

HLA DQA1 AND D1S80 POLYMORPHISMS IN THE POPULATION OF ANDALUSIA (SOUTHERN SPAIN).

Sanz P, Repetto G., Prieto V., Flores I.

Instituto Nacional de Toxicología, Sevilla, Spain.

INTRODUCTION

We report here the results of a survey of HLA DQA1 and D1S80 polymorphisms studied by the polymerase chain reaction (PCR) in blood samples from people living in Andalusia (Southern Spain). A population data base for both loci has been established for purposes of forensic identity testing.

MATERIAL AND METHODS

Population Sample: Blood was obtained from 174 unrelated Caucasian individuals living in our region, selected from casework.

Samples of 3 μ L liquid blood were deposited on clean sterile cotton tissue (20x20 mm) in a Petri dish, dried at room temperature. Once dried, cotton pieces were wrapped in aluminium foil and stored at -20°C until use.

DNA extraction: DNA for PCR analysis was isolated from blood stains by chelating resin extraction (Imidoacetic Acid, Sigma) according to Cetus Corporation technique (AmpliType User Guide, Version 2).

Extracted DNA was quantified spectrofluorometrically in a DNA fluorometer, TKO, Heofer Scientific Instruments.

Fluorescence reagent: Bis-benzimide (Hoescht 33258, Sigma).

Amplification and typing: Perkin Elmer Cetus DNA Thermal Cycler 480

DQA1: Amplitype HLA DQA1 Forensic DNA Amplification and Typing kit (Perkin Elmer). PCR product was detected by hybridisation with allele specific oligonucleotide probes

D1S80: The D1S80 DNA Amplification Reagent Set (Perkin Elmer) was used, PCR product was detected by flat bed SDS-PAGE followed by silver staining (gel: 8-18% SDS PAA; gel buffer: 0.12 M Tris-Acetate pH 6.4; electrode buffers: anode: 0.3 M Tris-Acetate, 4 g/L SDS, pH 6.4, cathode: 0.08 M Tris, 0.8 M Tricine, 4 g/L SDS, pH 7.1; Electrophoretic conditions: 400V, 100 mA, 30 W, 50-55 min).

STATISTICS

- Allele frequencies: calculated from numbers of each genotype in the sample set.
- Expected numbers of genotype frequencies were calculated under the assumption of Hardy-Weinberg expectation and possible divergences calculated by standard Chi-square test.
- Heterozygote frequency (=allele diversity): (Nei and Roychoudhury, 1974)

$$h = n [1 - \sum(n_i/n)^2] / (n-1)$$
, where: $n_1, n_2 \dots n_i$ = allele counts, n = number of chromosomes; Standard error: $\sqrt{h(1-h)/N}$, where h = expected heterozygote frequency, N = number of individuals.
- Lower and upper intervals of 95% confidence (LCL, UCL) for individual allele and genotype frequencies data were calculated taking into account a normal distribution or exactly calculated assuming a binomial distribution, depending on n_i values. (Domenech-Massons, 1990).
- Comparison between the different Caucasian populations was performed by pairwise comparisons in the Chi-squared test of homogeneity.
- Power of discrimination: $1 - \sum (P_i)^2$ (P_i = frequency of each genotype).

RESULTS**TABLE I DQA1 ALLELE FREQUENCIES**

ALLELE	FREQUENCY	95% LCL	95% UCL
1.1	0.2011	0.1590	0.2432
1.2	0.1207	0.0864	0.1550
1.3	0.0805	0.0519	0.1091
2	0.1466	0.1094	0.1838
3	0.1322	0.0965	0.1679
4	0.3190	0.1655	0.4725

N = 174 individuals

H-W equilibrium (15 degrees of freedom) $X^2 = 15.9912$

Homozygosity (n = 36) : 0.207

Heterozygosity (n = 138) : 0.793

Expected heterozygosity : 0.800 ± 0.095

Power of discrimination = 0.984

All but 1.2, 1.2 genotype were encountered in the population sample.

