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PCR-SBT Characterization of A New HLA Allele: A*02:548

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Introduction

The human Major Histocompatibility Complex (MHC) lies within the short arm of chromosome 6 and is responsible for the production of Human Leukocyte Antigens (HLA) [1]. The HLA genes are the most polymorphic genes in the human genome, encoding over 13000 allelic variants [2]. Characterization of HLA alleles is very important in the success of hematopoietic stem cell transplantation (HSCT) used to treat forms of blood and bone marrow cancers, for HLA-related diseases and for solid organ transplantation [3].

Genotyping of HLA-A, B, C, DRB1, DQB1, DQA1 and DPB1 at level of high resolution is a very important step for unrelated hematopoietic stem cell transplantation to minimize acute Graft versus Host Disease (aGvHD) [1,4].

Currently, there are several methods we can apply to determine HLA alleles in high resolution such as PCR-SSO, PCR-SSP and PCR-SBT. The final one is the method of choice to investigate HLA alleles giving the possibility of analyzing each of them base by base.

The following is a report of cases in which a new HLA-allele has been characterized using the PCR-SBT method.

Material and Method

DNA extraction

Genomic DNA, from our volunteer bone marrow donor peripheral blood, has been extracted using an automatic system Maxwell 16 (Promega).

Typing by PCR-SBT

Typing has been performed by PCR-SBT using Allele SEQR HLA-A (Celera) for exons 2, 3 and 4 according to the manufacturers' instructions. Typing has been perfomed again using Protrans S4 HLA-A for allele separation according to the manufacturers' instructions. The products were reconstituted with 25 µl of Hi-Di Formamide and loaded on the ABI Prism 310 (PE) and analysed using SBTEngine.

Results

During HLA-A PCR-SBT typing for exons 2, 3 and 4, we found a sequence largely homologous to A*02:01 with a mutation at position 367 (Exon 3) a C instead of a T. The full HLA type was found to be: HLA-A*02: new, *33:03; B*15:03, *38:01; C*02:02, *12:03; DRB1*11:01, 13:02 (Allele SEQR HLA-A Celera Corporation). We repeated this sequence in isolation from the second allele using allele specific primers (Protrans S4 HLA-A CE). Sequencing was performed in both directions and the data analyzed by SBTengine 3.5.2.2346 software. The novel allele was found to have, at exon 3, a Histidine instead of a Tyrosine codon 99 TAT→CAT (Figure 1).

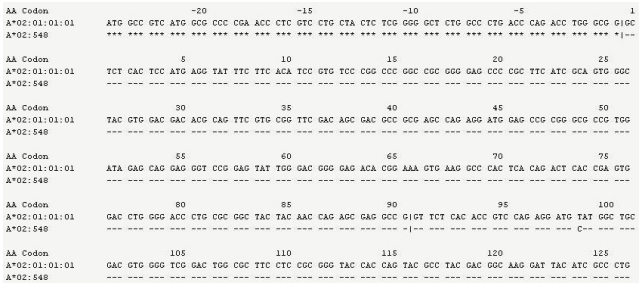

Figure 1: Alignment of the new allele against A*02:01:01:01 that was found to be most homologous to the new one. Dashes (-) represent identical sequence to A*02:548. The new A*02:548 has one nucleotide difference found at codon 99 (T>C), resulting in a coding change (Tyr>Hist).

This allele was then submitted to the WHO Nomenclature Committee via the IMGT/HLA Database for naming and was assigned the official name A*02:548. The name HLA-A*02:548 has been officially assigned by the World Health Organization (WHO) nomenclature committee in November 2014. This follows the agreed policy that subject to the conditions stated in the most recent nomenclature report [5].

Discussion

The described mutation is not unique but it has been observed in the alleles HLA-A*30:10 and A*30:88 but never in A*02 [2].

In our opinion PCR-SBT method is really useful both for genotyping HLA alleles and for the determination of new alleles. However, using a commercial kit we are able to amplify only exons 2, 3 and 4 for Class I and exon 2 for Class II so, inevitably, all those changes falling in introns and exons not amplified will be lost.

Since HLA genotyping is essential for unrelated hematopoietic stem cell transplantation, we consider the introduction of the Next Generation Sequencing (NGS) to be very helpful. This has the effect of sequencing the full length of each HLA locus improves HLA matching between donor and recipient while also preserving HLA new alleles.

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