HLA-Cw*0740, a new allele mistyped by generic sequencing and identified by allelic separation

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INTRODUCTION

Sequence-based typing (SBT) is the preferred method for allele level matching in unrelated bone marrow transplantation. Most SBT strategies currently use generic amplification followed by nucleotide sequencing. HLA class I analysis employs the use of sequences within exons 2 and 3. Due to the nature of SBT analysis, the combinations of different pairs of alleles may give an ambiguous typing result. Generic SBT-PCR is not appropriate to define the cis/trans linkage of polymorphic sequence motifs. In addition, the HLA sequence database (1) is growing fast, and therefore mistyping of new alleles based on sequence mimicry may be an increasing problem when generic sequencing is used. In this report, we describe a new HLA-Cw*07 allele found during donor search for haematopoietic stem cell transplantation. This new allele, Cw*0740, could not be detected by generic SBT since its sequence pattern was identical to the one of the allele combination Cw*070101, 0410.

METHODS

The donor was initially typed as HLA-A2, 3; B44, 18; Cw4, 7; Bw4; BW6 (Histo Tray ABC 72, BAG, Lich, Germany) and did not reveal any unusual reaction patterns in the serological tests. Low and high resolution DNA-based typing was carried out using sequence-specific primers (Olerup SSP, GenoVision, Vienna, Austria) (2). The PCR-SSP result of the Cw*07 subtyping was anomalous and did not match any known allele (Olerup high resolution HLA-Cw* 07/M07). In fact, one primer pair detecting the triplets "TCG" at position 97 and "AGC" at position 289, specific for all Cw*07 alleles, did not show an amplification signal. For confirmation, we performed direct sequencing with a commercial SBT kit (AlleleSEQR HLA-A,B,C, Atria Genetics). The test uses generic primer pairs for the amplification of HLA-A/B exon 2, 3, 4 and HLA-Cw exon 2 and 3. Nucleotide sequencing was performed in both directions with an ABI 3100 DNA Sequencer (Applied Biosystems, Foster City, CA). For sequencing plate setup and automatic data transfer to the sequencing device we used an inhouse software program (Figure 1). Subsequent sequence analysis was done using the Assign 3.2.7 allele identification software (Conexio Genomics, Applecross, Australia). The genotype of the donor was defined as A*0201, 0301; B*1801, 4403; Cw*0410, 0701; DRB1*0405, 0701; DQB1*0202, 0302. Interestingly, direct sequencing of the HLA-Cw alleles gave a conclusive result of Cw*0701 in combination with the infrequent Cw*0410 allele. To obtain further information and clarify the unusual PCR-SSP pattern, HLA-Cw sequence-based typing was carried out with group-specific primers (S3, Protrans, Ketsch, Germany) according to the manufacturer`s protocol. After allele separation we identified Cw*04010101 and a new Cw*07. The exon 2 and 3 sequence of the new Cw*0740 (3) allele was identical to that of the Cw*070101 allele except for a single base substitution in codon 73, exchanging GCT to ACT. This mutation is responsible for one aminoacid substitution from alanine (polar) to threonine (unpolar). Residue 73 (Figure 2) is located in the α1 domain of the HLA class I molecule and forms the C pocket of the peptide-binding groove (4) involved in peptide binding. It indicates that the change at codon 73 affects the peptide preferences of the Cw*0740 allele. The nucleotide sequence of Cw*0740, aligned with Cw*04010101, Cw*0410 and Cw*070101 and the composed consensus sequences of Cw*0410, 070101 and Cw*04010101, 0740, is shown in Figure 3. The identical consensus sequence of Cw*0410, 070101 and Cw*04010101, 07new is the reason of the typing error. The sequence of the new allele was submitted to EMBL Nucleotide Sequence Database under the accession number AM261864. This case is an example that generic SBT may produce incorrect results. Group-specific SBT-PCR, as used by the Protrans S3 HLA-C kit, revealed this new allele.

