

## High-Resolution HLA-A Typing in Normal Iranian Population

Azadeh Hadadianpour<sup>1,2</sup>, Mohammad Hasan Samiee Aref<sup>1,2</sup> and Sirous Zeinali<sup>\*2,3</sup>

<sup>1</sup>Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran; <sup>2</sup>Kawsar Human Genetics Research Center, Tehran, Iran; <sup>3</sup>Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Received 22 April 2017; revised 26 July 2017; accepted 12 August 2017

### ABSTRACT

**Background:** Human leukocyte antigen (*HLA*) gene is a highly polymorphic region. HLA typing is required to match patients and donors for transplantation; therefore, development of HLA registries is necessary for finding HLA match donors. HLA system is highly informative, and numerous studies have been conducted on *HLA* allele distribution in different populations. **Methods:** In this study, 100 unrelated Iranian individuals were typed for HLA-A locus using sequence-based typing method. Samples were subjected to the PCR, followed by Sanger sequencing and software analysis. **Results:** A\*02:01 (13%) and A\*24:02 (12%) were the two most frequent alleles, while A\*01:14, A\*02:05, A\*02:11, A\*02:34, A\*02:50, A\*11:04, A\*23:02, A\*24:34, A\*25:01, A\*26:09, A\*26:43, A\*29:67, A\*30:54, A\*31:02, A\*31:66, A\*32:03, A\*32:04, A\*33:03, and A\*66:15 alleles had the least frequencies (1%). **Conclusion:** This is the first report of HLA-A allele level typing in a randomized population of Iran and can be useful for development of national registries of HLA-typed volunteer marrow donors and local cord blood banks. **DOI:** 10.22034/ibj.22.2.134

**Keywords:** HLA, Unrelated donors, Iran

**Corresponding Author:** Sirous Zeinali

Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran; Tel./Fax: (+98-21) 88939140 and 88939142;

E-mail: sirouszeinali@yahoo.com

### INTRODUCTION

The major histocompatibility complex (MHC) is a fascinating genetic region encoding human leukocyte antigens (HLA)<sup>[1]</sup>. MHC molecules show an impressive degree of polymorphism that may have been resulted from selective pressures and functional adaptation during the evolution<sup>[1]</sup>. This high-degree polymorphism is essential for MHC molecule role in the regulation of immune system. Highly polymorphic MHC molecules can accommodate various antigenic peptides from invading pathogens and present these molecules to the T lymphocytes. Therefore, specific immune responses, unique to each person<sup>[2]</sup>, are generated. HLA varieties among individuals are the major cause of organ and bone-marrow transplant rejection due to their ability to recognize self-from non-self-antigens<sup>[3]</sup>. The degree of donor and recipient HLA compatibility predicts the severity of graft versus host disease, the rate of

transplant success, and the quality of patient's survival<sup>[4]</sup>. High-resolution HLA typing at the allele level has significant functional implications for successful bone marrow trans-plantation and vaccine development<sup>[4,5]</sup>. Therefore, HLA typing has been used as an invaluable tool in population genetics analysis, studies of disease associations, and anthropological investigations<sup>[6]</sup>. HLA polymorphism in different populations and ethnic groups has been studied extensively, and results indicate that distinct populations have different allele frequencies and exhibit different patterns of linkage disequilibrium<sup>[7-10]</sup>.

PCR-sequence-based typing (SBT) is a method generally used for obtaining high-resolution 4-digit allele level for HLA-A. This technology employs PCR to amplify the locus of interest. Sanger sequencing is subsequently used to determine the nucleotide sequence of the PCR products<sup>[11]</sup>. The present study was designed to investigate HLA class I allele frequencies for *HLA-A* gene in a randomized

healthy population of Iran using the high-resolution SBT method. The results from this study can be useful for development of national registries of HLA-typed volunteer marrow donors and local cord blood banks.

## MATERIALS AND METHODS

### Sample

The present study includes 100 anonymous samples (50 male and 50 female) randomly collected from healthy unrelated individuals who referred to a private Medical Genetics Lab (Tehran, Iran) for genetic tests. These individuals did not suffer from any known disease and allergy, and they were not on any medications at the time. Participants were chosen based on the ethnic composition of Iran as follows: Persians (including Gilakis and Mazandarani;  $n = 50$ ), Baloch ( $n = 20$ ), Azerbaijanis ( $n = 16$ ), Kurds ( $n = 6$ ), Lurs (including Bakhtiari people;  $n = 6$ ), Arabs ( $n = 2$ ). After obtaining signed informed consents from all the participants, the study was initiated.

### HLA-A allele level typing

Blood samples were collected in EDTA tubes. Genomic DNA was purified from whole venous blood using salting-out extraction method<sup>[12]</sup>. Quantification of DNA samples was conducted using the Thermo NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA). High-quality DNA samples with the appropriate concentration of 20-30 ng/ $\mu$ L with the purity of A260/A280 around 1.6-1.8 were selected and were typed to four digits for HLA-A locus using commercial SBT Resolver<sup>TM</sup> Kit (Conexio-Illumina, USA) according to the manual's instruction. The results were analyzed by Assign-SBT v4.7 software from Conexio-Illumina.

