

## TECHNICAL NOTE

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# Distribution of HLA-DQA1, Polymarker, CSF1PO, vWA, TH01, TPOX, D16S539, D7S820, D13S317, and D5S818 Alleles in East Bengali and West Punjabi Populations from Indo-Pak Subcontinent

**REFERENCE:** Tahir MA, Herrera RJ, Khan AA, Kashyap VK, Duncan G, Barna C, Budowle B, Rowold DJ, Sinha S, Amjad M. Distribution of HLA-DQA1, polymarker, CSF1PO, vWA, TH01, TPOX, D16S539, D7S820, D13S317, and D5S818 alleles in East Bengali and West Punjabi populations from Indo-Pak Subcontinent. *J Forensic Sci* 2000;45(6):1320–1323.

**ABSTRACT:** Blood samples were collected from 115 individuals residing in the Pakistani state of West Punjab and 81 Bengali individuals residing in the state of East Bengal, India. These samples were analyzed for the loci HLA-DQA1, PM (LDLR, GYPA, HBGG, D7S8, and GC) and eight short tandem repeats: CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818. Departures from Hardy–Weinberg (HWE) were observed in Punjabi population at LDLR, TH01, D13S317, D5S818, and D16S539 and at CSF1PO and TH01 in Bengali population.

**KEYWORDS:** forensic science, DNA typing, population genetics, DQA1, LDLR, GYPA, HBGG, D7S8, Gc, short tandem repeat, CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, Bengal, Pakistan, Punjab

We present the distribution of allele frequencies for eight STR Loci (CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818, HLA-DQA1, and five polymarker loci (LDLR, GYPA, HBGG, D7S8, and GC) in samples from two populations, the east Bengali and west Punjabi residing in India and Pakistan respectively.

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Received 23 April 1999; and in revised form 7 Sept., 5 Nov., and 29 Dec. 1999; accepted 30 Dec. 1999.

## Materials and Methods

One hundred and fifteen blood samples from West Punjabi and 81 samples from East Bengali unrelated individuals were collected in EDTA containing tubes. DNA was extracted from the liquid blood by organic extraction followed by ethanol precipitation (1). The Perkin-Elmer Quantiblot Kit (D17ZI Probe) was used to quantify the extracted DNA according to manufacturer's instructions. HLA-DQA1 and PM Loci were amplified from the extracted DNA using the amplitype PM + DQA1, kits (Perkin-Elmer, Foster City, CA) as per manufacturer's instructions. STR Loci (CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818) were simultaneously amplified using the powerplex 1.2 kit (Promega Corporation, Madison, WI) per manufacturer's instructions.

The amplified products of amplitype PM and HLA-DQA1 loci were detected by reverse dot blot hybridization (Perkin-Elmer, Foster City, CA). Fluorescently tagged STR amplified DNA products were separated on 5% denaturing Polyacrylamide gels using a model S2 electrophoresis apparatus (Life Technologies, Bethesda, MD). Three microliters of each amplified DNA product and allelic ladder was mixed with blue dextran dye.

The individual samples were denatured at 95°C for 5 min and snap cooled on ice. The electrophoresis was performed for 2 h at 40 W, in 0.5X Tris Borate EDTA (TBE) buffer. Fluorescence detection was accomplished using the Genomix SC Scanner (Genomix Corporation, Foster City, CA). The allele type was determined by comparing the sample migration to a STR allelic ladder.

Frequencies for each allele were calculated directly from the genotypic classes in the sample set. Unbiased estimates for heterozygosity were obtained and possible departures from Hardy–Weinberg expectations (HWE) were determined by the homozygosity test and the exact test (3). An interclass correlation criterion (4) was performed to detect potential disequilibrium between loci. Power of discrimination and power of exclusion (5) were calculated for each locus in both sample populations. The degree of genetic distance between the Bengali and West Punjabi sample pop-

ulations were estimated by standard G-tests conducted on all pairwise comparisons of allelic frequencies. As part of G-test a 2XC contingency table was used to generate G-values (2000 shuffling experiments) to test for association between the various populations using Monte Carlo simulations. Association would imply that the two sample populations compared were homogeneous. In addition, the G-test was performed using the RXC Test of Independence (6–7).

