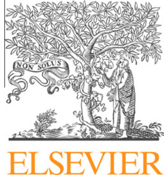
Human Immunology 77 (2016) 605–608



Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Identification of a novel HLA-A allele, HLA-A*01:195, in a UAE national

Wael Abdrabou [a,1], Ini-Isabée Witzel [a,1], Agnieszka Paduch [b], Guan Tay [c], Habiba Alsafar [a,d,*]

[a] Department of Biomedical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates
[b] Transplant Diagnostics, Thermo Fischer Scientific, Hennigsdorf, Germany
[c] Centre for Forensic Science, University of Western Australia, Crawley, Perth, Australia
[d] Khalifa University Center of Biotechnology, Khalifa University of Science, Technology & Research, Abu Dhabi, United Arab Emirates

A R T I C L E I N F O

Article history:
Received 14 November 2015
Revised 20 April 2016
Accepted 13 May 2016
Available online 14 May 2016

Keywords:
HLA-A*01:195
Novel HLA-A allele
Sequence based typing
Arab genetics

A B S T R A C T

A novel human leucocyte antigen (HLA)-A allele, HLA-A*01:195, was identified by sequence-based typing (SBT) in a UAE national subject. The novel allele is identical to its closest known allele, HLA-A*01:01:01:01, in exon 2, 3 and 4, except for a single nucleotide mutation of A to G at position 442 in exon 3 (codon 124 in the α2 domain of the α chain of the mature protein). This A to G mutation results in an amino acid change of isoleucine #124 to valine.

© 2016 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

1. Introduction

The human leukocyte antigen (HLA) genes are the most polymorphic genes in the human genome and play key roles in immunogenetics and histocompatibility. They are located in the major histocompatibility complex (MHC) on the short arm of chromosome 6 (6p21.3) and are grouped into class I and class II genes. The three major HLA class I genes, including HLA-A, -B and -C, encode glycoproteins that are expressed on platelets and most nucleated cells. Their main function is to present antigens to T lymphocytes to trigger the immune system, and they play a key role in the recognition of self and non-self antigens. Exons 2 and 3 of the HLA class I molecules express the peptide-binding motifs of the glycoproteins, and these are the regions where most of the new polymorphisms are found [1,2].

Advances in DNA sequencing techniques have significantly increased the resolution of typing of HLA alleles, which is of great importance in matching donors and recipients for transplants of hematopoietic stem cells and solid organ, particularly the kidneys, to reduce the risk of graft rejection [3,4]. The study of HLA alleles is also of great interest in the investigation of population genetics, as it can provide insights into demographic events throughout the history of human evolution, elucidating genetic relationships between different populations [5].

The data presented here is part of a larger study which involves the characterization of polymorphisms in individuals of Arabian ancestry. The subject in this study is a citizen of the United Arab Emirates (UAE) and is of Arabian ancestry. This population is an understudied one and is thought to comprise of tribes of Adnani and Qahtani lineages of Arabia [6]. Sequence-based typing (SBT) for HLA allele identification has been used to provide possible insights into novel mutations that may be associated with disease and for the characterization of haplotypes of the Major Histocompatibility Complex (MHC).

2. Materials and methods

One ml of saliva was collected from the study participant using the Oragene OGR-500 kit (DNA Genotek, Ottawa, Canada). Genomic DNA was extracted from saliva samples using the prepIT®L2P system (DNA Genotek, Ottawa, Canada) in accordance with the manufacturer's instructions. The HLA-A, -B and -C loci were amplified using the 9700 PCR system (Applied Biosystems, Foster City, CA, USA) and exons 2, 3 and 4 of each locus were sequenced using the SeCore HLA Kit (Invitrogen, Life Technologies, WI, USA). The manufacturer's protocol was followed, but reaction volumes were

Abbreviations: GSSP, group specific sequencing primer; HLA, human leukocyte antigen; IMGT, immunogenetics project; Ile, isoleucine; MHC, major histocompatibility complex; PCR, polymerase chain reaction; SBT, sequence-based typing; UAE, United Arab Emirates; Val, valine; WHO, World Health Organization.

