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Identification and Description Case Report of a New *HLA-A*33* Allele by Next Generation Sequencing Technology in a Brazilian Bone Marrow Donor

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Abstract

New A*33 allele has the closest match with *HLA-A*33:03:01:01*, except for a mismatch at position 270 in Exon 2. Instead of the expected T, an A was detected at this position. This information was included in the full Nomenclature report and contributed to the immunogenetic study.

Keywords: *HLA-A*33* • New allele • Next-Generation Sequencing (NGS) technology

Introduction

HLA genes encode surface proteins that recognize an individual's own antigens and distinguish them from antigens coming from foreign sources. HLA system is located on the short arm of chromosome 6, more specifically at 6p21 [1]. HLA genes are highly polymorphic, which explains the high specificity of the immune system. Development of new methodologies in molecular biology field allowed better identification of HLA polymorphism, resulting in great resolution for HLA typing. This has bring many improvements in therapeutics protocols and donor selection for patients candidates for a solid organ or Hematopoietic Stem Cells transplantation [2]. The implantation of NGS techniques along with bioinformatics tools provided an increase genotyping accuracy and reading length for obtaining complete sequences of HLA haplotypes [1,3].

Objective

Here we describe a novel HLA-A allele identified in a male donor of African ancestry common in Brazilian Mestizo population.

Results and Follow up

Initially, genomic DNA was isolated from peripheral blood using BIOPUR kit and HLA t c yping was performed using LABType® SSOP Kits (One Lambda, Canoga Park, CA). The donor genotype assignment was no match *HLA-A; HLA-B*08 HLA-B*44 and HLA-DRB1*1, HLA-DRB1*13*. In order to corroborate the results and to obtain a higher resolution in HLA genotyping, we first performed new genomic DNA isolation by salting-out method and the same result was achieved. The results were sequencing by NGS using AllType™ NGS Assay Kit (One Lambda - Thermo Fisher, Canoga Park, CA) on Ion platform (Ion Chef and Ion S5) at Xmol laboratory - Training and Support Center in Molecular Biology (Paraná, Brazil). The results were sequencing by software TypeStream Visual (TSV), version 1.1, catalog ALL-11LX_002_03, as well as document showing details of the analysis (Figure 1). NGS Custom Report presents the typing for 11 analyzed loci: HLA-A*02:01:01, A*33 (possible new); HLA-B*08:01:01, B*44:02:01; HLA-C*07:01:01, C*07:04:01; HLA-DRB1*01:01:01, DRB1*13:01:01; HLA-DRB3*01:01:02; HLA-DQA1*01:01, DQA1*01:03:01; HLA-DQB1*05:01, DQB1*06:03:01; HLA-DPA1*01:03:01, DPA1*01:03:01; HLA-DPB1*03:01:01, DPB1*04:01:01. The identification of a new allele *HLA-A*33* shows a mutation in Exon 2 (codon 66.3), thus being at the recognition site. The allele that most resembles is A*33:03:01:01, but instead of the expected T the consensus was an A. There was an amino acid change as well from Asparagine (Asn) to Lysine (Lys) (Figure 2). IMGT/HLA Database reference sequence confirms a mismatch in HLA-A*33:03:01:01 by a substitution of a T for an A at Exon 2 (Figure 2). The nucleotide sequence is available at GenBank accession number MK643128, IPD-IMGT/HLA Database submission HWS10054309 and the name A*33:183 has been officially assigned by World Health Organization (WHO) Nomenclature Committee in March 2019. This follows the agreed policy that, subject to the conditions stated in the most recent nomenclature report, names will be assigned to new sequences as they are identified. Lists of the new allele names were published in the following WHO nomenclature report [4].

