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ORIGINAL ARTICLE

Minor histocompatibility antigens HA-8 and PANE1 in the TUNISIAN population

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Abstract

Background: Minor histocompatibility antigens (mHAgs) are endogenous immunogenic peptides initially identified due to complications detected in several contexts of HLA geno-identical hematopoietic stem cell transplantation (HSCT). In this study, we chose to examine the molecular polymorphism of the mHAgs HA-8 and PANE1 in the Tunisian population.

Material and Methods: This study was conducted on 150 healthy and unrelated individuals. The DNA extraction and Sequence-Specific Primers PCR (PCR-SSP) methods were used for the molecular genotyping of the selected SNPs: *PUM3* (rs2173904) and *CENPM* (rs5758511).

Results: Our results show that, 94% of Tunisians are carriers of the PANE1R allele (immunogenic variant of the PANE1 mHAg) and 68% of Tunisians are carriers of the HA-8R allele (immunogenic variant of the HA-8 mHAg). Furthermore, this study shows that about 5% of the Tunisians are carrier of the PANE1R antigen and its HLA molecule of presentation (the PANE1R/HLA-A*0301 combination). However, only 2% of Tunisians are carrier of the HA-8R/HLA-A*0201 combination, that is, the HA8 immunogenic variant and its specific HLA molecule of presentation.

Conclusion: Our results are close to those reported in Caucasian, Asiatic, and African populations, this may be explained by the historical events experienced by Tunisia for millennia. These results could be used for further clinical and anthropological studies.

KEYWORDS
HA-8, hematopoietic stem cell transplantation, minor histocompatibility antigens, PANE1, SNP

1 | INTRODUCTION

Patients with hematologic malignancies benefit from an effective treatment called hematopoietic stem cell transplantation (HSCT). However, this treatment may be followed by some complications such as the Host vs. Graft (HvG), Graft vs. Host (GvH) reactions and, in cases where the GvH reaction produces adverse clinical symptoms, which usually include

symptomatic organ damage, it is considered to be Graft vs. Host Disease (GvHD) reaction (Ghimire et al., 2017; Nagasawa, 2021). The development of these immune reactions is generally due to the disparity between the donor and the recipient of the hematopoietic stem cell (HSCs) in the human leukocyte antigens (HLA) (Petersdorf, 2017a, 2017b). However, it is well documented that these complications could be developed even in patients who are HLA matched with their donors of HSCs. The elimination of the graft is achieved by immunogenic peptides presented on the surface of the grafted HSC following the triggering of a cascade of immune reactions directed by the recipient's T lymphocytes (Pabón et al., 2011; Summers et al., 2020; Turpeinen et al., 2013). Moreover, solid graft rejection reactions (renal transplant, skin transplant, etc.) even in the context of HLA geno-identity have been also associated with the disparity between the donor and the recipient of the target organ at the level of certain peptides encoded by autosomes or by gonosomes (Martin et al., 2017; Zorn & See, 2022). In addition, abortion could be caused by these antigens in case of disparity between the mother and her fetus, hence the need for on foeto-maternal allo-immunization (Linscheid & Petroff, 2013; Rizzuto & Erlebacher, 2022).

These peptides are derived from a range of endogenous proteins that exhibit genetic polymorphism between individuals and are called minor histocompatibility antigens (mHAgs). These immunogenic polymorphic peptides composed of 9 to 12 amino acids and encoded by non-HLA genes may drive a specific T-cell response when presented on the cell surface by HLA class I or II (Pietz et al., 2005; Posavec & Zunec, 2020). It is reported that the effect of these peptides depends on the presence of specific HLA antigens (Scheurer et al., 2022).

HA-8, PANE1, HA-1, HA2, and different mHAgs mismatching could increase the occurrence of immune complications (Spierings et al., 2013) making them interesting to explore in terms of improving patient outcomes (Feng et al., 2008; Spierings et al., 2013). In this context, HA-1 and HA-2 have been genotyped previously in Tunisian populations (Sellami et al., 2010), and in this study, HA-8 and PANE1, also known well by their immunodominant powers, are the mHAgs of interest (Spierings et al., 2007).

The HA-8 peptide consists of nine amino acids and is coded by the *PUM3* gene on the region p24.2 of chromosome 9. This gene has at least two allelic forms: HA-8R is the immunogenic form and HA-8P is the non-immunogenic form. Its expression is ubiquitous and the immunogenic peptide HA-8R has a high affinity to HLA-A*0201 molecules present on the surface of APCs (A. G. Brickner et al., 2001). The mechanism that could be at the origin of the non-presentation of the HA-8P peptide to this lineage could be explained by the weak interaction with TAP transport molecules (Posavec & Zunec, 2020).

