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Pattern of Genetic Diversity of ABO system in Moroccan Blood Donors Evidenced by Model-Based Bayesian Clustering

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ABSTRACT: *Introduction:* Historically, Morocco has known many successive conquests and invasions that have induced genetic changes in its autochthons population. It's known that blood groups are among the most polymorphic systems. The study of ABO blood groups showed that their distribution varied in different populations. The aim of this study is to analyze the diversity and genetic differentiation of ABO system in the Moroccan population.

Material and methods: Data of ABO system genetic polymorphism from previous study were analyzed using statistical approaches which are the classical and the Bayesian methods. The classical approach has been used to assess genetic differentiation by adopting multivariate analysis type: PCA (Principal Component Analysis) and the index of genetic differentiation F_{st}. The Bayesian approach was used to assess the genetic structure of ABO system in the Moroccan population compared to other countries.

Results: Within the studied Moroccan population, 10 ABO alleles and 21 genotypes were identified. The heterozygosis rate is about 0.74 and 0.72, respectively, for the expected and observed heterozygosis.

PCA analysis shows that the studied population forms 4 groups. Data of genetic distances confirm the presence of Morocco within a group formed by Kuwait, Spain and Jordan with low genetic distances of 1%, 1.8% and 2%, respectively. The Bayesian analysis shows that all the countries, except Germany, present 5 genetic pools. Besides Morocco and Kuwait that have been found to present 5 genetic pools with similar frequencies.

Conclusion: The Moroccan population studied exhibits similarity with the countries of the Middle East and the southwest of Europe.

KEYWORDS: ABO system, diversity, genetic differentiation, PCA, Bayesian approach.

1 INTRODUCTION

Studying the phenotypic and genetic polymorphisms allow to understand the diversity within the populations. It’s useful to display genetic frequencies variation in different ethnic groups and to calculate the genetic distances between world populations.

Blood groups are among the most polymorphic systems. Since the discovery of blood groups by Karl Landsteiner in 1901, more than 300 alleles listed in 35 systems have been described in the data base db RBC on the NCBI website [1], [2], [3]. The most important blood group system is the ABO system, given its role in blood transfusion and transplantation [4], [5], [6]. In addition, several studies have reported associations between ABO polymorphism and various diseases and infections [7], [8], [9], [10]. Interestingly, the polymorphism of ABO blood group is the first marker that was used in the genetic anthropology discipline. ABO blood groups studying shows that their distribution varies from population to another. For instance, B blood group forms about 27.32% in Kuwait while in Spain, it is about 8.33% [11], [12]. The phenotypic polymorphism ABO is due to a polymorphism in the ABO gene. ABO gene is located in the long arm of chromosome 9 (9q34). It’s represented by several alleles A, B and O. The most frequent ABO alleles in the world are: A_{101}; A_{102}, B_{101}, O_{01} and O_{02}. A and B alleles are codominant, while O alleles are recessive. Thus it is not possible to indentify genotypes from some phenotypes, which need direct genetic exploration using molecular biology methods.

Geneticists and Anthropologists are interested in morocco because of its geographic situation as crossroads between Europe, Asian middle-east and Africa.

Historically, Morocco knew many successive invasions such as Phoenicians, Carthaginians, Romans, Vandals and Arabs. The last invasion was from Europe with Portuguese, French and Spanish populations. All these civilizations have had deep cultural and genetic changes on its autochthon population.

Several studies have determined the phenotypic frequencies of ABO blood groups and their distribution in different regions of Morocco by determining genic frequencies through statistical estimation [13], [14]. Other anthropological studies have identified O alleles polymorphisms in Moroccan Berber Populations [15], [16]. However, no genetic data are available concerning A and B alleles.

Our aim is to determine and evaluate the ABO genetic polymorphism and composition in Moroccans comparatively to populations from other countries.

2 MATERIAL AND METHODS

The genotyping results of our previous study that concern 120 samples of Moroccan blood donors (Unpublished data), taken randomly, have been analyzed and compared to other countries using different statistical approaches.

2.1 GENETIC DIVERSITY

The assessment of the genetic variability has been estimated using standard parameters of diversity, including number of alleles, allele frequencies, observed heterozygosity (Ho) and non-biaised estimated heterozygosity.

This last parameter [17] has been used for a better genetic diversity representation of populations with small samples [18]. The set of these parameters has been calculated using GenALex programme. The deviation of Hardy-Weinberg equilibrium has been tested via Fis parameter [19], according to Weir & Cockerham (1984) [20]. Fis values and their significance have been calculated using Genepop Software, adopting the exact test [21].

