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Research Article

ABO Genotyping and Anthropology: A Study of The Population of NorthEastern Algeria.

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ABSTRACT

With the development of molecular biology techniques, several researchers have become interested in the study of genetic polymorphism of the ABO system. Our work consists in studying the genetic polymorphism of the ABO system in the population of the Constantine region in northeast Algeria, and to compare it with other populations. **Material and Methods:** This is a descriptive cross-sectional study of 57 blood donors from the Constantine region in north-eastern Algeria, the genotype of the ABO system was determined by the molecular biology technique, Polymerase Chain Reaction - Sequence specific primers (PCR-SSP). The calculation of genetic distances was performed by SPSS version 20 software. The principal component analysis and the ascending hierarchical classification were carried out with the R software. **Results:** The order of frequency of ABO genotypes is as follows: OO > AO > BO > AA > AB > BB with respectively: 45.62% > 21.05% > 15.78% > 10.53% > 5.26% > 1.75%, and the frequencies of the ABO alleles ; A1, A2, B, O1 and O2, are respectively: 17,54%, 6.14%, 12,28%, 58.77%, 5.26%. Our study population is very close genetically to other Maghrebian populations, and genetic distances are not always well correlated with geographical distances, but influenced by other factors resulting in more mixtures with some European and Asian populations than with others. **Conclusions:** The geographical locations of our study population, associated with several historical events, have contributed to its genetic identity.

Keywords: ABO System, Genotype, Molecular Biology, Anthropology.

INTRODUCTION

The genetic polymorphism of the ABO system reflects thousands of years of migration and intermingling between populations, and interaction with the natural and cultural environment, the current distribution of ABO alleles between different regions of the world is related in part to major epidemics and some infectious diseases, in addition to populations movements, as well as mutations and drifts, This has made this blood group system a real marker and genetic tracer of populations, and has aroused the interest of several researchers, not only to understand the mechanisms of interactions of this system with different diseases, but also in the field of biological anthropology, which ABO allele polymorphism has become an important tool participating in the study and understanding of human genetic diversity [1].

The existence of the science of Immunohematology, and the foundation of the bases and rules of transfusion medicine followed the discovery of the ABO system in 1900 by Karl Landsteiner, [2, 3], this system is classified No. 001 according to the International Society of Blood Transfusion (ISBT 2008), it includes 4 blood groups: A, B, O and AB, each of which is doubly defined by the presence or absence of A and or B antigen on the surface of red blood cells, and by the presence or absence of corresponding serum antibodies. Each antigenic specificity is also assigned a number, A: 001, B: 002, AB: 003 [4].

Our work, whose objective is the study of the genetic polymorphism of the ABO system in the population of the Constantine region in north-eastern Algeria, using molecular biology technique, allowing to determine the allelic and genotypic frequencies of this system, to establish a database for the genetic markers of this population allowing its anthropogenetic characterization, in order to enrich and deepen our knowledge on the genetic diversity at the scale of this region, but also to estimate the genetic distances between our study population and other Maghrebian, and world populations.

Material and Methods

This is a descriptive cross-sectional study to investigate genetic polymorphism of the ABO system in the population of the Constantine region in northeastern Algeria, by molecular biology technique (PCR-SSP). It involves a sampling of 57 blood donors collected at the level of the Blood transfusion center of the University Hospital Center of Constantine, selected in a random way independently of sex and age. All blood donors presumed to be healthy and whose geographical origin was NorthEast Algeria were included in the study after obtaining informed consent.

Two EDTA tubes per individual were filled with 05 ml of whole blood. The first tube was used for ABO blood grouping by hemagglutination using manual techniques on a plate and on a microfiltration gel, carried out in the hours following the collection. The second tube, intended for ABO genotyping by molecular biology, is used directly for DNA extraction, and if this extraction is delayed, the whole blood is frozen at (-40°C) until its use.

Two processes have been used for DNA extraction based on kit-based extraction techniques, the QuickGene-Mini80 Nucleic Acid Extraction and Purification System [5], and the Maxwell® 16 Blood DNA Purification System [6].

