Int J Mol Epidemiol Genet 2012;3(2):179-183
www.ijmeg.org /ISSN1948-1756/IJMEG1106004

Original Article

Distribution of polymorphisms IL4 -590 C/T and IL4 RP2 in the human populations of Madeira, Azores, Portugal, Cape Verde and Guinea-Bissau

Anabela G Berenguer[1], Rita A Câmara[2], António D Brehm[1], Susana Oliveira[2], Ana T Fernandes[1]

[1]Human Genetics Laboratory, University of Madeira, 9000-390 Funchal, Portugal; [2]Imunoallergology Unit, Central Hospital of Funchal, 9000-514 Funchal, Portugal

Received June 24, 2011; accepted April 23, 2012; Epub May 10, 2012; Published May 30, 2012

Abstract: The IL4 gene is located on chromosome 5q23.3-31.2. Polymorphisms within this cytokine gene, like the derivative allele T of IL4-590, have been reported as being associated to elevated IgE serum levels and asthma. In the present work, the allelic and genotypic frequency of the IL4-590 and IL4 RP2 polymorphisms was carried out in 599 individuals from Madeira, Azores, Portugal mainland, Cape Verde and Guinea-Bissau and in a sample of 101 asthmatics from Madeira population. In all populations the polymorphisms were in LD and presented a significant dissimilar allelic and genotypic distribution ($p<0.05$) except between mainland Portugal and Madeira when compared to Azores. Significant differences regarding both loci were found between Madeira population and the group of asthmatics. Genotype 183183TT frequency is higher for African populations while 253253CC prevails in Caucasian populations. The existence of a Hardy-Weinberg Disequilibrium in Guinea-Bissau population not observed in neutral markers leads to the hypothesis of natural selection occurring in these loci probably associated to a rapid population growth an hypothesis strengthened by neutral STRs D5S818 and CSF1PO gene diversity.

Keywords: IL4-590, IL4 RP2, D5S818, CSF1PO, Asthma, Madeira, Azores, Portugal mainland, Cape Verde, Guinea-Bissau

Introduction

The Interleukin 4 (IL4) cytokine mediates a variety of interactions among components of the immune system. It induces immature effector T cells to assume a Th2 phenotype and also B-cells to undergo immunoglobulin type-switching and secretion of IgE [1].

The IL4 gene located on chromosome 5q23.3-31.2 presents the IL4-590 (C-590T) single nucleotide polymorphism (SNP) on the promoter region of the gene [2]. Phylogenetic studies indicate that this polymorphism belongs to a conserved region in all primates except for humans. The derivative allele T has been related to elevated serum levels of IgE and asthma, a complex disease affecting the worldwide population mainly on developed countries. High frequencies of this allele may be the result of positive selection [1] perhaps justified by an association between IL4 -590T allele and elevated anti-malarial IgG levels [3].

Polymorphism RP2 is a repetitive sequence located at the second intron of IL4 gene. This polymorphism can be classified as a VNTR as it consists of a 70 bp repetitive motif unit generally appearing in two or three copies sized 183 and 253 bp, respectively [2].

The Atlantic Islands of Madeira, Azores and Cape Verde were colonized by the Portuguese in the 15th and 16th centuries and received different levels of sub-saharan slave contribution from the African coast of Guinea [4]. Studies using mtDNA show that more than 20% of Madeira haplotypes belong to sub-Saharan haplogroups contrarily to the 8.7% found at Azores [5] but these results are completely different to Y chromosome where no sub-saharan lineages were found [6].

The data obtained for Cape Verde through chromosome Y studies indicate no more than a 15.9% influence from Guinea-Bissau (the putative place of origin of its sub-Saharan popula-

tion) while the European contribution through male lineages is about 53.5% [7], contrasting to mtDNA studies where 93% holds sub-Saharan haplogroups [8].

Considering the prevalence of asthma and atopy, the results from Cape Verde and Madeira presented a significant difference of atopy in the population: from 9% in Cape Verde to 54% in Madeira, and active asthma between 7% in Cape Verde and 14.6% in Madeira. The significant variation of asthma prevalence found in several populations remains in discussion, but although genetics linked to ethnicity seems to play a role, it will be strongly modulated by environmental variables and lifestyle [9]. In the last few years in Cape Verde the atopy prevalence raised threefold although the prevalence of atopic asthma increased with the growth of the tourism industry and the improvement of standards of living of the local population [9].

Given the importance of IL4 as an immune-regulatory gene it is of great interest to investigate the genetic background of populations for polymorphisms within the gene proved to influence its expression.

