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Genetic Typing of HLA Class II Genes in Swedish Populations: Application to Forensic Analysis

REFERENCE: Allen, M., Saldeen, T., Pettersson, U., and Gyllensten, U., **"Genetic Typing of HLA Class II Genes in Swedish Populations: Application to Forensic Analysis,"** *Journal of Forensic Sciences*, JFSCA, Vol. 38, No. 3, May 1993, pp. 554–570.

ABSTRACT: In an attempt to determine the value of DNA based typing of HLA class II loci to forensic analysis, allele and genotype frequencies at DQA1, DQB1, DPB1, and DRB1 were determined in samples from two Swedish populations using hybridization with sequence specific oligonucleotides to PCR amplified DNA. Significant allele frequency differences were observed at the DQB1 and DRB1 loci between the two populations, as well as between one of the Swedish and a Norwegian population. The average heterozygosity varies between 0.74 to 0.91 and the power of discrimination between 0.90 to 0.98, with the highest values obtained for the DRB1 locus. The probability of genotype identity by chance differs on average 2% between the populations. When applied to a paternity case with one parent deceased and a criminal case, typing of class II loci proved in both cases informative. Analyses of DR and DQ genes does not increase the power of discrimination, due to strong linkage, but offers through the reconstruction of putative haplotypes an internal control for the consistency of the typing results at several loci. Typing of the DRB1 and DPB1 loci was found to result in an approximate combined average probability of genotype identity by chance of one in a thousand.

KEYWORDS: pathology and biology, genetic typing, DNA, HLA Class II genes, PCR

The rapid development of recombinant DNA techniques during the past years has made possible the genetic typing of many types of biological evidence materials found at the scene of a crime. The first molecular genetic technique developed for typing of biological evidence was based on the analysis of hypervariable regions of the genome [*1–3*]. Analyses of such regions result in very high discrimination powers but require to be successful high-molecular weight or only partly degraded DNA. The introduction of the polymerase chain reaction (PCR) [*4–7*] has made possible the analysis of additional types of evidence material containing highly degraded or extremely small amounts of DNA, such as that found in single hairs, bits of bone, blood stains, semen mixed with vaginal epithelial cells and tissue found under nails [*8–10*].

In order to analyze such materials, however, the PCR has to be targeted to relatively short segments of DNA, encompassing extensive allelic polymorphism. The HLA class II loci are among the most polymorphic protein coding sequences known in the human genome and provide a potentially valuable set of markers for typing of forensic samples. The HLA region, spanning approximately 1 Mb of the short arm of chromosome 6,

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consist of the subregions DP, DQ and DR, with the DP subregion closest to the centromere and the DR subregion closest to the telomere. Each of these subregions contain loci encoding one α chain and one β chain, with the exception of the DR subregion, which encode several β chains. The genes in the DR and DQ regions are closely linked, while recombination between these regions and the DP region has been reported [*11–14*].

To determine the value of HLA typing for individual identification it is necessary to obtain data on the genotype frequencies in the populations of interest. In this study we describe the allele and genotype frequencies at four HLA class II genes in two swedish populations. The purpose of the study was to determine the extent of genetic variation between swedish populations, and estimate the power of discrimination and probability of genotype identity by chance based on the analysis of four class II loci.

Materials and Methods

Populations/Samples

Blood samples were collected from 178 healthy donors from the city of Lund in southern Sweden and 86 individuals from Stockholm in the middle of Sweden. For the paternity case blood samples were obtained from the mother and the child while paraffin-embedded tissue samples were supplied from the deceased putative father. Biological evidence and reference samples from a criminal case where supplied by the Swedish Forensic laboratory. In this case the victim was raped and the evidence material consisted of two genital hairs found among the pubic hairs of the victim. As reference material from the two suspects and the victim, freshly plucked hairs were supplied. DNA from homozygous typing cells lines were used as typing controls for the different alleles [*13*].

DNA Extraction

DNA was extracted by mixing 0.5 mL peripheral blood cells with 0.5 mL lysis solution (0.32 M sucrose, 10 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$ and 1% Triton X-100). The cells were washed in the lysis solution and pelleted by centrifugation twice. The nucleated cells were resuspended in 0.5 mL PCR buffer (50 mM KCl, 10 mM Tris-HCl pH (8.3), 1.5 mM $MgCl_2$, 0.1 mg/mL gelatin, 0.45% Nonidet P 40 and 0.45% Tween 20), 10 μL proteinase K (10 mg/mL) and 3 μl 1M DTT were added and the samples incubated for 1 hour at 55°C. The proteinase K was heat inactivated at 95°C for 10 min and 25 μL from the solution was used without further purification in the PCR. DNA extraction from hair was done by digestion of an 1 cm portion of the hair including the root in 100 μL of the buffer above and 3 μL proteinase K (10 mg/mL). The samples were incubated for 3 h at 56°C, the proteinase K was thereafter inactivated in 94°C for 10 min [*15*].

Approximately 50 mg of the paraffin-embedded tissue material was cut into small pieces and incubated in Xylene for 24 h to remove the paraffin. The material was pelleted and washed successively with 95%, 80% and 70% ethanol and then dried. The pellet was digested overnight in the buffer above with the addition of 12.5 μL proteinase K (10 mg/mL). After digestion the samples were extracted with phenol/chloroform, precipitated in 0.3 M NaAc and the DNA resuspended in 50 μL TE buffer [*16*].

PCR Amplification

PCR amplification was performed in a 100 μL reaction containing 50 mM KCl, 10 mM Tris-HCl pH 8.4, 1.5 mM $MgCl_2$, 0.5% each of Nonidet P 40 and Tween 20, 50 pmol of each primer and 2.5 units Taq DNA polymerase. Each dATP, dCTP, dGTP and dTTP was present at 200 μM (800 μM total dNTP). Either 25 μL, equivalent to about one microgram human genomic DNA, 20 μL of single hair digest, or 5 μL of DNA from

paraffin-embedded tissue was used in each PCR. To raise the efficiency of the PCR reaction, glycerol to a final concentration of 10% was added. The size of the amplication products were estimated by electrophoresis in a gel containing 3% Nusieve (FMC) and 1% regular agarose. The gel was stained with ethidium bromide and the fluorescense was photographed under UV light.

SSO Typing

Twenty microliters of amplified PCR product (~200 ng) was denaturated in 200 μL of 0.4 N NaOH, 25 mM EDTA for 20 min and 50 μL applied to each of four pre-wetted BioDyne B membranes (Pall corp.), using a dot blot apparatus (Bio Rad). These filters do not require DNA immobilization by UV irradiation and can be reused multiple times after stripping in 80°C water with 1% SDS. The filters were hybridized to oligonucleotide probes labeled with horseradish peroxidase (HRP) at their 5′ ends, at a concentration of 1 pmol per mL hybridization solution. After the washing, hybridization was detected using the chemiluminescent detection system (ECL, Amersham) by placing the filters in the ECL solution for 1 min and then exposing them to Kodak Xomat-S X-ray film for 1 to 5 min.

