KEEPING UP WITH NEW HLA ALLELES – A TWO-TIER STRATEGY TO DIFFERENTIATE 'AMBIGUITY CAUSING ALLELES'

WELSH BLOOD SERVICE
GWASANAETH GWAED CYMRU

WELSH TRANSPLANTATION AND IMMUNOGENETICS LABORATORY

J. DOWNING, J. THOMPSON AND C. DARKE

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Introduction

Five-locus HLA typing (HLA-A, -B, -C, -DRB1 and -DQB1) is performed by PCR-SSP prior to enrolling haematopoetic stem cell (HSC) donor volunteers on the Welsh Bone Marrow Donor Registry.

We strive to assure that HLA typing is carried out essentially to the level of the first two digits of the allele name, generally equivalent to 'split' serological specificity.

'Ambiguity causing' alleles

Since new alleles are constantly being discovered we regularly review our PCR-SSP mixtures to confirm that all alleles are being detected.

The amplification specificity of each mixture is reviewed yearly against up-to-date nucleotide sequence alignments by manual assessment and by use of Helmburg's SCORE program.

Some new alleles simply extend our existing allele groups, while others require only small modifications to PCR-SSP mixture design to ensure their unambiguous detection.

However, some 'ambiguity causing alleles' prevent unequivocal assignments at the two digit level that can only be corrected by designing new PCR-SSP mixtures. For example: A*2301/3/5-8 cannot be differentiated from HLA-A*2413 and A*0301-4/7/9 cannot be differentiated from A*6819 in the presence of A*68.

Generally, the 'ambiguity causing alleles' are thought to be 'low frequency' in our primarily north-western European Caucasoid HSC donor population. However, accurate population frequency data is not known for these alleles.

Strategy

Consequently, in order to minimise the initial number of PCR-SSP mixtures required to HLA type HSC donors for HLA-A, B, C typing, we use a two-tier typing strategy.

Firstly each HSC donor is typed using our 126 well-established 'in-house' PCR-SSP mixtures. This allows unambiguous '2-digit' typing in just over half of HSC donors.

However, in the remainder an unambiguous assignment is not possible, for at least one locus. These are tested against 30 supplemental PCR-SSP mixtures designed to differentiate the 'ambiguity causing alleles'.

Practical application

We have now HLA typed 3,361 donors using the above strategy. 1,798 donors were assigned an unambiguous two-digit allele HLA-A, B, C, DRB1 and DQB1 type.

1,568 donors (46.57%) underwent supplemental typing.

We have identified one case where supplemental typing showed the donor type to be the less frequent 'ambiguity causing' allele. The donor initially typed as HLA-A*02 (0202/05/08/14/47/52), *03, but this amplification pattern could also be assigned as A*03, *030103. This is due to similarity between A*02 and A*030103 nucleotide sequence at the primer annealing sites used in the PCR-SSP mixture used to detect A*02. A*030103 has been detected in our donor population with an estimated phenotype and gene frequency of <0.02% and <0.00007 respectively.

On supplemental typing, the mixtures used to differentiate A*02 from A*030103 showed the presence of A*030103. This was confirmed by a further five PCR-SSP mixtures that detect A*030103. Thus the donor type was found to be A*030103, *03. If it had been assumed that the more frequent allele was present, i.e. A*02, this donor would have been incorrectly assigned as A*02, *03.

No other cases of rare 'ambiguity causing alleles' have been identified in the 3,361.

Discussion

Eventually this strategy will enable us to make judgements about the detection of rare 'ambiguity causing alleles' based on their frequency in our local blood donor population. Thus, it may be acceptable to 'ignore' such alleles with a frequency of, e.g., <1/10,000 donors (allele frequency of < 0.00005), while ensuring that their detection is guaranteed at the confirmatory typing stage.

Thus, we will type 10,000 donors, after which time the detection of particular unidentified rare 'ambiguity causing alleles' may be dropped from our HSC donor typing sets. Further mixtures can easily be added to our supplemental typing set to resolve further new 'ambiguity causing alleles' as they are identified.

This represents an efficient typing strategy as only those donors that are not able to be unambiguously assigned with a low resolution two-digit HLA type go through to supplementary typing stage. Thus, the average number PCR-SSP reactions used for each donor is kept to a minimum, helping to reduce typing costs, whilst maintaining high quality typing.