Association studies between mutations and STR allow favorable mutations to be detected. If the frequency of the selected allele will increase, the same is expected for the frequency of the STR allele that is on the same genomic region as the selected allele but this will depend on the number of alleles and overall heterozygosity of the locus [10].

This study aims to determinate the frequency for both IL4-590 and IL4 RP2 polymorphisms in the Atlantic Islands of Madeira, Azores and Cabo-Verde as well as mainland Portugal (Western Europe) and Guinea-Bissau (West African coast), to compare the populations by analysing the distribution of these two polymorphisms and observing if there is local selection on this genomic region and if these mutations behave favorably. Additionally by analyzing a group of asthmatics from Madeira Island we intent to assess the importance of both loci on detecting asthma predisposition in the Madeira population.

Material and methods

DNA samples from 599 unrelated male subjects from the following populations were used: Madeira (n=110), Azores (n=116), Portugal Mainland (n=106), Cape Verde (n=152) and Guinea-Bissau (n=115). A sample of asthmatics (n=101) from the immunoalergology consultation at Dr. Nélio Mendonça Hospital, Funchal, was also analyzed.

The IL4-590 SNP (rs 2243250) was analyzed by real-time PCR using the 7300 System SDS Software v1.4. (Applied Biosystems). The IL4 RP2 VNTR was analyzed according to Mout and colleagues [2].

Comparison between each pair of populations was done by Fisher's exact test using ARLEQUIN vs.3.01. Statistical significance was defined as $p < 0.05$.

The average gene diversity of both loci was assessed using previously published data for two STRs - D5S818 and CSF1PO in chromosome 5 - for Madeira [11], Azores [12], Cape Verde [13] and Guinea-Bissau [14] excluding the double heterozygous individuals in both IL4 and STRs polymorphisms, in a whole of 159 individual from all populations. Rst values were determined by FSTAT 2.9.3.2.

Results

Genotyping and allelic frequencies for all populations are shown in **Table 1**. All studied populations are in Hardy-Weinberg equilibrium for both loci except for Guinea-Bissau ($p=0.00764$) since it presents a significant lower frequency of heterozygous for the IL4-590 locus (0.261) than expected (0.361). For all populations IL4-590 and IL4 RP2 loci were found to be in linkage disequilibrium (LD). All pairs of populations present significant differences ($P<0.05$) for both polymorphisms except in case of Madeira and mainland Portugal when compared with Azores but only in the case of IL4-590.

Both -590T and 183 allelic frequencies are higher for Guinea-Bissau population (0.765/0.517), while Madeira presents the lowest frequencies (0.105/0.105). Genotypic TT frequency varies decreasingly from 0.635 in Guinea-Bissau to 0.009 in Madeira and Azores whereas genotype 183/183 varies from 0.252 in Guinea-Bissau to 0.027 in Madeira population.

When both IL4-590 and II4 RP2 polymorphism were considered, nine genotypes were found.

Table 1. Distribution of allelic and genotypic frequencies, p value for HWE and LD for IL4-590 and RP2 polymorphisms for five populations: Madeira Asthmatics (MA);Madeira (M); Azores (A); Portugal Mainland (P); Cabo-Verde(CV) and Guinea-Bissau(GB)

	MA	M	A	P	CV	GB
N	101	110	116	106	152	115
C	0,792	0,895	0,871	0,802	0,408	0,235
T	0,208	0,105	0,129	0,198	0,592	0,765
CC	0,624	0,800	0,750	0,651	0,191	0,104
CT	0,337	0,191	0,241	0,302	0,434	0,261
TT	0,039	0,009	0,009	0,047	0,375	0,635
HWE	1.00000	1.00000	0.68945	0.54906	0.24029	0.00764
253	0,817	0,895	0,871	0,722	0,572	0,483
183	0,183	0,105	0,129	0,278	0,428	0,517
253/253	0,673	0,818	0,776	0,557	0,335	0,217
183/253	0,287	0,155	0,190	0,330	0,474	0,530
183/183	0,040	0,027	0,034	0,113	0,191	0,252
HWE	0.73635	0.09135	0.09331	0.08841	0.74169	0.57814
253/253CC	0,624	0,791	0,741	0,547	0,184	0,070
253/253CT	0,050	0,027	0,034	0,009	0,105	0,061
253/253TT	-	-	-	-	0,046	0,087
183/253CC	-	-	0,009	0,085	0,007	0,035
183/253CT	0,287	0,145	0,181	0,226	0,296	0,191
183/253TT	-	0,009	-	0,019	0,171	0,304
183/183CC	-	0,009	-	0,019	-	-
183/183CT	-	0,018	0,017	0,066	0,033	0,009
183/183TT	0,040	-	0,017	0,028	0,158	0,243
LD (p)	0,000	0,000	0,000	0,000	0,000	0,000

Genotype 253253CC is mainly found in Madeira (0.791) and Azores (0.741) while at Guinea-Bissau 183183TT appears in a higher frequency (0.243). In Cape Verde the highest frequency is observed for genotype 183253CT (0.296).

