Sequence-Based HLA Typing of 1,000 Young Donors on the Welsh Bone Marrow Donor Registry

J. PEPPERALL, J. JOHNSON, J. STREET & C. DARKE

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

Introduction

Almost since its inception, in 1989, Welsh Bone Marrow Donor Registry volunteer donors have been HLA-A, B, DRB1, DQB1 typed by PCR-SSP (C typed since 2001) essentially at the 1st field/specificity level.

Thus, some 90% of the Registry’s 54,463 currently active donors (December 2011) are fully and accurately 5-loci typed, albeit at ‘low resolution’.

To assess technical and service practicalities we have recently undertaken the HLA sequence-based typing of young volunteer donors at registration.

Donors

1,000 subjects of <25 years of age at registration were tested. All were blood donors resident in Wales and most came from a north-western European Caucasoid (NEC) general population.

HLA-A, B, C, DRB1, DQB1 sequence-based typing

This was performed on a 48 capillary Applied Biosystems 3730 DNA Analyzer with Data Collection Software v3.1.1 using SeCore kits, GSSPs to resolve ambiguities and uTYPE software for data analysis (Life Technologies).

Exons 2-4 were sequenced for HLA- A, B, C, exons 2 and 3 for DQB1 and exon 2 for DRB1 alleles.

Population genetics analyses

These analyses included Hardy-Weinberg equilibrium, carriage, allele and haplotype frequencies (HF) and linkage disequilibrium (LD) parameters and their significance.

Results

- Hardy-Weinberg equilibrium showed a good fit for all 5 loci (all p values >0.05).
- Allele family frequencies and their high frequency (>1.0%) HLA-A-B-DRB1 haplotypes were all typical of a north-western European Caucasoid population.
- The HLA-A*02 family showed most alleles, viz., A*02:01-03, 05-07, 11, 13, 38, 97, followed by DQB1*06- 06:01-04, 09, 14, 16, 27; B*15-15:01, 03, 07, 16-18, 24 and DRB1*13-13:01-03, 05, 10, 20, 36.
- Eight examples of alleles with no official serological specificity or confirmatory sequence were identified, e.g. A*01:37, C*06:07, DQB1*06:27, together with a single ‘null’ allele, viz, A*68:11N.
- Six examples of likely novel alleles were also identified - these were: 1 HLA-A*01, 1 B*27 and 4 DQB1 alleles.
- Noteworthy low frequency haplotypes (HF <0.5%), with significant positive LD values (corrected p < 0.001) included: A*02:11-B*40:06 B*15:18-C*07:04 DRB1*08:03-DQB1*06:01 DRB1*16:02-DQB1*05:02 A*03:01-B*51:01-DRB1*04:08 and A*11:01-B*39:06-DRB1*01:03.

Comment

This endeavour has established successful sequence-based typing for our haemopoietic stem cell donors and will help inform future strategies for high resolution typing at donor registration.