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Original Synthesis Report

HLA Genes in Atlantic Celtic populations: Are Celts Iberians?

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Abstract- Atlantic Europe populations were analyzed with HLA genes in order to establish their relationship among themselves and with other populations. Standard genetic and statistical software analyses were used. Celtic populations (British Isles and French Bretons) have genetically been found close together: Irish, Welsh, Orkney Islanders (Scottish), French Bretons, Galicians, Spanish Basques, Portuguese, cluster together in DA genetic distances, correspondence analysis and Neighbour Joining dendrograms. Genetics have been shown by itself not suffice to determine populations migration/relatedness. Aristotle and Herodotus placed Celts in Iberia and R1b chromosome Y marker is high in Iberia and all Celtic European populations above mentioned (probably stemming from Iberian Ice refugee after Last Glaciation) and Ancient Celt language (Gaelic) is being translated from Iberian-Tartesian language: these suggest that Celts and Iberians, so named by Classic authors, constitute the same population. On the other hand, a) R1b gene analysis of Canary Islands ancient inhabitants (Guanches), b) abundant Iberian scripts are also found in Canary Islands, c) a established North Africa/Iberia ancient gene flow, and d) no evidence of demic diffusion from eastern to western Mediterranean according to human ancient skeleton studies is noticed in Mesolithic/Neolithic transition: these facts suggest that ancient Canary Islanders may be included within the Iberian/Celtic population. Our conclusions are that: 1) Celts are concentrated in Atlantic Europe, 2) Iberians and Celts mentioned by classic authors most probably refer to the same population living in Iberian Peninsula (Spain/Portugal); in addition, North African Berbers and ancient Canary Islanders also belong to this group 3) Postulated farmers demic diffusion in a East to West Mediterranean direction never existed.

Keywords: Celts, Iberians, Picts, Scottish, Orkney Islands, Irish, British (English), French Bretons, Welsh, Basques, Galicians, Canary Islands, Mediterranean demic diffusion, Gaelic language.

Introduction

Human leukocyte antigen (HLA) complex comprises a group of very polymorphic loci, which have been used mostly in medical practice and research. HLA compatibility is routinely used for choosing donor/recipient couples in transplantation, for studying autoimmune genetic epidemiology and other diseases (HLA linkage to disease). These genes are also useful in anthropological and forensic studies (Riley and Olerup, 1992; Arnaiz-Villena et al. 2001a).

In modern times (XIX century) Celts have been defined as people that spread from Center Europe (Hallstat and La Tene culture). They had expanded from this original place (France, Germany and Austria, Alps area) to many other places throughout all Continent up to nowadays Turkey (Oppenheimer, 2007). Thus, Encyclopaedia Britannica defines Celt as a member of an early Indo-European people who spread over much of Europe from 2nd millennium BCE to the 1st century BCE. These Celt groups eventually ranged from the British Isles and northern Spain to as far east as Transylvania, the Black Sea costs and Galatia, in Anatolia, and were later partially included within Roman Empire (Encyclopaedia Britannica, 2015).

This view came from a wrong interpretation of Herodotus and Aristotle (Oppenheimer, 2007) writings about Celts. Herodotus stated that Danube (Ister) River "begins from the Celts live in the city of Pyrenne" and "Celts are placed outside the Pillars of Heracles (Strait of Gibraltar) and neighbor with Kynesians (Cynetes, at extreme Southwest of Iberia)" (*Histories* 2.11) (Oppenheimer, 2007). Also, Aristotle placed Danube origin at Pyrenne mountain (Hubert, 2000). Altogether, according to Herodotus and Aristotle are unequivocally indicating that the Celts original place was in Iberian Peninsula; Herodotus was mistaken on where Danube River started (i.e.: it really starts in Black Forest, Germany, and not in Mount Pyrenees) (see Discussion, "The Origin of Celts").

On the other hand, haplogroups R1b of Y-chromosome is the most common and the most frequent of western Europe, reaching its maximum frequency in North Spain, Wales, Scotland, Ireland and Brittany (www.eupedia.com/europe/Haplogroup_R1b_Y-DNA.shtml#distribution, Oppenheimer, 2007). Therefore, this fact is an effect of inhabitants of North Iberia (part of Mediterranean Ice-Age refuge) going northwards to

Brittany (France), England, Wales, Scotland and Ireland about regression of ice by about 10,000 year BC (Oppenheimer, 2007). Nowadays, populations speaking Celt languages are: Galician (Northwest Spain), Welsh, Irish, Bretons, Scottish, English, Cornish and also at Man Island.

Figure 1. Localization of Galicia in NW Spain

Part of First Canary Islanders (Guanches) have been included within these Atlantic First Populations (Arnaiz-Villenaet al. 2015). First Canary Inhabitants (Guanches) have a mixed anthropological ancestry (Braem, 2010; González, 1992). Some Guanches were described as being tall, blue-eyed and with robust complexion (like Europeans) and other appeared to bear of a more graciles skeletum and were more similar to Mediterraneans (Braem, 2010; González, 1992). 10 % of Guanche mummies bore R1b2 and P (xR1a and R1b1b2) Y chromosome marker (Fregel et al. 2009). This Guanche marker could come from Iberian Peninsula as reported for northern Atlantic populations after Last Ice Age retreat (Oppenheimer, 2007), and also may have gone from Iberia southwards to Canary Islands (Fregel et al. 2009; Arnaiz-Villena et al. 2015). This is supported because widespread ancient Iberian language rock scripts have been found at Canary Islands (Lanzarote, Fuerteventura and El Hierro) (Arnaiz-Villena and Alonso-Garcia, 2001a; Arnaiz-Villena et al. 2015). Thus, it is possible that part of First Canary Island Inhabitants may be included within Atlantic European populations.