DQA1 Amplification and Typing

A 242 bp fragment from the second exon was amplified with the primer pair GH 26 and GH 27 during 35 cycles of PCR (45 s at 94°C, 45 s at 55°C and 45 s at 72°C) [*17*]. PCR products were hybridized to a set of eight oligonucleotides (GH 75, GH 68, GH 67, GH 66, GH 88, GH 76, GH 89 and GH 77). These probes do not distinguish between the 0401, 0501 and 0601 alleles. Primer and probe sequences as well as hybridization and washing conditions have been described [*17–20*]. The forensic cases were analyzed with the Amplitype™ HLA-DQα Forensic kit (Perkin-Elmer/Cetus) using the reverse dot blot technique [*21,22*].

DQB1 Amplification and Typing

The second exon of DQB1 was amplified using either the primer pairs GH 28 and GH 29, which amplifies both DQB1 and DQB2, or using DB 130 and DB 131, which preferentially amplify the DQB1. The samples were subjected to 35 cycles of PCR (1 min at 94°C, 1 min at 55°C and 1 min at 72°C) and the products hybridized to 16 HRP labeled probes (DB 80, DB 54, DB 105, DB 53, DB 107, DB 78, DB 114, DB 115, DB 162, UG 82, DB 51, DB 69, DB 158, DB 55, DB 110 and DB 79). These probes can not distinguish between the 0401 and 0402 alleles. Primer and probe sequences, as well as hybridization and washing conditions have been described [*20,23*].

DPB1 Amplification and Typing

The samples were amplified using the primers UG 19 and UG 21 for 35 cycles in a two step cycle (1 min at 94°C and 1 min at 65°C). Amplified products were typed using 16 probes (DB 10, DB 11, DB 12, DB 22, DB 13, DB 14, DB 59, DB 17, DB 18, DB 19, DB 20, DB 62, DB 63, DB 40, DB 41 and DB 123). This collection of typing oligonucleotides can not distinguish the alleles 0901 from 1701 and 0801 from 1601. Primer and probe sequences as well as hybridization and washing conditions have been described [*20,24,25*].

DRB1 Amplification and Typing

The primer pair GH 46 and GH 50 was used to amplify the DRB1-5 loci for 35 cycles (1 min at 94°C, 1 min at 55°C and 1 min at 72°C). The amplified 272 bp fragment was hybridized to each of 19 probes (CRX 60, GH 105, GH 104, GH 59, CRX 06, GH 122, CRX 62, CRX 23, CRX 35, CRX 49, GH 102, CRX 63, GH 111, CRX 34, CRX 04, GH 56, CRX 68, CRX 61 and CRX 12). A DRB1 specific amplification was performed using the primers GH 46 and CRX 37 for 35 cycles (1 min at 94°C, 1 min at 55°C and 1 min at 72°C). Under these conditions DRB1 alleles on DR 2, 7 and 9 haplotypes will not amplify. The 297 bp DRB1 fragment was hybridized to a second typing panel containing 8 probes (GH 125, CRX 50, CRX 53, CRX 15, CRX 56, CRX 57 and CRX 12). These oligonucleotide probes do not distinguish the DR 2 subtypes or the allele 0403 from 0406. Conditions for hybridization and washing, as well as primer and probe sequences have been described [*20,26*].

Statistical Calculations

Expected genotype frequencies were calculated on the basis of the estimated allele frequencies under the assumption of Hardy-Weinberg equilibrium. Frequency values and G - tests for allele frequency heterogeneity among populations were calculated using the program JMP 1.05 (SAS Institute Inc.). Average heterozygosity was calculated as $H = [1 - \Sigma x_i^2][n/n - 1]$ where x_i is the frequency of the ith allele, and n = sample size [*27*]. The power of discrimination (P.D.) was calculated as $Pd = 1 - \Sigma p_i^2$, where p_i^2 is the frequency of the ith genotype [*28*].

Results

DQA1

The most common alleles in both Swedish populations are the 0102, 0301 and 04-06 with frequencies from 0.21 to 0.26 (Table 1). All 21 expected genotypes were found. No statistically significant allele frequency difference was found between the two populations ($G = 3.7$, df = 5, P = N.S.). Neither were any allele frequency differences found between the Swedish and a Norwegian population ($G = 7.1$, df = 5, P = N.S.) [*29*] or a North American caucasian population ($G = 10.2$, df = 5, $P = 0.07$) [*19*]. The average heterozygosity values and the estimated power of discrimination are very similar for the two populations (Table 5). The observed genotype distribution conforms to the proportions expected under the assumption of Hardy-Weinberg equilibrium ($G = 5.9$, df = 18, P = N.S. for the southern Swedish population and $G = 6.8$, df = 16, P = N.S. for

TABLE 1—*Allele and genotype frequencies at the DQA1 locus. Expected genotype frequencies were calculated under the assumption of Hardy-Weinberg equilibrium.*

DQA1	Southern Sweden Frequency		Mid Sweden Frequency	
ALLELE	N	(%)	N	(%)
0101	36	(10.2)	23	(13.7)
0102	88	(24.8)	36	(21.4)
0103	22	(6.2)	14	(8.3)
0201	31	(8.8)	12	(7.1)
0301	85	(24.0)	45	(26.8)
04–06	92	(26.0)	38	(22.6)
Σ N	354		168	