For the control of the concentration and purity of the genomic DNA, the assay was performed by spectrophotometry using a "NanoDrop 2000c Thermo scientific". The concentration of DNA was determined by measuring the optical density (OD) at 260 nm (A260), it is expressed in ng/µl according to the following formula: [DNA] ng/µl = OD 260 x 50 x 100.

A measurement of OD at 280 nm and calculation of the A260/A280 ratio, which must be approximately 1.8 in order to qualify the extracted DNA as pure.

ABO genotyping was performed using the PCR-SSP technique to search for the five common ABO alleles, and rare ABO allele variants, which enables amplification of defined DNA sequences [7, 8], and the evaluation of the result is performed by agarose gel electrophoresis. The system is able to clearly distinguish the most frequent alleles: A1, A2, B, O1 and O2, using in addition to the negative controls and primers allowing the amplification of an internal control, eight specific primers corresponding to the polymorphisms; 261delG, 261G, 802G>A, 802G, 803G>C, 803G, 1061delC, 1061C, as well as several couples of specific primers for the search of weak variants of ABO alleles, the different alleles A2, A3, Aweak, Abant, B3, Bx, Bweak, Bel, cisAB, B(A) and several O alleles [9].

Calculation of genetic distances between the populations selected for comparison of ABO allelic frequencies was performed using SPSS version 20 software. Principal component analysis and hierarchical ascending classification were performed by R software using the FactoMiner package [10, 11].

Results

The average age of our sample was 36.05 years with extremes from 19 to 52 years, the sample was composed of 85.96% men 14,04% women, with a M/F sex ratio of 6.12, this distribution is consistent with that usually observed in our blood donors, but is not representative of the general population, influenced by the nature of the blood donors that our blood transfusion centers receive, which are mostly men, this does not affect the representativeness of our sample in relation to the general population, because the expression and genetic transmission of the ABO alleles are independent of sex. Phenotype A is present in 31,58% of subjects, 17.53% of subjects are phenotype B, 5.26% are AB, and phenotype O is present in 45.62% of subjects. Following the analysis of our sample by molecular biology, the frequencies of ABO genotypes given in table 1 showed that the O1O1 genotype is the most frequent followed by the BO1 genotype; while none of the A2A2, A2O2, and O2O2 genotypes were detected in this population.

Table 1. ABO genotype frequencies :

Phenotypes	N	Genotypes	N	%

A	18	A_1A_1	04	07,02
		A_1A_2	02	03,51
		A_1O_1	07	12,28
		A_1O_2	01	01.75
		A_2O_1	04	07,02
		A_2O_2	00	00
		A_2A_2	00	00
B	10		01	01.75
		BB BO_1	08	14.03
		BO_2	01	01,75
AB	03			
		A_1B	02	03,51
O	26	A_2B	01	01,75
		O_1O_1	22	38,60
	57	O_1O_2	04	07,02
Total		O_2O_2	00	00
		Total	57	100

In our study 47,37% of individuals are homozygous for the studied alleles, of which 7,02% A1A1, 1,75% BB, and 38,60% O1O1. While 52,63% are heterozygous. In our study population, the frequency of AA genotype is estimated at 10.53%, AO 21,05%, BB genotype which is the least frequent of the observed genotypes its frequency is 1,75%, 15,78% for BO genotype, 5,26% for AB, and 45,62% for OO genotype the most frequent of the observed genotypes. The order of frequencies of the five common alleles identified in our study, ABO*A1, ABO*A2, ABO* B1, ABO*O.01 and ABO*O.02, presented in Table 2, being as follows: O1 > A1 >B > A2 > O2. These results show that the O allele is in the majority in this population with an overall frequency of (64.03%), followed by the A allele (23.68%), and finally the B allele is the least frequent (12.28%).