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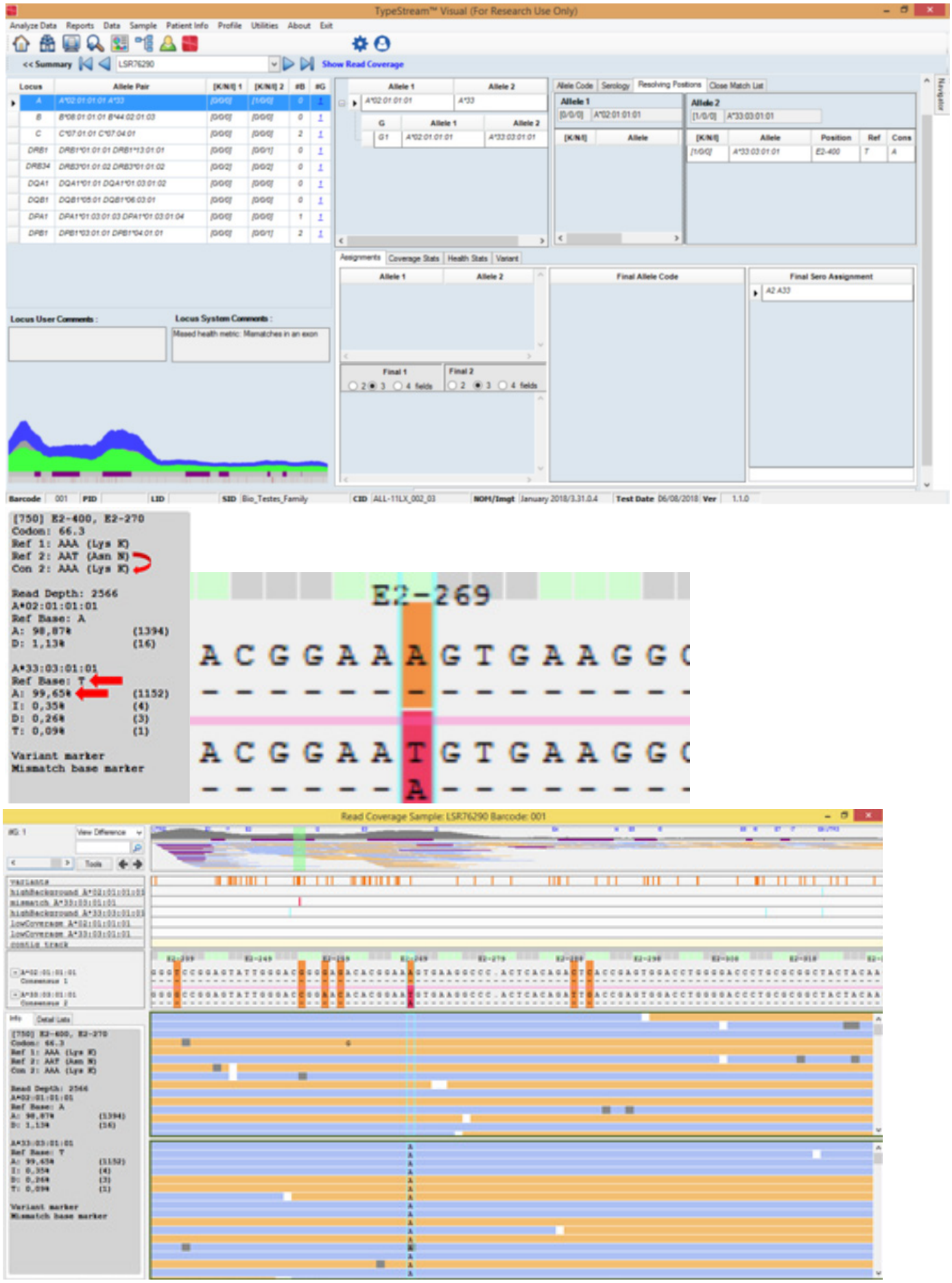

Figure 1. HLA-A analysis screen showing a mismatch in exon.