When average gene diversity is considered for D5S818 and CSF1PO STRs from chromosome 5 a significant LD association between each pair of loci was found except between D5S818 and CSF1PO (0.27077). The allelic distribution for D5S818 and CSF1PO within 253253CC, 183183TT and 253253TT genotypes is shown in **Figure 1**. Average gene diversity for STRs D5S818 and CSF1PO is higher for 253253 TT (0.841667) followed by 183183 TT (0.783019) and 253253 CC (0.740385). Estimated value of Rst over all samples for both STR loci D5S818 and CSF1PO was 0.071 and 0.167, respectively.

When comparing the group of asthmatics with the Madeira population, significant differences were found for both IL4 -590 and IL4 RP2 loci regarding genotype (p=0.010 and p=0.048) and allele distribution (p=0.005 and p=0.025) respectively.

Discussion

In this study, significant inter-populational differences regarding both IL4-590 and IL4 RP2 polymorphisms indicate a clearly distinct genetic background amongst all studied populations. However, genotype 183183TT seems to prevail in African populations (Guinea-Bissau and Cape-Verde) while 253253CC appears in a higher frequency among typically Caucasian populations (Madeira, Azores and Portugal mainland).

Nevertheless significant differences were found within populations of Guinea-Bissau and Cape

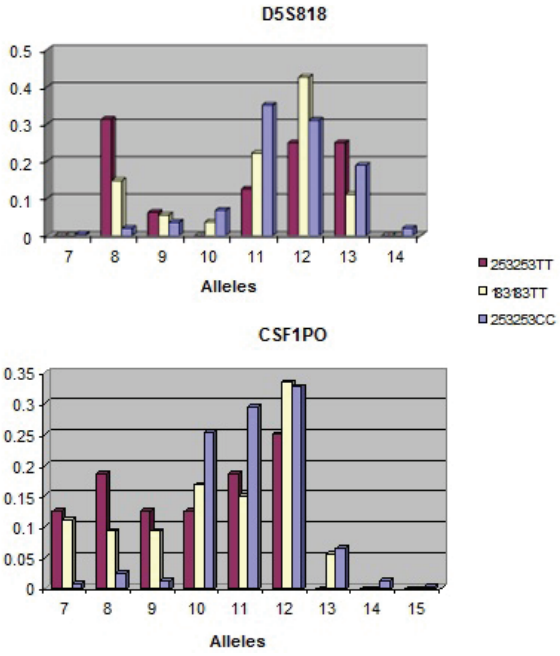

Figure 1. Allelic distribution for D5S818 and CSF1PO within 253253CC (n=248), 183183TT (n=54) and 253253TT (n=16) genotypes.

Verde. This last population presents the highest frequency of genotype 183253CT (0.296), maybe as the result of the high level of admixture in this population that can be explained by the extensive male Caucasian contribution to its genetic background [7].

Guinea-Bissau is in Hardy-Weinberg disequilibrium for the IL4 -590 locus possibly due to natural selection affecting the IL4 gene since the same population was found to be in Hardy-Weinberg equilibrium when neutral STRs were used [14].

It is known that malaria is a growing pathology affecting African populations [15]. Recent studies among the Fulani from Mali showed a higher prevalence of *Plasmodium falciparum* among the carriers of the T allele. It seems that the persistence of infection among T carriers may result in the production of anti-malarial antibodies [16]. Therefore the high frequency for TT genotype in Guinea-Bissau may come as a possible protective defence mechanism against malaria infection.

Phylogenetic studies indicate IL4-590 polymorphism belongs to a conserved region in all primates except for humans [1]. Therefore allele C is though to be the ancestral while T allele the derived. LD between both IL4-590 and IL4 RP2 suggests allele 253 is linked to C allele and therefore one should expect this to be the ancestral haplotype. Thus genotype 253253CC should present the highest average gene diversity for both studied STRs. However genotype 253253TT holds the highest value. A possible natural selective process of an advantageous allele (most likely T allele from IL4-590 locus) might have been responsible for this particular pattern. The relatively high Rst value for CSF1PO nearby STR locus (0.167) seems to strengthen this hypothesis since high Rst values are likely to mark genomic regions that have been subjected to selection [10].