TABLE 1—*Continued.*

Genotype	Observed		Expected		Observed		Expected	
	N	(%)	N	(%)	N	(%)	N	(%)
0101/0101	1	(0.6)	1.8	(1.0)	3	(3.6)	1.6	(1.9)
0101/0102	11	(6.2)	8.9	(5.0)	4	(4.8)	4.9	(5.9)
0101/0103	1	(0.6)	2.2	(1.3)	1	(1.2)	1.9	(2.3)
0101/0201	2	(1.1)	3.2	(1.8)	1	(1.2)	1.6	(2.0)
0101/0301	7	(4.0)	8.6	(4.9)	7	(8.3)	6.2	(7.3)
0101/04–06	13	(7.3)	9.4	(5.3)	4	(4.8)	5.2	(6.2)
0102/0102	11	(6.2)	10.9	(6.2)	5	(5.9)	3.8	(4.6)
0102/0103	6	(3.4)	5.5	(3.1)	4	(4.8)	3.0	(3.6)
0102/0201	7	(4.0)	7.7	(4.4)	2	(2.4)	2.6	(3.1)
0102/0301	23	(13.0)	21.1	(11.9)	8	(9.5)	9.6	(11.5)
0102/04–06	19	(10.7)	22.9	(12.9)	8	(9.5)	8.1	(9.7)
0103/0103	0	(0.0)	0.7	(0.4)	1	(1.2)	0.6	(0.7)
0103/0201	2	(1.1)	1.9	(1.1)	0	(0.0)	1.0	(1.2)
0103/0301	4	(2.3)	5.3	(3.0)	3	(3.6)	3.7	(4.5)
0103/04–06	9	(5.1)	5.7	(3.2)	4	(4.8)	3.2	(3.8)
0201/0201	2	(1.1)	1.4	(0.8)	2	(2.4)	0.4	(0.5)
0201/0301	6	(3.4)	7.4	(4.2)	4	(4.8)	3.2	(3.8)
0201/04–06	10	(5.6)	8.0	(4.6)	1	(1.2)	2.7	(3.2)
0301/0301	14	(7.9)	10.2	(5.8)	5	(5.9)	6.0	(7.2)
0301/04–06	17	(9.6)	22.1	(12.5)	13	(15.5)	10.2	(12.1)
04–06/04–06	12	(6.8)	12.0	(6.8)	4	(4.8)	4.3	(5.1)

TABLE 2—*Allele and genotype frequencies at the DQB1 locus. Expected genotype frequencies were calculated under the assumption of Hardy-Weinberg equilibrium.*

DQB1	Southern Sweden Frequency		Mid Sweden Frequency	
ALLELE	N	(%)	N	(%)
0201	61	(17.5)	19	(12.7)
0301	75	(21.6)	19	(12.7)
0302	47	(13.5)	25	(16.7)
0303	10	(2.9)	11	(7.3)
0401/0402	13	(3.7)	8	(5.3)
0501	32	(9.2)	16	(10.7)
0502	1	(0.3)	0	(0.0)
0503	5	(1.4)	4	(2.7)
0504	1	(0.3)	3	(2.0)
0601	3	(0.9)	0	(0.0)
0602	55	(15.8)	26	(17.3)
0603	19	(5.5)	13	(8.7)
0604	20	(5.7)	5	(3.3)
0605	6	(1.7)	1	(0.7)
Σ N	348		150	

Genotype	Observed		Expected		Observed		Expected	
	N	(%)	N	(%)	N	(%)	N	(%)
0201/0201	4	(2.3)	5.3	(3.1)	0	(0.0)	1.2	(1.6)
0201/0301	12	(6.9)	13.1	(7.6)	1	(1.3)	2.4	(3.2)
0201/0302	9	(5.2)	8.2	(4.7)	4	(5.3)	3.2	(4.2)
0201/0303	3	(1.7)	1.8	(1.0)	3	(4.0)	1.4	(1.9)
0201/0401,0402	4	(2.3)	2.3	(1.3)	3	(4.0)	1.0	(1.3)
0201/0501	5	(2.9)	5.6	(3.2)	0	(0.0)	2.0	(2.7)
0201/0503	2	(1.1)	0.9	(0.5)	1	(1.3)	0.5	(0.7)
0201/0504	0	(0.0)	0.2	(0.1)	1	(1.3)	0.4	(0.5)
0201/0601	1	(0.6)	0.5	(0.3)	0	(0.0)	0.0	(0.0)
0201/0602	10	(5.7)	9.6	(5.5)	3	(4.0)	3.3	(4.4)
0201/0603	4	(2.3)	3.3	(1.9)	1	(1.3)	1.6	(2.2)
0201/0604	3	(1.7)	3.5	(2.0)	2	(2.7)	0.6	(0.8)
0301/0301	6	(3.4)	8.1	(4.6)	1	(1.3)	1.2	(1.6)
0301/0302	12	(6.9)	10.1	(5.8)	5	(6.7)	3.2	(4.2)

the population from the middle of Sweden. Genotypes with observed numbers lower than two were combined in to one group). When tested separately, there is no significant deviation of homo- and heterozygote proportions from the expected proportions in either of the two samples (G = 0.2, df = 1, P = N.S.).

DQB1

Fourteen different DQB1 alleles were found in the two Swedish populations with the most frequent alleles being the 0201, 0301, 0302 and 0602 (Table 2). In the two populations, 51 of the possible 105 different genotypes were found. The only genotypes that are not possible to distinguish with these probes are the genotypes 0602/0604 and 0603/0605. The allele frequency comparison indicate a marginally significant difference between the two populations (G = 23.5, df = 13, P = 0.036). When observed classes with less than five are combined the difference is still significant (G = 21.4, df = 11, P = 0.030). The allele frequencies for the population from the middle of Sweden are not significantly different from those of a Norwegian population (G = 17.8, df = 10, P = 0.059) [*29*]. However, the allele frequencies for the southern Swedish population were found to be significally different from those of the Norwegian population both when all the alleles are compared (G = 31.2, df = 13, P = 0.003) and when groups with observed numbers lower than five are combined (G = 25.1, df = 10, P = 0.005). The population