PANE1 is composed of 10 amino acids and is coded by *CENPM* region on 22q.13.2 chromosome. This gene has two allelic forms: PANE1 R is the immunogenic form and PANE1R* is the non-immunogenic peptide resulting from the replacement of the Arginine (Arg) by a stop codon. PANE1R has a strong affinity with HLA-A*0301. In the case of HSCT, when the recipient expresses the immunogenic peptide PANE1R the donor's T lymphocytes attack the recipient's cells since they recognize it as non-self, which induces the occurrence of GvHD. This antigen has a limited distribution in hematopoietic B lymphoid cells which suggests that the GVL effect may be significant for this antigen without GvHD (Brickner et al., 2006).

This study aims to examine the allele, genotype, and phenotype frequencies of the minor histocompatibility antigens HA-8 and PANE1 as well as the estimation of the prevalence of the combinations HA-8R/HLA-A*0201 and PANE1R/HLA-A*0301 in a Tunisian group of healthy subjects. Consequently, this investigation will give us information about the percentage of Tunisians grafted of HSCs who may be at the risk to develop post graft complications.

2 | MATERIAL AND METHODS

2.1 | Ethical compliance and sample collection

For the purposes of this study, a total of 150 healthy and unrelated Tunisians from different regions of Tunisia were enrolled at the National Blood Transfusion Center of Tunis (CNTS). This study was approved by the Faculty of Medicine of Tunis local ethical committee, Tunis; Tunisia, and all the participants in this study reported their consent.

2.2 | Genomic extraction and genotyping

Genomic DNAs were obtained from peripheral blood samples using the salting-out method (Miller et al., 1988) and subsequently typed using polymerase chain reaction with sequence-specific primers (PCR-SSP). Genotyping was performed by primers previously published (Spierings et al., 2006) and verified on (http://genome.ucsc.edu) (Table 1). In a total volume of 10 μl, we performed multiple PCRs that contained 100 ng of genomic DNA, 1 μl of 10 PCR Buffer (Promega), 1.5 mM of MgCl2 (Promega), 200 μM of each dNTP. The concentration of specific primers (detecting the specific immunogenic or non-immunogenic allele) was 0.2 μM for HA-8 (ID: AL832239) and 0.06 μM for PANE1 (ID: BC000705). The concentration of control primers (detecting a sequence of the Human Growth

TABLE 1 Characteristics of HA-8 and PANE1 primers used for genotyping (Spierings et al., 2006)

Gene polymorphisms	Primers	Primer melting temperature (tm)	Amplicon size (bp)
HA-8[a] * G/C (R/P)	F: 5′TGCAGTCAGCAGATCACCG3′	56.7	187
	R: 3′CTTCTGGGCAACAGTTATGGA5′	60.1	
	F: 5′TGCAGTCAGCAGATCACCC3′	56.7	187
	R: 3′CTTCTGGGCAACAGTTATGGA5′	60.1	
PANE1[b] * G/A (R/R*)	F: 5′AGGCAAGTCCCACACTCG3′	54.1	207
	R: 3′AATGGGGTAAATGACGTGCTG5′	62	
	F: 5′CAGGCAAGTCCCACACTCA3′	54.1	207
	R: 3′AATGGGGTAAATGACGTGCTG5′	62	

Abbreviations: bp, base pair; F, Forward; P*, Non-immunogenic peptide; R, Reverse; R, Immunogenic peptide; R*, Non-immunogenic peptide; Tm, Primer melting temperature.

[a]HA-8 (ID: AL832239);

[b]PANE1 (ID: BC000705).