Both IL4 -590 and IL4 RP2 can be useful genetics markers to detect asthma predisposition in Madeira Population. In addition, LD between both loci may lead to a possible synergic action contributing to the pathofisiology of asthma.

According to these results a new approach must be used for populations with African background, especially in the developing populations. A high frequency of the allele T associated with social and cultural development could determine changes in the atopy and asthma in a near future in these populations.

Acknowledgments

Anabela G Berenguer is a recipient of a PhD scholarship from Fundação para a Ciência e Tecnologia (FCT) with the reference SFRH/BD/31273/2006.

Dr. Ana T Fernandes was deceased while the manuscript was in press.

Address correspondence to: Dr. António Brehm, Human Genetics Laboratory, University of Madeira, 9000-390 Funchal, Portugal Tel: 351 291 705380; Fax: 351 291705399; E-mail: lgh@uma.pt

References

[1] Rockman MV, Hahn MW, Soranzo N, Goldstein DB, Wray GA. Positive selection on a human-specific transcription factor binding site regulating IL4 expression. Current Biology 2003; 13: 2118-2123.

[2] Mout R, Willenze R, Landegent JE. Repeat polymorphism in the interleukin-4 gene (IL-4). Nucleic Acids Res 1991; 19: 3763.

[3] Luoni G, Verra F, Arca B, Sirima BS, Troye-Blomberg M, Coluzzi M, Kwiatkowski D,

Modiano D. Antimalarial antibody levels and IL4 polymorphism in the Fulani of West Africa. Genes Immun 2001; 2: 411-414.
[4] Russel-Wood AJ. The Portuguese empire 1415-1808. A World on the Move. Baltimore, Johns Hopkins University Press 1998; pp: 1-7.
[5] Brehm A, Pereira L, Kivisild T, Amorim A. Mitochondrial portraits of the Madeira and Açores archipelagos witness different genetic pools of its settlers. Hum Genet 2003; 114: 77-86.
[6] Gonçalves R, Freitas A, Branco M, Rosa A, Fernandes AT, Zhivotovsky LA, Underhill PA, Kivisild T, Brehm A. Y-chromosome lineages from Portugal, Madeira and Açores record elements of Sephardim and Berber ancestry. Annals of Human Genetics 2005; 69: 443-454.
[7] Gonçalves R, Rosa A, Freitas A, Fernandes A, Kivisild T, Villems R, Brehm A. Y-chromosome lineages in Cabo Verde Islands witness the diverse geographic origin of its first male settlers. Hum Genet 2003; 113: 467-472.
[8] Brehm A, Pereira L, Bandelt HJ, Prata MJ, Amorim A. Mitochondrial portrait of the Cabo Verde Archipelago: The Senegambiam outpost of Atlantic Slave Trade. Ann Hum Genet 2002; 66: 49-60.
[9] Rosado-Pinto J and Almeida MM. Epidemiology of asthma in schoolchildren in Portuguese speaking regions. Revue française d'allergologie et d'immunologie Clinique 2005; 45: 547-549.
[10] Kayser M, Brauer S, Stoneking M. A Genome Scan to Detect Candidate Regions Influenced by Local Natural Selection in Human Populations. Mol Biol Evol 2003; 20: 893-900.
[11] Fernandes AT, Brehm A, Alves C, Gusmão L, Amorim A. Genetic profile of the Madeira Archipelago population using the new PowerPlex16 System kit. Forensic Sci Int 2002; 125: 281-283.
[12] Velosa RG, Fernandes AT, Brehm A. Genetic profile of the Açores Archipelago population using the new PowerPlex 16 system kit. Forensic Sci Int 2002; 129: 68-71.
[13] Fernandes AT, Velosa R, Jesus J, Carracedo A, Brehm A. Genetic Differentiation of the Cabo Verde Archipelago Population Analyzed by STR Polymorphisms. Ann Hum Genet 2003; 67: 340 -347.
[14] Gonçalves R, Jesus J, Fernandes AT, Brehm A. Genetic profile of a multi-ethnic population from Guiné-Bissau (west African coast) using the new PowerPlex1 16 System kit. Forensic Science International 2002; 129: 78-80.
[15] Hartl DL. The origin of malaria: mixed messages from genetic diversity. Nature Reviews Microbiology 2004; 2: 15-22.
[16] Vafa M, Maiga B, Berzins K, Hayano M, Bereczky S, Dolo A, Daou M, Arama C, Kouriba B, Farnert A, Doumbo OK, Troye BM. Associations between the IL-4 -590 T allele and Plasmodium falciparum infection prevalence in asymptomatic Fulani of Mali. Microbes and Infection 2007; 9: 1043-1048.