TABLE 2—*Continued.*

0301/0303	4	(2.3)	2.1	(1.2)	3	(4.0)	1.4	(1.9)
0301/0401,0402	1	(0.6)	2.8	(1.6)	1	(1.3)	1.0	(1.3)
0301/0501	10	(5.7)	6.9	(4.0)	2	(2.7)	2.0	(2.7)
0301/0601	1	(0.6)	0.6	(0.4)	0	(0.0)	0.0	(0.0)
0301/0602	15	(8.6)	11.9	(6.8)	3	(4.0)	3.3	(4.0)
0301/0603	4	(2.3)	4.1	(2.3)	1	(1.3)	1.6	(2.2)
0301/0604	4	(2.3)	4.3	(2.5)	1	(1.3)	0.6	(0.8)
0302/0302	4	(2.3)	3.2	(1.8)	1	(1.3)	2.1	(2.8)
0302/0303	0	(0.0)	1.4	(0.8)	3	(4.0)	1.8	(2.4)
0302/0401	2	(1.1)	1.8	(1.0)	1	(1.3)	1.3	(1.8)
0302/0501	3	(1.7)	4.3	(2.5)	2	(2.7)	2.7	(3.6)
0302/0503	0	(0.0)	0.7	(0.4)	2	(2.7)	0.7	(0.9)
0302/0602	5	(2.9)	7.4	(4.3)	3	(4.0)	4.3	(5.8)
0302/0603	3	(1.7)	2.6	(1.5)	3	(4.0)	2.2	(2.9)
0302/0604	5	(2.9)	2.7	(1.5)	0	(0.0)	2.2	(2.9)
0303/0602	2	(1.1)	1.6	(0.9)	2	(2.7)	1.9	(2.5)
0303/0605	1	(0.6)	0.2	(0.1)	0	(0.0)	0.1	(0.1)
0401,0402/0501	2	(1.1)	1.2	(0.7)	1	(1.3)	0.8	(1.1)
0401,0402/0602	1	(0.6)	2.1	(1.2)	1	(1.3)	1.4	(1.8)
0401,0402/0603	3	(1.7)	0.7	(0.4)	1	(1.3)	0.7	(0.9)
0501/0501	1	(0.6)	1.5	(0.8)	3	(4.0)	0.9	(1.1)
0501/0502	1	(0.6)	0.1	(0.1)	0	(0.0)	0.0	(0.0)
0501/0602	5	(2.9)	5.0	(2.9)	2	(2.7)	2.8	(3.7)
0501/0603	1	(0.6)	1.7	(1.0)	1	(1.3)	1.4	(1.8)
0501/0604	1	(0.6)	1.8	(1.0)	2	(2.7)	0.5	(0.7)
0501/0605	2	(1.1)	0.6	(0.3)	0	(0.0)	0.1	(0.1)
0503/0503	1	(0.6)	0.0	(0.0)	0	(0.0)	0.1	(0.1)
0503/0602	1	(0.6)	0.8	(0.4)	1	(1.3)	0.7	(0.9)
0504/0602	1	(0.6)	0.2	(0.1)	2	(2.7)	0.5	(0.7)
0601/0604	1	(0.6)	0.2	(0.1)	0	(0.0)	0.0	(0.0)
0602/0602	6	(3.4)	4.3	(2.5)	2	(2.7)	2.2	(3.0)
0602/0603	3	(1.7)	3.0	(1.7)	4	(5.3)	2.3	(3.0)
0602/0605	0	(0.0)	0.9	(0.5)	1	(1.3)	0.2	(0.2)
0603/0603	0	(0.0)	0.5	(0.3)	1	(1.3)	0.6	(0.7)
0603/0604	1	(0.6)	1.1	(0.6)	0	(0.0)	0.4	(0.6)
0604/0604	1	(0.6)	0.6	(0.3)	0	(0.0)	0.1	(0.1)
0604/0605	3	(1.7)	0.3	(0.2)	0	(0.0)	0.0	(0.0)

TABLE 3—*Allele and genotype frequencies at the DPB1 locus. Expected genotype frequencies were calculated under the assumption of Hardy-Weinberg equilibrium.*

DPB1	Southern Sweden Frequency	
ALLELE	N	(%)
0101	17	(4.8)
0201	50	(14.2)
0202	3	(0.9)
0301	53	(15.0)
0401	157	(44.6)
0402	38	(10.8)
0501	6	(1.7)
0601	11	(3.1)
0701	1	(0.3)
0801/1601	3	(0.9)
0901/1701	6	(1.7)
1001	2	(0.6)
1301	1	(0.3)
1401	1	(0.3)
1501	1	(0.3)
1901	2	(0.6)
Σ N	352	

Genotype	Observed		Expected	
	N	(%)	N	(%)
0101/0101	1	(0.6)	0.4	(0.2)
0101/0201	3	(1.7)	2.4	(1.4)
0101/0301	2	(1.1)	2.6	(1.4)
0101/0401	4	(2.3)	7.6	(4.3)
0101/0402	4	(2.3)	1.8	(1.0)
0101/0501	1	(0.6)	0.3	(0.2)
0101/1901	1	(0.6)	0.1	(0.1)
0201/0201	3	(1.7)	3.5	(2.0)
0201/0301	12	(6.8)	7.5	(4.3)
0201/0401	20	(11.4)	22.3	(12.7)
0201/0402	5	(2.8)	5.4	(3.1)
0201/0601	3	(1.7)	1.6	(0.9)
0201/0801,1601	1	(0.6)	0.4	(0.2)
0202/0401	2	(1.1)	1.3	(0.8)
0202/0501	1	(0.6)	0.0	(0.0)

from southern Sweden has a slightly lower average heterozygosity (0.86) than the population from mid Sweden (0.89) (Table 5). The observed genotype distribution conforms to the proportions expected under the assumption of Hardy-Weinberg equilibrium ($G = 6.8$, df = 29, P = N.S. for the southern Swedish population and $G = 10.6$, df = 21, P = N.S. for the population from the middle of Sweden. To avoid the problem with small absolute numbers in the statistical comparison where alleles with frequencies under five % combined in one allele group. Genotypes with observed numbers lower than two were then combined into one group.) There was no significant deviation of overall homo- and heterozygote proportions from expected values in either of the two samples ($G = 0.4$, df = 1, P = N.S.).

DPB1

Only the sample from southern Sweden was typed for DPB1 (Table 3). The 0401 allele was the most frequent allele (0.45) and this and the 0201, 0301 and 0402 alleles were

TABLE 3—*Continued.*

0301/0301	2	(1.1)	4.0	(2.3)
0301/0401	29	(16.5)	23.6	(13.4)
0301/0402	3	(1.7)	5.7	(3.2)
0301/0501	1	(0.6)	0.9	(0.5)
0301/0601	2	(1.1)	1.6	(0.9)
0401/0401	35	(19.9)	35	(19.9)
0401/0402	15	(8.5)	16.9	(9.6)
0401/0501	3	(1.7)	2.7	(1.5)
0401/0601	3	(1.7)	4.9	(2.8)
0401/0701	1	(0.6)	0.4	(0.2)
0401/0801,1601	1	(0.6)	1.3	(0.8)
0401/0901,1701	4	(2.3)	2.7	(1.5)
0401/1001	2	(1.1)	0.9	(0.5)
0401/1301	1	(0.6)	0.4	(0.2)
0401/1401	1	(0.6)	0.4	(0.2)
0401/1501	1	(0.6)	0.4	(0.2)
0402/0402	4	(2.3)	2.0	(1.2)
0402/0601	1	(0.6)	1.2	(0.7)
0402/0901,1701	1	(0.6)	0.6	(0.4)
0402/1901	1	(0.6)	0.2	(0.1)
0601/0801,1601	1	(0.6)	0.1	(0.1)
0601/0901,1701	1	(0.6)	0.2	(0.1)

TABLE 4—*Allele and genotype frequencies at the DRB1 locus. Expected genotype frequencies were calculated under the assumption of Hardy-Weinberg equilibrium.*

