HLA-DQα PCR SYSTEM: FREQUENCIES OF A SOUTH BAVARIAN POPULATION AND FAMILY DATA

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INTRODUCTION

- The HLA-DQα locus and the analysis of amplified DNA by using non-radioactive-labelled oligonucleotide probes in dot-blot-form have first been described by Saiki et al. (1986).
- Further developement of the polymerase-chain-reaction followed (Saiki et al., 1988).
- Ultimately, Cetus Corporation, Emeryville, introduced it`s AmpliType® Type HLA-DQα kit (1989).
- The aim of our random test was to collect data about the distribution of HLA-DQα alleles and phenotypes in a South Bavarian population and to compare the obtained data with other statistics already described in relevant literature.
- Furthermore, the exploration of family data for the same population was intended, same as the determination of the single exclusion chance for non-fathers (SECNF).

MATERIALS and METHODS:

Whole blood from 213 unrelated individuals out of a South Bavarian population and 11 families with altogether 15 children.

DNA extraction: sodium dodecyl sulfate (SDS)/proteinase K digestion
phenol / chlorophorm / isoamylalcohol (24:24:1) extraction
ethanol precipitation
This is done by inserting 2 ml of whole blood.
OR:
Applicaftion of the QIAmp Blood Kit from Qiagen according to the manuacturer`s instructions in-serting 250 µl of whole blood.

DNA amplification: Amplification was carried out using the HLA-DQα Forensic DNA Amplification and Typing Kit from AmpliType, Cetus, according to the manufacturer`s instructions (AmpliType™ User Guide, 1990). It took place in the GeneAmp PCR System 9600 from Perkin Elmer running the following programme for 32 cycles:

- Denaturation at 94°C for 20 sec.
- Primer-Template-Annealing at 60°C for 10 sec.
- Primer Extension at 72°C for 20 sec.

HLA-DQα Typing: Dot blot hybridisation and colour developement was managed according to the manufacturer`s instructions.

RESULTS

ALLELE FREQUENCIES:

We examined 213 individuals from a South Bavarian population, which means 426 alleles. The allele distribution was as follows:

1.1:	45 x	= 10.56%
1.2:	79 x	= 18.54%
1.3:	34 x	= 7.98%
2:	57 x	= 13.38%
3:	68 x	= 15.96%
4:	143 x	= 33.57%

FIGURE 1: A COMPARISON OF OBSERVED ALLELE FREQUENCIES IN VARIOUS POPULATIONS:

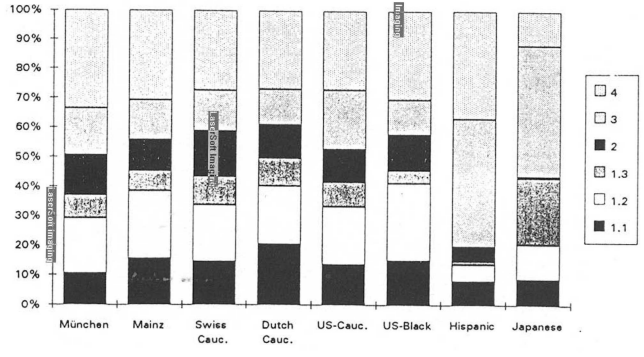

TABLE 1: OBSERVED AND EXPECTED PHENOTYPE FREQUENCIES IN OUR POPULATION SAMPLE:

Genotype	n	observed %	expected %	n	χ^2
1.1,1.1	3	1.41	1.12	2.38	0.0767
1.1,1.2	8	3.76	3.92	8.34	0.0694
1.1,1.3	5	2.35	1.69	3.59	0.2593
1.1,2	8	3.76	2.83	6.02	0.3053
1.1,3	5	2.35	3.37	7.18	0.3115
1.1,4	13	6.10	7.09	15.11	0.1378
1.2,1.2	7	3.29	3.44	7.33	0.0677
1.2,1.3	8	3.76	2.96	6.31	0.2139
1.2,2	10	4.69	4.96	10.57	0.0145
1.2,3	14	6.57	5.92	12.61	0.0719
1.2,4	25	11.74	12.45	26.52	0.0408
1.3,1.3	0	0.00	0.64	1.36	0.6370
1.3,2	1	0.47	2.14	4.55	1.3000
1.3,3	6	2.82	2.55	5.43	0.0284
1.3,4	14	6.57	5.36	11.41	0.2753
2,2	6	2.82	1.79	3.81	0.5887
2,3	9	4.23	4.27	9.10	0.0005
2,4	17	7.98	8.98	19.13	0.1117
3,3	4	1.88	2.55	5.43	0.1762
3,4	26	12.21	10.72	22.83	0.2072
4,4	24	11.27	11.27	24.00	0.0000
total:	213			213.01	χ^2 4.8938 0.995>p>0.990 df 15

The expected phenotype frequencies were calculated according to the Hardy-Weinberg-equilibrium from the allele frequencies.

The Power of Discrimination (PD) was 0.929.

TABLE 3: FAMILY DATA FROM 7 FAMILIES* WITH ALTOGETHER 9 CHILDREN:

Parents:	n	Children: 1.1,1.2	1.2,4	1.3,3	2,4	3,3	3,4	4,4
1.1,1.1x1.2,4	1	1	-	-	-	-	-	-
1.1,4x2,2	1	-	-	-	1	-	-	-
1.1,4x3,4	1	-	-	-	-	-	-	1
1.2,4x4,4	1	-	1	-	-	-	-	2
1.3,2x3.4	1	-	-	1	-	-	-	-
2,3x3,4	1	-	-	-	-	-	1	-
3,3x3,4	1	-	-	-	-	1	-	-

* We examined another 4 families where the putative father was excluded from paternity in two other systems in each case; so these families were not taken into account.

SECNF: 0.5986

SUMMARY:

- The distribution of HLA-DQα subtypes found for a South Bavarian population sample is in good agreement with the Hardy-Weinberg-equilibrium.
- The power of discrimination is 0.929.
- In comparison with other population samples the differences varied from uninconsiderable (other German or Central European populations being compared) to significant (populations from other continents being compared).
- The segregation of HLA-DQα subtypes in 7 South Bavarian families with altogether 9 children was in accordance with an assumed autosomal codominant mode of inheritence.
- SECNF being 0.5986, the HLA-DQα system can be considered to be an informative marker for paternity testing.

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