## RESULTS

In this study, 100 DNA samples from unrelated normal individuals were typed for HLA class I, using a reliable and an efficient high-resolution typing method. A total of 39 different alleles were detected for HLA-A locus in Iranian population. The most frequent allele groups were A\*02, A\*24, A\*01, and A\*03 with the frequency above 10%, and the least frequent allele groups were found to be A\*66 and A\*25 (1%). The complete list of alleles with their frequencies is shown in Table 1. The most frequent alleles were A\*02:01 (13%) and A\*24:02 (12%), followed by A\*01:01 (9%) and A\*03:01 (9%). However, the least frequent alleles were A\*01:14, A\*02:05, A\*02:11, A\*02:34, A\*02:50,

A\*11:04, A\*23:02, A\*24:34, A\*25:01, A\*26:09, A\*26:43, A\*29:67, A\*30:54, A\*31:02, A\*31:66, A\*32:03, A\*32:04, A\*33:03, and A\*66:15; all with the frequency of 1%. Most of the samples were heterozygote, while some alleles were observed as homozygous such as A\*02:05:01, A\*26.01, and A\*01:01:01.

## DISCUSSION

Numerous studies have focused on the distribution of HLA alleles in different populations due to its functional polymorphism. However, few studies are concerned with HLA allele frequencies in Iranian populations and mostly have drawn the attention to polymorphism in HLA class II loci<sup>[8,13]</sup> and to allelic association with diseases<sup>[14]</sup>. Most of the data presented in these reports were obtained by low-resolution HLA typing methods such as PCR-SSP<sup>[15,16]</sup>. Using molecular HLA typing technique, similar studies have reported A\*02011 (20.2%) and A\*11011 (14.6%) as the most frequent alleles in Baloch as well as A\*24:02 (17.6%) and A\*02:01 (12.5%) as the most frequent alleles in Kurd populations<sup>[17,18]</sup>. However, this is the first report of HLA-A allele level typing in a randomized Iranian population including different ethnic groups.

The results indicated that the distribution of HLA-A alleles in Iranian population was mostly similar to Caucasians. For instance, the most common alleles found in Caucasians were A\*02 (27%) and -A\*24 (10.8%)<sup>[19]</sup>. Furthermore, HLA-A\*02 (15.45-30.65%), -A\*11 (16.66-30.72%), and -A\*24 (11.03-17.07%) were the first three frequent alleles of HLA-A locus in six different Chinese populations<sup>[7]</sup>. Similarly, A\*02 (29%) and A\*24 (22.4%) had the highest frequencies in Korean population<sup>[10]</sup>. HLA-A\*0201 and -A\*0101 have also been reported as the most frequent alleles in a Berber population from North Morocco, named Metalsa<sup>[20]</sup>. Comparing to the HLA-A allele distribution in Armenian population, the most frequent HLA class I alleles were HLA-A\*0201 (15.5%), -A\*0101 (12.5%), and -A\*2402 (12%), while HLA-A\*25, -A\*34, -A\*66, and -A\*74 were rare allele groups with a frequency of less than 1%. All of these findings support the genetic similarity between these neighboring and related countries<sup>[9]</sup>.

Information provided by allele level HLA typing is of high importance for the bone marrow transplant community and vaccine studies. Furthermore, these kinds of research focusing on the allele and haplotype frequencies in Iranian population could be useful for finding the best matched unrelated donors in Iran.

**Table 1.** HLA-A allele frequencies obtained in the Iranian population (n = 100)

HLA-A alleles	Allele frequencies (%)
A*01:01	9
A*01:02	2
A*01:14	1
A*02:01	13
A*02:02	2
A*02:05	1
A*02:11	1
A*02:34	1
A*02:50	1
A*03:01	9
A*03:02	2
A*11:01	5
A*11:04	1
A*23:01	2
A*23:02	1
A*24:02	12
A*24:03	2
A*24:34	1
A*25:01	1
A*26:01	5
A*26:09	1
A*26:43	1
A*29:01	2
A*29:67	1
A*30:01	5
A*30:54	1
A*31:01	4
A*31:02	1
A*31:66	1
A*32:01	4
A*32:03	1
A*32:04	1
A*33:01	3
A*33:03	1
A*66:15	1

The data of this study may be used as a reference by Iran's hematopoietic stem cell donor bank and local registries to evaluate the immunogenetic profile of populations in specific regions and can impact investigations for unrelated bone marrow donors. However, further studies are required on other HLA loci and also on a larger sample size to achieve more precise results for development of HLA registries and cord blood banks.

### ACKNOWLEDGMENTS

This research was supported by Kawsar Human Genetics Research Center (Tehran, Iran). We thank our colleagues at Kawsar Human Genetics Research Center who provided insight and expertise that greatly assisted the research.