2.2 GENETIC DIFFERENTIATION

The classical approach has been used to evaluate the genetic differentiation, adopting PCA and Fst differentiation index and using Past and GenAlex Softwares respectively [22].

The establishment of distance dendogram has been realized using UPGMA Algorithm via Treeview Programme.

2.3 BAYESIAN ANALYSIS APPROACH

The genetic structure of ABO system of the Moroccan population compared to other countries was assessed by Bayesian approach.

First, the assumed number of ancestral genetic populations K was defined. Then each sample was assigned to its original population. The analysis was run using a model with Admixture and correlated allele frequencies, with the assumed number of genetic K clusters varying from 1 to 10, ten replicate runs per K value, a burning period length of 200,000, and a post burning simulation length of 1,000,000.

The identification of the most probable number of genetic ancestral populations, amongst the 10 K tested (K=1 to K=10), was established using ΔK parameter [23].

3 RESULTS

3.1 GENETIC DIVERSITY

Table 1 and 2 summarizes the genetic diversity parameters scored at the ABO loci in the Moroccan population and in some selected countries.

Table 1: Allele frequencies and sample size by population

	Allele	**Morocco** (*n=110*)	**Spain** (*n=108*)	**Germany** (*n=1335*)	**England** (*n=98*)	**Jordan** (*n=150*)	**Kuwait** **(***n=166*)	**India** (*n=100***)**	**China** (*n=125*)	**Japan** (*n=1000*)
	A_{101}	0.113	0.273	0.219	0.138	0.200	0.111	0.155	0.012	0.057
	A_{102}	0.021	-	-	0.015	0.007	0.018	0.010	0.180	0.222
A	**A_{104}**	-	-	-	-	-	-	-	-	0.005
	Av	-	-	-	0.005	-	-	-	-	-
	$A1^{-2}$	-	-	-	-	0.013	-	-	-	-
	A_{201}	0.058	0.019	0.064	0.056	0.053	0.030	0.020	-	0.001
	A_{204}	-	-	-	-	-	-	-	-	0.002
	A_{207}	-	-	-	-	-	0.006	-	-	-
B	**B_{101}**	0.121	0.088	0.100	0.128	0.130	0.163	0.265	0.168	0.164
	B_{102}	-	-	-	-	-	-	-	-	0.008
	B_{108}	-	-	-	-	-	0.003	-	-	-
	O_{01}	0.233	0.324	0.596	0.408	0.393	0.310	0.320	0.384	0.282
	O_{02}	0.346	0.259	-	0.163	0.203	0.250	0.145	0.248	0.257
	O_{03}	0.0083	0.037	0.021	0.010	-	0.003	0.085	-	-
	O_{05}	-	-	-	-	-	-	-	-	0.001
	O_{06}	-	-	-	-	-	-	-	-	0.003
O	**O_{v}**	-	-	-	0.077	-	-	-	0.008	-
	O_{103}	-	-	-	-	-	0.003	-	-	-
	O_{107}	0.0042	-	-	-	-	-	-	-	-
	O_{109}	-	-	-	-	-	0.081	-	-	-
	O_{207}	-	-	-	-	-	0.003	-	-	-
	O_{208}	-	-	-	-	-	0.003	-	-	-
	O_{209}	-	-	-	-	-	0.006	-	-	-
	O_{217}	-	-	-	-	-	0.003	-	-	-
	O_{305}	-	-	-	-	-	0.003	-	-	-
	O_{306}	0.0042	-	-	-	-	0.006	-	-	-
	O_{nv}	0.0042	-	-	-	-	-	-	-	-
Reference		Unpublished data	[12]	[27]	[25]	[34]	[35]	[36]	[25]	[26]

Table 2: Summary of private alleles by population

Population	Angleterre	Jordanie
Allèle	A_v	A_1^{-2}
Fréquence	0.0051	0.0133

Japon				
B_{102}	A_{104}	A_{204}	O_{06}	O_{05}
0.008	0.0045	0.0020	0.0030	0.0005

Kuwait									
B_{108}	A_{207}	O_{109}	O_{208}	O_{209}	O_{217}	O_{305}	O_{207}	O_{103}	O_{306}
0.0030	0.0060	0.0813	0.0030	0.0060	0.0030	0.0030	0.0030	0.0030	0.0060

Maroc		
O_{306}	O_{nv}	O_{107}
0.0042	0.0042	0.0042

In Morocco, 10 alleles have been revealed, whereas only 5 are found at the German population. The highest number of alleles has been detected at the population of Kuwait.