In the present work, we have studied a population of Galicia in Northwest Spain (Fig. 1) in order to: 1) Determine the HLA class I (A and B) and class II (DRB1 and DQB1) allelic Galician lineages (hereafter “alleles” for simplicity) and specific HLA haplotypes by using PCR-Luminex and DNA sequencing, 2) Compare the Galician HLA allele frequencies with those of Atlantic Europe and other worldwide populations (Table 1) in order to establish origins in Galician by calculating genetic distances, Neighbour Joining (NJ) dendrograms and Correspondence analyses and finally, 3) Relating HLA Galician gene profile to other populations of Atlantic Europe (i.e.: Welsh, Irish, Bretons, Scottish and English).

Material and Methods

Samples

HLA-A, -B, -DRB1 of one hundred and twenty five unrelated healthy Galician individuals were typed.Samples were taken from unrelated blood donors volunteers at the Santiago de Compostela, Blood Center, Galicia, Spain. All subjects were born in the same region (Galicia, Spain; Fig. 1) and their four grandparents had been born in the same area. We compare our HLA data with those of other Atlantic Celt populations, Mediterraneans and other populations (Table 1).

HLA typing

HLA class I (-A, -B, -C) and class II (-DR) typing was done by a two-step microlymphocytotoxicity technique on T or B lymphocytes, respectively, using the 10th and 11th International Histocompatibility Workshop and local reagents (Arnaiz-Villena et al. 1997).Other HLA DNA technologies were used when necessary (Arnaiz-Villena el al. 2009).

Selecting Haplotypes

HLA haplotypes were selected for populations relatedness calculations, according to Degos and Dausset haplotypes assignation to historical European populations. These haplotypes have been used for calculation in the present work (Degos and Dausset, 1974).

Statistical Analysis

Statistical analysis was performed with Arlequin v3.0 software provided by Excoffier and Slatkin (Schneider et al. 2000). In summary, this program calculates HLA-DRB1 and –DQB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between *n* alleles at *n* different loci. Their level of significance (p) for 2 x 2 comparisons was determined as previously described (Imanishi et al. 1992c; Imanishi et al. 1992a). In addition, the most frequent complete extended haplotypes were deduced from: 1) the 2 HLA loci haplotype frequencies (Imanishi et al. 1992c; Imanishi et al. 1992a) 2) the previously described haplotypes in other populations (Imanishi et al. 1992a); and 3) haplotypes if they appeared in two or more individuals and the alternative haplotype was well defined (Imanishi et al. 1992c; Imanishi et al. 1992a). In order to compare phenotype and haplotype HLA frequencies with other populations, the reference tables of the 11th and 12th International HLA Workshops were used (Imanishi et al. 1992b; Clayton and Lonjou, 1997) (Table 1). Due to Galician HLA profile was typed in low resolution, that of worldwide populations were converted in low resolution. Phylogenetic trees (dendrograms) were constructed with the haplotypic frequencies using the Neighbor-Joining (NJ) method (Saitou andNei, 1987) with the genetic distances between populations (DA) (Nei, 1972), using DISPAN software comprising the programs GNKDST and TREEVIEW (Nei, 1973; Nei et al. 1983). Correspondence analysis in three dimensions and its bidimensional representation was carried out by using the Vista v5.05 computer program (Young and Bann, 1996). Correspondence analysis consists of a geometric technique that may be used for displaying a global view of the relationships among population according to HLA (or other) haplotypic (or allele) frequencies. This methodology is based on the genetic distances (DA) variance among populations (similar to the classical principal components methodology) and on a statistical visualization of the differences.

Table 1. Populations used in this study.

Populations	N	Ref.	Populations	N	Ref.
Algerians	106	(Arnaiz-Villena et al. 1997)	Irish	1000	(Gonzalez-Galarza et al. 2011)
Armenians	97	(Imanishi et al. 1992a)	Japanese	893	(Imanishi et al. 1992a)
Austrians	93	(Imanishi et al. 1992a)	Madeirans	185	(Spínola et al. 2006)
Azorean	43	(Gonzalez-Galarza et al. 2011)	Orkney Islands (Scotland)	158	(Winney et al. 2012)
British (NW England)	298	(Gonzalez-Galarza et al. 2011)	Portuguese	50	(Spínola et al. 2002)
French (Rennes)	244	(Imanishi et al. 1992a)	South African Blacks	89	(Imanishi et al. 1992a)
Chuvash	82	(Arnaiz-Villena et al. 2003)	Spanish Basques	75	(Sanchez-Velasco et al. 2003)
Danes	122	(Imanishi et al. 1992a)	Swiss	116	(Clayton and Lonjou, 1997)
Finns	102	(Clayton and Lonjou, 1997)	Wales	1798	(Gonzalez-Galarza et al. 2011)
Galician (NW Spain)	125	*Present study*			

Results

HLA Allele Frequencies Found in Galician Population: Comparison with Other Populations

The expected and observed gene frequency values for HLA-A, -B and -DRB1 loci do not differ significantly and the population is found in Hardy-Weinberg equilibrium, (data not shown). Table 2 shows the HLA allele frequencies found in the sampled population. Eighteen different HLA-A and thirty three different class I HLA-B alleles have been found. Nine HLA-A alleles and eight HLA-B alleles had frequencies higher than 4% (-A*01, -A*02, -A*03, -A*11, -A*23, -A*24, -A*29, -A*30 -A*68, -B*07, -B*08, -B*18, -B*35, -B*44, -B*49, -B*51, -B*65,). Thirteen different class II HLA-DRB1 alleles have been found. Only seven of them had frequencies higher than 4% (-DRB1*01, -DRB1*03, -DRB1*04, -DRB1*07, -DRB1*11, -DRB1*13, -DRB1*15).

