

# AN ANALYSIS OF 12,746 HLA-B;C HAPLOTYPES



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## Introduction

Since the advent of the Welsh Bone Marrow Donor Registry (WBMDR) in 1989, we have HLA-A, B, DR and DQ typed our blood donor panel members at registration.

However, HLA-C matching has now emerged as an important factor in successful unrelated stem cell transplantation. Furthermore, it is apparent that even with good HLA-B matching and the presence of strong linkage disequilibrium between HLA-B and HLA-C alleles, the probability of achieving a contingent HLA-C match is less than 50%.

This together with the WBMDR's continuing need to provide a service consistent with the timely provision of donor stem cells prompted us (in January 2000) to instigate additional HLA-C typing at the time of donor registration.

Here we present an analysis of HLA-C alleles and HLA-B, C 'haplotypes' in a contiguous population of 6,373 random unrelated blood donors resident in Wales and recruited onto the WBMDR.

## Methods

Five-locus HLA typing (A, B, C, DRB1 (3/4/5), DQB1) was done by PCR-SSP at the 'two-digit' level as a minimum.

Formal population genetics analysis included Hardy-Weinberg and homozygosity analysis, phenotype and maximum likelihood gene and haplotype frequencies and various linkage disequilibrium ( $\Delta$ ) parameters (Journal of Experimental and Clinical Immunogenetics, 1998, 15, 69).

## Results

*Hardy-Weinberg and homozygosity analysis.* These showed an excellent goodness-of-fit to that expected (all p-values > 0.25).

*HLA-B and C phenotype and gene frequencies.* 35 HLA-B and 15 HLA-C alleles were found. The alleles and their frequencies were all typical of a northern European Caucasoid population.

*Linkage disequilibrium between HLA-B and C alleles.* Formal population genetics analysis identified 39 HLA-B;C haplotypes that showed significant  $\Delta$  values (all corrected p values < 0.05).

*Further identification of HLA-B; C haplotypes.* The 39 haplotypes identified by the formal analysis were used as 'reference haplotypes' to assign likely B,C haplotypes to each phenotype, e.g. B8, B44; Cw5, Cw7 was assigned the haplotypes B8; Cw7/B44; Cw5; 82.5% of phenotypes completely matched these associations.

The remaining 1,115 phenotypes were examined by 2x2 tables between each HLA-B and C allele; a further 17 significant (uncorr. p < 0.05) associations were found.

These were added to the reference haplotypes and the phenotypes reassessed for their likely B;C haplotypes. Phenotypes complying fully with the extended list of haplotypes were removed and the remainder reanalysed.

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This iterative process was continued until no further significant B;C associations were identified. 250 phenotypes remained, after 70 reference haplotypes were found. These were generally assigned a single reference haplotype and their remaining B;C alleles were considered a further haplotype.

164 distinct HLA-B;C haplotypes (Table 1). A total of 164 different B;C haplotypes were identified. The most common, with a frequency of > 0.05, were: B8,Cw7; B7,Cw7; B44,Cw5; B60,Cw10 and B35,Cw4; 46 B;C haplotypes had a frequency of < 0.0001 and 7 low frequency HLA-B alleles were seen only in association with one C allele, e.g. B56; Cw1.

In 17 instances B alleles were seen with the same C allele in >85% of haplotypes, e.g. B8;Cw7 constituted 99.9% of B8-bearing haplotypes while B58;Cw7 constituted 85.9% of B58 haplotypes.

The most 'promiscuous' B alleles were B44 - found with 13 different C alleles; B51- found with 12 C alleles; B27 and B35- found with 11 C alleles and B7- found with 10 different C alleles.

**Table 1. Examples of the distribution of HLA-B;C haplotypes and, for each HLA-B allele, the proportion of HLA-C-bearing haplotypes. Full data tables are available from CD.**

Haplotype <sup>a</sup>	No. found	Frequency	Percent HLA-C-bearing haplotypes
B51/Cw1	57	0.00447	11.45
B51/Cw2	32	0.00251	6.43
B51/Cw9	12	0.00094	2.41
B51/Cw10	2	0.00016	0.40
B51/Cw4	22	0.00173	4.42
B51/Cw5	12	0.00094	2.41
B51/Cw6	14	0.00110	2.81
B51/Cw7	8	0.00063	1.61
B51/Cw8	0	0.00000	0.00
B51/Cw12	5	0.00039	1.00
B51/Cw14	88	0.00690	17.67
B51/Cw15	219	0.01718	43.98
B51/Cw16	27	0.00212	5.42
B51/Cw17	0	0.00000	0.00
B51/Cw18	0	0.00000	0.00
B52/Cw1	0	0.00000	0.00
B52/Cw2	0	0.00000	0.00
B52/Cw9	0	0.00000	0.00
B52/Cw10	0	0.00000	0.00
B52/Cw4	1	0.00008	2.33
B52/Cw5	0	0.00000	0.00
B52/Cw6	1	0.00008	2.33
B52/Cw7	1	0.00008	2.33
B52/Cw8	0	0.00000	0.00
B52/Cw12	39	0.00306	90.70
B52/Cw14	0	0.00000	0.00
B52/Cw15	1	0.00008	2.33
B52/Cw16	0	0.00000	0.00

<sup>a</sup> Specificities derived from equivalent allele groups from PCR-SSP.

## Comment

This comprehensive view of HLA-B;C haplotypes and their distribution is of considerable use in: compiling selection strategies for potential HLA-C matched stem cell donors; the routine validation of HLA-class I phenotypes; and the detection of new and rare HLA class I alleles.