* Corresponding author at: Department of Biomedical Engineering, Khalifa University, PO Box 127788, Abu Dhabi, United Arab Emirates.

E-mail address: Habiba.Alsafar@kustar.ac.ae (H. Alsafar).

[1] Equally-contributing as a first author.

http://dx.doi.org/10.1016/j.humimm.2016.05.010
0198-8859/© 2016 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

adjusted as described here. The purified template DNA was diluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) to 20 ng/μl; 50 ng DNA was mixed with 9.9 μl locus-specific amplification mix and 0.1 μl FastStart Taq Polymerase for PCR amplification. Three μl of the PCR product were used for agarose gel electrophoresis to confirm the presence of amplified HLA-A, -B and -C loci based on band size. The remainder of the PCR product was treated with 2 μl ExoSAP-IT. Cycle sequencing was carried out using 1.3 μl ExoSAP-treated DNA and 4.2 μl cycle sequencing mix, using a separate reaction for each cycle sequencing primer to amplify exon 2, 3 and 4 in the forward and the reverse directions. The amplified products were precipitated using 1 μl precipitation buffer and 20 μl 100% Ethanol; and washed with 70% Ethanol. DNA pellets were resuspended in 10 μl HiDi Formamide and denatured. Capillary gel electrophoresis was carried out on the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using POP-7™ polymer and a 12 s injection. Sequences were analyzed using One Lambda's uTYPE® v6.0 software (One Lambda, Canoga Park, CA, USA).

The uTYPE® software was unable to find a perfect allele match for the HLA-A locus due to a novel heterozygous site in exon 3. To confirm these observations, exon 3 was re-amplified in another independent PCR reaction and the product of this reaction was sequenced following the same procedure described earlier. To identify the novel allele containing the mutation, the group specific sequencing primers (GSSP) Z5 and Z6 (Secore GSSP Kit, Invitrogen, Life Technologies, WI, USA) were used to re-sequence HLA-A exon 3. The GSSPs were used according to the manufacturer's protocol, without any alterations to reaction volumes. The MHC class II gene HLA-DRB1 was also sequenced for this individual, using the Secore DRB1 Locus Sequencing Kit (Invitrogen, Life Technologies, WI, USA), following the manufacturer's protocol. PolyPhen-2, an online bioinformatics tool for annotating coding non-synonymous SNPs, was used to visualize the amino residue subject for change in three dimension [7]. The online tools SIFT and I-Mutant were used to predict the effect of the amino acid substitution on the function and structure stability of the protein, respectively [8,9].

SIFT prediction is based on the degree amino acid residues are conserved in alignments derived from closely related sequences that are collected through PSI-BLAST. The SIFT prediction score is the scaled probability of an Amino Acid Substitution (AAS) being tolerated. ASS ranges from 0 to 1 and substitutions with scores that fall below 0.05 are predicted to affect protein function. To assess confidence in its prediction, SIFT also calculates the conservation value for the position where the substitution takes place. This value ranges from zero, when all 20 amino acids are observed at this position in various alignments, to log2 (20) (=4.32), when only one amino acid is observed at that position in all alignments. Positions with high conservation value are confirmed intolerant to substitutions [8].

In contrast, I-Mutant; a support vector machine (SVM)-based tool; uses the free energy change ($\Delta\Delta G$) upon single point mutation as a method for prediction. $\Delta\Delta G$ is calculated by ΔG (New Protein) – ΔG of (Wild Type) in kcal/mol. Mutations with $\Delta G < -0.5$ kcal/mol are considered to be destabilizing mutations, those with $\Delta\Delta G > 0.5$ kcal/mol are stabilizing, and those with ($-0.5 < \Delta\Delta G < 0.5$ kcal/mol) are considered neutral mutations [9].