One type of analysis was done in order to compare Galician HLA haplotype frequencies with other World population haplotype frequencies; it was done with three loci haplotypes, HLA-A-B-DRB1. Low resolution data was shown to give informative results for several datasets because of HLA haplotype structure is quasi-specific for populations and the very high loci polymorphisms (Degos and Dausset, 1974; Arnaiz-Villena et al. 2017).

HLA-A, -B and -DRB1 Extended Haplotype Analysis in Galician: Comparison with Other Populations

Haplotypes between different HLA *loci* were estimated in Galician population (Table 3). The most probable five *loci* haplotype (A-B-C-DRB1-DQB1) were calculated. The most frequent combinations are characteristic of Atlantic European and Mediterranean populations (Table 3). Five Atlantic European three loci haplotypes are found in Galician population that represents 8.09% of all calculated haplotypes; five Mediterranean three loci haplotypes are found in this population that represents 7.96% of all calculated haplotypes. Only two European three loci haplotypes are found in this population and they represent 2.45%. Thus, Galicians show HLA relationships with Mediterranean and with Atlantic European populations.

Table 2. Allele frequencies in Galician population

Allele	Allele frequencies (%)	Allele	Allelefrequencies (%)
	HLA-A		HLA-B
01	9.01	49	4.8
02	27.04	50	2.81
03	13.34	51	10.18
11	5.85	52	0.35
23	5.26	53	1.05
24	9.6	55	0.81
25	1.52	56	0.46
26	3.63	57	3.27
29	4.68	58	1.99
30	4.21	60	2.69
31	3.04	61	1.75
32	3.51	62	3.75
33	3.04	63	0.23
34	0.35	64	1.99
66	0.46	65	5.5
68	4.91	71	0.35
69	0.35	72	0.58
74	0.11	76	0.11
	HLA-B	78	0.11
07	8.54		HLA-DRB1
08	5.73	01	12.17
13	1.4	03	8.54
18	6.32	04	12.52
27	2.57	07	20.72
35	10.65	08	2.92
37	1.63	09	1.17
38	1.4	10	1.87
39	0.93	11	11.7
41	0.7	12	1.28
42	0.11	13	13.81
44	15.1	14	2.57
45	1.75	15	9.36
47	0.23	16	1.28

Table 3. Haplotype frequencies of Galician population

Haplotype	HF (%)	Haplotype Origin
A*01-B*08-DRB1*03[a]	2.61	Mediterranean
A*02-B*44-DRB1*07[b]	1.94	Atlantic Europe
A*29-B*44-DRB1*07[c]	1.74	Atlantic Europe
A*02-B*07-DRB1*15[d]	1.58	Mediterranean
A*33-B*65-DRB1*01[e]	1.51	Atlantic Europe
A*02-B*44-DRB1*13[f]	1.46	Atlantic Europe
A*02-B*51-DRB1*07[g]	1.44	Atlantic Europe
A*02-B*18-DRB1*11[h]	1.32	Mediterranean
A*03-B*35-DRB1*01[i]	1.31	European
A*23-B*44-DRB1*07[j]	1.31	Mediterranean
A*02-B*35-DRB1*07[k]	1.29	European
A*03-B*07-DRB1*15[l]	1.14	Mediterranean

[a] Found in Cretans (1.1%), Macedonians (4.9%), Palestinians (0.9%), Pas Valley (1.1%), SpanishBasques (4.0%), Chuvash (1.2%) and Madeira (2.6%) (Arnaiz-Villena et al. 1999; Arnaiz-Villena et al. 2001a; Arnaiz-Villena et al. 2001b; Sanchez-Velasco et al. 1999; Arnaiz-Villena et al. 2003; Arnaiz-Villena et al. 2009; Sanchez-Velasco et al. 2003).

[b] Found in Macedonians (0.6%), Cabuernigos (2.2%), Cantabrian (1.8%) and Madeira (1.4) (Arnaiz-Villena et al. 2001a; Sanchez-Velasco et al. 2004b; Sanchez-Velasco et al. 2004a; Arnaiz-Villena et al. 2009)

[c] Found in Cabuernigo (2.2%), Pas Valley (2.7%), SpanishBasques (5.3%) and Madeira (1.4%) (Sanchez-Velasco et al. 2004b; Sanchez-Velasco et al. 1999; Sanchez-Velasco et al. 2003; Arnaiz-Villena et al. 2009).

[d] Found in Chuvash (1.2%), Cretans (0.4%), Tuvinians (2.0%), Macedonians (0.6%), Cabuernigos (2.2%), Pasiegos (3.8%) and Alpujarras (2.0%) (Arnaiz-Villena et al. 2003; Arnaiz-Villena et al. 1999; Martinez-Laso et al. 2001; Arnaiz-Villena et al. 2001a; Sanchez-Velasco et al. 2004b; Sanchez-Velasco et al. 1999; Longas et al. 2012).

[e] Found in Madeira (2.3%) (Arnaiz-Villena et al. 2009).

[f] Found in a population of Portugal Bragança (2.1%) and Portugal Viana do Castelo (1.8%)(Gonzalez-Galarza et al. 2011) .

[g] Found in Azores Oriental (2.6%), Portugal Viana do Castelo (1.5%) and Portugal Bragança (1.3%) (Spínola et al. 2005; Gonzalez-Galarza et al. 2011).

