Antropologia Portuguesa

Volume 20/21
2003/2004

Departamento de Antropologia | Universidade de Coimbra

Population genetics of 5 STR loci (CD4, TPO, VWA, F13A1 and MBPB) in S. Miguel Island (Azores, Portugal) [1]

Catarina Silva[1,2]; Paulo Castro-Oliveira[2]; Rafael Montiel[2]; Manuela Lima[2]

1 Serviço de Genética Médica
Hospital de Dona Estefânia
1169-045 Lisboa, Portugal
2 Centro de Investigação em Recursos Naturais
Universidade dos Açores
9501-801 Ponta Delgada, S. Miguel, Açores, Portugal
catarina.carneiro@sapo.pt

Abstract A study of five Short Tandem Repeat (STR) loci was undertaken using samples of unrelated individuals with Azorean ancestry, born in the island of S. Miguel. Markers were analysed by PCR, followed by PAGE (CD4, TPO and VWA) or using a Genetic Analyser (F13A1 and MBPB). The results obtained revealed that two of the markers studied (VWA and MBPB) were not in agreement with the Hardy-Weinberg expectations. Population differentiation tests demonstrated a higher number of significant differences observed between S. Miguel and samples from the North and South of Portugal than between S. Miguel and Centre Portugal. The Neighbour-Joining tree showed a clear separation between sub-Saharan populations and all the other populations. Further analyses suggest genetic affinities between S. Miguel and one of the North African samples. These results are in agreement with mtDNA data as well as with historical reports in which the contribution of Moorish slaves to the founding population of S. Miguel is frequently evoked.

Key-words S. Miguel (Azores); population genetics; STRs; PCR; population differentiation.

Resumo Um estudo sistematizado de cinco *loci* do tipo *Short Tandem Repeat* (STR) foi desenvolvido, usando amostras de indivíduos não-aparentados com ascendência açoriana, naturais da ilha de S. Miguel. Os marcadores genéticos foram amplificados por PCR e analisados em PAGE (CD4, TPO e VWA) ou recorrendo a um *Genetic Analyser* (F13A1 e MBPB). Os resultados obtidos revelaram que

[1] Este artigo foi aceite para publicação em 2003.

dois dos marcadores estudados (VWA e MBPB) não se encontravam de acordo com o formalismo de Hardy-Weinberg. Testes de diferenciação populacional demonstraram um maior número de diferenças significativas entre S. Miguel e o Norte e Sul de Portugal do que entre S. Miguel e o Centro de Portugal. A árvore genética, construída pelo método de *Neighbour-Joining*, evidencia uma separação clara entre as populações sub-sarianas e as restantes populações. Outras análises efectuadas sugerem uma afinidade genética entre S. Miguel e uma das populações do Norte de África. Estes resultados vão ao encontro quer dos dados obtidos para o mtDNA quer aos relatos históricos nos quais a contribuição de escravos mouriscos para a população fundadora de S. Miguel é frequentemente evocada.

Palavras-chave S. Miguel (Açores); genética populacional; STRs; PCR; diferenciação populacional.

Introduction

The Azores is an archipelago formed by 9 islands, clustered in 3 geographical groups (Centre, Western and Eastern). It is located in the North Atlantic Ocean, approximately 2000 Km away from Mainland Portugal. With a global area of 2344 km^2 and 243 895 inhabitants (SREA, 2001), the Azores were uninhabited when first discovered by the Portuguese in 1432. The island of S. Miguel, included in the Western group, is the more populated (131.510 according to the census of 2001 (SREA, 2001)) and was one of the first islands to be settled (Matos, 1989). The contribution of the several populations involved in the settlement process it is not clear, mostly because the historical sources do not provide sufficient information. Nevertheless, a consensus exists on the fact that the main group of settlers originated from mainland Portugal and from Madeira Island.

Although a great investment is taking place in the genetic profiling of several European populations, the genetic characterization of the Azorean populations is recent. The first studies concerned classical markers namely enzymatic polymorphisms (see *eg.* Santos *et al.*, 1992; Santos and Amorim, 1994). The pioneer use of DNA markers for the Azorean populations was undertaken only by 1998 (Corte-Real *et al.*, 1998; Prata *et al.*, 1998).

