

IDENTIFICATION OF HLA-CW*0409N AND ITS DISTRIBUTION IN BLOOD DONORS RESIDENT IN WALES



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Introduction

Null alleles occur throughout the HLA genes manifested as absence of, or significantly reduced, cell surface HLA molecule expression.

Cw*0409N was the first null allele to be found in the HLA-C gene.

The lack of Cw*0409N cell surface expression is caused by a point deletion at nucleotide position 1095 in exon 7 (Balas et al. (2001) *Tissue Antigens*, **59**, 95). This results in a reading frame shift that adds 32 amino acids at the C-terminus of the HLA-Cw4 heavy chain (Zhigang et al. (2002) *Human Immunology*, **63**, 295) resulting in an elongated heavy chain that is not expressed at the cell surface.

Identification

During the routine update of primer mixtures for 'high resolution' HLA-C typing by PCR-SSP we designed mixtures to identify the Cw*0409N allele. Details of the mixtures are shown in Table 1.

Table 1. Primer mixtures for Cw*0409N

Primer mixture name	Primer name	5'-3' nucleotide sequence	Annealing position
D415	Cw5'1095-AAA	GAGTCTCTCATCGC-TTGTA	1075-1095
	Cw3' EX8	CCCACACACAGGC-AGCTGT	1103-1121
D416	Cw5'1095-AAG	GAGTCTCTCATCGC-TTGTAAG	1075-1095
	Cw3' EX8	CCCACACACAGGC-AGCTGT	1103-1121

The PCR-SSP mixture D416 amplified Cw*0409N.

Mixture D415 amplified all HLA-C alleles except Cw*0409N and Cw*0701-0715.

As part of their quality control, the mixtures were validated on DNA from the two original Cw*0409N cells (CTM6991383, 13W09501) and on 96 random donors from the Welsh Bone Marrow Donor Registry (WBMDR).

Unexpectedly, one donor (LD) gave a reproducible positive amplification with mixture D416 but was not amplified by D415, indicating the presence of Cw*0409N.

Serological typing

Serological HLA-A, B and C typing (including 18 Cw4 and 10 Cw7 antisera) confirmed the PCR-SSP-based phenotype except that LD was clearly Cw4 negative. The reactivity of LD's cells was normal. A family study showed the Cw*0409N-bearing haplotype was: A*02; B*44; DRB1*0701; DQB1*0202.

Further cases

We searched 8,412 phenotypes on the WBMDR for the likely haplotype, A*02; B*44; Cw*04.

187 donors had A*02; B*44; Cw*04. Those that had Cw*04 with a B allele other than B*44, e.g. B*35, *53 and also had a C allele known to be associated with B*44, e.g. Cw*05, *16, *07, were discarded.

The 87 remaining subjects and a 'control' group of 100 donors possessing Cw*04 without B*44, were tested for Cw*0409N.

Cw*0409N was found in 11 out of the 87, but none of the 100 'controls'.

Thus the minimum phenotype and gene frequencies for Cw*0409N in our blood donor population are 0.001308 and 0.000654 respectively.

10 of the 11 Cw*0409N donors were A*0201 and 1 was A*0205; all 11 were B*4403 and DRB1*0701.

Inspection of the 87 subjects showed that 31 (35.6%) possessed A*23.

Family studies on one of the 11 Cw*0409N subjects found the Cw*0409N bearing haplotype to be: A*23; B*4403; Cw*0409N; DRB1*0701; DQB1*0202.

Comments

From these studies it appears that Cw*0409N is usually present on a haplotype with A*0201 or A*23; B*4403; DRB1*0701 and DQB1*0202 and that this allele has phenotype and gene frequencies of not less than 0.001308 and 0.000654, respectively, in the largely northern European Caucasoid blood donor population resident in Wales.

Failure to identify a null allele could have serious implications in transplantation particularly bone marrow transplantation if a patient with a null allele is matched with a donor who has an expressed version of that allele.

Cw*0409N appears to have a higher frequency than other HLA-null alleles.