FIGURE 1 Allele, genotype and phenotype frequencies for HA-8[a] and PANE1[b] loci in 150 Healthy and unrelated individuals. [a]HA-8 (ID: AL832239); [b]PANE1 (ID: BC000705)
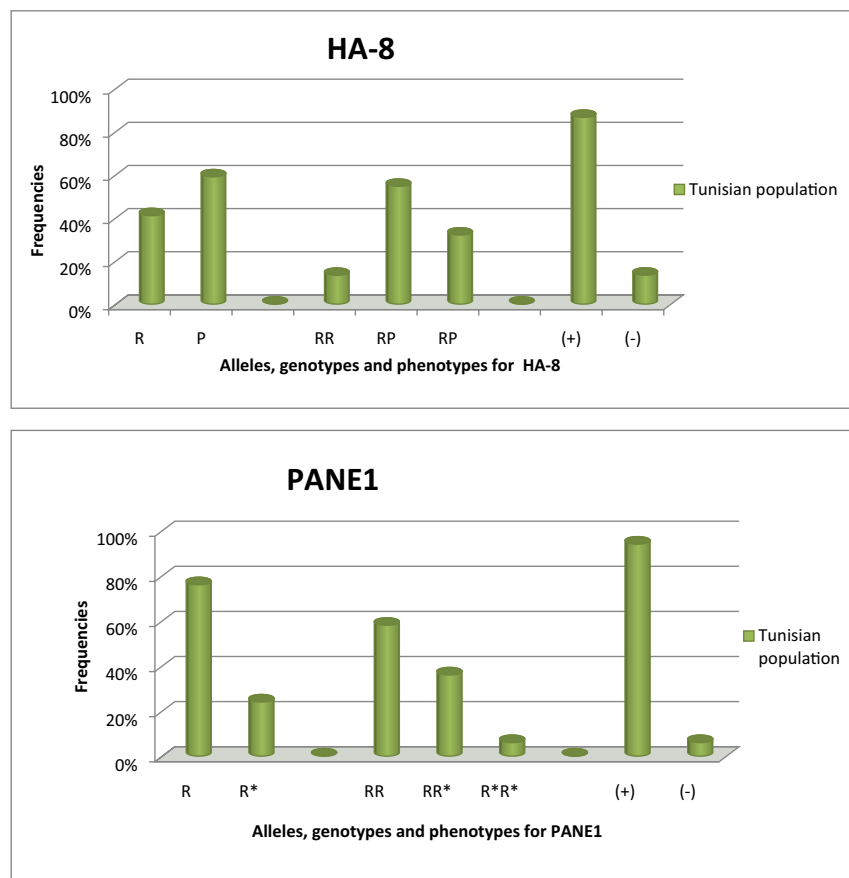
Hormone (GH1 [ID: 2688] allele)) was 0.18 μM and 0.2 μM, respectively, for HA-8 and PANE1. However, Taq polymerase concentration (Promega) was 0.5 U for HA-8 and 0.15 U for PANE1. Amplicons were visualized on agarose gels (2%) stained with ethidium bromide. Clear bands in the correct positions indicate the presence of an immunogenic and/or non-immunogenic allele. This latter assignment was simply a matter of determining the presence or absence of a specific band, that is, whether there was allele-specific amplification. Indeed, band-specific amplification showed that the amplified DNA contained the allele defined by the primer pair used in PCR. The PCR products obtained have an amplification size specific to the primer oligomers that could be easily differentiated from a non-specific amplification and adjusted well transfer (Table 1). Then, we determined sample genotypes based on assigned phenotypes, established by the assessed alleles. Allele-specific PCR-SSP was established for the alleles of the HA-8 and PANE1 mHAgs. The internal control primers showed a band of 709 bp. Sample genotype was

TABLE 2 HA-8 and PANE1 allele frequencies distribution, unrelated individuals in a range of ethnic groups

Population/ allele	Tunisian Present study (N = 150) (%)	Asiatic (Spierings et al., 2007) (N = 305) (%)	African (Spierings et al., 2007) (N = 162) (%)	Caucasian (Spierings et al., 2007) (N = 2011) (%)	Mexican (Spierings et al., 2007) (N = 119) (%)	Cap-colored (Spierings et al., 2007) (N = 65) (%)	Mulatto (Spierings et al., 2007) (N = 23) (%)
HA-8[a] R	41	39.7	35.8	45	50.8	29.4	32.6
HA-8[a] P	59	60.3	64.2	55	49.2	70.6	67.4
PANE1[b] R	76	71.2	94.7	69.9	82.5	83.6	63.3
PANE1[b] R*	24	28.8	8.4	38.8	19.7	25.8	13

Abbreviations: N, Number; P, Non-immunogenic peptide; R, Immunogenic peptide; R*, Non-immunogenic peptide.
[a]HA-8 (ID: AL832239);
[b]PANE1 (ID: BC000705).