[h] Found in Macedonians (4.1%), Palestinians (0.9%) and Cantabrian (1.2%) (Arnaiz-Villena et al. 2001a; Arnaiz-Villena et al. 2001b; Sanchez-Velasco et al. 2004a).

[i] Found in a Chuvashian population (5.5%) (Arnaiz-Villena et al. 2003).

[j] Found in Macedonians (0.6%), Palestinians (1.2%), Portugal Aveiro (1.5%) and Portugal Guarda (1.4%) (Arnaiz-Villena et al. 2001a; Arnaiz-Villena et al. 2001b; Gonzalez-Galarza et al. 2011).

[k] Found in a German population (0.35%) (Müller et al. 2003).

[l] Found in Macedonians (0.8%), Palestinians (0.6%), Chuvash (4.9%), Cabuernigo (2.9%), Cantabrian (2.4%), Pas Valley (6.5%), Madeira (1.4%) and Alpujarras (0.8%) (Arnaiz-Villena et al. 2001a; Arnaiz-Villena et al. 2001b; Arnaiz-Villena et al. 2003; Sanchez-Velasco et al. 2004b; Sanchez-Velasco et al. 2004a; Sanchez-Velasco et al. 1999; Arnaiz-Villena et al. 2009; Longas et al. 2012).

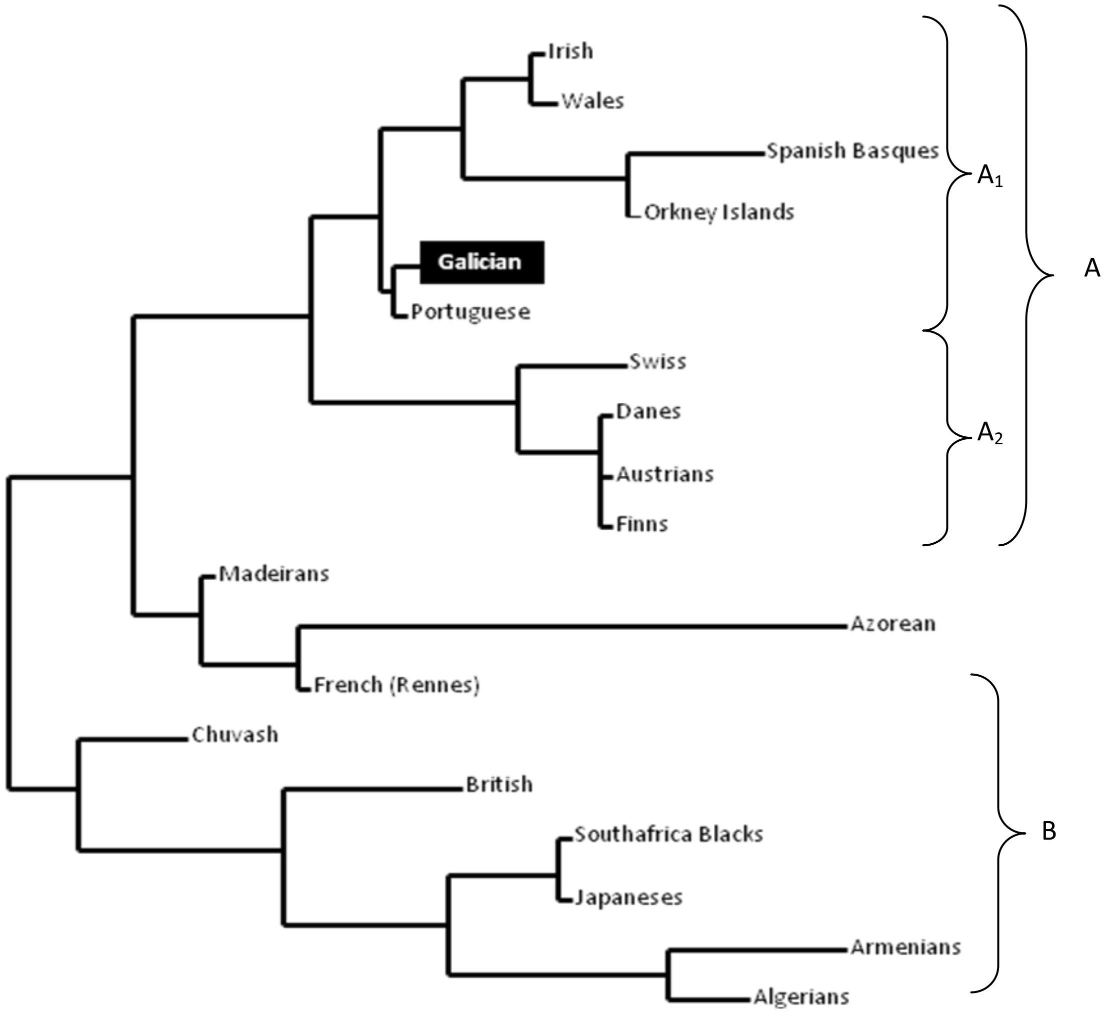

Figure 2. Neighbour-Joining dendrogram.