Otherwise, observed and estimated heterozygosity in Moroccan population of blood donors showed 0.724 and 0.740 values respectively [Table 3].

Table 3: Summary of genetic diversity parameters by population

	N_a	N_e	H_o	H_{eu}	F
Morocco (*n=110*)	10	3.80	0.724	0.740	0.018
Spain (*n=108*)	6	3.90	0.731	0.747	0.016
Germany (*n=1335*)	5	2.40	0.578	0.583	0.009
England (*n=98*)	9	4.20	0.755	0.766	0.009
Jordan (*n=150*)	7	3.91	0.740	0.746	0.005
Kuwait (*n=166*)	16	4.86	0.723	0.797	0.090
India (*n=100***)**	7	4.44	0.830	0.778	-0.072
China (*n=125*)	6	3.71	0.784	0.733	-0.074
Japan (*n=1000*)	11	4.44	0.759	0.775	0.021
Mean	8.11	3.96	0.736	0.741	0.003
SE	1.16	0.23	0.023	0.021	0.017

N_a: Number of Different Alleles, N_e = Number of Effective Alleles, H_o = Observed Heterozygosity, H_{eu} = Unbiased Expected Heterozygosity, F = Fixation Index

3.2 GENETIC STRUCTURE

3.2.1 CLASSICAL ANALYSIS

The results of PCA analysis, based on allele frequencies, have been demonstrated in a two-dimensional diagram. The two axes represent 56.74% and 24.27% of the variation [Figure 1]. This analysis allowed us to distinguish four groups. The first contains China and Japan. While the second, contains Morocco, Kuwait and Spain. The third comprises England, India and Jordan. The last group is formed only by Germany.

