AN ITALIAN COLLABORATIVE STUDY ON THE HLA-DQA1 LOCUS (GEFI's "GARDA 2" PROJECT)

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INTRODUCTION

In October 1991 the GEFI (Gruppo Ematologi Forensi Italiani) proposed a collaborative study on the use of AmpliType HLA-DQα Forensic DNA Amplification and Typing Kit (Parkin Elmer Cetus) for individual typing at the HLA-DQA1 locus (six alleles). The general recommendation for each participating group was to choose for the analysis a sample of at least 50 unrelated individuals, resident in the laboratory region. Five blind control samples from the Tenth International Histocompatibility Workshop were forwarded to each group. Twenty-three laboratories sent results in the deadline (see list below). We show here an analysis of the genetic heterogeneity between samples. A laboratory sent a selected subsample from its database and was excluded. The total number of considered individuals was 1623.

RESULTS

1) Heterogeneity of phenotype frequencies

About 2/3 of the expected values for the 21 phenotypes times 22 laboratories (= 462 cells) were lower than 2.0, making it impractical to test the equality of phenotypic proportions among laboratories. However, we did compute the χ^2s, in order to obtain a rough representation of the variation between samples. Two laboratories had a χ^2 value disproportionately higher than the mean; these were the Sardinian sample and a laboratory that had mistyped one control phenotype (L9).

2) Hardy-Weinberg equilibrium within laboratories

Owing to the small expected frequencies of the majority of phenotypes, we applied the method of Smith (*Ann.Hum.Genet.*: **50**, 163, 1986), which covers expectations even smaller than 0.5, to test each sample for accordance to the square law. The L9 sample was out of equilibrium (P>99.9%); a second laboratory had too high an accordance (P>99%). Another laboratory was above the level of 95% probability of deviation (but in a collection of about 20 independent random samples this should happen by chance just once).

3) Heterogeneity of gene frequency

The contingency table of the 6 alleles times 22 laboratories (grandtotal=3246 genes) was easily tested for heterogeneity of gene frequency, since no cell had an expected value less than 5.0. There was an overall high degree of heterogeneity (P>99.5%),

which, however, was due mainly to the Sardinian sample. After removing this sample the probability of heterogeneity was included between 90 and 95%.

4) Heterogeneity of the system reduced to 4 alleles

The typing method itself suggested to check the heterogeneity of the system after the frequencies of the alleles 1.1, 1.2 and 1.3 were combined in a single value. This analysis confirmed the peculiarity of the Sardinian allele frequencies. Two laboratories were now out of H.W. equilibrium: L9 (because of an excess of phenotypes 3 and 1/4) and L4 (with an excess of phenotypes 1/4).

5) Heterogeneity for the allele 4

Since the most represented phenotypes included the allele 4, it was meaningful to check the heterogeneity of this allele against all others (i.e. treating the system as a diallelic locus with three genotypes: 4/4, 4/- and -/-). None of the possible heterogeneity sources (phenotypic total, within-samples square law and between-samples gene frequency) was significant; however, there was a general slight tendency to deviate from H.W. equilibrium (P about 90%), towards an excess of heterozygotes.

6) Table of Italian (Sardinia excluded) allele frequencies

Allele	Gene count	Gene frequency
DQA1.1	496	0.167
DQA1.2	534	0.180
DQA1.3	181	0.061
DQA2	396	0.133
DQA3	232	0.078
DQA4	1133	0.381
TOTAL (1486 ind.)	**2972**	**1.000**

CONCLUSIONS

With the exception of one laboratory, which had mistyped a control sample and was very far from H.W. equilibrium (and for these reasons was excluded from the computation of the Italian allele frequencies), all other data were self-consistent (21 laboratories scattered on the entire national territory. The Sardinian sample was highly different from the other Italian samples. These were rather heterogeneous (P near 95%), probably reflecting a genetic differentiation within Italy, but not at the extent to invalidate the computation of the overall frequencies (see table above). The global sample was in H.W. equilibrium, so that the genotype frequencies may be estimated by the square law.

We should mention that there seems to be an excess of heterozygotes 1.1/4 (or 1/4 or -/4) in several laboratories. The frequency of the 1.1/4 phenotype in the global sample,

considered against the sum of all other phenotypes, was higher than expected (O=229, E=199, P=97%). This is even more significant, since the stratification of the population (or the heterogeneity of gene frequency) works in the opposite direction (i.e. towards an excess of homozygotes). We cannot distinguish, however, if this effect is due to an intrinsic methodological difficulty of the typing kit or it corresponds to a true genetic phenomenon. In any case, the effect is small and should be confirmed by further studies.

LIST OF PARTICIPATING LABORATORIES

Ist. Medicina Legale, Univ. **Ancona**; Ist. Medicina Legale, Univ. **Bari**; Ist. Medicina Legale, Univ. **Bologna**; Ist. Medicina Legale, Univ. **Brescia**; Ist. Medicina Legale, Univ. **Camerino**; Ist. Medicina Legale, Univ. **Ferrara**; Ist. Medicina Legale, Univ. **Genova**; Centro Genetica Medica, Immunopatologia, **Ozieri**; Ist. Medicina Legale, Univ. **Padova**; Ist. Medicina Legale, Univ. **Palermo**; Ist. Medicina Legale, Univ. **Parma**; Ist. Medicina Legale, Univ. **Pavia**; Dip. Biomedicina, sez. Medicina Legale, Univ. **Pisa**; Dip. Sanità Pubblica e Biostatistica, Univ. **Pisa**; Ist. Medicina Legale, Univ. **Roma** "Cattolica"; Ist. Medicina Legale, Univ. **Roma** "La Sapienza"; Ist. Genetica Medica, Univ. **Roma** "La Sapienza"; Servizio Polizia Scientifica, Polizia di Stato, **Roma**; Centro Nazionale Tresfusione Sangue, CRI, **Roma**; Dip. Biologia, Univ. **Trieste**; Ist. Medicina Legale, Univ. **Siena**; Ist. Medicina Legale, Univ. **Terni**; Ist. Medicina Legale, Univ. **Verona**.