**Results and Discussion**

Tables 1 and 2 show the allelic frequencies for HLA-DQA1, PM and eight STR loci from 115 Punjabi and 81 Bengali individuals. Differences in sample count within the data are due to failed typing. The observed heterozygosities for HLA-DQA1 and PM loci vary from 30 to 72% for Punjabi and 36 to 87% for Bengali population (Table 1). The observed heterozygosity values for STR loci vary from 59 to 77% for Punjabi and 63 to 87% for Bengali population (Table 3). Heterozygosity observed in these populations demonstrates high degree of polymorphism associated with these loci.

In both the Bengali and Punjabi groups, the HLA-DQA1 locus displays the highest power of discrimination (PD) value (0.939 and 0.937 respectively) and the D7S8 locus, the lowest (0.504 and 0.507) (Table 1). Power of exclusion (PE) values are the highest for two loci (0.656 for HLA-DQA1 in Bengali and 0.631 for vWA in Punjabi populations) (Tables 1 and 2). Lowest PE value is exhib-

ited by D7S8 locus for both populations (0.141 in Bengali and 0.142 in the Punjabi).

Departure from Hardy-Weinberg (HWE) was observed in each population set (Tables 1 to 3). The Punjabi sample population demonstrated departure at the LDLR locus and the STR loci, THO1, D13S317, D16S539, and D5S818. The Bengali population exhibited departure at CSF1PO and THO1 loci. These observations could very well be due to the custom of inter-family (cousins) marriages leading to excessive homozygosity. We recognize that sample number, especially for vWA (*n* = 43), is small, however, we believe it is important that we provide the reader with the information so that they could use it to contribute to the further studies.

An interclass correlation test was performed on the HLA-DQA1/PM and STR loci. All possible pairwise comparisons were done. This comparison indicated departure at the GYP A/HBGG and D7S8/DQA1 loci for Punjabi population. Similarly, in the Bengali population, departure was observed at the LDLR/HBGG and D7S8/DQA1 loci. Departure for the STR loci was observed at D7S820/D13S317 and D16S539/D13S317 for the Punjabi population. The Bengali population demonstrated departure at 4 loci combinations, CSF1PO/D13S317, TPOX/vWA, TPOX/D16S539, and THO1/D13S317. No comparison was made between the STR and HLA-DQA1/PM loci. Punjabi/Bengali populations were statistically similar. Comparison of African-Americans with Punjabi and Bengali sample populations at 14 loci showed similar allele frequency at 3 loci for Punjabi and 2 loci

TABLE 1—PM and HLA-DQA1 allele frequencies in Bengali (n = 81) and Punjabi (n = 115) populations.

Allele	LDLR		GYPA		HBGG		D7S8		Gc						
	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi					
A	0.463	0.443	0.586	0.610	0.426	0.500	0.784	0.781	0.321	0.289					
B	0.537	0.557	0.414	0.390	0.574	0.500	0.216	0.219	0.179	0.149					
C					0	0			0.500	0.561					
HLA-DQA1															
	Bengali	Punjabi													
1.1	0.173	0.127													
1.2	0.130	0.118													
1.3	0.142	0.180													
2	0.290	0.149													
3	0.086	0.075													
4.1	0.080	0.325													
4.2/4.3	0.099	0.026													
Extract Test			HLA-DQA1		LDLR		GYPA		HBGG		D7S8		Gc		
	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	Bengali	Punjabi	
P. E.*	0.926	0.438	1.000	0.002	0.502	1.000	0.074	0.262	0.744	0.173	0.847	0.125	0.306		
	0.656	0.620	0.187	0.186	0.184	0.181	0.185	0.188	0.141	0.142	0.330	0.306			
Heterozygosity Test						Power of Discrimination									
Observed				Expected				Observed				Expected			
Bengali		Punjabi		Bengali		Punjabi		Bengali		Punjabi		Bengali		Punjabi	
HLA-DQA1	0.865	0.722	0.830	0.806	0.939	0.937	0.948	0.936							
LDLR	0.506	0.635	0.500	0.495	0.619	0.519	0.624	0.622							
GYPA	0.530	0.478	0.393	0.477	0.593	0.609	0.617	0.612							
HBGG	0.396	0.557	0.492	0.502	0.652	0.589	0.619	0.625							
D7S8	0.358	0.304	0.341	0.347	0.504	0.507	0.505	0.509							
Gc	0.667	0.539	0.619	0.581	0.763	0.752	0.778	0.752							

\* P.E. = Power of exclusion.