Neighbour-Joining (NJ) dendrogram constructed with HLA-A-B-DRB1 haplotype frequencies (low resolution) showing relatedness between Galician and other World populations. Boostrap values are 100%.For references see Table 1. Haplotypes used for each population: Algerians: A*01-B*57-DRB1*07 (1.9%), A*30-B*18-DRB1*03 (1.5%), A*33-B*14-DRB1*01 (1.5%), A*02-B*35-DRB1*11 (1.5%), A*02-B*35-DRB1*03 (1.5%); Azorean: A*24-B*07-DRB1*15 (3.8%), A*29-B*44-DRB1*07 (3.8%), A*33-B*14-DRB1*01 (3.8%) A*01-B*08-DRB1*03 (2.6%), A*01-B*08-DRB1*04 (2.6%), A*02-B*18-DRB1*11 (2.6%); British: A*01-B*08-DRB1*03 (9.5%), A*03-B*07-DRB1*15 (4.20%), A*02-B*44-DRB1*04 (3.9%), A*02-B*07-DRB1*15 (3.80%), A*29-B*44-DRB1*07 (2.20%); Chuvash: A*03-B*35-DRB1*01 (5.50%), A*03-B*07-DRB1*15 (4.90%), A*24-B*07-DRB1*15 (4.30%), A-68-B*35-DRB1*08 (3.70%), A*11-B*35-DRB1*07 (3%); Irish: A*01-B*08-DRB1*03 (9%), A*03-B*07-DRB1*15 (4.9%), A*02-B*44-DRB1*04 (4.1%), A*02-B*07-DRB1*15 (3%), A*29-B*44-DRB1*07 (2.1%); Madeira: A*02-B51-DRB1*13 (2.70%), A*01-B*08-DRB1*03 (2.4%), A*02-B*08-DRB1*03 (2.30%), A*29-B*44-DRB1*01 (1.90%), A*24-B*14-DRB1*07 (1.60%); Orkney Islands: A*01-B*08-DRB1* 03 (5.1%), A*02-B*07-DRB1*15 (5.1%), A*02-B*44-DRB1*04 (4.4%), A*29-B*44-DRB1*07 (4.4%), A*02-B*15-DRB1*04 (1.3%); Portuguese: A*01-B*44-DRB1*07 (4.5%), A*01-B*07-DRB1*03 (3%), A*02-B*07-DRB1*15 (3%), A*02-B*51-DRB1*08 (3%), A*68-B*45-DRB1*4 (3%); Spanish Basques: A*29-B*44-DRB1*07 (4.30%), A*01-B*08-DRB1*03 (4%), A*02-B*44-DRB1*03 (3.30%), A*02-B*44-DRB1*15 (2.70%), A*11-B*44-DRB1*07 (2.70%); Wales: A*01-B*08-DRB1*03

(10.10%), A*02-B*44-DRB1*04 (5.10%), A*03-B*07-DRB1*05 (3%), A*02-B*07-DRB1*15 (2%), A*02-B*44-DRB1*07 (1.80%); Finn: A*01-B*08-DRB1*03 (6.3%), A*02-B*27-DRB1*01 (5.3%), A*02-B*62-DRB1*04 (5.3%), A*03-B*18-DRB1*04 (4.8%), A*02-B*62-DRB1*13 (3.4%); Swiss: A*03-B*07-DRB1*02 (4.5%), A*01-B*08-DRB1*07 (4.3%), A*02-B*44-DRB1*07 (2.9%), A*03-B*18-DRB1*13 (1.2%), A*30-B*18-DRB1*03 (1.2%); South African Blacks: A*23-B*07-DRB1*11 (5.1%), A*30-B*42-DRB1*3 (4.5%), A*29-B*44-DRB1*11 (3.4%), A*02-B*70-DRB1*11 (3%), A*31-B*70-DRB1*03 (2.2%); Armenian: A*33-B*14-DRB1*01 (3.6%), A*03-B*07-DRB1*04 (3.1%), A*03-B*35-DRB1*04 (3%), A*24-B*18-DRB1*11 (2.1%), A*02-B*51-DRB1*11 (2.1%); Austrians: A*01-B*08-DRB1*03 (6.4%), A*02-B*44-DRB1*11 (3.2%), A*03-B*07-DRB1*15 (3.2%), A*24-B*35-DRB1*11 (2.7%), A*02-B*08-DRB1*03 (2.2%); Dane: A*01-B*08-DRB1*03 (6.1%), A*03-B*07-DRB1*15 (3.8%), A*02-B*44-DRB1*07 (2.9%), A*02-B*60-DRB1*13 (2.5%), A*02-B*44-DRB1*04 (2%); French (Rennes): A*01-B*08-DRB1*03 (3.7%), A*29-B*44-DRB1*07 (2.3%), A*03-B*07-DRB1*15 (2.1%), A*33-B*14-DRB1*01 (2%), A*02-B*44-DRB1*13 (1.4%); Japanese: A*24-B*52-DRB1*15 (8.2%), A*33-B*44-DRB1*13 (5.2%), A*24-B*07-DRB1*01 (3.6%). A*24-B*54-DRB1*04 (2.3%), A*02-B*46-DRB1*08 (2.2%).

The NJ relatedness dendrogram based on HLA-A-B-DRB1 haplotype frequencies (Fig.2) separates populations in two well-differentiated clusters. One of them (Cluster A) groups European populations with Galician (present study), Portuguese (Spínola et al. 2002), Orkney Islands (Winney et al. 2012), Spanish Basques (Sanchez-Velasco et al. 2003), Wales (Gonzalez-Galarza et al. 2011), Irish (Gonzalez-Galarza et al. 2011), Swiss (Clayton and Lonjou, 1997), Danes (Imanishi et al. 1992a), Austrians (Imanishi et al. 1992a) and Finns (Clayton and Lonjou, 1997). The other cluster (Cluster B) mostly groups non-European populations Chuvash (Arnaiz-Villenaet al. 2003), British (Gonzalez-Galarza et al. 2011), South African Blacks (Imanishi et al. 1992a), Japanese (Imanishi et al. 1992a), Armenians (Imanishi et al. 1992a) and Algerians(Arnaiz-Villenaet al. 1995). The European populations cluster (cluster A) is, also, divided in two subclusters (cluster A_1 and A_2). The first (cluster A_1) groups Atlantic European populations (Galician, Portuguese, Orkney Islands, Spanish Basques, Wales and Irish). The second subcluster (cluster A_2) groups Center European populations (Swiss, Danes, Austrians and Finns).