Short Tandem Repeats (STRs) are widely used as effective tools for individual and population genetic characterisation, as well as to examine genetic relationships between populations. Among other features, their

ubiquity in the human genome, their high polymorphism and the possibility of amplification by PCR techniques, made possible a large implantation of this kind of markers in genetic studies. The purposes underlying this study were: 1) To report on the allelic frequencies of five STR loci (CD4, TPO, VWA, F13A1 and MBPB) in the Island of S. Miguel; 2) To establish a comparative study, aiming to investigate the presence of genetic differentiation between the present sample and other populations; and 3) To calculate genetic distances and to construct genetic trees aiming to: (i) understand the genetic context of the S. Miguel population relatively to other populations; (ii) infer aspects of the population history of this Island.

The historical reports on the peopling of the Azorean Archipelago allow us to predict that each island or group of islands may present specific genetic characteristics, since there are indications of a differential contribution from distinct groups of settlers. The results obtained in the present study together with others obtained for the remaining islands should contribute to the genetic characterisation of the Azorean populations, and allow advances in the understanding of its structure. Furthermore, this study provides new data that can be used for the improvement of a specific database for this population.

Methodology

Sampling

Buccal swabs were collected from unrelated individuals born in S. Miguel Island (Azores), with Azorean ancestry confirmed up until the third generation (great-grandparents born in the Azorean Archipelago).

DNA markers and loci

In the present study, five STR loci were analysed. Four of them are characterized by a tetrameric repeat motif and one (CD4) is characterized by a pentameric repeat motif. The motifs and respective locations of these loci are as follows:

– CD4: (AAAAG)n, surface of the antigen cd4 gene (12p12-pter);
– TPO: (AATG)n, intron 10 of the thyroid peroxidase gene (2p23-2pter);

– VWA: (TCTA)n, intron 40 of the von Willebrand factor gene (12p12-pter);
– F13A1: (AAAG)n, intron 1 of the human coagulation factor XIII, A1 polypeptide (6p24-p25);
– MBPB: (TGGA)n, myelin basic protein gene, locus B (18q23-qter).

DNA extraction

DNA extraction was performed according to the chelating resin method, as described by Lareu *et al.* (1994).

PCR amplification

Amplification of the markers in study was made accordingly to primers and conditions described in Table 1.

Table 1. Amplification primers and conditions for the markers under study (n = 35 cycles).

Markers	Primers	Pre-denaturation	Denaturation	Annealing	Elongation	Final Extension
CD4	Edwards *et al.* (1991)	94º C, 5 min	95º C, 30 s	58º C, 1 min	72º C, 1 min	72º C, 7 min
TPO	Anker *et al.* (1992)	94º C, 5 min	95º C, 30 s	60º C, 1 min	72º C, 1 min	72º C, 7 min
VWA	Kimpton *et al.* (1992)	95º C, 5 min	95º C, 30 s	54º C, 1 min	72º C, 1 min	60º C, 30 min
F13A1	Polymeropoulos *et al.* (1991)	95º C, 11 min	95º C, 30 s	58º C, 1 min	72º C, 1 min	60º C, 45 min
MBPB	Gusmão *et al.* (1996)	95º C, 2 min	95º C, 30 s	58º C, 1 min	72º C, 1 min	60º C, 30 min

Electrophoresis

For CD4, TPO and VWA, PCR products were separated on a discontinuous horizontal electrophoresis system as described by Luis and Caeiro (1995). Visualisation was undertaken by the silver staining method according to Budowle *et al.* (1991) and genotyping was carried out through side-by-side comparison, using previously typed samples as references. For F13A1 and MBPB, an ABI 310 Genetic Analyser from Perkin-Elmer was used. Fragment sizes were determined automatically using the GeneScan software, also from Perkin-Elmer.

Analyses

All statistical analyses were undertaken using the following statistical packages: ARLEQUIN version 2000 (Schneider *et al.*, 2000); GENEPOP version 3 (Raymond and Rousset, 1995a); PHYLYP version 3.5c (Felsenstein, 1993); SPSS 11.0 (SPSS Inc., 1989-1999).

Intra-population analyses

Allele frequencies were estimated and values for the expected number of heterozygotes were evaluated for each marker. Hardy-Weinberg equilibrium (HWE) was tested by an exact test (Guo and Thompson, 1992), for all markers. For markers yielding p-values lower than 0.01, a score test was performed to evaluate the hypothesis of a homozygotes excess (Rousset and Raymond, 1995).