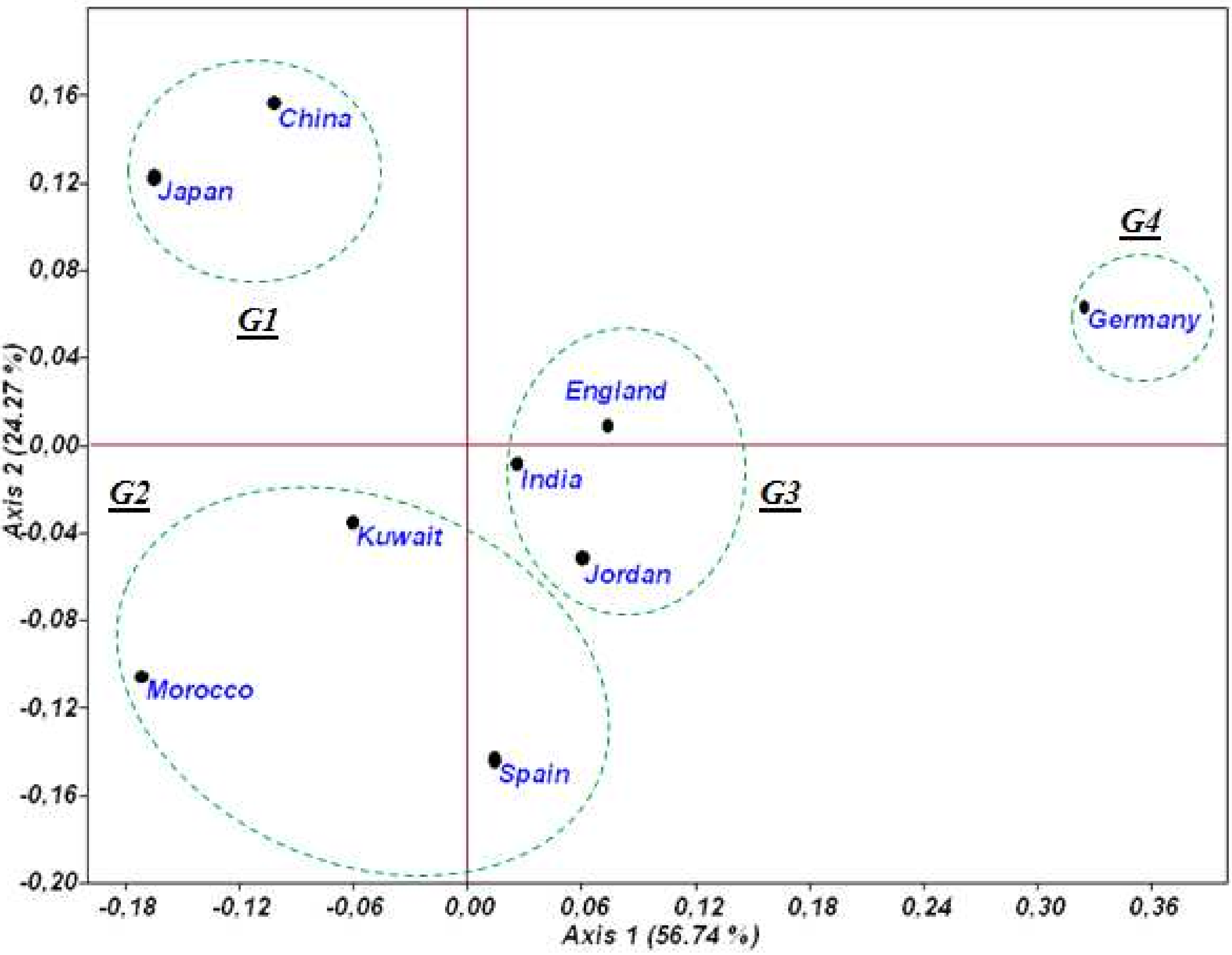

Figure1: PCA analysis among the populations defined according to their ABO alleles

The genetic distances analysis showed 3 groups [Figure 2 & Table 4]. The first group is a fusion of 2 groups, comprising 6 populations which are Morocco, Kuwait, Spain, Jordan, England and India. The remaining groups are unchanged.

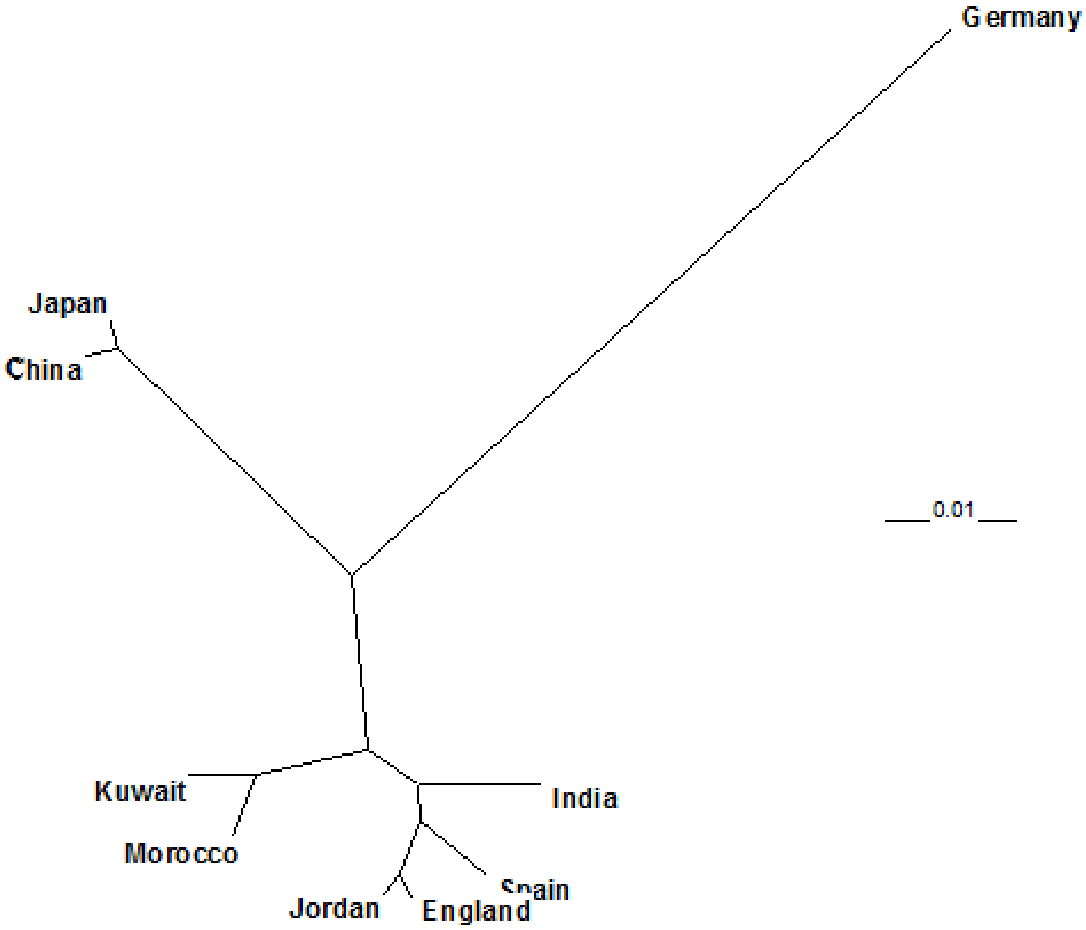

Figure 2: Genetic relationships among the populations defined according to their ABO alleles