DRB1	Southern Sweden Frequency		Mid Sweden Frequency	
ALLELE	N	(%)	N	(%)
0101	30	(8.8)	18	(11.1)
0103	1	(0.3)	1	(0.6)
0301	38	(11.2)	17	(10.5)
0303	1	(0.3)	0	(0.0)
0401	55	(16.2)	27	(16.7)
0402	3	(0.9)	1	(0.6)
0403/0406	0	(0.0)	4	(2.5)
0404	15	(4.4)	8	(4.9)
0407	1	(0.3)	1	(0.6)
0408	6	(1.8)	2	(1.2)
0701	31	(9.1)	10	(6.2)
0801	13	(3.8)	8	(4.9)
0802	2	(0.6)	2	(1.2)
0803	1	(0.3)	1	(0.6)
0901	1	(0.3)	3	(1.9)
1001	1	(0.3)	2	(1.2)
1101	15	(4.4)	4	(2.5)
1102	0	(0.0)	1	(0.6)
1104	6	(1.8)	0	(0.0)
1201	4	(1.2)	1	(0.6)
1202	1	(0.3)	0	(0.0)
1301	15	(4.4)	14	(8.6)
1302	22	(6.5)	5	(3.1)
1303	6	(1.8)	0	(0.0)
1401	5	(1.5)	4	(2.5)
1402	1	(0.3)	1	(0.6)
DR2	66	(19.4)	27	(16.7)
Σ N	340		162	

TABLE 4—*Continued.*

Genotype	Observed		Expected		Observed		Expected	
	N	(%)	N	(%)	N	(%)	N	(%)
0101/0101	0	(0.0)	1.3	(0.8)	2	(2.5)	1.0	(1.2)
0101/0301	3	(1.8)	3.3	(2.0)	1	(1.2)	1.9	(2.3)
0101/0401	5	(2.9)	4.9	(2.8)	4	(4.9)	3.0	(3.7)
0101/0404	1	(0.6)	1.3	(0.8)	1	(1.2)	0.9	(1.1)
0101/0407	1	(0.6)	0.1	(0.0)	0	(0.0)	0.1	(0.1)
0101/0701	1	(0.6)	2.7	(1.6)	1	(1.2)	1.1	(1.4)
0101/0801	2	(1.2)	1.1	(0.7)	1	(1.2)	0.9	(1.1)
0101/1001	0	(0.0)	0.1	(0.1)	1	(1.2)	0.2	(0.3)
0101/1101	3	(1.8)	1.3	(0.8)	0	(0.0)	0.4	(0.5)
0101/1104	2	(1.2)	0.5	(0.3)	0	(0.0)	0.0	(0.0)
0101/1301	1	(0.6)	1.3	(0.8)	1	(1.2)	1.5	(1.9)
0101/1302	1	(0.6)	1.9	(1.1)	0	(0.0)	0.6	(0.7)
0101/1402	0	(0.0)	0.1	(0.1)	1	(1.2)	0.1	(0.1)
0101/DR2	10	(5.9)	5.8	(3.4)	3	(3.7)	3.0	(3.7)
0103/0802	0	(0.0)	0.0	(0.0)	1	(1.2)	0.0	(0.0)
0103/1104	1	(0.6)	0.0	(0.0)	0	(0.0)	0.0	(0.0)
0301/0301	1	(0.6)	2.1	(1.2)	0	(0.0)	0.9	(1.1)
0301/0401	7	(4.1)	6.1	(3.6)	6	(7.4)	2.8	(3.5)
0301/0404	2	(1.2)	1.7	(1.0)	0	(0.0)	0.8	(1.0)
0301/0408	1	(0.6)	0.7	(0.4)	0	(0.0)	0.2	(0.3)
0301/0701	4	(2.3)	3.5	(2.0)	0	(0.0)	1.0	(1.3)
0301/0801	2	(1.2)	1.4	(0.8)	3	(3.7)	0.8	(1.0)
0301/0901	0	(0.0)	0.1	(0.1)	1	(1.2)	0.3	(0.4)
0301/1101	1	(0.6)	1.7	(1.0)	0	(0.0)	0.4	(0.5)
0301/1201	2	(1.2)	0.4	(0.3)	0	(0.0)	0.1	(0.1)
0301/1301	2	(1.2)	1.7	(1.0)	1	(1.2)	1.5	(1.8)
0301/1302	2	(1.2)	2.5	(1.4)	2	(2.5)	0.5	(0.6)
0301/1401	2	(1.2)	0.6	(0.3)	1	(1.2)	0.4	(0.5)
0301/1402	1	(0.6)	0.1	(0.1)	0	(0.0)	0.1	(0.1)
0301/DR2	7	(4.1)	7.4	(4.3)	2	(2.5)	2.8	(3.5)
0303/1303	1	(0.6)	0.0	(0.0)	0	(0.0)	0.0	(0.0)
0401/0401	8	(4.7)	4.4	(2.6)	2	(2.5)	2.3	(2.8)
0401/0402	1	(0.6)	0.5	(0.3)	0	(0.0)	0.2	(0.2)
0401/0404	4	(2.3)	2.4	(1.4)	1	(1.2)	1.3	(1.6)
0401/0701	4	(2.3)	5.0	(2.9)	3	(3.7)	1.7	(2.0)
0401/0801	1	(0.6)	2.1	(1.2)	1	(1.2)	1.3	(1.6)
0401/1101	1	(0.6)	2.4	(1.4)	2	(2.5)	0.7	(0.8)
0401/1102	0	(0.0)	0.0	(0.0)	1	(1.2)	0.2	(0.2)
0401/1202	1	(0.6)	0.2	(0.1)	0	(0.0)	0.2	(0.2)
0401/1301	3	(1.8)	2.4	(1.4)	2	(2.5)	2.3	(2.9)
0401/1302	3	(1.8)	3.6	(2.1)	1	(1.2)	0.8	(1.0)
0401/DR2	9	(5.3)	10.7	(6.3)	2	(2.5)	4.5	(5.6)
0402/0404	0	(0.0)	1.3	(0.8)	1	(1.2)	0.0	(0.1)
0402/1101	1	(0.6)	0.1	(0.1)	0	(0.0)	0.0	(0.0)
0402/1302	1	(0.6)	0.2	(0.1)	0	(0.0)	0.0	(0.0)
0403,0406/0901	0	(0.0)	0.0	(0.0)	1	(1.2)	0.1	(0.1)
0403,0406/1201	0	(0.0)	0.0	(0.0)	1	(1.2)	0.0	(0.0)
0403,0406/1301	0	(0.0)	0.0	(0.0)	1	(1.2)	0.3	(0.4)
0403,0406/1401	0	(0.0)	0.0	(0.0)	1	(1.2)	0.1	(0.1)
0404/0407	0	(0.0)	0.0	(0.0)	1	(1.2)	0.0	(0.1)
0404/0408	1	(0.6)	0.3	(0.2)	0	(0.0)	0.1	(0.1)
0404/0701	2	(1.2)	1.4	(0.8)	0	(0.0)	0.5	(0.6)
0404/1301	1	(0.6)	0.7	(0.4)	1	(1.2)	0.7	(0.8)
0404/1303	1	(0.6)	0.3	(0.2)	0	(0.0)	0.0	(0.0)
0404/1401	0	(0.0)	0.2	(0.1)	1	(1.2)	0.2	(0.2)
0404/DR2	3	(1.8)	2.9	(1.7)	2	(2.5)	1.3	(1.6)
0408/0801	1	(0.6)	0.2	(0.1)	1	(1.2)	0.1	(0.1)
0408/1302	2	(1.2)	0.4	(0.2)	0	(0.0)	0.1	(0.1)
0408/DR2	1	(0.6)	1.2	(0.7)	1	(1.2)	0.3	(0.4)