Inter-population analyses

For all markers, a population differentiation exact test (Raymond and Rousset, 1995b) using allelic frequencies was carried out, to compare data obtained in this study with data available for other European and African populations. Genetic distances between populations (Cavalli-Sforza and Edwards, 1967; Reynolds *et al.*, 1983) were estimated from allelic frequencies of markers TPO and VWA and were used to construct a Neighbour-Joining (Saitou and Nei, 1987) tree; the robustness of the tree was accessed by means of 10 000 bootstrap replications (Felsenstein, 1985). The number of markers used for tree reconstruction was limited by data availability (only for TPO and VWA a set of population data common to both markers could be compiled). The matrix resulting from the genetic distances between populations was represented in a bi-dimensional chart by means of Multidimensional Scaling (MDS) analysis.

Results and Discussion

Intra-population analyses

In Table 2 the allelic frequencies obtained for each marker are reported. Values for observed and expected heterozygosity and values of significance for the Hardy-Weinberg Equilibrium (HWE) are also presented.

Two out of the five markers analysed (VWA and MBPB) presented significant values, for the HWE test. The score tests computed for these two systems showed that differences between observed and expected heterozygosis were significant only for MBPB (p=0.009). This result demonstrates an excess of homozygotes in our sample. Although the number of individuals typed for this marker can be considered acceptable as representative of the sub-population analysed in this study it is, nevertheless, reduced (n=32). Thus, the implications of this finding will have to be substantiated with an enlargement of the sample.

Table 2. Allelic frequencies, heterozygosity values and p-values for the HWE, for the five markers analysed in S. Miguel Island.

Allele	CD4 (n = 113)	TPO (n = 102)	VWA (n = 69)	F13A1 (n = 37)	MBPB (n = 32)
3.2	-	-	-	0.0405	-
5	0.4646	-	-	0.1757	-
6	0.2389	0.0049	-	0.3649	-
7	0.0044	0.0098	-	0.4054	0.4531
8	0.0044	0.4608	-	-	-
9	0.0044	0.1569	-	-	0.1719
10	0.2257	0.0245	-	-	0.0781
11	0.0044	0.3284	-	0.0135	0.1719
12	0.0221	0.0147	-	-	0.1094
13	0.0265	-	-	-	0.0156
14	0.0044	-	0.1377	-	-
15	-	-	0.1159	-	-
16	-	-	0.2101	-	-
17	-	-	0.2826	-	-
18	-	-	0.2029	-	-
19	-	-	0.0507	-	-
H_0	0.646	0.686	0.826	0.649	0.531
H_e	0.752	0.732	0.808	0.683	0.818
p	0.163	0.056	0.003*	0.795	0.007*

H_0 - Observed heterozigosity; H_e - Expected heterozigosity; p - Hardy-Weinberg equilibrium (exact test probability based on 3000 dememorization steps); * - significant values ($p \leq 0.01$).

Table 3. Exact test of population differentiation between S. Miguel and several populations.

S. Miguel *vs.*														
CD4			**TPO**			**VWA**			**F13A1**			**MBPB**		
NPortugal[1]	0.002	+	NPortugal[1]	0.004	+	NPortugal[1]	0.834	-	NPortugal[4]	0.197	-	NPortugal[2]	0.000	+
CPortugal3[22]	0.002	+	CPortugal2[11]	0.044	-	CPortugal1[7]	0.906	-	CPortugal1[7]	0.273	-	S. Tomé[5]	0.000	+
SPortugal[22]	0.000	+	SPortugal[22]	0.003	+	CPortugal2[11]	0.774	-	Madeira[8]	0.302	-	Germany[2]	0.002	+
Madeira 2[23]	0.002	+	Madeira2[23]	0.009	+	SPortugal[22]	0.227	-	Spain[20]	0.145	-	Dinamarca[2]	0.001	+
S. Tomé[5]	0.000	+	C.Verde[12]	0.000	+	Madeira 1[8]	0.576	-	Italy[21]	0.356	-	USA[2]	0.094	-
Flemish[6]	0.000	+	S. Tomé[5]	0.000	+	Madeira 2[23]	0.158	-	M.Arabs 2[18]	0.000	+	Eskimos[2]	0.002	+
Germans[19]	0.000	+	Guiné[24]	0.000	+	Spain[20]	0.458	-	M.Berbers[18]	0.000	+			
Moroccans[19]	0.000	+	M.Arabs 1[13]	0.027	-	Italy[21]	0.519	-	S. Tomé[5]	0.000	+			
			N.Berbers[13]	0.027	-	M.Arabs 1[13]	0.163	-	Flemish[6]	0.398	-			
			Galicia[14]	0.007	+	N.Berbers[13]	0.008	+	Goa[9]	0.000	+			
			Astúrias[15]	0.000	+	M.Arabs 2[18]	0.163	-	Philippines[10]	0.000	+			
						M.Berbers[18]	0.052	-						
						Angola[16]	0.003	+						
						C.Verde[12]	0.071	-						
						Mozambique[17]	0.006	+						
						S. Tomé[5]	0.000	+						
						Guiné[24]	0.005	+						
						Galicia[14]	0.919	-						
						Astúrias[15]	0.521	-						
						Goa[9]	0.269	-						
						Philippines[10]	0.499	-						