**CONFLICT OF INTEREST.** None declared.

### REFERENCES

- Hughes AL, Nei M. Maintenance of MHC polymorphism. *Nature* 1992; **355**(6359): 402-403.
- Prugnotte F, Manica A, Charpentier M, Guégan JF, Guernier V, Balloux F. Pathogen-driven selection and worldwide HLA class I diversity. *Current biology* 2005; **15**(11): 1022-1027.
- Soiffer RJ. Hematopoietic Stem Cell Transplantation. Second edition. Germany: Springer Science & Business Media; 2008.
- Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, Fernandez-Vina M, Flomenberg N, Horowitz M, Hurley CK, Noreen H, Oudshoorn M, Petersdorf E, Setterholm M, Spellman S, Weisdorf D, Williams TM, Anasetti C. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**(13): 4576-4583.
- Stern LJ, Calvo-Calle JM. HLA-DR: molecular insights and vaccine design. *Current pharmaceutical design* 2009; **15**(28): 3249-3261.
- Buhler S, Sanchez-Mazas A. HLA DNA sequence variation among human populations: molecular signatures of demographic and selective events. *PloS one* 2011; **6**(2): e14643.
- Pan Q, Fan S, Wang X, Zhao X, Pan M, Wang C, Shen J. The distribution of HLA-A, -B, and -DRB1 alleles and haplotypes in inhabitants of Guizhou Province of China. *Journal of biomedical research* 2011; **25**(5): 328-334.
- Amirzargar A<sup>1</sup>, Mytilineos J, Farjadian S, Doroudchi M, Scherer S, Opelz G, Ghaderi A. Human leukocyte antigen class II allele frequencies and haplotype association in Iranian normal population. *Human immunology* 2001; **62**(11): 1234-1238.

9. Matevosyan L, Chattopadhyay S, Madelian V, Avagyan S, Nazaretyan M, Hyussian A, Vardapetyan E, Arutunyan R, Jordan F. HLA-A, HLA-B, and HLA-DRB1 allele distribution in a large Armenian population sample. *Tissue antigens* 2011; **78**(1): 21-30.
10. Whang DH, Yang YS, Hong HK. Allele and haplotype frequencies of human leukocyte antigen-A, -B, and -DR loci in Koreans: DNA typing of 1,500 cord blood units. *Korean journal of laboratory medicine* 2008; **28**(6): 465-474.
11. Adams SD, Barracchini KC, Simonis TB, Stroncek D, Marincola FM. High throughput HLA sequence-based typing (SBT) utilizing the ABI Prism 3700 DNA Analyzer. *Tumori* 2001; **87**(2): S40-S43.
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids research* 1988; **16**(3): 1215.
13. Farjadian S, Ota M, Inoko H, Ghaderi A. The genetic relationship among Iranian ethnic groups: an anthropological view based on HLA class II gene polymorphism. *Molecular biology reports* 2009; **36**(7): 1943-1950.
14. Amirzargar AA, Khosravi F, Dianat SS, Alimoghaddam K, Ghavamzadeh F, Ansari-pour B, Moradi B, Nikbin B. Association of HLA class II allele and haplotype frequencies with chronic myelogenous leukemia and age-at-onset of the disease. *Pathology and oncology research* 2007; **13**(1): 47-51.
15. Ghashghaie A, Alimoghaddam K, Ostadi MR, Ghaffari H, Khansari L, Sadraee M, Mirrasekhian E, Mohyedin I, Raoofi F, Noori A, Yaghmaian H, Eshaghi H, Ghavamzadeh A. Allele frequencies of HLA class-I loci in the normal Iranian population. *International journal of hematology-oncology and stem cell research*, 2009; **3**(2): 18-20.
16. Shaiegan M, Yari F, Abolghasemi H, Bagheri N, Paridar M, Heidari A, Kaskani SH, Sabaghi F, Dikloo F, Zaman-Vaziri M. Allele frequencies of HLA-A, B and DRB1 among people of Fars ethnicity living in Tehran. *Iranian journal of blood and cancer* 2011; **3**(4): 55-59.
17. Farjadian S, Naruse T, Kawata H, Ghaderi A, Bahram S, Inoko H. Molecular analysis of HLA allele frequencies and haplotypes in Baloch of Iran compared with related populations of Pakistan. *Tissue antigens* 2004; **64**(5): 581-587.
18. Amirzargar A, Rey D, Muñoz E, Palacio-Grüber J, Nikbin B, Nicknam H, Khosravi F, Joshghan H, Areces C, Enríquez-de-Salamanca M, Martínez-Quiles N, Martín-Villa JM, Arnaiz-Villena A. Kurds HLA genes: its implications in transplantation and pharmacogenomics. *Open medicine journal* 2015; **2**(1): 43-47.
19. Bardi MS, Jarduli LR, Jorge AJ, Camargo RB, Carneiro FP, Gelinski JR, Silva RA, Lavado EL. HLA-A, B and DRB1 allele and haplotype frequencies in volunteer bone marrow donors from the north of Parana State. *Revista brasileira de hematologia e hemoterapia* 2012; **34**(1): 25-30.
20. Piancatelli D, Canossi A, Aureli A, Oumhani K, Del Beato T, Di Rocco M, Liberatore G, Tessitore A, Witter K, El Aouad R, Adorno D. Human leukocyte antigen-A, -B, and -Cw polymorphism in a Berber population from North Morocco using sequence-based typing. *Tissue antigens* 2004; **63**(2): 158-172.