P-XA-RP2-C4B-CYP21A2-TNXB*) of the RCCX region of chromosome (Chr.) 6p21.3 and the strategy for *C4A* and *C4B* determination of the *C4* gene by the PCR amplification method. The white box indicates the structures of the *RP1*, *CYP21A2*, and *C4B* genes; the black box represents the *C4A*, *CYP21A1P*, *XA*, and *RP2* genes. Sizes of the genes from the ATG start codon to the TGA stop codon, including *RP1*, *C4A*, *CYP21A1P*, *XA*, *RP2*, *C4B*, *CYP21A2*, and *TNXB*, in the RCCX module of the figure, are based on the sequences of GenBank accession nos. AL049547 and AF019413. The presence of *C4A* (the long gene, 20.4 kb) or *C4B* (the short gene, 14.1 kb) depends on the presence of HERV-K (C4), the endogenous 6.7-kb retroviral sequence, in intron 9. The locations of residues at position 1101–1106 in exon 26 are marked with a solid circle (●) in both the *C4A* and *C4B* genes. The boundary of the bimodular form consisting of both the long and short RCCX modules is also shown. Solid arrows indicate the orientation of transcription, and dashed arrows show the location of the primers for PCR amplification. A 121-bp deletion in exon 36 of the *TNXA* gene is marked with an asterisk (*). Top: scale in kilobases (kb), with the *TNXB* gene starting at 0. Bottom: restriction digestion analysis of *Nla*IV restriction fragments on a secondary PCR product amplified with the primer pair, C4-25inF/C4-27inR, in the region (nucleotides 95,959–96,495; GenBank accession no. AL049547) of residues 1101–1106 for the *C4A* and *C4B* genes, scaled by EMBL/GenBank/DDBJ accession no. AL049547. Each 'N' below the scale indicates an *Nla*IV restriction site.

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