The p-value is showed and significant differences (p≤0.01) were marked with a plus (+) symbol. Populations from: 1 - Pereira et al., 1997; 2 - Gusmão et al., 1996; 3 - Pinheiro et al., 1996; 4 - Miranda et al., 1998; 5 - Gusmão et al., 2001; 6 - Mertens et al., 1997; 7 - Souto et al., 1998; 8 - Corte-Real et al., 1999; 9 - Geada et al., 1996; 10 - Halos et al., 1998; 11 - Anjos et al., 2000; 12 - Dios et al., 1998; 13 - Pérez-Lezaum et al., 2000; 14 - Luis and Caeiro, 1995; 15 - Marco et al., 1999; 16 - Corte-Real et al., 1998; 17 - Corte-Real et al., 2000; 18 - Bosh et al., 2001; 19 - Brinkmann et al., 1998; 20 - Martín et al., 1996; 21 - Dobosz et al., 1996; 22 - Fernandes and Brehm, 2002; 23 - Fernandes et al., 2002; 24 - Gonçalves et al., 2002.

Inter-population analyses

Population differentiation tests were performed using all 5 markers (Table 3). Markers CD4, TPO and MBPB were the ones showing more differentiation between populations whereas for VWA only 5 out of the 21 populations analysed presented a significant differentiation relatively to our sample.

On what concerns the results obtained in the comparisons made with mainland Portugal, and considering all markers, a higher number of significant differences was observed between S. Miguel and the samples from North and South Portugal. These results contrast with the situation verified for Centre Portugal where only a significant result was obtained for marker CD4. However, no differences were detected between the three regions of Mainland Portugal (data not shown).

A Neighbour-Joining tree built using markers TPO and VWA is presented in Figure 1. Although population differentiation tests were carried out using geographically distinct subpopulations of Mainland Portugal (North, Center and South), the topology of the genetic trees reconstructed by using these subpopulations demonstrated a very poor statistical support, as assessed by bootstrap analysis (results not shown). Therefore the genetic tree presented in this work was obtained using the polled data for Mainland Portugal.

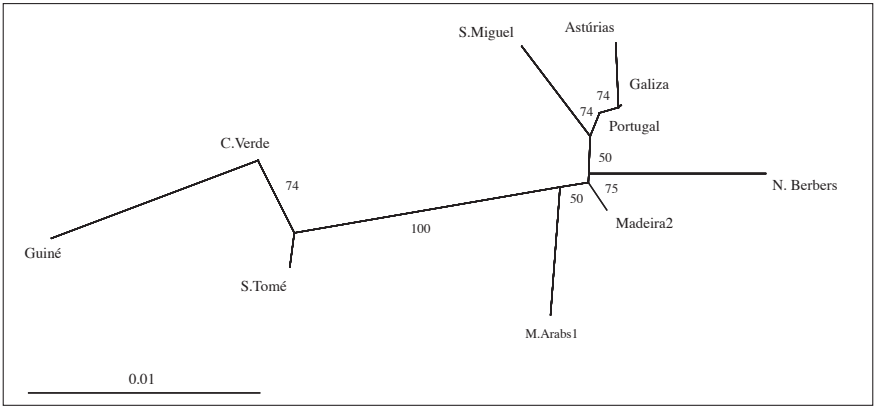

Figure1. Neighbour-Joining tree (Saitou and Nei, 1987) constructed from Cavalli-Sforza and Edwards (1967) genetic distances, using markers TPO and VWA. Numbers in branches are bootstrap probabilities for 10 000 resamplings. Reynolds et al. (1983) genetic distances produced a tree with essentially the same topology, but with lower bootstrap values.

