

SEQUENCE, GENETICS, SEROLOGY AND FREQUENCY OF A NEW HLA-B ALLELE B*4031



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Identification and nucleotide sequence

The finding of an unexpected amplification pattern (B*08+, B*40+ ['B60 allele group'], Bw4-/Bw6+, but B*44+) during routine PCR-SSP-based HLA typing of a blood donor (RG) on the Welsh Bone Marrow Donor Registry (WBMDR) identified, by nucleotide sequencing of exons 2 and 3, a new HLA allele - B*4031 (cell identification 33692 – B40'RG'; accession number AJ271160).

This allele differs from B*4001 by two nucleotide substitutions in exon 3 (positions 363 (G to C) and 412 (A to G)).

Amino acid substitutions

This results in two amino acid differences between B*4001 and B*4031 products (Arg to Ser at position 97 and Asn to Asp at position 114 (β -strand, $\alpha 2$ domain)).

Confirmation by PCR-SSP

Both base substitutions were confirmed by PCR-SSP; substitution at position 363 using primers L280 5'-GCTACG TGGACGACACGCT-3' and R15R 5'-CACGTGCGAG CCGTACATG-3' (these are not specific; other alleles amplified include: B*2707/11, B*4002/3/5/8/9/13/15/16/18/19/24/27/29/31/32/35, B*4102/4) and position 412 using L280 and R285 5'-CGTCGTAGGCGTACTGGTC-3' (other alleles amplified include: B*2706/21/22, B*4009/18/24/31/32/33, B*4104, B*4425).

HLA-B*4031-bearing haplotype

A family study showed B*4031 to be on the haplotype:

HLA-A*03; B*4031; Cw*0304; DRB1*0404/32; DRB4*0101/3/5; DQA1*03; DQB1*0302.

Serology of the HLA-B*4031 specificity

Serology was done with 45 well-characterised sera, with a B60 or B60 and B61 component. 41 of these were known to be stimulated, during pregnancy, by HLA-B7 (n=10), B13 (1), B41 (1), B44 (2), B50 (2), B60 (24) or B61 (1).

RG's cells reacted with all 45 sera, including three that contained antibodies towards B60 but not B61. The reactivity of these sera was not confounded by RG's second (B8 [Bw6]) specificity. However, we were unable to serologically confirm the Bw6 motif of B*4031, although RG was Bw4 negative with 8 Bw4 antisera.

Frequency of B*4031 in blood donors resident in Wales

The assignment of HLA-B*40 was reviewed in 24,990 WBMDR donors HLA-A,B typed by 'low resolution' PCR-SSP.

B*40 was identified in 14.6% of donors and no further examples of B*4031 were found.

B*4031 was differentiated from B*40 in all but 2.6% (B*40,B*44+) of donors.

Thus, B*4031 has an estimated phenotype frequency of 0.004% and gene frequency of 0.00002, in our local northern European Caucasoid blood donor population.

EBV-transformed B-cell line

An EBV-transformed B-cell line is available from the B*4031 propositus. Contact our Dr Helen Bass at helen.bass@wbs.wales.nhs.uk

Summary

- B*4031 was found in a northern European Caucasoid blood donor on the Welsh Bone Marrow Donor Registry.
- B*4031 differs from B*4001 by two nucleotides in exon 3 (positions 363 (G to C) and 412 (A to G)).
- B*4001 and B*4031 products have two amino acid differences (Arg > Ser at position 97 and Asn > Asp at position 114).
- B*4031 is on a haplotype with A*03; Cw*0304; DRB1*0404/32; DQB1*0302.
- B*4031's serological specificity is HLA-B60.
- B*4031 has a gene frequency of <0.0001 (0.00002) in northern European Caucasoid blood donors.
- B*4031 has an available B-cell line.