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HLA-A, -B, -C, -DR, -DQ typing in a population group of Senegal: distribution of HLA antigens and HLA-DRB1*13 and DRB1*11 subtyping by PCR using sequence-specific primers (PCR-SSP)

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One-hundred-and sixteen Senegalese Serere were typed for HLA antigens and compared with other ethnic groups in Gambia. We did not find significant differences (Fisher's exact test; P<0.01) in the HLA antigens distribution between the Serere and Mandinka groups in Senegal and the Serere, Mandinka and Wolof in The Gambia. The most common HLA haplotypes found (P<0.01; Chi square with Yates' correction) were: A1, B8; A2, B51; A32, B44; A33, B58; A2, Cw2; A2, Cw4; A33, Cw3; A2, DR17; A10, DR10; B35, Cw4; B53, Cw6; B57, Cw3; B65, Cw8; B50, DR15; B52, DR4; Cw2, DR17; DR7, DQ2; DR18, DQ4. The HLA-DRB1*13 and DRB1*11 alleles were subtyped by PCR-SSP and the frequencies of these alleles in the studied

Key words: HLA antigen – antigen and gene frequencies – DRB1 alleles – polymerase chain reaction – sequence specific primers

Table 1.
HLA class I gene frequencies in Serere and Mandinka[a] (Senegal) and other ethnic groups (Gambia)[b]

HLA antigen	Serere Senegal n=116	Mandinka[a] Senegal n=103	Mandinka[b] Gambia n=318	Wolof Gambia n=109	HLA antigen	Serere Senegal n=116	Mandinka Senegal n=103	Mandinka Gambia n=318	Wolof Gambia n=109
	gf[c]	gf	gf	gf		gf	gf	gf	gf
A1	0.0351	0.0490	0.0683	0.0055	B7	0.0174[e]	0.0440	0.0566	0.0961
A2	0.1440	0.1860	0.1134	0.1987	B8	0.0396[d,e]	0.0600	0.1011	0.1276
A3	0.0087	0.0580	0.0403	0.0371	B13	0.0000	–	0.0080	0.0045
A10	0.0762	–	0.0990	0.0845	B14	0.0570	0.0160	0.0465	0.0232
A11	0.043	–	0.0000	0.0000	B15	0.0043	–	0.0140	0.0045
A23	0.0441[d]	0.1800	0.1386	0.1066	B17	0.1133	–	0.1044	0.1380
A24	0.0000	0.0100	0.0015	0.0045	B18	0.0531	0.0450	0.0207	0.0518
A29	0.0087	0.0490	0.0222	0.0140	B27	0.0000	0.0190	0.0110	0.0186
A30	0.1192	0.1500	0.1514	0.1219	B35	0.1390	0.1390	0.1717	0.1485
A31	0.0396[d]	0.0190	0.0045	0.0000	B37	0.0000	0.0100	0.0015	0.0000
A32	0.0174	0.0190	0.0268	0.0518	B38	0.0043	–	0.0000	0.0000
A33	0.0623[d]	0.1030	0.1242	0.0961	B39	0.0043	0.0290	0.0080	0.0140
A36	0.0043	–	0.0000	0.0000	B40	0.0000	–	0.0030	0.0045
A43	0.0000	–	0.0000	0.0000	B41	0.0000	–	0.0176	0.0232
A74	0.0218	–	0.0000	0.0000	B42	0.0174	0.0290	0.0268	0.0423
Blank	0.2759	0.0400	0.0981	0.2029	B44	0.0043	0.0580	0.0156	0.0140
Cw1	0.043	0.0490	0.0253	0.0140	B45	0.0000	0.0100	0.0156	0.0140
Cw2	0.0531	0.0760	0.0818	0.0813	B49	0.0130	0.0530	0.0434	0.0140
Cw3	0.0441[d,e]	–	0.1585	0.1705	B50	0.0043	0.0050	0.0289	0.0232
Cw4	0.1490	0.1810	0.1403	0.1016	B51	0.0351	0.0300	0.0237	0.0140
Cw5	0.0130	0.0290	0.0222	0.0471	B52	0.0306	0.0490	0.0030	0.0045
Cw6	0.0531	0.0600	0.0818	0.0715	B53	0.1541	0.0400	0.1479	0.0571
Cw7	0.0998	0.1250	0.0906	0.1168	B59	0.0000	–	–	–
Cw8	0.0669	–	0.0418	0.0232	B70	0.0000[d,e]	0.0950	0.0769	0.0715
Blank	0.5167	0.3210	0.3577	0.3740	Blank	0.3089	0.0410	0.0446	0.0864

[a] Dard et al. (18)
[b] Allsopp et al. (3)
[c] gf=gene frequency
P<0.01 Comparison between Serere (Senegal) and Gambian ethnic groups:
[d] Serere (Senegal) vs. Mandinka
[e] Serere (Senegal) vs. Wolof

dard microlymphocytotoxicity test previously reported (11). The sera were provided from commercial sources in Terasaki Black HLA-ABC (72) and DR (60) Trays (Lagitre®, Milan, Italy). Each sample was designated using the WHO Nomenclature Committee for factors of the HLA system (12).