TABLE 2—Bengali and Punjabi STR allele frequencies.

Bengali STR Allele Frequencies								
No. Repeat (Allele)	CSF1PO <i>n</i> = 80	TPOX <i>n</i> = 80	THO1 <i>n</i> = 79	vWA <i>n</i> = 76	D16S539 <i>n</i> = 80	D7S820 <i>n</i> = 79	D13S317 <i>n</i> = 75	D5S818 <i>n</i> = 76
6			0.213					
7		0.006	0.200			0.013	0.020	
8	0.019	0.377	0.144		0.063	0.215	0.160	0.006
9	0.012	0.093	0.319		0.206	0.051	0.087	0.057
9.3			0.113					
10	0.235	0.099	0.013		0.094	0.259	0.107	0.120
11	0.247	0.414			0.275	0.215	0.293	0.361
12	0.420	0.012			0.250	0.215	0.213	0.329
13	0.062			0.007	0.106	0.032	0.100	0.121
14				0.171	0.006		0.020	0.006
15	0.006			0.079				
16				0.250				
17				0.237				
18				0.184				
19				0.066				
20				0.007				
P. E.	0.456	0.409	0.570	0.618	0.598	0.584	0.635	0.496
Exact Test	0.036	0.660	<10 <sup>-3</sup>	0.581	0.928	0.640	0.084	0.379
Punjabi STR Allele Frequencies								
No. Repeat (Allele)	CSF1PO <i>n</i> = 82	TPOX <i>n</i> = 97	THO1 <i>n</i> = 97	vWA <i>n</i> = 43	D16S539 <i>n</i> = 90	D7S820 <i>n</i> = 88	D13S317 <i>n</i> = 91	D5S818 <i>n</i> = 91
6			0.232					
7		0.005	0.170					
8	0.006	0.397	0.134		0.039	0.244	0.137	0.005
9	0.018	0.119	0.289		0.133	0.068	0.066	0.044
9.3			0.165					
10	0.177	0.098	0.010		0.122	0.239	0.093	0.099
11	0.355	0.340			0.400	0.216	0.258	0.363
12	0.366	0.041			0.161	0.199	0.341	0.341
13	0.091				0.122	0.023	0.093	0.143
14				0.140	0.017	0.006	0.011	0.005
15	0.006			0.128	0.006	0.006		
16				0.151				
17				0.267				
18				0.221				
19				0.081				
20				0.012				
P. E.	0.466	0.456	0.582	0.631	0.568	0.587	0.575	0.482
Exact Test	0.824	0.664	0.010	0.195	0.003	0.360	<10 <sup>-3</sup>	<10 <sup>-3</sup>

P.E. = Power of exclusion.

TABLE 3—Observed and expected Power of Discrimination and heterozygosity values for STR loci from Punjabi and Bengali populations.

	Power of Discrimination				Heterozygosity			
	Punjabi		Bengali		Observed		Expected	
	PD (Obs.)	PD (Exp.)	PD (Obs.)	PD (Exp.)	Punjabi	Bengali	Punjabi	Bengali
CSF1PO	0.866	0.868	0.858	0.862	0.695	0.790	0.718	0.786
TPOX	0.858	0.860	0.844	0.830	0.691	0.638	0.705	0.674
THO1	0.917	0.922	0.893	0.918	0.670	0.760	0.792	0.786
vWA	0.925	0.940	0.932	0.935	0.744	0.750	0.824	0.812
D16S539	0.894	0.919	0.921	0.928	0.768	0.812	0.769	0.800
D7S820	0.922	0.924	0.907	0.923	0.739	0.873	0.797	0.795
D13S317	0.910	0.916	0.928	0.941	0.600	0.747	0.780	0.819
D5S818	0.864	0.875	0.872	0.883	0.593	0.720	0.724	0.734
Total	>0.999	>0.999	>0.999	>0.999				

\* Unbiased test.

for Bengali populations respectively (data not shown). In comparison of Caucasians to Punjabi 9 out of 14 loci were statistically similar, while a comparison between Bengali's and Caucasian revealed 6 statistically similar loci out of 14 (Data not shown). This indicates that, statistically, both Punjabi and Bengali populations are more similar to each other than to Caucasians or African Americans. This is supported in literature with data from allozymes and recently, SNP's (Single-nucleotide-polymorphism) (8–10).

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