TABLE 4—*Continued.*

Genotype	Observed		Expected		Observed		Expected	
0701/0701	2	(1.2)	1.4	(0.8)	2	(2.5)	0.3	(0.4)
0701/0801	1	(0.6)	1.2	(0.7)	0	(0.0)	0.5	(0.6)
0701/0802	1	(0.6)	0.2	(0.1)	0	(0.0)	0.1	(0.1)
0701/1001	1	(0.6)	0.1	(0.0)	0	(0.0)	0.1	(0.1)
0701/1101	3	(1.8)	1.4	(0.8)	0	(0.0)	0.2	(0.3)
0701/1301	2	(1.2)	1.4	(0.8)	0	(0.0)	0.9	(1.1)
0701/1302	2	(1.2)	2.0	(1.2)	1	(1.2)	0.3	(0.4)
0701/1303	1	(0.6)	0.5	(0.3)	0	(0.0)	0.0	(0.0)
0701/DR2	5	(2.9)	6.0	(3.5)	1	(1.2)	1.7	(2.1)
0801/0802	1	(0.6)	0.1	(0.0)	0	(0.0)	0.1	(0.1)
0801/1101	0	(0.0)	0.6	(0.3)	1	(1.2)	0.2	(0.2)
0801/1301	2	(1.2)	0.6	(0.3)	1	(1.2)	0.7	(0.8)
0801/DR2	3	(1.8)	2.5	(1.5)	0	(0.0)	1.3	(1.6)
0802/DR2	0	(0.0)	0.4	(0.2)	1	(1.2)	0.3	(0.4)
0803/DR2	1	(0.6)	0.2	(0.1)	1	(1.2)	0.2	(0.2)
0901/1302	1	(0.6)	0.1	(0.0)	0	(0.0)	0.1	(0.1)
0901/DR2	0	(0.0)	0.2	(0.1)	1	(1.2)	0.5	(0.6)
1001/DR2	0	(0.0)	0.2	(0.1)	1	(1.2)	0.3	(0.4)
1101/1101	1	(0.6)	0.3	(0.2)	0	(0.0)	0.0	(0.1)
1101/1302	1	(0.6)	1.0	(0.6)	0	(0.0)	0.1	(0.1)
1101/1303	1	(0.6)	0.3	(0.2)	0	(0.0)	0.0	(0.0)
1101/DR2	2	(1.2)	2.9	(1.7)	1	(1.2)	0.7	(0.8)
1104/1201	1	(0.6)	0.1	(0.0)	0	(0.0)	0.0	(0.0)
1104/DR2	2	(1.2)	1.2	(0.7)	0	(0.0)	0.0	(0.0)
1201/DR2	1	(0.6)	0.8	(0.4)	0	(0.0)	0.2	(0.2)
1301/1301	0	(0.0)	0.3	(0.2)	1	(1.2)	0.6	(0.7)
1301/1302	1	(0.6)	1.0	(0.6)	0	(0.0)	0.4	(0.5)
1301/DR2	3	(1.8)	2.9	(1.7)	5	(6.2)	2.3	(2.9)
1302/1302	1	(0.6)	0.7	(0.4)	0	(0.0)	0.0	(0.0)
1302/1303	1	(0.6)	0.4	(0.2)	0	(0.0)	0.0	(0.0)
1302/DR2	5	(2.9)	4.3	(2.5)	1	(1.2)	0.8	(1.0)
1303/DR2	1	(0.6)	1.2	(0.7)	0	(0.0)	0.0	(0.0)
1401/1401	1	(0.6)	0.0	(0.0)	0	(0.0)	0.0	(0.1)
1401/DR2	1	(0.6)	1.0	(0.6)	1	(1.2)	0.7	(0.8)
DR2/DR2	6	(3.5)	6.4	(3.8)	2	(2.5)	2.3	(2.8)

present at a frequency higher than 0.10. Only 37 of the 136 possible genotypes were detected. The DPB1 genotype 0201/0501 cannot be distinguished from 0402/1901 using this typing approach. One allele was found with a hybridization pattern identical to that described and denoted DP20 by Fernandez-Vina et al. [*30*]. Sequencing of this allele is underway and it has therefore not been included in our calculations. A comparison with a North American caucasian population [*31*] revealed no significant allele frequency differences, even when the alleles are grouped to avoid observed values below five ($G = 11.6$, df = 8, P = N.S.). The average heterozygosity and discrimination power are lower than for the other loci, because of the skewed allele frequency distribution (Table 5). The observed genotype distribution conforms to the proportions expected under the assumption of Hardy-Weinberg equilibrium ($G = 5.1$, df = 14, P = N.S. for the southern Swedish population). No significant deviation from expected homo- and heterozygote proportions was found ($G = 0.2$, df = 1, P = N.S.).

DRB1

Twenty-seven different DRB1 alleles and 93 out of a total of 378 possible genotypes were found in the two populations (Table 4). Several genotype combinations at the DRB1

TABLE 5—*Average heterozygosity and discrimination power for the two Swedish populations.*

	Average Heterozygosity		Discrimination Power	
	South SW.	Mid SW.	South SW.	Mid SW.
LOCUS				
DQA1	0.79	0.80	0.93	0.93
DQB1	0.86	0.89	0.96	0.97
DPB1	0.74	—	0.90	—
DRB1	0.90	0.91	0.98	0.97

locus are not possible to distinguish, with the set of oligonucleotide probes used. However most of these are rare, for example, 0404/1302, 0408/1301 and 0402/1402, with a frequency of 0.004, 1101/1301, 1102/1305 and 1104/1302, with a frequency of 0.006. The 0403 or 0406 allele was not found in the population from southern Sweden. The allele frequency comparison indicate statistically significant differences between the two populations when all allelic classes are included ($G = 39.3$, $df = 26$, $P = 0.046$), as well as when alleles with an observed number of less than five are combined ($G = 25.3$, $df = 14$, $P = 0.032$). Again, no significant allele frequency differences were observed between the population from the middle of Sweden and the Norwegian population ($G = 27.1$, $df = 20$, $P =$ N.S.) [*28*]. However, the allele frequency differences between the southern Swedish population and the Norwegian population were statistically significant both when all alleles were compared ($G = 53.6$, $df = 24$, $P = 0.001$) and when alleles were combined to avoid the problem with small expectancy values ($G = 33.5$, $df = 15$, $P = 0.004$). The average heterozygosity and the discrimination powers are high for both populations (Table 5). The observed genotype distribution conforms to the proportions expected under the assumption of Hardy-Weinberg equilibrium ($G = 6.9$, $df = 23$, $P =$ N.S. for the southern Swedish population and $G = 10.4$, $df = 18$, $P =$ N.S. for the population from the middle of Sweden). No significant deviation from expected homo- and heterozygote proportions were observed ($G = 0.3$, $df = 1$, $P =$ N.S.).