CONCLUSION

In summary, a novel HLA-Cw allele is described which was detected only by group-specific SBT and assigned the name HLA-Cw*0740. The new allele contains an amino acid change in the antigen binding site of the protein, which potentially has contact with the bound peptides and may alter its antigen-binding properties. This work demonstrates the usefulness of different HLA typing techniques of patients waiting for HSCT and their prospective donors.
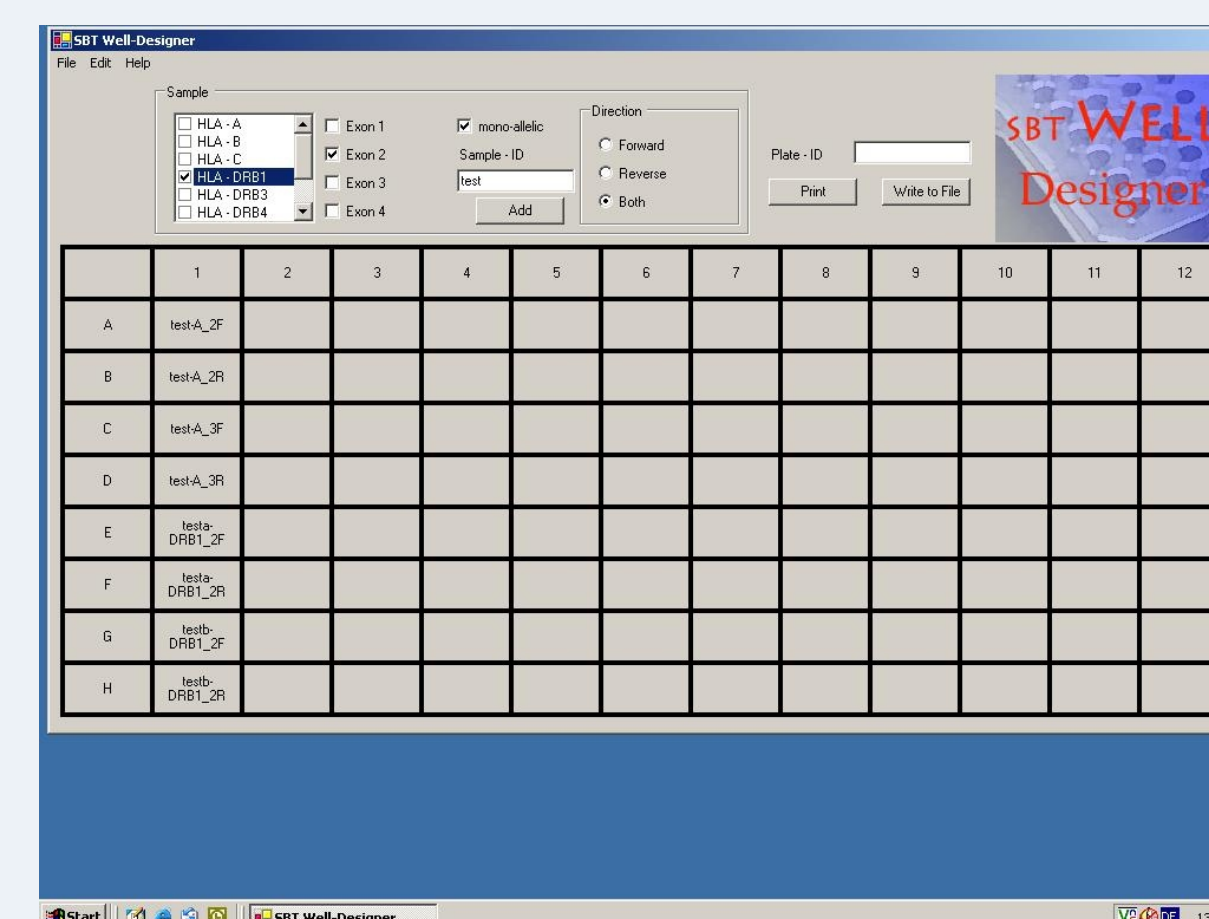

Figure 1 Illustration of the SBT- Well Designer data entry sheet.

We have developed a freeware computer software program that enables easy setup of sequencing plates and automatic data transfer to the sequencing device. In the area of the sample field, the parameters of a sample batch can be defined. On the left, you can select the genes and exons which will be examined and determine weather both alleles will be tested in one well or in two separate wells (mono- allelic). The sample ID has to be entered and the direction of the sequencing primer must be chosen: forward, reverse or both. By clicking the "Add" button, the sample batch can be individually arranged on the sequencing plate. After definition of the plate ID, the document can be printed and saved as *.plt file. The plt- file can by imported into the Data Collection Software of the ABI 3100 DNA- Sequencer after sequencing-setup is finished. The software helps saving time and avoids typing errors. The SBT-Well Designer Software is available on request or via download: http://www.bioinformatiker.at/software.html

Figure 2
Nucleotide alignment of Codon 65-75 of the new Cw*0740 allele in comparison to the consensus of all other Cw*07 alleles. Dashes indicate the homology of the sequence with Cw*010201. Codon 73.1 is a marker SNP for CW*07 alleles and commonly used in PCR-SSP kits.

Figure 3
Sequence alignment of the new Cw*0740 allele with nucleotide exchange marked in grey.
The exon 2 nucleotide sequence of the reference sequence Cw*010201 as well as Cw*04010101, Cw*0410, Cw*070101 and Cw*0740 is shown. Two consensus sequences are composed of sequences 3 + 4 (Cw*0410, 070101) and sequences 2 + 5 (Cw*04010101, 0740). Mixed bases are displayed with IUB codes. Dashes (-) indicate sequence identity. The nucleotides are numbered according to the IMGT/HLA Database (1). The sequence of Cw*0740 is identical to that of Cw*070101 except for position 289 G>A . This mutation is liable for the identical consensus sequence of combination Cw*04010101, 0740 versus Cw*0410, 070101. Identification of the new Cw*0740 allele was not possible by generic sequencing.

References

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