The tree shows two main clusters, one formed exclusively by sub--Saharan populations and the other containing North African and European populations. This separation is strongly supported by bootstrap analysis (probability of 100%). In the group formed by North African and European populations, the North African samples appear to be the more differentiated. However the topology of this group is difficult to clarify because in the juncture points that involve North African populations, the bootstrap values are relatively low (50%). The fact of this tree is unrooted, makes difficult the evaluation of the positioning of each population included in the main European cluster relatively to the African populations. Thus, the positioning of S. Miguel and the evaluation of its affinities with European and African populations is not straightforward.

Using genetic distances we performed a Multidimensional Scaling (MDS) analysis (Helgason *et al.*, 2001). The result clearly shows an affinity between S. Miguel and the Moroccan Arabs (Figure 2). When this analysis was performed with the subdivisions of Mainland Portugal, S. Miguel showed proximity with the sample of South Portugal and Moroccan Arabs (Figure 3).

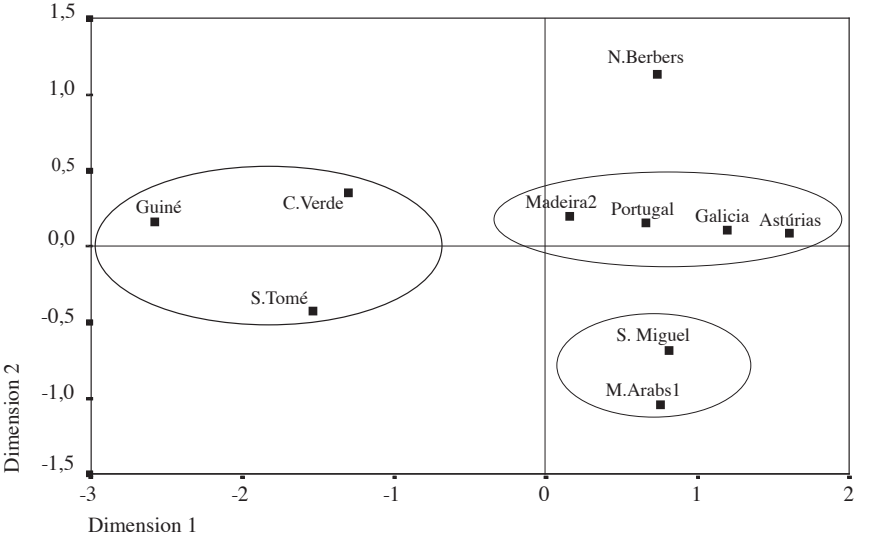

Figure 2. Multidimensional scaling chart of genetic distances.

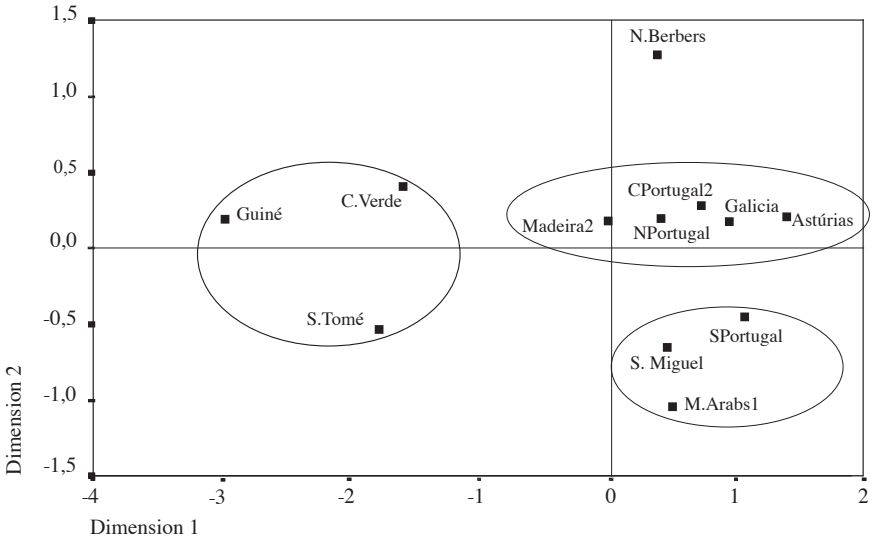

Figure 3. Multidimensional scaling chart of genetic distances.