Table 4: Geographic group pairwise comparisons

	Morocco	Spain	Germany	England	Jordan	Kuwait	India	China	Japan
Morocco	**0**	0.108	**0.498**	0.174	0.125	0.063	0.211	0.154	0.145
Spain	0.018	**0**	0.215	0.094	0.035	0.086	0.133	0.240	0.245
Germany	**0.095**	0.052	**0**	0.083	0.104	0.253	0.188	0.302	0.443
England	0.026	0.015	0.027	**0**	0.024	0.073	0.090	0.124	0.201
Jordan	0.020	0.006	0.031	0.004	**0**	0.051	0.079	0.147	0.196
Kuwait	0.010	0.013	**0.057**	0.010	0.008	**0**	0.090	0.100	0.131
India	0.030	0.020	**0.047**	0.013	0.012	0.012	**0**	0.175	0.213
China	0.025	0.037	**0.068**	0.020	0.024	0.016	0.026	**0**	0.025
Japan	0.022	0.034	**0.084**	0.027	0.028	0.017	0.027	0.005	**0**

Above diagonal: Nei's (1972) genetic distance. Below diagonal: Pairwise genetic differentiation index (Fst)

3.2.2 BAYESIAN ANALYSIS APPROACH

The Bayesian approach showed that the accurate representation of the ABO genetic structure of the analyzed populations, was observed for K =2 (DK = 168.475) and K =5 (DK = 87.986) [Figure3a]. For k=2 and k=5, a high rate of admixture was showed for all analyzed populations.

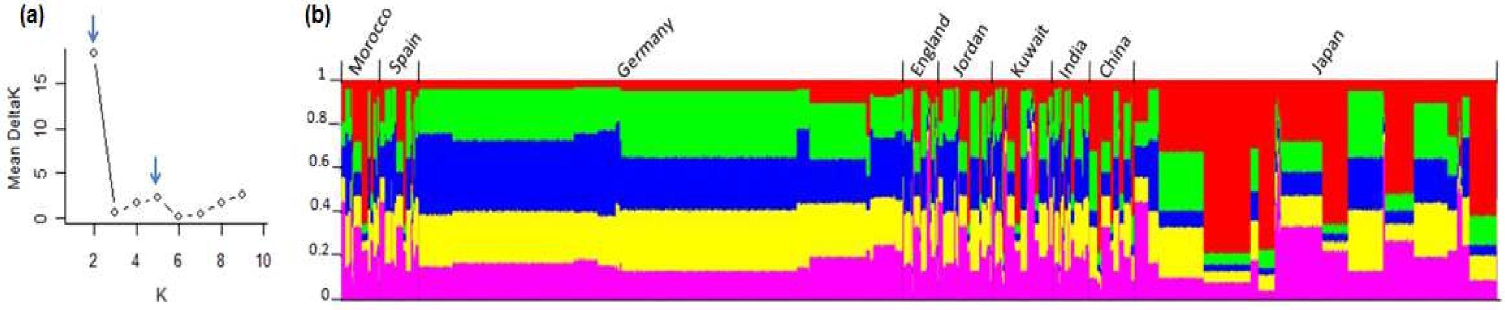

Figure3. The genetic relationships among the 9 countries estimated using STRUCTURE

(a) The ΔK (Evanno et al., 2005) was plotted against various values of K suggesting K=5 as the most likely number of clusters, (b) assignation of individuals to the genetic clusters at K=5. The y-axis shows the likelihood distance and the x-axis shows the individual (blood donor) and subgroup indicated by color. Each color represents one gene pool.

Thus, all countries present the 5 genetic clusters with nearest probability [Table 5 and figure 3b]. Except Germany, of which the first cluster (red) is less frequent (0.053), all other countries present the 5 genetic clusters with nearest assignation probability. Moreover, Morocco and Kuwait present the 5 clusters with a similar probability.