Casework Examples

Paternity Case

Since the only available material from the putative father was paraffin embedded tissue samples from the autopsy, paternity testing using southern blot analysis would be difficult or impossible. DNA from the biopsis proved difficult to amplify, especially with the DRB1 specific primer pair, presumably due to degradation or chemical modification of the DNA. The typing results shows that the suspected man can be excluded as a possible father at three of the four different class II loci (DQA1, DQB1 and DRB1) (Fig. 1). The child has a DQA1*0102 allele not received from the mother and that was not detected in the sample from the presumptive father. At the DQB1 locus the child has a 0602 allele not received from the mother and that is not present in the sample from the man. At the DRB1 locus the child has a DR2 allele that was not found in the presumptive father. In addition, the reconstruction of putative haplotypes shows that the man can be excluded because the child has the haplotype DRB1*DR2, DQB1*0602 and DQA1*0102, that can not be found in the mother or the presumptive father.

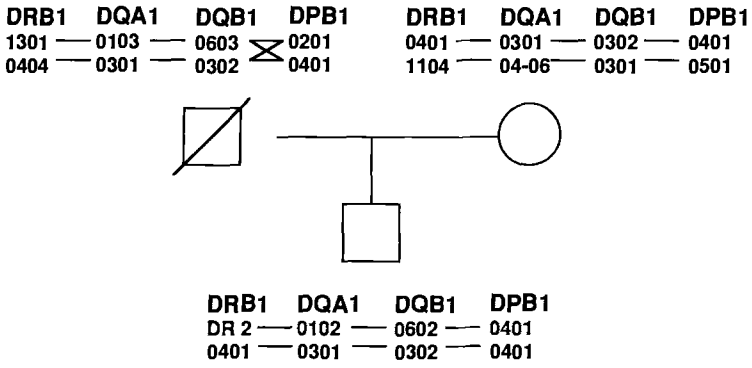

FIG. 1—*Results of typing of DQA1, DQB1, DPB1 and DRB1 in a paternity case.*

Forensic Case

This was a rape case where the evidence material consisted of two genital hairs found among the pubic hair of the victim. The reference material also consisted of freshly plucked hairs from the two suspects. The results of the typing of DQA1 and DPB1 are shown in Fig. 2 and Fig. 3, respectively. The two suspects and the victim have different genotypes at all four loci (Fig. 4) Thus, it is possible to determine if the evidence material can be derived from any of the two suspects. The alleles detected in evidence No. 1 are identical to those of suspect No. 2 at all four loci (DQA1 0102/0401, DQB1 0301/0605, DPB1 0401/new and DRB1 1101/1302) (Fig. 4.). Both evidence No. 1 and suspect No. 2 carry the DPB1*0401 allele and also a new allele, as indicated by a hybridization pattern not previously described (Fig. 3). The probability of genotype identity by chance is 10.7% for DQA1 and 0.6% for DRB1. Since no frequency estimates are available for the DPB1 genotype with the "new" allele and the DQB1 genotype 0301/0605, these probabilities

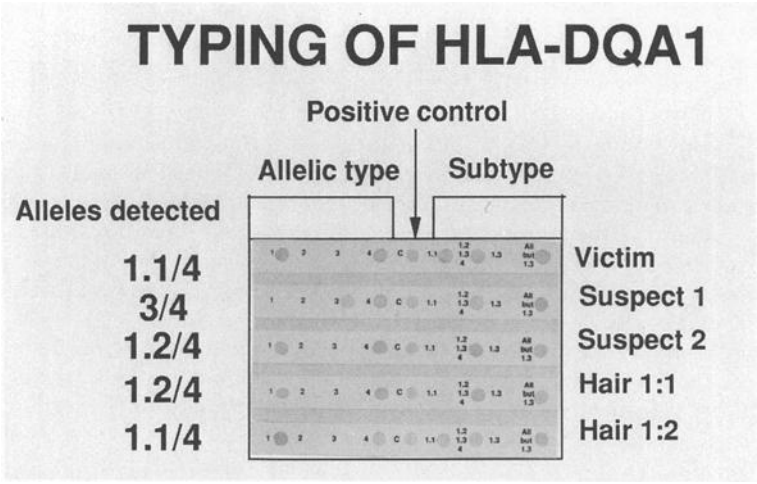

FIG. 2—*Results of typing DQA1 in a forensic case. The typing was performed using the reverse dot blot technique and the Amplitype kit (Perkin-Elmer/Cetus).*

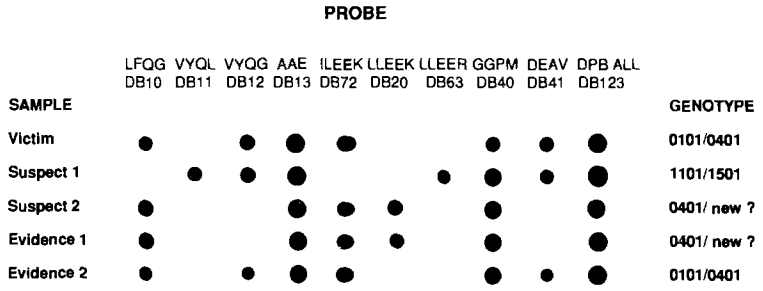

FIG. 3—*Results of typing of DPB1 in a forensic case. The sample from suspect No. 2 and that from evidence material No. 1 both indicate the presence of the DPB1*0401 allele and the presence of a previously unidentified allele (see probe DB 20).*

	LOCUS			
SAMPLE	DQA1	DQB1	DPB1	DRB1
Victim	0101/04-06	0201/0501	0101/0401	0101/0301
Suspect 1	0301/04-06	0201/0201	1101/1501	0301/0408
Suspect 2	0102/04-06	0301/0605	0401/NEW	1101/1302
Evidence 1	0102/04-06	0301/0605	0401/NEW	1101/1302
Evidence 2	0101/04-06	0201/0501	0101/0401	0101/0301

FIG. 4—*Summary of the results of typing four class II genes in a criminal case.*

are uncertain but are likely to be less than 0.3 and 0.5%, respectively. The power of discrimination for the allelic combinations of suspect 2 is 0.999 985 using the genotype frequency values for the Swedish populations. Evidence material No. 2 has the same allelic combinations as that of the victim and it can not be excluded that it may be derived from the victim herself.