Table 2. ABO allele frequencies :

Alleles	N	%
A_1	20	17,54
A_2	07	6,14

B	14	12,28
O_1	67	58.77
O_2	06	5.26
Total	114	100

Comparison of the ABO allele frequencies of our study population with other North African and world populations represented in Table 3, shows that the A1 allele is less frequent in North African populations compared to Asian and European populations. Its highest frequency was recorded in the Spanish population (Spa1: Spain; 27.30%) [18]. As for the A2 allele, our results show that it is generally more frequent in North Africans compared to Asian and European populations. The highest frequency of the O1 allele was recorded in the Basque population (Spa2: Basques; 69.30%) [12], the lowest frequency was observed in the Germans (Ger: German; 36.62%) [15], whereas this German population recorded a particularly high frequency of the O2 allele (22.08%) compared to the other populations.

Table 3. Comparison of ABO allele frequencies with other populations

Population	N	A_1	A_2	*B*	O_1	O_2	Other
NE. Alg	57	0.1754	0.0614	0.1228	0.5877	0.0526	/
Mor [12]	69	0.145	0.072	0.145	0.594	0.022	0.022
Mau [12]	30	0.117	0.067	0.133	0.633	0.017	0.033
W.S [12]	66	0.152	0.076	0.152	0.599	0.015	0.008
Kwt [13]	166	0.1296	0.0301	0.1627	0.5603	00	0.1175
Chn [14]	834	0.2134	0.0024	0.2086	0.5731	0,0012	0.0012
Ger [15]	113367	0.2063	0.0687	0.095	0.3662	0.2208	0.043
Cor [16]	222	0.2725	00	0.1982	0.5293	00	/
Ita [17]	232	0.142	0.023	0.153	0.650	0.015	0.017
Pol [18]	170	0.223	0.041	0.168	0.544	0.023	/

Spa1 [18]	108	0.273	0.019	0.088	0.583	0.037	/
Spa2 [12]	165	0.194	0.024	0.027	0.693	0.009	0.052
Ind [19]	201	0.165	0.02	0.2650	0.455	0.095	0.005
Jord [20]	150	0.220	0.05	0.130	0.600	0	/

Abbreviations: NE.Alg, North-East Algeria; Mor, Morocco; Mau, Mauritania; W.S, Western Sahara; Kwt, Kuwait; Chn, China; Ger, Germany; Cor, Korea; Ita, Italy; Pol, Poland; Spa1, Spain; Spa2, Basque population; Ind, India; Jord, Jordan.

The study of the genetic distances between our study population and the populations chosen for comparison represented in Table 4 and Table 5, shows that our study population is genetically very close to the Maghrebian populations with an average distance of (0.0413), especially Western Sahara (0.025), Morocco (0.028) and Mauritania (0.071), while the most distant populations are Asian, with an average distance of (0.2450), and the average distance from European populations is (0.2404).

Table 4. Genetic distances matrix by ABO alleles distribution

	NE.Alg	Mor	Mau	W.S	Kwt	Chn	Ger
NE.Alg	**,000**						
Mor	**,028**	**,000**					
Mau	**,071**	**,012**	**,000**				
W.S	**,025**	**,010**	**,030**	**,000**			
Kwt	**,374**	**,276**	**,228**	**,354**	**,000**		
Chn	**,233**	**,318**	**,366**	**,322**	**,400**	**,000**	
Ger	**,625**	**,707**	**,807**	**,742**	**,935**	**,980**	**,000**
Cor	**,341**	**,474**	**,567**	**,471**	**,566**	**,040**	**1,000**
Ita	**,108**	**,121**	**,104**	**,141**	**,228**	**,111**	**,914**
Pol	**,061**	**,131**	**,207**	**,123**	**,387**	**,081**	**,691**
Spa1	**,200**	**,349**	**,430**	**,348**	**,558**	**,129**	**,806**
Spa2	**,208**	**,249**	**,226**	**,288**	**,270**	**,277**	**,941**
Ind	**,237**	**,290**	**,350**	**,305**	**,406**	**,140**	**,697**

Jord	**,040**	**,097**	**,158**	**,086**	**,400**	**,138**	**,765**

Table 5. Genetic distances matrix of other populations by ABO alleles distribution

Cor	Ita	Pol	Spa1	Spa2	Ind	Jord
Cor	**,000**					
Ita	**,273**	**,000**				
Pol	**,113**	**,123**	**,000**			
Spa1	**,088**	**,235**	**,084**	**,000**		
Spa2	**,366**	**,135**	**,232**	**,178**	**,000**	
Ind	**,237**	**,204**	**,153**	**,349**	**,524**	**,000**
Jord	**,185**	**,117**	**,017**	**,091**	**,166**	**,272**	**,000**