Table5: Probability of meeting the country in five gene pool

	Cl1	Cl2	Cl3	Cl4	Cl5
Morocco	0.289	0.145	0.155	0.149	0.261
Spain	0.17	0.176	0.211	0.187	0.255
Germany	**0.053**	0.249	0.283	0.253	0.162
England	0.153	0.18	0.198	0.185	0.284
Jordan	0.164	0.189	0.211	0.194	0.242
Kuwait	0.211	0.153	0.156	0.157	0.323
India	0.165	0.189	0.222	0.21	0.214
China	0.337	0.179	0.115	0.169	0.2
Japan	0.388	0.158	0.111	0.15	0.194

4 DISCUSSION

Our results display the presence in Morocco of three alleles A_{101}, A_{102} and A_{201} with the proportion of A_{101}/A_{102}= 5.38 and A_{101}/A_{201}=1.95; while in Japan the frequency of ABO*A_{102} is 23.5% and 18%, at the Chinese population [24]. ABO*A_{201} allele is either absent i.e. within the Chinese or rare as for the Japanese (0,1%) [25], [26]. Comparatively ABO*A_{102} allele is absent at the Spanish and the German [27], [12].

Our previous study revealed that O_{02} allele is more frequent than O_{01} at a proportion of O_{02}/O_{01}= 1.5, this value is similar to that reported by Roubinet et al. (O_{02}/O_{01} = 1.8), while it equals 1 in Ivory Coast and at Afro-American communities [28], [15]. The frequency of O_{02} and A_{201} alleles shows a similarity between the Moroccan, the Spanish and Kuwaiti populations. Moreover, the presence of O_{306} allele within the Moroccan population also illustrates a similarity with Kuwait.

Our previous study has identified 10 alleles at the Moroccan population. This result should be reconsidered after DNA sequencing of O individuals with an unidentified variant. Roubinet *et al*. (2004) confirmed the presence of 9 O alleles at the Berber Moroccan population [15]. The diversity of the Moroccan population could be explained by different migration movements that Morocco has historically experienced.

The genetic migration reduces endogamy which leads to increased genetic variability and heterozygosity [29]. The most genetic diversity has been identified at the Kuwaiti population with 16 ABO alleles; 10 of them are private alleles.

Germany presents only 5 alleles and the least level of heterozygosity. Furthermore, the most value of the observed heterozygoty is that of the Chinese and Indian populations with 0.830 and 0.784, respectively [Table 3]. In Morocco, the values of observed and expected hererozygosity are 0.740 and 0.724, respectively.

PCA Analysis displays four populations groups. The results of genetic distance confirm that Moroccan population is in the same group of Kuwait, Spain, and Jordan with genetic distances 0.010, 0.018 and 0.020, respectively.

Several studies have confirmed this genetic convergence that may be explained by a gene flux, according to different migration movements [30], [31], [32].

Bekada et al. (2013) have confirmed that nearly 20% and 35% of the Maghreb genetic pool is from the Middle-East, and 5% and 30%, from Europe, especially the Iberic Peninsula, of maternal (ADNmt) and paternal(Y-ADN) lineages [33].

Botigué et al. (2013) have reported that gene flux from North-Africa contributes to the increase of diversity and differentiation in the South-West European population [32].

The Asian gene flux, especially from Indian and Pakistani populations, towards North-Africa is explained by the common shared history before and after Islamization, supports arguably the presence of Morocco in the same genetic group, as England, a country showing a large presence of Indian and Pakistani citizens.

Bayesian analysis showed that, apart from Gremany, of which the first (red) cluster is less frequent (0.053), all other countries present the 5 genetic clusters with nearest assignation probability. Moreover, Morocco and Kuwait present 5 clusters with a similar probability.

The rate of ABO system admixture between different countries illustrates that there is an important gene flux between populations despite geographic origins.

5 CONCLUSION

The Moroccan population studied exhibits similarity with countries of the Middle East (Jordan and Kuwait) and the southwest of Europe (Spain). Furthermore, the crossing rate and merging for the ABO system between countries shows that it is difficult to talk about genetic border between populations.

AUTHORSHIP CONTRIBUTIONS

FZ and HA: Contribution to achievement of the statistical analysis, manuscript drafting, study conception and design, data acquisition, data analysis and interpretation and revision of the manuscript.

NH: Contribution to manuscript drafting, conception and design, data acquisition, data analysis and interpretation, manuscript revision and critical revision of the intellectual content.

MM, HE, ZB and KB participated in study design and data acquisition and manuscript revision.

All Authors approved the final version of the manuscript to be published.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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