TABLE II D1S80 ALLELE FREQUENCIES

ALLELE	FREQUENCY	95% UCL
16	0.0030	0.0089
17	0.0059	0.0142
18	0.1923	0.2352
20	0.0266	0.0441
21	0.0444	0.0668
22	0.0444	0.0668
23	0.0296	0.0480
24	0.3521	0.4041
25	0.0444	0.0668
26	0.0118	0.0236
27	0.0148	0.0279
28	0.0473	0.0704
29	0.0917	0.1231
30	0.0089	0.0191
31	0.0414	0.0631
32	0.0207	0.0362
33	0.0059	0.0142
34	0.0030	0.0089
36	0.0030	0.0089
37	0.0059	0.0142
40	0.0030	0.0089

N = 166 individuals

H-W equilibrium (36 degrees of freedom) $X^2 = 201.9817$

Homozygosity (n = 30) : 0.177

Heterozygosity (n = 139) : 0.822

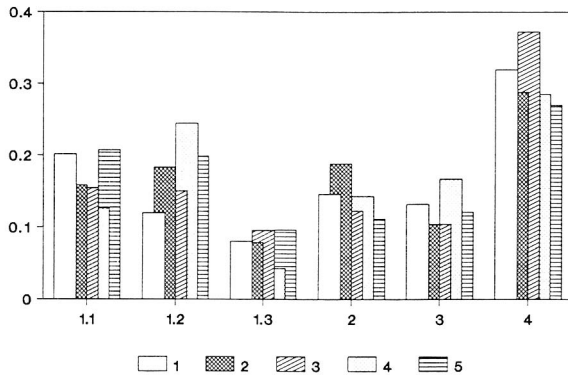
Expected heterozygosity : 0.820 ± 0.0008

Power of discrimination = 0.972

57 different genotypes and 21 alleles were observed in the population sample.

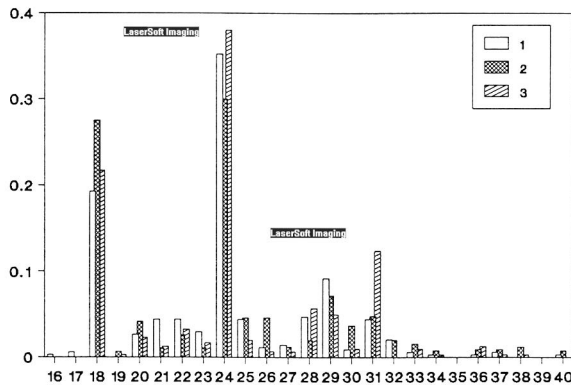
According to the results of statistic calculations, a divergence of genotype frequencies from Hardy-Weinberg expectation was found ($X^2 = 201.9817$ is much higher than tabulated value for 36 freedom degrees). This anomaly can be due to the high polymorphism of the population which probably requires a larger sample set, or another statistic strategy to be undertaken for calculations. Nevertheless Hardy-Weinberg equilibrium can be accepted in a population which is known to be at equilibrium for many other genetic characters (Bertranpetit, 1993, personal communication).

FIG. 1.- COMPARISON BETWEEN CAUCASIAN POPULATIONS: DQA1



- 1: Andalusia, Spain.
 2: Spain (region unspecified), Lorente et al., 1992.
 3: Catalonia, Spain, Gene et al., 1992.
 4: USA, McClure and Schart, 1991.
 5: Holland, Kloosterman et al., 1992.

FIG. 2.- COMPARISON BETWEEN CAUCASIAN POPULATIONS: D1S80



- 1: Andalusia, Spain.
 2: USA, Cetus Corporation, 1990.
 3: Holland, Kloosterman et al., 1992.

REFERENCES

- Cetus Corporation (1990) AmpliType User Guide, Emeryville.
- Domenech-Massons J.M. (1990) Métodos Estadísticos en Ciencias de la Salud. Barcelona.
- Gene M., Huguet E., Carracedo A., Mezquita J. (1992) Study of the HLA DQ α polymorphism in the population of Catalonia (Spain). Advances in Forensic Haemogenetics, 4. Ed. Rittner C. and Schneider P.M., Springer-Verlag.
- Kloosterman A.D., Daselaar P., Budowle B., Riley E.L. (1992) Population Genetic Study on the HLA DQ α and the D1S80 Locus in Dutch Population. Proceedings from the Third International Symposium on Human Identification.
- Lorente J.A., Lorente M., Budowle B., Corney C.T., Villanueva E. (1992) HLA DQ α Types in the Spanish Population. Proceedings from the Third International Symposium on Human Identification.
- McClure G, Schart S. (1991) Unpublished Communication. Cetus Corporation.
- Nei M., Roychoudhury A.K. (1974) Sampling Variances of Heterozygosity and Genetic Distance. Genetics, 76, 379-390.