In summary, the affinity with one of the North African population included in the analysis (MArabs1) is put forward by the MDS. Furthermore, this affinity is supported by the fact that the S. Miguel sample showed relatively high frequencies of alleles 8 and 9 from TPO and 15 from VWA. In fact, these alleles are representative of gene flow between European and African populations (Dios *et al.*, 2001). Also, mitochondrial DNA data generated by our research group shows the presence of North-African lineages in the population of S. Miguel (Santos *et al.*, 2003). This evidence agrees with historical reports regarding the contribution of Moorish slaves to the founding population of S. Miguel. The inclusion in this study of other populations that potentially contributed to the Azorean gene pool may clarify the relationships observed in the genetic trees.

This study provides new data concerning the genetic variability of the Azores and allows the inference of aspects on the settlement history of the Azorean populations. Nevertheless, to fully access the genetic profile of the Azorean populations it will be necessary to increase both the sample size and the set of markers used, as well as to integrate data from other genetic systems (mtDNA and Y chromosome polymorphisms). Furthermore, the use of non-genetic data in the inference of the population history of the Azores Islands is a priority, since specific issues concern-

ing the origins of the Azorean populations can only be addressed using biodemographic data, currently being systematized in a large population database (Alves *et al.*, 2000). As fully demonstrated by several authors (see for ex., Lasker, 1954; Mielke and Swedlund, 1993) this kind of data will surely provide high quality information that can strongly support the genetic variation found.

Acknowledgements

The authors wish to thank all the DNA donors who kindly accepted to participate in this work. This work was partially supported by PRI (Projecto Regional Integrado sobre a Doença de Machado-Joseph) and by CIRN (Centro de Investigação em Recursos Naturais), from the University of the Azores.

Bibliography

Alves, C.; Lima, M.; Silva, C.; Santos, C.; Mayer, F. M.; Labelle, E.; Smith, M. T. 2000. Biodemographic profile of an isolated population: framing of a genealogical database and preliminary results for Flores Island. *In:* Varela, T. (ed.). *Investigaciones en biodiversidad humana*. Santiago de Compostela, Servicio de Publicacións e Intercambio Científico, Universidad de Santiago de Compostela: 804-809.

Anjos, M. J.; Carvalho, M.; Andrade, L.; Corte-Real, F.; Vieira, D. N.; Vide, M. C. 2000. Allele frequencies of STR multiplex systems in two Portuguese population samples. *Progress in Forensic Genetics*, 8: 208-211.

Anker, R.; Steinbrueck, T.; Donis-Keller, H. 1992. Tetranucleotide repeat polymorphism at the human thyroid peroxidase (hTPO) locus. *Human Molecular Genetics*, 1: 137.

Bosh, E.; Clarimón, J.; Pérez-Lezaun, A.; Calafell, F. 2001. STR data for 21 loci in northwestern Africa. *Forensic Science International*, 116: 41-51.

Brinkmann, B.; Junge, A.; Meyer, E.; Wiegand, P. 1998. Population genetic diversity in relation to microsatellite heterogeneity. *Human Mutation*, 11: 135-144.

Budowle, B.; Chakraborty, R.; Giusti, A. M.; Eisenberg, A. J.; Allen, R. C.1991. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. *American Journal of Humam Genetics*, 48: 137-144.

Cavalli-Sforza, L. L.; Edwards, A. W. F. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution*, 32: 550-570.

Corte-Real, F.; Andrade, L.; Carvalho, M.; Anjos, M. J.; Gamero, J.; Vieira, D. N.; Carracedo, A.; Vide, M. C. 2000. Comparative analysis of STR data for Portuguese spoken countries. *Progress in Forensic Genetics*, 8: 212-214.

Corte-Real, F.; Anjos, M. J.; Carvalho, M.; Andrade, L.; Souto, L.; Mesquita, J.; Medeiros, A.; Pita da Silva, J.; Vieira, D. N.; Vide, M. C. 1998. Frequências alélicas de STRs na população açoriana. *In*: Vieira, D. N.; Rebelo, A.; Corte-Real, F. (Coord.). *Temas de medicina legal*. Lousã, Centros de Estudos de Pós-Graduação em Medicina-Legal: 441-446.

Corte-Real, F.; Souto, L.; Anjos, M. J.; Carvalho, M.; Vieira, D. N.; Carracedo, A.; Vide, M. C. 1999. Population distribution of six PCR-amplified loci in Madeira Archipelago (Portugal). *Forensic Science International*, 100: 93-99.

Dios, S.; Luis, J. R.; Caeiro, B.; Teixeira Ribeiro, J. C. 1998. TPOX, HUMVWA31/A, HUMTH01, CYP19, D5S373, D8S323, D8S344, D8S345: STR database for a West African population. *Progress in Forensic Genetics*, 7: 267-269.

