

HA-1 TYPING IN UNRELATED BONE MARROW PANEL DONORS RESIDENT IN WALES



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Introduction

The minor histocompatibility antigen, HA-1, is derived from a nonapeptide, termed HA-1^H, encoded by the KIA A0223 gene located on chromosome 19. The HA-1^H peptide, but not its allelic counterpart HA-1^R, has high affinity for HLA-A2 and is a target for T cell-mediated allorecognition in bone marrow transplantation. We have recently introduced HA-1 typing by PCR-SSP to allow HA-1 to be considered in donor selection prior to allogeneic bone marrow transplantation.

Methods

The PCR-SSP method was as previously described (Wilke et al. (1998) Tissue Antigens, 52, 312) with a minor modification to one primer to suit our reaction conditions (Thomas et al. (1996) Tissue Antigens, 48, 586). The primer mixtures were validated with two reference DNA samples, Ha05 (HA-1^{HH}) and DoHe (HA-1^{RR}), kindly supplied by M. Wilke (Leiden) and 166 random unrelated volunteers from the Welsh Bone Marrow Donor Registry (WBMDR).

Results

The reference DNA samples typed as expected and, of the 166 random donors, 30 (18.07%) were homozygous for HA-1^H, 53 (31.93%) were homozygous for HA-1^R and the remaining 83 (50.0 %) were HA-1^{HR} heterozygous. HA-1 typing of three samples is shown in Figure 1.

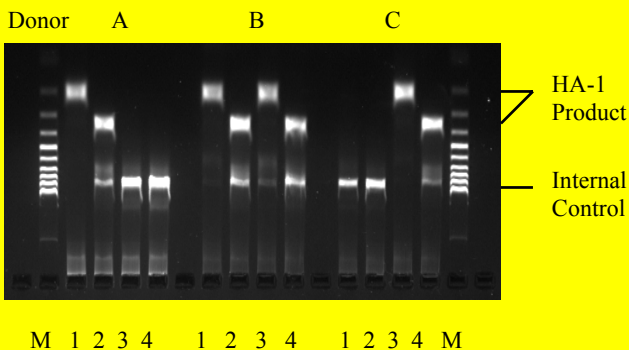


Figure 1. HA-1 typing by PCR-SSP of three donors typing as HA-1^{HH} (donor A), HA-1^{RR} (donor B) and HA-1^{HR} (donor C). Four PCR-SSP mixtures were used, two were HA-1^H specific (tracks 1 and 2), and two were HA-1^R specific (tracks 3 and 4). M, 100 bp DNA ladder (Helena BioSciences, UK).

HA-1 phenotype and gene frequencies

The phenotype and gene frequencies (derived by direct counting) for HA-1^H and HA-1^R were 68.07% and 0.43072, and 81.93% and 0.56928, respectively. The distribution of HA-1 phenotypes was consistent with those found in other Caucasoid populations (Tseng et al. (1998) Tissue Antigens, 52, 305).

Hardy-Weinberg and homozygosity analysis

The expected phenotype frequencies, from Hardy-Weinberg equilibrium (Table 1) and homozygosity analysis (Table 2), did not differ significantly from those observed in our study (all $p > 0.78$).

Table 1. Summary of Hardy-Weinberg analysis (n = 166)

HA-1 type	Total observed	Total expected	Chi-square
HH	30	30.859	0.024
HR	83	81.283	0.036
RR	53	53.858	0.014

Total chi-square = 0.074, for 1 degree of freedom $p = 0.782202$

Table 2. Summary of homozygosity analysis

HA-1	Number observed	Number expected	% homozygous in allele/null combination	Corrected Allele number of homozygotes
HH	30	30.86	99.65	29
RR	53	53.86	99.73	52

Total number of observed homozygotes : 83

Total expected homozygotes : 84.73

Proportion observed : 49.85%

Expected homozygosity (Watterson's F value) : 50.88%

Chi-square for goodness-of-fit : 0.03, $p = 0.853915$.

Conclusion

These findings support the validity of the typing method that will be used locally for bone marrow donor selection and typing of volunteer bone marrow donors at the time of registration on the WBMDR.