TABLE 3 HA-8 and PANE1 genotype frequencies distribution in unrelated individuals in a range of ethnic groups

Population/ genotype	Tunisian Present study (N = 150) (%)	Asiatic (Spierings et al., 2007) (N = 305) (%)	African (Spierings et al., 2007) (N = 162) (%)	Caucasian (Spierings et al., 2007) (N = 2011) (%)	Mexicain (Spierings et al., 2007) (N = 119) (%)	Cap-colored (Spierings et al., 2007) (N = 65) (%)	Mulatto (Spierings et al., 2007) (N = 123) (%)
HA-8[a] RR	13.33	16.1	11.7	19.7	25	11.1	8.7
HA-8[a] RP	54.6	47.1	48.1	50.4	51.7	36.5	47.8
HA-8[a] PP	32	36.8	40.1	29.8	23.3	52.4	43.5
PANE1[b] RR	58	49	90.7	47.2	68.3	70.3	50
PANE1[b] RR*	36	44.4	8.1	45.3	28.3	26.6	27.3
PANE1[b] R*R*	6	6.6	1.2	7.4	3.3	3.1	22.7

Abbreviations: N, Number; P, Non-immunogenic peptide; R, Immunogenic peptide; R*, Non-immunogenic peptide.
[a]HA-8 (ID: AL832239);
[b]PANE1 (ID: BC000705).

determined based on assigned alleles and subsequently, a positive phenotype was assigned to samples that present heterozygous or homozygous for the dominant allele.

2.3 | Statistical analysis

Statistical analysis such as allele frequencies and Hardy–Weinberg equilibrium were estimated using the Thesias version 3.1.

3 | RESULTS

The Hardy–Weinberg equilibrium analysis for the two antigens showed that the Tunisian population is in equilibrium since the calculated χ^2 value is lower than the theoretical χ^2 (at 5% and ddl = 1), and the difference is not significant ($p \geq .05$).

In the present study, we determined the allele, genotype, and phenotype loci in the group of 150 healthy and unrelated individuals. The HA-8 antigen was present in the Tunisian population with an allele frequency of 41% for HA-8R and 59% for HA-8P ($p < .05$; IC 95% ± 0.07).

Genotype frequencies were 13% for RR, 55% for RP, and 32% for PP. Among the test group, 68% of samples showed HA-8-positive phenotype (Figure 1). The frequency of PANE1R allele in the main group was 76% and it was 24% for PANE1R*. Genotype frequencies were 58% for RR, 36% for **RR***, and 6% for **R*R***. PANE1-positive phenotype was found in 94% of the samples (Figure 1). Then, to better characterize our population, we compared our results with those reported in other major ethnic groups (Tables 2 and 3). Finally, we estimated the probability that a Tunisian hematopoietic stem cell (HSC) recipient could be HA-8 (R or P)/HLA-A*0201 or PANE1 (R or R*)/

HLA-A*0301 (Table 4) based on previously published data on the Tunisian HLA frequencies (Ayed et al., 2004).

4 | DISCUSSION

Minor histocompatibility antigens are endogenous immunogenic peptides identified after the development of post-transplant complications in HLA identical HSCT (Martin et al., 2017). These mHAgs are presented on the cell surface in association with HLA molecules. Post-transplant complications such as HvG, GvH, and GvHD are the result of a mismatch between the donor and the recipient at the level of these antigens by triggering a series of immune reactions. Nevertheless, this mismatch could induce beneficial effects such as graft-versus-tumor (GvT) or leukemic (GvL) cells. Furthermore, in the case of HLA-identical solid organ transplantation, this mismatch could induce graft rejection (Akatsuka et al., 2007; Falkenburg et al., 2003; Nagasawa, 2021).

In this study, we have studied the molecular polymorphisms of HA-8 and PANE1 antigens in a group of healthy and unrelated Tunisian subjects. This allowed us to evaluate the allelic and genotypic frequencies and to determine the phenotypic frequencies of these antigens in the selected group.

Given that, previous data clearly showed that alleles and genotypes frequencies for HA-8 and PANE1 do not deviate significantly from the frequencies described in Caucasian population (Spierings et al., 2003, 2007) (Tables 2 and 3). Based on minor allele frequencies (MAF), we deduced that HA-8 marker is more polymorphic than the PANE1 with rates of 41% for HA-8R and 24% for PANE1R*. Allele frequencies were 41% for HA-8R, 59% for HA-8P, and do not differ from Caucasian (45%, 55%), Asiatic (39.7%, 60.3%), and African populations (35.8%, 64.2%). In addition, allelic

TABLE 4 Probabilities of various status HA-8/HLA-A*0201 and PANE1/HLA-A*0301 among Tunisian population

HSCT context		Recipient HA-8[a] state	
		HA8 (+) (A)	HA8 (−) (B)
Recipient HLA-A[c] *0201 state	HLA-A*0201 (+) (E)	0.02747 [= p(A)*p(E)]	0.10325 [= p(B)*p(E)]
	HLA-A*0201 (−) (F)	0.33825 [= p(A)*p(F)]	0.48675 [= p(B)*p(F)]

HSCT context		Recipient PANE1[b] state	
		PANE1 (+) (A)	PANE1 (−) (B)
Recipient HLA-A[c] *0301 state	HLA-A*0301 (+) (E)	0.05092 [= p(A)*p(E)]	0.01608 [= p(B)*p(E)]
	HLA-A*0301 (−) (F)	0.70908 [= p(A)*p(F)]	0.22392 [= p(B)*p(F)]

Abbreviations: HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation.