Extraction and PCR amplification primers

Genomic DNA were isolated from lymphocytes obtained from anti-coagulated blood (ACD). The DNA were prepared according to the salting-out method (13) or phenol-chloroform extraction procedure.

The HLA-DRB1*11 and DRB1*13 alleles were determined by PCR-SSP as described (14, 15). The primer solutions were supplied ready to be used by Dynal France®. Each tube in the Dynal SSP set contains 250 µl of a primer solution consisting of a specific primer mix, i.e. the allele-group-specific primers, as well as a control primer pair matching nonallelic sequences.

Two sets of primer solutions were used, the first to assign 8 subtypes of DRB1*13 allele (1301-1308) and the second, 10 subtypes of DRB1*11 allele (1101 - 1110).

PCR subtyping of DRB1*13 and DRB1*11 alleles

For DRB1*13 subtyping, 8 separate PCR reactions were performed per DNA sample and 10 for DRB1*11 according to the manufacturer's recommendations. Each PCR reaction mixture (10 µl) consisted of 5 µl of the primer solutions supplied and 5 µl of PCR pre-mixture containing 35 µl of PCR solution, 30 µl of genomic DNA and 1.1µl of AmpliTaq 5 U/µl (Perkin-Elmer Cetus Corporation).

PCR amplifications were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Instru-

Table 2.
HLA class II antigen and gene frequencies in Senegalese Serere ethnic group

DR Locus (n=113)	antigen frequency	gene frequency
DR1	0.0957	0.0490
DR4	0.0957	0.0490
DR7	0.0609	0.0309
DR8	0.0522	0.0264
DR9	0.2000	0.1056
DR10	0.0609	0.0309
DR11	0.1826	0.0959
DR12	0.0000	0.0000
DR13	0.1913	0.1007
DR16	0.0174	0.0087
DR17	0.0696	0.0354
DR18	0.0435	0.0220
Blank		0.3022
DQ Locus (n=114)		
DQ1	0.6579	0.4151
DQ2	0.2895	0.1571
DQ3	0.7105	0.4620
DQ4	0.439	0.0222
Blank		0.0000

ments). The PCR cycling conditions were as follows: denaturation at 94°C for 2 min followed by 10 cycles of denaturation 94°C for 45 s, annealing and extension at 64°C for 75 s followed finally by 20 cycles of denaturation at 94°C for 45 s, annealing at 61°C for 60 s and extension at 72°C for 45 s.

Gel electrophoresis

The absence or presence of PCR products was visualized by agarose gel electrophoresis. The PCR reactions mixtures were loaded in 3-mm wide slots in 2% (w/v) agarose gels (BRL) pre-stained with ethidium bromide (1 µg/ml gel).

Minigels (8.5×10 cm) were run for 15 min at 15 V/cm in 0.5×TBE buffer (89 mM Tris base / 89 mM boric acid / 2 mM EDTA, pH 8.0).

Gels were examined under UV illumination and documented by photography.

Table 3.
HLA-A, -B, -C, -DR, and DQ haplotype frequencies in Senegalese Serere

Haplotype	Haplotye frequency	Chi-square	P-value	Yates	P-value
A1 B8	0.0170	21.42	p<0.001	15.55	p<0.001
A1 B39	0.0043	13.61	p<0.001	2.91	
A1 B63	0.0043	13.61	p<0.001	2.91	
A2 B51	0.0250	10.22	p<0.01	7.74	p<0.01
A3 B57	0.0043	27.99	p<0.001	6.50	
A11 B18	0.0043	8.74	p<0.01	1.71	
A23 B63	0.0043	10.69	p<0.01	2.19	
A32 B44	0.0043	28.24	p<0.001	6.56	p<0.01
A33 B58	0.0279	9.11	p<0.01	7.08	p<0.001
A36 B8	0.0043	11.99	p<0.001	2.51	
A2 Cw2	0.0379	15.92	p<0.001	13.29	p<0.001
A2 Cw4	0.0643	12.22	p<0.001	10.63	p<0.01
A33 Cw3	0.0161	8.04	p<0.01	5.42	
A2 DR17	0.0257	10.26	p<0.01	7.88	p<0.001