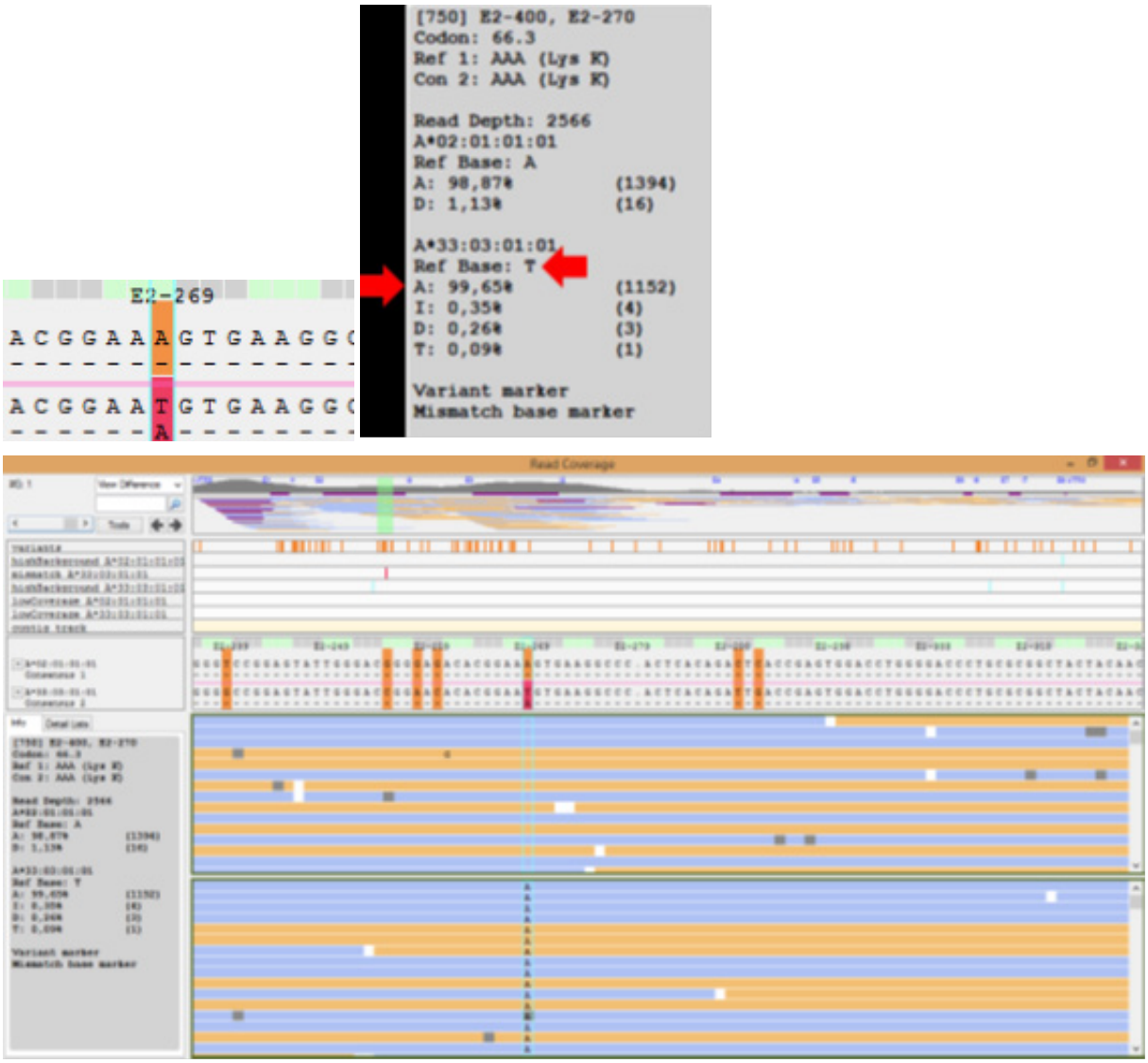

Figure 2. Detailed analysis of the mutation.

Conclusion

This information was included in the full Nomenclature report and contributed to the immunogenetic study.

Author Contributions

FZP, GD, CG and MM conceived and designed the study. GD, CG and MM performed the allele analysis. FZP and SMK wrote the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of Interest

The authors have declared no conflicting interests.

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