3. Results

Sequence analysis identified the individual's HLA haplotype as HLA-B*37 and B*57, HLA-C*01 and C*06, HLA-DRB1*07 and DRB1*10. The HLA sequence analysis software could not call the HLA-A alleles and identified a novel mutation at a heterozygous site in exon 3 at nucleotide 442. The closest HLA-A allele matches were HLA-A*01:01:01:01 and A*03:01:01:01, which both have an adenine (A) base at position 442. To resolve which allele contained the novel mutation to a guanine (G) at position 442, GSSPs were used to re-sequence HLA-A exon 3. Based on the resultant sequences, the individual's HLA-A alleles were identified as the known A*03:01:01:01, which contains an A at position 442, and a novel variant of A*01:01:01:01 that contains the G at position 442. The A to G nucleotide change in codon 124 of the α2 domain of the α chain results a coding change in the mature HLA-A protein, altering the common isoleucine #124 to a valine (Fig. 1).

SIFT showed that the novel I124V substitution has an intolerance score of 0.03, while the median sequence conservation at this location was 3.56 for 399 sequences used in the analysis. It was also predicted by I-Mutant that the substitution has a destabilizing effect on the structure stability of HLA-A protein with a $\Delta\Delta G$ value of -1.15 kcal/mol.

The novel HLA-A*01 allele was submitted to the IMGT/HLA database and designated HLA-A*01:195 by the WHO Nomenclature Committee in August 2015. It was also submitted to the GenBank database and was assigned Accession Number KT220195.

4. Discussion

The list of new HLA alleles continues to grow with the continuing advancements in DNA analysis technologies and diagnostic laboratory techniques. At this time, approximately 10,041 HLA class I alleles, including 3192 HLA-A alleles, have been submitted to the IMGT/HLA database [10,11]. The HLA-A gene is particularly interesting as it is one of the most rapidly evolving human genes. It is one of the key MHC loci that has to be matched to ensure compatibility between tissue and bone marrow transplant donors and recipients. Further, variants of these genes have been linked to the risk and progression of various human diseases.

While conducting a study of the MHC complex in a cohort of UAE subjects, a novel variant of the HLA-A*01 allele was identified in a subject of Arabian descent from the city of Al-Ain (Fig. 2). The observed single nucleotide mutation that distinguishes this new allele from its closest match, the common HLA-A*01:01:01:01 allele, is an A to G mutation at nucleotide 442, codon 124 in α2 domain of the α chain of the mature HLA protein, resulting in an amino acid change of isoleucine #124 to valine.

The mutation that gives rise to this novel HLA-A allele was identified in exon 3; intronic sequences were not considered in this study.

The affected α2 domain form the extracellular portion of the heavy α chain of HLA-A protein with α1 and α3 domains. The α1 and α2 domains are the most polymorphic domains and together, they form a platform of β-pleated sheet topped with α helices with a long peptide binding groove in between the helices that is the antigen recognition site that is contacted by T-cell receptors [2]. The single nucleotide mutation identified in this study causes a coding change, altering the commonly expressed isoleucine to a valine. Both these amino acids have aliphatic sidechains that are considered non-reactive, and are thought to play a role in substrate recognition or ligand binding; they rarely play a role in protein function (Fig. 3). Contrary to the assumption that this mutation may therefore merely alter the peptide-binding specificity of the individual's HLA-A protein, the online tools SIFT and I-Mutant predicted that the amino acid change in question is deleterious for the protein function and has a destabilizing effect on the protein structure.

HLA-A*01:195 allele is a variation of its closest match, HLA-A*01:01 and was generated through new mutation. The latter is commonly found in Caucasians and Orientals [9]. According to Immune Epitope Database Analysis Resource and the Allele

Download English Version:

https://daneshyari.com/en/article/3349503

Download Persian Version:

https://daneshyari.com/article/3349503

Daneshyari.com