Table 4.
Antigen and gene frequencies (%) of HLA-DRB1*13 (1301–1308) Subtypes in Dielmo inhabitants (n=113)

HLA-DRB1*13 subtypes	Antigen Frequency	Gene Frequency
DRB1*1301	2.65	1.333
DRB1*1302	2.65	1.333
DRB1*1303	0.88	0.442
DRB1*1304	15.00	7.804
DRB1*1305	0.00	0.00
DRB1*1306	1.76	0.883
DRB1*1307	0.00	0.000
DRB1*1308	0.88	0.442
DRB1*13 blank	1.76	0.883

Table 5.
Antigen and gene frequencies (%) of HLA-DRB1*11 (1101 1110) Subtypes in Dielmo inhabitants (n=113)

HLA-DRB1*11 subtypes	Antigen Frequency	Gene Frequency
DRB1*1101	5.30	2.680
DRB1*1102	18.5	9.720
DRB1*1103	0.00	0.000
DRB1*1104	0.00	0.000
DRB1*1105	0.00	0.000
DRB1*1106	0.00	0.000
DRB1*1107	0.00	0.000
DRB1*1108	0.00	0.000
DRB1*1109	0.00	0.000
DRB1*1110	0.00	0.000
DRB1*11 blank	2.60	1.308

Statistics

The analysis of serological results was done using conventional immunogenetic methods. Antigen frequency (af) was obtained by direct count. Gene frequencies (gf) were calculated from the antigen frequencies (af) by Bernstein's formula: $gf=1-(1-af)^{1/2}$.

The frequency of the "blank" gene was obtained by subtracting the total sum of defined gene frequencies from the unit. The gene frequencies in the Serere and Mandinka from Senegal and different ethnic groups in The Gambia (Mandinka and Wolof) were compared using the chi-square test and Fisher's exact test. Significant p-values have been corrected with factors (Fc) depending on the number of comparisons (Fc=100). Haplotype frequencies were computed from 2×2 tables of the phenotype frequencies using the formula of Mattiuz et al. (16) and their significance was evaluated by the chi-square test with Yates' correction.

Results

HLA class I and class II typing

The HLA-A, -B, -C gene frequencies in the Serere (Senegal) present some differences when compared with that of the Mandinka and Wolof ethnic groups in The Gambia (Table 1). The following HLA-A and B antigens: A11, A36, A43, A74 and B38 were not found in The Gambia. The "African" antigen HLA-B70 (3) was absent in the Serere (Senegal) and present in the two Gambian ethnic groups.

The frequencies of HLA-B8 and Cw3 were higher but not significantly in Mandinka and Wolof compared with the Serere. HLA-B53 was the most common HLA-B antigen in the Gambian Mandinka. There were no significant gene frequencies differences (Fisher's exact test; P>0.01) between Senegalese Serere, Wolof and Mandinka.

The HLA class II antigen and gene frequencies in the Senegalese Serere are listed in Table 2. The following HLA-DR antigens: DR9, DR13 and DR11 had the highest gene frequencies respectively 10.5, 10 and 9.6% among the Senegalese Serere.

The most common HLA haplotypes among Senegalese Serere are presented in Table 3. Seventeen (17) haplotypes showed significant associations

Table 6.
Frequencies (%) of HLA-DRB1*11 and 13 subtypes in 7 negroïd ethnic groups: SEN SER: Senegalese Sereres; SEN MAN: Senegalese Mandinka; GAM: Gambians; SAB: South African Blacks; BUS: Bushman (San); HOT: Hottentot (Khoi); AAM: African Americans. [a]Dard et al. (18). [b]Hill et al. (5)

	SEN SER	[a]SEN MAN	[b]GAM	[a]SAB	[a]BUS	[a]HOT	[a]AAM
n=	113	172	3152	84	79	91	132
1101	5.3	9.3	6.5	21.0	1.3	1.1	8.2
1102	18.5*	7.4	–	2.4	3.4	1.6	3.8
1103	0.0	–	–	0.6	–	–	0.8
1104	0.0	0.6	–	–	–	–	0.4
1301	2.6	4.7	3.6	3.6	11.6	5.5	4.2
1302	2.6	5.6	16.4	9.6	11.6	12.9	8.1
1303	0.8	1.7	2.2	0.6	–	–	3.0
1304	15.0	25.3	27.3	–	–	–	0.4
1305	0.0	0.9	–	–	–	–	–

* P<0.01, Chi 2 with Yates' correction

($p<0.01$; Chi-square Yates' correction) between phenotypes from two loci.