Correspondence analyses based on HLA-A-B-DRB1 haplotype frequencies (Fig.3) shows similar results. Also, two clusters are clearly defined according to first dimension that explains most of the variability among populations. The first cluster groups all European populations. This cluster is divided in Atlantic European populations (Galician, Portuguese, Irish, Wales, Orkney Islands, Spanish Basques, Britons, Madeirans, Azoreans and British) and in Center European populations (Swiss, Finns, Austrians and Danes). The second cluster groups all non-European populations such as Armenians, Algerians, Japanese, Chuvash and South African Blacks.

This effect is more evident in Table 4 which shows Galician closest genetic distance are the Atlantic European populations (Portuguese, Orkney Islands, Madeira, Spanish Basques, French (Rennes), Irish and Wales). The rest of European populations (Danes, British, Finns, Austrians and Swiss) are grouped together separately from Atlantic European populations and from the non-European populations.

Figure 3. Correspondence analysis.
Correspondence analysis with HLA-A-B-DRB1 haplotype frequencies (low resolution) showing relatedness between Galician and other World populations. See footnote of Fig. 2.

Table 4. Genetic distance (DA) between Galician (Spain) and Other World populations.

Population	Genetic Distances (DA) (x10^$^{-2}$)
Portuguese	0.2
Orkney Islands	0.52
Madeira	0.7
SpanishBasques	0.89
French (Rennes)	1.09
Irish	1.11
Wales	1.41
Danes	1.43
British	1.46
Finns	1.47
Austrians	1.49
Swiss	1.74
Chuvash	2.01
South AfricanBlacks	2.22
Japaneses	2.22
Azorean	3.44
Algerians	3.7
Armenians	4

Discussion

HLA Genetics of Atlantic European Populations

In the present work Galician population (Spain) HLA profile was compared with that of other European populations, particularly regarding to complete HLA haplotypes. Also, Atlantic Celt populations (i.e.: these who speak Celtic languages: Galician (North East Spain), Welsh, Irish, Bretons, Scottish, English, Cornish and Man Island) were included in the comparisons.

Y chromosome marker R1b reaches his maximum frequency in North Spain, Brittany, Wales, England, Scotland and Ireland (www.eupedia.com/europe/Haplogroup_R1b_Y-DNA.shtml#distribution; Oppenheimer, 2007). This suggests an ancestry relationship between populations inhabiting Atlantic Europe façade. Also, 10% of Guanche (Canary Islands) aboriginal samples bore R1b Y chromosome marker (Fregelet al. 2009; Arnaiz-Villena et al. 2015). Thus, European Atlantic populations spreading from Canary Islands to Scotland bore R1b Y chromosome marker in a relative high frequency.

These data are concordant with our HLA results: NJ dendrogram (Fig. 2) and Correspondence Analysis (Fig. 3) show that Atlantic Celts (Galician, Portuguese, Spanish Basques, French Bretons (Rennes), Irish, Wales, Orkney Islands and British) are grouped together in the same cluster. On the other hand, five of the twelve most frequent haplotypes in our Galician populations are found in other Atlantic Celt populations (Table 3 and footnote).British people have had more admixture from Central Europeans (Oppenheimer, 2007)

Also, there are genetic evidences that Sahara desertification led a gene flow northwards into Iberia (Arnaiz-Villena et al., 2002; Arnaiz-Villena and Lubell 2000; Jakes et al. 1997; Currat et al. 2010; Campmajo and Crabol 2009; Botigué et al. 2013). However, data from Iberian skeletons and their dental diet marks show that demic (human) agriculture diffusion from Middle East to Iberian Mesolithic/Neolithic transition does not exist (Arnaiz-Villena et al. 2002; Arnaiz-Villena and Lubell, 2000; Jakes et al., 1997; Currat et al. 2010; Campmajo and Crabol, 2009; Botigué et al. 2013).

In contrast Iberia/North Africa genetic/cultural exchange is well documented (Arnaiz-Villena et al., 2002; Arnaiz-Villena and Lubell 2000; Jakes et al. 1997; Currat et al. 2010; Campmajo and Crabol 2009; Botigué et al. 2013).

The Origin of Celts took place in Iberia according to Classical Historians

Current view about Celts origin has been that their homeland was Central Europe and that they spread to West and East Europe reaching nowadays Turkey (Oppenheimer, 2007). However, Classical historians pointed out that Origin of Celts was in Iberia.

Herodotus in his *Histories* (Oppenheimer, 2007) compared the length of Nile River with the length of Danube (Ister) River (Oppenheimer, 2007). He added that Danube (Ister) River began at the city of Pyrene in the country of Celts (Keltoi); he also asserted Celts country was outside of Pillars of Heracles (Gibraltar Straight) close to the Cynetes - Kynesians living place (South Portugal; Fig. 4) (Oppenheimer, 2007). Herodotus meant Pyrenees Mountains when he quoted “the city of Pyrene” (Fig. 4); also Aristotle (Meteorology 1.13) explained that Pyrene was a mountain and not a city (Huber, 2000). It is concluded that Herodotus did not know where Danube River started; however, it was clear that the country (Origin) of Celts was placed at Iberian Peninsula (Fig. 4).