Dios, S.; Luis, J. R.; Carril, J. C.; Caeiro, B. 2001. Sub-Saharan genetic contribution in Morocco: microsatellite DNA analysis. *Human Biology*, 73(5): 675-688.

Dobosz, M.; Pescarmona, M.; Moscetti, A.; Caglià, A.; D'Aloja, E.; Grimaldi, L.; Pascali, V. L. 1996. Allele frequencies of VWA, FES/FPS, FXIIIA1 and D21S11 in an Italian population sample. *Advances in Forensic Haemogenetics*, 6: 526-527.

Edwards, M. C.; Clemens, P. R.; Tristan, M.; Pizzuti, A.; Gibbs, R. A. 1991. Pentanucleotide repeat length polymorphism at the human CD4 locus. *Nucleic Acids Research,* 19(7): 4791.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39(4): 783-791.

Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package).* Version 3.5c. Distributed by the author. Seattle, Department of Genetics, University of Washington.

Fernandes, A. T.; Brehm, A. 2002. Population data of five STRs in three regions from Portugal. *Forensic Science International*, 129: 72-74.

Fernandes, A. T.; Brehm, A.; Alves, C.; Gusmão, L.; Amorim, A. 2002. Genetic profile of the Madeira Archipelago population using the new PowerPlex®16 System kit. *Forensic Science International*, 125: 281-283.

Geada, H.; Espinheira, R.; Ribeiro, T.; Reys, L. 1996. Population genetics of D1S80, HUMVWFA31/A and HUMF13A1 from Portugal and Goa (India). *Advances in Forensic Haemogenetics*, 6: 465-467.

Gonçalves, R.; Jesus, J.; Fernandes, A. T.; Brehm, A. 2002. Population data of five STRs in three regions from Portugal. *Forensic Science International*, 129: 78-80.

Guo, S. W.; Thompson, E. A. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, 48: 361-372.

Gusmão, L.; Amorim, A.; Prata, M. J.; Pereira, L.; Lareu, M. V.; Carracedo, A. 1996. Failed PCR amplifications of MBP-STR alleles due to polymorphism in the primer annealing region. *International Journal of Legal Medicine*, 108: 313-315.

Gusmão, L.; Prata, M. J.; Miranda, C.; Trovoada, M. J.; Amorim, A. 2001. STR data from S. Tomé e Príncipe (Gulf of Guinea, West Africa). *Forensic Science International*, 116: 53-54.

Halos, S. C.; Fortuno III, E. S.; Ferreon, A. C. M.; Chu, J. Y.; Miranda, J.; Harada, S.; Benecke, M. 1998. Allele frequency distributions of the polymorphic STR loci HUMVWA, HUMFES, HUMF13A01 and the VNTR D1S80 in a Filipino population from Metro Manila. *International Journal of Legal Medicine*, 111: 224-226.

Helgason, A.; Hickey, E.; Coodacre, S.; Bosnes, V.; Stefánsson, K.; Ward, R.; Sykes, B. 2001. mtDNA and the Islands of the North Atlantic: estimating the proportions of Norse and Gaelic ancestry. *American Journal of Human Genetics*, 68(3): 723-737.

Kimpton, C.; Walton, A.; Gill, P. 1992. A further tetranucleotide repeat polymorphism in the vWF gene. *Human Molecular Genetics*, 1(4): 287.

Lareu, M. V.; Phillips, C. P.; Carracedo, A.; Lincoln, P. J.; Syndercombe Court, D.; Thomson, J. A. 1994. Investigation of the STR locus HUMTH01 using PCR and two electrophoresis formats: UK and Galician Caucasian population surveys and usefulness in paternity investigations. *Forensic Science International*, 66: 41-52.

Lasker, G. W. 1954. Human evolution in contemporary communities. *Southwestern Journal of Anthropology*, 10: 353-65.

Luis, J. R.; Caeiro, B. 1995. Application of two STRs (VWF and TPO) to human population profiling: survey in Galicia. *Human Biology*, 67(5): 789-795.

Marco P. N.; Marttinez-Jarreta, B.; Martinez, E. A.; Sanchis, A. P.; Fonseca, R. H. 1999. Allele frequency distribution of the STR loci HUMTPOX, HUMTH01, HUMVWA in Asturias (North Spain). *Journal of Forensic Sciences*, 44(2): 389-391.