The results of the Principal Component Analysis (PCA) presented in figure 1, illustrates the comparison of our population with other North African and world populations, based on the frequencies of ABO A1, A2, B, O1, and O2 alleles. This study shows a global variability of 63.27%, and that the first component representing 31.71% of the variation, is mainly determined by the O2 allele which is positively correlated to this axis, but also by the O1 allele which is negatively correlated to this axis, which opposes our study population, with the German, Indian and Polish populations to the other populations studied. The second component explains 31.56% of the variation, and is positively correlated with the A2 allele, while the A1 allele seems to have a negative correlation with the second axis, which opposes all the North African populations in addition to Kuwait grouped in the positive part at the top of the second vector, to the Asian populations, the other European populations have taken divergent positions on either side of this axis.

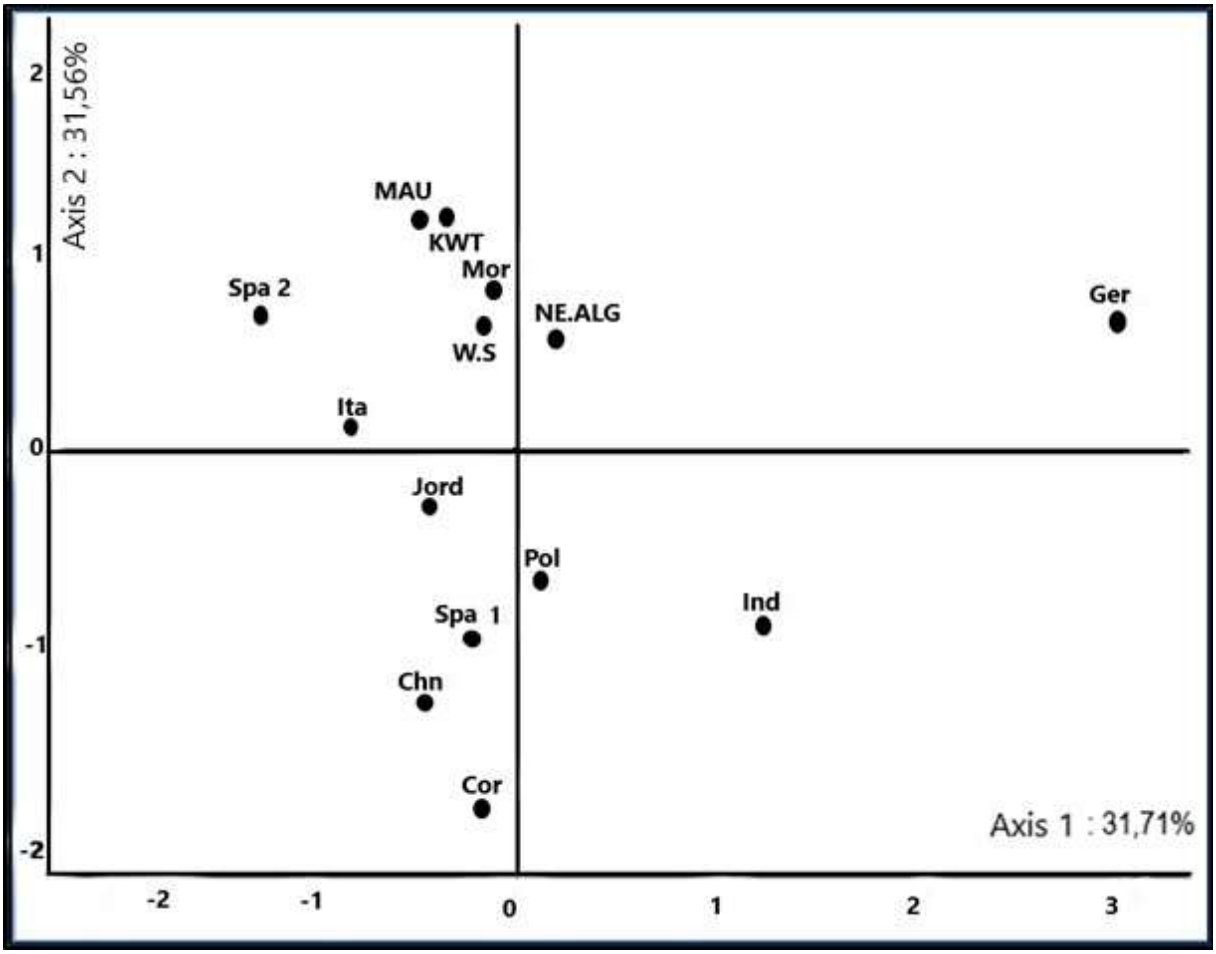

Figure 1. Principal Component Analysis

The population that expresses the O2 allele more is located further to the right of the first vector, while the population that expresses the A2 allele more is located further up the second vector.

The analysis of the dendrogram presented in figure 2, obtained following the ascending hierarchical classification, allowed us to note the grouping of the populations chosen for the comparison in three essential clusters according to their closeness in function of the distribution of ABO alleles, the first cluster groups Italy, Kuwait, the Basque population (Spa2), and all the North African populations, the second cluster groups Poland, Spain and all the Asian populations except India, the third cluster groups Germany and India.