In XIX century, Henri d’Arbois de Jubainville (Jones, 2008) ignored Herodotus-Aristotle above mentioned geographical references and he paid attention only to "Celts are placed on the Danube River fountains" that in XIX century were correctly placed in Centre Europe and not in Pyrenees Mountains. Thus, Jubainville ignoring Herodotus and Aristotle references and wrongly established that Origin of Celts was Central Europe (Oppenheimer, 2007; Arnaiz-Villena and Alonso-Garcia, 2001b). La Tene and Hallstat Central Europe cultures were wrongly assimilated as Celts. However, Celts speaking populations have always been placed in Atlantic Europe and may also include Iberians, part of First Canary Islands Inhabitants, Brittany (France) and British Islanders; In fact, Prof. Koch (Koch, 2009; Koch, 2011) from Bristol is assimilating Iberian-Tartesian language with ancient Gaelic language; also, R1b genetic marker high frequencies location also includes Iberian and Canary Islands (Fregel et al. 2009; Arnaiz-Villena et al. 2015).

Atlantic Celts according to History and HLA and Y chromosome Genes

South Europe and Mediterranean Area was a refuge for human populations during Last Ice Age that also received Saharan People and Culture (Arnaiz-Villena et al., 2002). Iberian Peninsula and Southwest France were included. After ice retreat, populations coming from Basque region went northwards to North France and British Isles (Oppenheimer, 2007), i.e.: Brittany, England, Wales, Scotland and Ireland (Oppenheimer, 2007). According to Y chromosome R1b marker, all these populations would come from northern Iberia and Spanish and French Basque Country Areas; HLA genetic data analyses in these Atlantic Celts populations is coincidental with these of R1b one (present paper, Figs 2 and 3; Table 4).

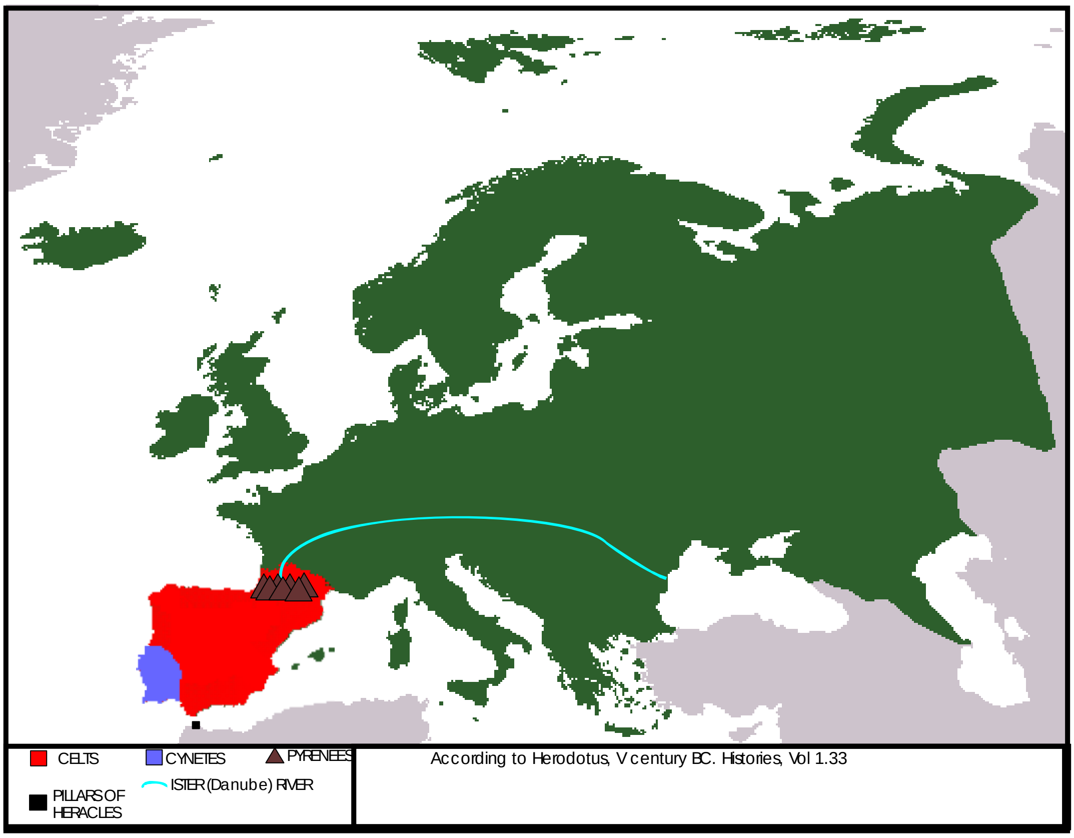

Figure 4. Map of Europe according to Herodotus.

Herodotus placed Celts further than "Pyrene" (Mt. Pyrenees), where he wrongly believed that Ister River (Danube) started. He also quoted "Celts were outside Heracles' Pillars" (Gibraltar Straight) and close to another population, "Kynesians" (Cynetes, in South Portugal). Therefore, Celts were placed by Herodotus in Iberia. However, Henri d'Arbois de Jubainville (Oppenheimer, 2007; Arnaiz-Villena and Alonso-Garcia, 2001b) made a mistake in XIX century AD: he ignored all details that Herodotus gave and only took care of Danube true origin (Central Europe). Thus, Jubainville localized Celts by taking care the only one Herodotus mistake and ignored his other correct infornations (Gibraltar Straight, Pyrene and Cynete southwest Iberian population).

In addition, Celtic languages spread from South Iberian Peninsula to Scotland, including Britsih Isles and Brittany (France) (Cunliffe, 2012). Current Celtic-speaking populations must be considered Atlantic Celts, as defined in the present paper (Mc Evoy et al. 2004). Also, translations had been made from Tartessian to ancient Celtic (Gaelic) languages as Irish, Scottish and Welsh (Koch, 2009, 2011). Moreover, names of Scottish Picts and Celts ("keltoi") populations mean a similar concept:painted (Picts,Wikipedia 2017) or tattooed and "keltoi"or "khelt-er",the latter meaning soot in Basque-Iberian language (Arnaiz-Villena and Alonso-Garcia, 2001b).Thus ,both Picts and Celts are not proper names, but common ones referring to painted bodies.