Martín, P.; Alonso, A.; Budowle, B.; Albarrán, C.; Garcia, O.; Sancho, M. 1996. Spanish population data on 13 PCR-based systems. *Advances in Forensic Haemogenetics*, 6: 578-580.

Matos, A. T. 1989. Povoamento e colonização dos Açores. *Portugal no Mundo*, 12: 185-188.

Mertens, G.; Mommers, N.; Heylen, H.; Gielis, M.; Muylle, L.; Vandenberghe, A. 1997. Allele frequencies of nine STR systems in the Flemish population and application in parentage testing. *International Journal of Legal Medicine*, 110: 177-180.

Mielke, J. H.; Swedlund, A. C. 1993. Historical demography and population structure. *In*: Lasker, G. W.; Mascie-Taylor, C. G. N. (eds.). *Research strategies in human biology*. Cambridge, Cambridge University Press: 140-185.

Miranda, C.; Prata, M. J.; Amorim, A. 1998. Population genetics of the F13A1 STR polymorphism in North Portugal and S. Tomé e Príncipe. *Progress in Forensic Genetics*, 7: 338-340.

Pereira, L.; Gusmão, L.; Amorim, A.; Prata, M. J.; Silva, F.; Bessa, I.; Santos, M. T. 1997. Population and segregation data of the STRs CD4, FES/FPS, MBP (Locus B), TH01, TP53, TPO and vWA31/A in North Portugal. *Proceedings from the Seventh International Symposium on Human Identification (1996)*: 193.

Pérez-Lezaun, A.; Calafell, F.; Bosch, E.; Mateu, E.; Gusmão, L.; Amorim, A.; Benchemsi, N.; Bertranpetit, J. 2000. Allele frequencies of 13 short tandem repeats in population samples from the Iberian Peninsula and Northern Africa. *International Journal of Legal Medicine*, 113: 208-214.

Pinheiro, M. F.; Pontes, M. L.; Gené, M.; Huguet, E.; da Costa, J. P. 1996. Population study of 3 STR loci in the North of Portugal. *Advances in Forensic Haemogenetics*, 6: 601-603.

Polymeropoulos, M. H.; Rath, D. S.; Xiao, H.; Merril, C. R. 1991. Tetranucleotide repeat polymorphism at the human coagulation factor XIII: a subunit gene (F13A1). *Nucleic Acids Research*, 19: 4306.

Prata, M. J.; Gusmão, L.; Mota, P.; Silva, D.; Amorim, A. 1998. Genetic profiling of S. Miguel Island (Azores). *Progress in Forensic Genetics*, 7: 294-296.

Raymond, M.; Rousset, F. 1995a. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86: 248-249.

Raymond, M.; Rousset, F. 1995b. An exact test for population differentiation. *Evolution*, 49: 1280-1283.

Reynolds, J. B.; Weir, B. S.; Cockerham, C. C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, 105: 767-779.

Rousset, F.; Raymond, M. 1995. Testing heterozygote excess and deficiency. *Genetics*, 140(4): 1413-1419.

Saitou, N.; Nei, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4): 406-425.

Santos, C.; Lima, M.; Montiel, R.; Angles, N.; Pires, L.; Abade, A.; Aluja, M. P. 2003 Genetic structure and origin of peopling in the Azores Islands (Portugal): the view from mtDNA. *Annals of Human Genetics*, 67(Pt5): 433-456.

Santos, M. F.; Amorim, A. 1994. Population studies of genetic markers in Portugal – a comparison between S. Miguel (Azores) and Northern Portugal. *In: 3rd International Workshop on Machado-Joseph disease*. S. Miguel, 7-9 April 1994 [Abstract].

Santos, M. F.; Prata, M. J.; Amorim, A.; Almeida, U. M.; Santos, M. T. 1992. Ilha de São Miguel: caracterização genética preliminar. *XXVII Jornadas Luso--Espanholas de Genética*, Badajoz [Abstract].

Schneider, S.; Roessly, D.; Excoffier, L. 2000. *ARLEQUIN ver. 2000: a software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Souto, L.; Amorim, A.; Vide, M. C. 1998. Population and segregation data on the multiplex system (TH01, VWA, FES, F13A1) from Central Portugal. *Progress in Forensic Genetics*, 7: 363-365.

SPSS Inc., 1989-1999. *SPSS for Windows 98* - Release 11.0. Chicago.

SREA (Serviço Regional de Estatística dos Açores) 2001. http://srea.ine.pt/ [Acedido em 01-12-2001].