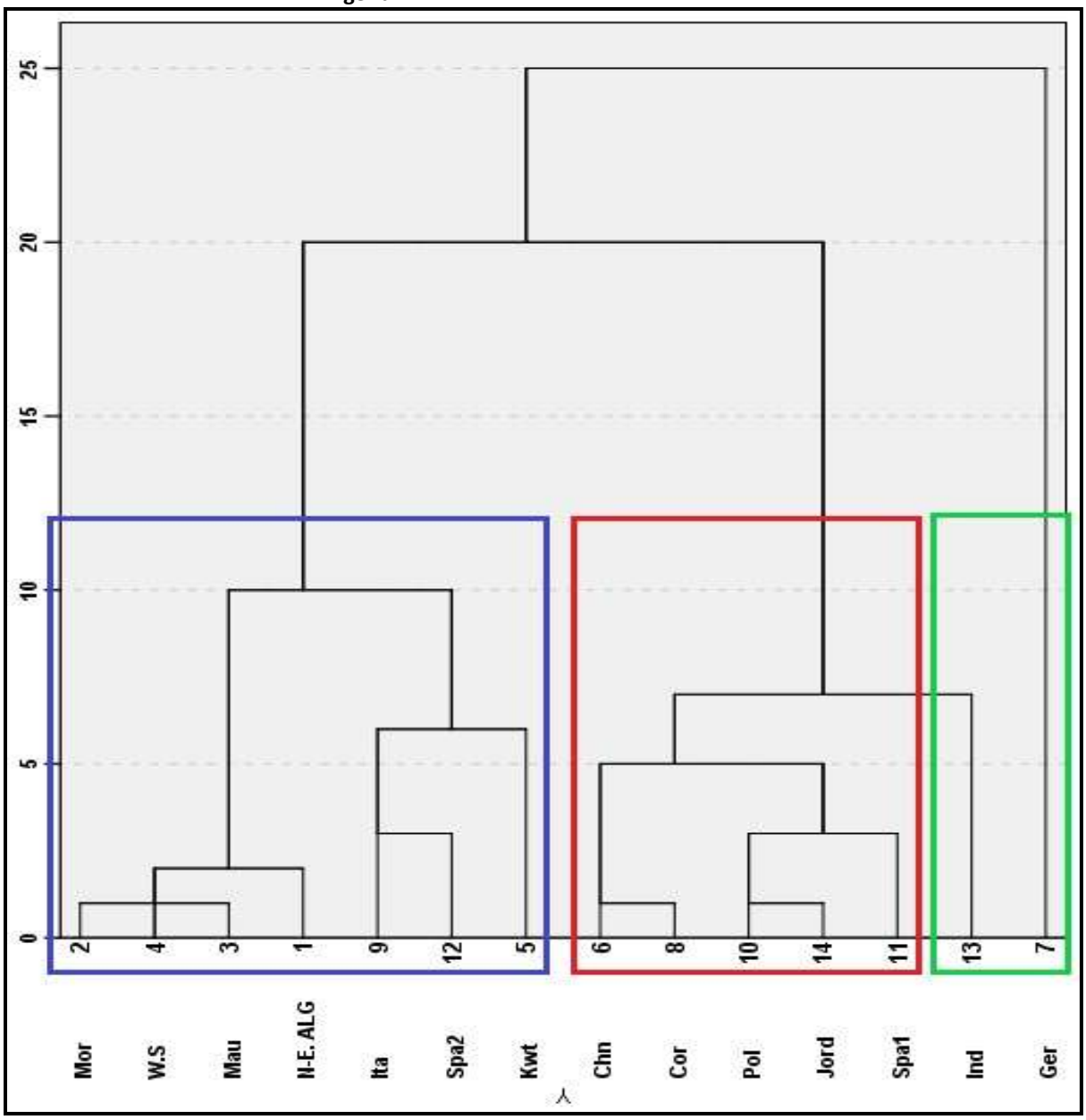

Figure 2. Ascending hierarchical classification of the distribution of ABO alleles.

The Hierarchical Ascending Classification, gave a dendrogram that shows the grouping of populations into three essential clusters according to their expressions of ABO alleles.

Discussion

The order of the observed genotype frequencies in our population is as follows: OO > AO > BO > AA > AB > BB. This is not consistent with the genotypes observed in the other populations compared, where the AA genotype was less frequent than the AB genotype.

The comparison of ABO allele frequencies between the different selected world populations shows that the A2 allele seems to be a marker of the North African populations, which express it with an average frequency of (0.0691%), and which exceeds all the frequencies of the other populations selected for the comparison, with an average expression of (0.0351) in Europeans and (0.0205) in Asians. Only the German population [15] expressed a relatively high frequency of this allele compared to the other European populations.

This comparison shows that our population is genetically true to its geographic location, noted by a low level of genetic heterogeneity with North African populations compared to the other populations studied, and a mean distance of (0.0413). This finding was better illustrated by Principal Component Analysis (Figure 1), which shows a clustering of the Maghrebi populations, which took positions close to the two axes, and the trees obtained by hierarchical clustering (Figure 2) also consolidate this finding with nodes between Maghrebi populations whose heights are relatively closer. While Asians are the most distant from our population, the German population[15] showed the highest genetic distance (0.625), linked to the anthropological characteristics of this population responsible for its singularity, and marked by its strong endogamy, sharing of a single language, traditions and religion, with little contact with North African populations, Unlike the Spanish populations [12, 18], the Italian population [17] and the Polish population [18], the latter is considered by our study to be the closest European population genetically to our population, with a genetic distance of (0.061). This is linked to the greater historical mixing of these populations with North African populations, compared with the German population.

Asian populations showed a very high B allele expression compared to other populations, with Jordan [20] being the closest Asian population to ours with a genetic distance of (0.040), while Kuwait [13] is the furthest Asian population from ours (0.374). This shows that the populations movements and mixtures between the Maghrebi populations in general and our study population in particular, were more pronounced with some countries of the European and Asian continents compared to other countries belonging to the same continents, which made that the genetic distances are not perfectly correlated to the geographical distances, but much more influenced by other historical factors such as colonizations, trade, Islamic conquests ... etc. and this is well demonstrated in the dendrogram of the ascending hierarchical classification, where the distance of the heights of the nodes between the populations is the composition of the three groups constituting this tree, is not perfectly correlated to the geographical distance, except for group 01 (Figure 2) composed of the Maghrebian populations in addition to Italy, Kuwait and the Basques. In the absence of genetic studies on sub-Saharan and African populations in general, we were unable to position our study population and estimate genetic distances from populations on the African continent.

Conclusion

The geographical location of our population at the gates of Africa, and on the southern shore of the Mediterranean, associated with several historical events such as invasions, trade, and Islamic

conquests, have been at the origin of many movements and population mixtures that our region has experienced, and thus contributing to the complexity and genetic identity of our population.

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