Discussion

In this study genotypes at four different class II loci were deduced from the pattern of hybridization of oligonucleotide probes to amplified DNA. This method for typing of HLA allelic variation has the advantage that actual coding sequence information is being studied, rather than the presence of restriction sites in flanking intron sequences. Therefore, new alleles characterized by mutations occurring within the framework of allelic polymorphisms are likely to be detected. Indeed, a putative new allele was identified in one individual, based on a previously undetected hybrization pattern. However, the employed method is unable to resolve the individual alleles for certain genotypes where the allelic phase of different polymorphic regions is not known. This problem is due to the fact that individual alleles are deduced from the hybridization pattern of a number of sequence specific probes and very few alleles can be identified by the hybridization of a single allele specific probe. Thus, the obtained hybridization pattern may be identical for several different genotype combinations. The number of alleles that can not be resolved varies between loci, with the highest number for the DRB1 [8] and the lowest

number for the DQB1 and DPB1 [3]. In total, less than 1% of the individuals typed had allelic combinations that could not be resolved. This problem is different from that of misclassification of alleles, such as the comigration of VNTR alleles with relatively small migration differences. In the case of the class II loci, ambiguities can be easily identified from the pattern of hybridization of oligonucleotide probes. Also, the allelic status of individuals with unresolved genotypes can in most cases be determined by allele-specific amplification followed by oligonucleotide typing.

The possibility of biases in the typing procedure, as well as the existence of significant population subdivision within each locality, were examined by comparing the observed genotype distribution to binomial expectations. At none of the four loci, or in any of the two populations, was there a significant deviation from expected proportions. The observed homo- and heterozygote proportions also conform to binomial expectations at all the studied loci; thus there are no indications of systematic typing errors such as allelic drop-out, that would result in an excess of homozygotes.

The two Swedish populations shows notable allele frequency differences both at the DQB1 and DRB1 loci. No differences were observed at the DQA1 locus for the six alleles studied; however, differences may exist for some of the additional seven alleles recently reported to exist at this locus [13]. The allele frequencies at the DPB1 locus was studied only in one of the two populations and comparisons with a North American caucasian population did not indicate significant allele frequency differences. The statistical power for detecting significant differences is, however, lower for this locus due to the skewed allele frequency distribution.

Significant allele frequency differences were found between the southern Swedish population and a Norwegian population at the DQB1 and DRB1 loci, while no such differences were detected between the Norwegian population and that from the middle of Sweden. Possibly, these patterns of genetic differentiation are likely to reflect the origin and exchange of genetic material, with the southern Swedish locality being closer to Continental Europe.

Taken together these results indicate microgeographic differentiation at some of the class II genes, with possible implications for the use of these loci in forensic medicine. Substantial genotype frequency differences may exist in the face of relatively small allele frequency differences and affect the estimates of probability of genotype identity by chance, and power of discrimination (P.D.). However, the largest difference in genotype frequences between the two Swedish populations is 6% (DQA1 0301/04-06 and DQB1 0201/0301). In general, the error introduced by microgeographic heterogeneity in estimating P.D. is less than this value and can be estimated by the mean genotype frequency difference between the two populations (DQA1 2%; DQB1 1.5%; DRB1 1%). At present, these values may be used in individual forensic cases as guidelines for the error introduced by ethnic variation within northern Scandinavia to the P.D. values. Further studies will show whether these ranges are applicable also to other populations.

The usefulness of these loci to forensic analysis varies, with the lowest discrimination power and average heterozygosity for the DPB1 locus and the highest for the DRB1 locus. Due to the linkage between the DQ and DR regions the discrimination power can not be increased by a combined analysis of several DR and DQ loci. Analysis of 266 independent chromosomes showed no recombination between the DRB1, DQA1 and DQB1 loci [32]. However, there is only loose linkage with a recombination fraction of about 0.008 between the DQB1 and the DPB1 [32]. An approximation of the discrimination power resulting from a combined analysis of DPB1 and either a DR or a DQ locus can be obtained by multiplying the values of the two individual loci (DQA1-DPB1 = 0.007; DQB1-DPB1 = 0.003; DRB1-DPB1 = 0.002). Thus, the probability of genotype identity by chance for a combined analysis of two the most suited pair of class II loci is on average 2 to 7 in a 1000.

The close linkage between some class II loci may in certain cases present an advantage, by providing an internal control for the typing results between loci. For instance, in the paternity case the putative haplotypes of the mother fit two common haplotypes, one of which (DRB1*0401, DQA1*0301, DQB1*0302) was transmitted to the child (Fig. 1). Consequently, the child is likely to have received a chromsome with the allelic constitution DRB1*DR2, DQA1*0102, DQB1*0602 from the biological father. The putative chromosomal constitution of the potential father can also be deduced, although they can not be confirmed since no segregation data is available. However, none of the possible haplotypes in the putative father fits those of the child. Similarly, for the forensic case, the putative haplotype constitution can be inferred and compared between the different samples. In the samples from the Swedish population we found 40 different putative haplotypes (unpublished). An earlier study of five different ethnic groups revealed more than 70 different haplotype combinations for the DRB1-DQA1-DQB1 loci, including the 40 haplotypes found in the Swedish population [*33*,*34*].

As previously noted, the class II loci differ with regard to the frequency of genotypes whose allelic combinations can not be resolved. However, the pattern of polymorphism at class II loci, with few amino acid residues unique to each allele and most polymorphism common to several alleles may cause typing ambiguities when samples with mixed genotypes, such as prenatal samples with materal cells, rape samples with a mixture of epithelial cells and sperm, nailsscrapes from forensic cases and mixed blood samples are analyzed. Unless the material from one genotype predominate, it may be difficult to determine unambiguously the allelic constitution in a sample. This problem is most severe for the DRB1 and DPB1, where the allelic variation appears to be generated in part by recombinational mechanisms, and least severe for the DQA1 locus where more allele-specific polymorphisms are found. However, the presence of individual alleles in a sample, independent of the relative frequency can be detected by allele-specific amplification [*Allen et al. in preparation*, *35*].

Acknowledgments

We are grateful to H. Erlich for the HRP labeled oligonucleotides, M. Sandberg for the DNA samples from the southern of Sweden and H. Luthman for the DNA samples from the middle of Sweden. This work was supported by a Fellowship from Knut and Alice Wallenberg Foundation, grants from the Beijer Foundation and the Swedish Natural Sciences Council.

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