Frequencies of DRB1*13 and DRB1*11 subtypes

One hundred thirteen serere subjects were serologically typed for HLA-DR13 and DR11. The gene frequencies of DR13 and DR11 were shown to be respectively 10 and 9.6%. The HLA-DRB1*13 and DRB1*11 subtypes are listed in Tables 4 and 5. HLA-DRB1*1304 was the most common allele at 15.0%, followed by DRB1*1301 and 1302, 2.6% for the two alleles. There were no carriers of HLA-DRB1*1305 and 1307 alleles in this population.

Only 2 subtypes out of the detectable 10 DR11 alleles were observed. HLA-DRB1*1102 was the most common allele found (18.5%) and the second, HLA-DRB1*1101 at 5.3%.

In both alleles, HLA-DRB1*13 and DRB1*11, there were some DNAs we did not succeed in determining: 2.6% of DRB1*11 and 1.7% of DRB1*13 by PCR-SSP because of the false extra bands even if we repeated the amplification.

Discussion

In this present study, we investigate the HLA class I and class II frequencies of the Serere ethnic group and compare the class I genes with that of other African groups.

The gene frequencies of the following HLA-antigens A23, A31, A33, B52, B53, B70 and Cw3, significantly different between some of the ethnic groups (Chi-square with Yates' correction; $P<0.01$), became insignificant when the Fisher's exact test was used for comparisons. A high frequency of "blank" was found particularly in the HLA class I in Senegalese Serere..

The HLA antigens DR13 and DR11 were also highly represented in North Americans Blacks (17), this may be a support to a common origin of these populations. The gene frequencies of DR13 and DR11 were identical between Senegalese Serere and Mandinka, 10% and 9.6% (18).

The DR11 frequency was twice as high among Colombian Blacks as among Senegalese Serere or Mandinka (19).

The haplotype: A36, B53 characteristic in North American Blacks (17) was not found in Senegalese Serere while the most common Serere haplotype: A1, B8 was absent in Colombian Blacks (19).

The HLA-DRB1*13 allele was more polymorphic in the Senegalese Serere than in Japanese population (20). The high frequency of DRB1*1304 subtype was observed among Senegalese Mandinka (21) and among Gambians (5). The HLA-DRB1*11 allele showed less genetic diversity than the DRB1*13 allele.

The DRB1*1102 allele frequency of the Senegalese Serere was significantly different from the other African ethnic groups (Table 6).

We consider it very important to investigate the DRB1 alleles for a better knowledge of the molecular analysis of the associations HLA - malaria peptides with the aim of developing efficient vaccines subunits.

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References

1. Du Toit ED, MacGregor KJ, Taljaard DG, Oudshoorn M. HLA-A, B, C, DR and DQ polymorphisms in three South African populations groups: South African Negroes, Cape Coloureds and South African Caucasoids. *Tissue Antigens* 1988: **31**: 109-125.
2. Okoye RC, Williams E, Alonso A et al. HLA polymorphisms in Nigerians. *Tissue Antigens* 1985: **25**: 142-155.
3. Allsopp CEM, Harding RM, Taylor C et al. Interethnic genetic differentiation in Africa: HLA class I antigens in The Gambia. *Am J Hum Genet* 1992: **50**: 411-421.
4. Colin Stine O, Dover GJ, Zhu D, Kirby DS. The evolution of two West African populations. *J Mol Evol* 1992: 34: 336-344.
5. Hill AVS, Allsopp CEM, Kwiatskowski D et al. Extensive genetic diversity in the HLA class II region of Africans with a focally predominant allele, DRB1*1304. *Proc Natl Acad Sci U S A* 1992: **89**: 2277-2281.
6. Hill AVS, Elvin J, Willis AC et al. Molecular analysis of the association of HLA-B53 and resistance to severe malaria. *Nature* 1992: **360**: 434-439.
7. Abel L, Cot M, Mulder L, Carnevale P, Feingold J. Segregation analysis detects a major gene controlling blood infection levels in human malaria. *Am J Hum Genet* 1992: **50**: 1308 - 1317.
8. Trape JF, Rogier C, Konate L et al. The Dielmo project: a longitudinal study of natural malaria infection and the mechanisms of protective immunity living in a holoendemic area of Senegal. *Am J Trop Med Hyg* 1994: **51**: 123-137.
9. Vartdal F, Gauderna G, Funderud S, Bratlie A et al. HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation - a fast and reliable technique. *Tissue Antigens* 1986: **28**: 301-312.
10. Vartdal F, Bratlie A, Gauderna G, Funderud S et al. Microcytotoxic HLA typing of cells directly isolated from blood by means of antibody-coated microspheres. *Transplant Proc* 1987: **19**: 655-657.