On the other hand, it is feasible that Iberians and Paleo North African populations (Arnaiz-Villena et al., 2002; Arnaiz-Villena and Lubell, 2000; Jakes et al., 1997; Currat et al. 2010, Campmajo and Crabol, 2009; Botigué et al. 2013) migrated also to Iberia and to Canary Islands (Arnaiz-Villena et al. 2015). There are two anthropological types of Guanches (First Canary Islands Inhabitants) (Braem, 2010; González, 1992). One of them would be tall with thick cranium and bone building; the other more gracile one would be of Mediterranean-like body building (Braem, 2010; González, 1992). Also, ancient rock Iberian scripts (wrongly named "Latin inscriptions") found in Fuerteventura (Canary Island), Lanzarote and El Hierro support ancient Iberian gene impact to Canary Island (Arnaiz-Villena et al. 2015). So it is possible that part of First Canary Inhabitants were Atlantic Celts from Iberia, together with North Africans which in turn had in part populated Iberia from Africa after the establishment of Sahara Desert (Arnaiz-Villena et al., 2002; Arnaiz-Villena and Lubell, 2000; Jakes et al., 1997; Arnaiz-Villena et al., 2001a; Currat et al. 2010, Campmajo and Crabol, 2009; Botigué et al. 2013).

In conclusion, the Origin of Celts is placed at Iberian Peninsula and not at Cental Europe, as it has been believed (Fig. 5). Also, Classic Iberians and Celts may be the same population (Arnaiz-Villena and Alonso-Garcia, 2001b). Also, according to genetics, Atlantic European populations i.e.: Orkney Islands, Scottish, Irish, British, Bretons, Iberians (Basques, Galicians), Guanches and Berbers may have been originated from ancient Celt/Iberian populations (Fig. 5). Finally, a common cultural substratum (spoken and written language) may have existed in Atlantic European populations from Canary Islands to British Isles; this is based on Canary Islands Iberian Scripts (Arnaiz-Villena et al. 2015) and Iberian-Gaelic languages relationships (Koch 2009; 2011).

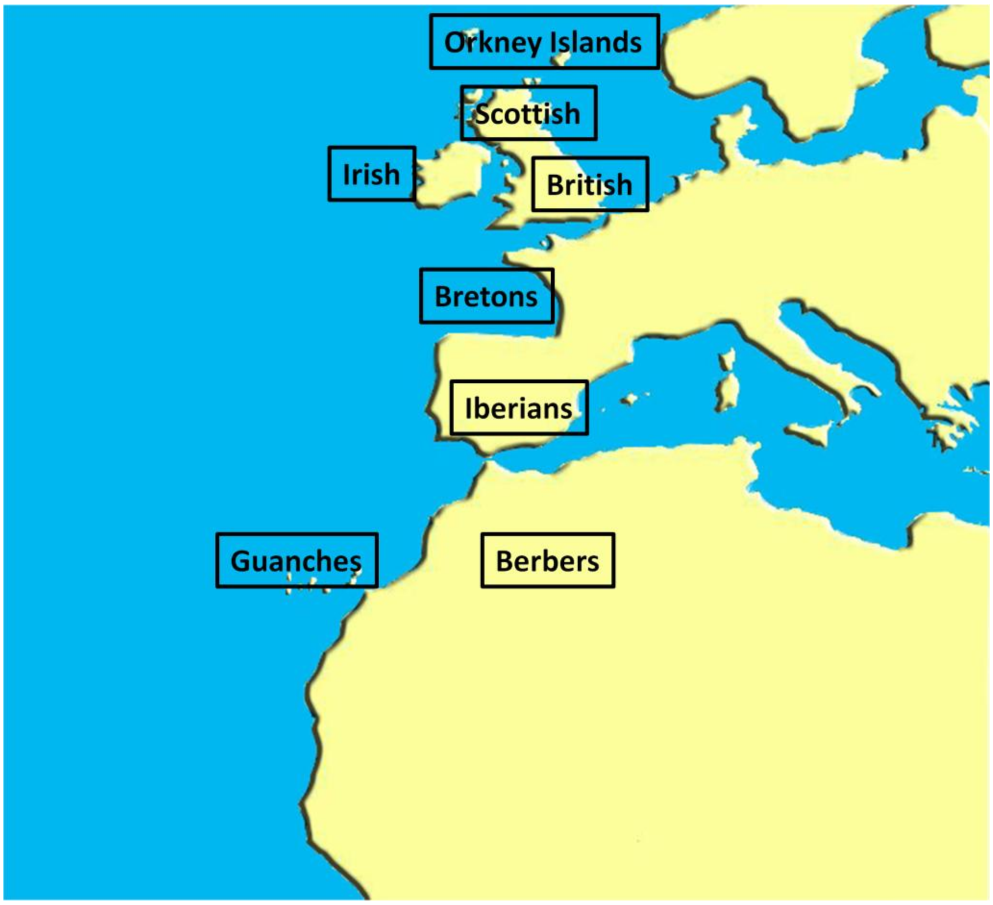

Figure 5.
Map of West Europe and North West Africa with populations who may have been originated form ancient Celt-Iberian populations: Orkney Islands, Scottish, Irish, British, Bretons, Iberians (Basques,Galicians), Guanches and Berbers.

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