[a]HA-8 (ID: AL832239);

[b]PANE1 (ID: BC000705);

[c]HLA-A (ID:3105).

frequencies were 76% for PANE1R, and 24% for PANE1R* in our population that resembled the Caucasian population and were different from those of the Mexican (82.5%, 19.7%) and African (94.7, 8.4%) populations. Furthermore, genotypes distributions analysis, revealed that the homozygous genotype RR for PANE1 and the heterozygous genotype RP for HA-8 were the predominant genotypes in our cohort, with frequencies of 58% and 55%, respectively, which is in concordance with the Caucasian population (47.2% and 50.4%), but showed substantial differences compared to the African population especially for PANE1RR (90.7%). These results are in agreement with other results that affirm the heterogeneity of the Tunisian population throughout the historical events experienced by Tunisia for millennia (Ayed et al., 2004; Hajjej et al., 2006, 2011; Sellami et al., 2008). In fact, Tunisia occupies a strategic geographical position, which attracted various civilizations to be installed there since thousands of years such as the Berbers, the Phoenicians, the Romans, the Capsians, the Iberians, the Bedouins, the Arabs, the Andalusians (Elkamel et al., 2017; Kefi et al., 2015; Rando et al., 1998).

In addition, we found that the HA-8 antigen was present in 68%, against 94% for PANE1 in our cohort study. Clinically, the importance given to the HA-8 antigen at the expense of PANE1 could be explained by the frequency of allogeneic reactions that could be triggered following the recognition of each antigen by alloreactive T lymphocytes, (in subjects positive for the HLA ligand specific to each antigen). At this level, it should be remembered that HLA-A*0201 and HLA-A*0301 are the specific molecules crucial for HA-8 and PANE1 mHAgs presentation to immunocompetent alloreactive lymphocytes. In the case of HLA geno-identical allogeneic HSC, the T lymphocytes of 32% of the donors who are HLA-A*0201-positive, but HA-8 negative (HA-8P) could attack their recipients expressing the immunogenic variant HA-8R. Conversely, only T cells from 6% of HLA-A*0301 positive, but PANE1 negative (PANE1R*) donors could attack their hosts expressing the immunogenic variant PANE1R, since it is almost present in most Tunisians.

Based on previously published data on the HLA system of Tunisians (Ayed et al., 2004), we estimated the probability that a Tunisian HSC recipient could be either HA-8(R or P)/HLA-A*0201 or PANE1 (R or R*)/HLA-A*0301 (Table 4). This estimation may help clinicians to have a general idea of the proportion of Tunisian recipients who might develop a GvH reaction after HSCT. In this context, it's known that HLA-A*0201, and HLA-A*0301 are two of the specific molecules required for the presentation of HA-8, and PANE1 antigens to T cells, respectively (Pierce et al., 2001; Tseng et al., 1998). These HLA ligands were the most frequent alleles among HLA-A class subtypes in the Tunisian population, with an overall frequencies of

approximately 17.5% for HLA-A*0201 and 6.7% for HLA-A*0301 (Ayed et al., 2004). Based on 100 HSC recipients, our results highlight that 5 individuals carry the immunogenic peptide PANE1R and the HLA*A0301 molecule and only two individuals carry the immunogenic peptide HA-8R and the HLA*A0201 molecule (Table 4).

5 | CONCLUSION AND FUTURE PERSPECTIVES

To the best of our knowledge, this study is the first to examine HA-8 and PANE1 polymorphisms in North Africa. Our findings will serve as a database for future further studies to explore the association between the occurrence of GvH and minor histocompatibility antigens in Tunisian/North African hematopoietic stem cell transplant recipients.

CONFLICT OF INTEREST
The authors all report that they have no conflicts of interest in this study.

DATA AVAILABILITY STATEMENT
Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

ETHICS APPROVAL
This project was approved by the Faculty of Medicine of Tunis local ethical committee, Tunis; Tunisia.

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