

# Role of HLA antigens in Rh (D) alloimmunized pregnant women from Mumbai, Maharashtra, India

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Immunogenetic studies in various diseases provide potential genetic markers. We have studied the incidence of HLA A, B, C, DR and DQ loci antigen in Rh (D) antigen isoimmunized mothers compared to those nonimmunized isoimmunized Rh negative mothers. Seventy six mothers who were immunized to Rh (D) antigen due to pregnancy (responders) and fifty four mothers who did not develop Rh (D) isoimmunization despite positive pregnancies (nonresponders) were selected for the study. Standard methods of serological HLA typing, ABO and Rh (D) groups, and screening for Rh D antibodies were used. 392 unrelated individuals from the population were compared as controls. In addition 45 unrelated individuals from the same population were typed for HLA DRB and DQB gene using PCR-SSP kits. The genotype frequencies of HLA A2, A3, A28, B13, B17, B35, B52, B60, Cw2, Cw6, DR4, and DQ3 were significantly increased, while the frequencies of the HLA A11, A29, A31, B7, B37, B51, Cw1 and DR9 were decreased in the responder women when compared to the non-responder women. HLA A30 (19) split antigen was not identified in immunized women while HLA A23 (9) split antigen was not identified in non immunized women. HLA A3, B17, Cw2 and DR4 showed a significant relative risk among the immunized responder women. When compared with Rh immunized women (responders) reported from USA, England and Hungary the phenotype frequencies of HLA A11, A24, A28, B5, B17, B40, DR2 and DR5 were increased while HLA A23, B8, B18, and DR6 were decreased in the Indian Rh immunized women. Two locus haplotype frequency analysis observed among the responders women revealed that among the significant haplotypes expressed A2-B5, B7-Cw1, DR2-DQ1 were highly significant haplotypes in positive linkage, while A1-B5, and A1-B7 were in significant negative linkage disequilibrium. The haplotype frequencies were  $\leq$  one when these common haplotypes were compared with control population. Thus in the present study it is evident that the inheritance of HLA A3, B17, Cw2 and DR4 increases the relative risk factor by 2-6 times among Indian Rh isoimmunized women. Further, it is evident that there are significant differences in the observed HLA antigen frequencies and two locus haplotypes in Rh isoimmunized women when compared to women from USA, UK and Hungary due to extreme HLA polymorphism in different populations of the world.

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## 1. Introduction

The Rh (D) antigen is a potent immunogen but only a small portion of Rh (D) negative pregnant individuals respond to it (Mollison *et al* 1993). Several factors are

known to influence Rh immunization. In our earlier report (Gupte and Kulkarni 1994) we suggested that ABO incompatibility and Rh-negative pregnancies have lesser impact than the parity of the women in Rh immunization. Transplacental hemorrhage is the major

**Keywords.** Haplotype frequency; HLA antigen; Indian women; linkage disequilibrium; Rh (D) isoimmunization

source of Rh immunization (Woodrow 1970; Clarke and McConnel 1972). Before the discovery of anti D immunoglobulins, Walker (1958) and Boggs *et al* (1963) reported a 4.8% incidence of Rh immunization among Rh negative mothers. In India, about 5% of the population are Rh (D) negative and 1.7% of Rh (D) negative pregnant women develop Rh antibodies (Gupte and Kulkarni 1994). Since majority of the patients are not covered by health insurance and some Rh (D) negative mothers are unable to buy costly anti D immunoglobulins, we are still far from eradicating Rh alloimmunization from India.

Immunogenetic studies in many diseases have provided potential genetic markers for immune response studies (Tiwari and Terasaki 1985). During the international workshop held in 1972 no association between HLA and immune response to Rh (D) antigen was reported (Petrayani *et al* 1974). Later studies have revealed positive as well as negative correlation with class I and class II antigens (Van Rood *et al* 1975; Murray *et al* 1976; Darke 1977; Darke *et al* 1983; Kruskall *et al* 1990) with Rh (D) isoimmunization from different parts of the world. Further, HLA phenotyping of women known to have anti-D antibodies early in pregnancy seems to be an effective way to assess the probability of severe haemolytic disease of the New Born (Hilden *et al* 1995; Neppert *et al* 1999). As there is a fairly high incidence of haemolytic disease of the new born among Indian women and a significant difference in the HLA antigen and haplotype frequencies in different populations, and a paucity of data on the impact of Rh (D) isoimmunization in Indian women we carried out the present study.

## 2. Materials and methods

### 2.1 Samples

One hundred and thirty Rh (D) negative women attending the antenatal clinic at Nowrosjee Wadia Maternity Hospital, Mumbai between 1994–1998 were selected for this study. They were divided into responders and non-responders for the Rh antigen D depending on whether they developed demonstrable anti D antibody after one or more pregnancies with Rh (D) positive fetus. They were immunologically separated into two groups as follows: Group I: Seventy-six Rh (D) immunized women (responders) were referred to the hospital from small towns and the mothers were not aware of their Rh status. Group II: Fifty four non-immunized pregnant women were retrospectively selected from the hospital out of which 41 had more than 2 Rh (D) positive pregnancies but were not administered prophylactic anti-D and yet did not respond to D antigen. Thirteen women who had large (> 10 ml) fetal cell leakage during the first pregnancy formed the remaining of non-responders group.

These patients also did not receive anti-D immunoglobulin and they were assessed for isoimmunization every week up to 3 months after delivery. From the same ethnic population 392 unrelated individuals were compared as control (Shankarkumar *et al* 1999).

### 2.2 Serology

ABO and Rh (D) grouping were performed in the samples collected by the standard method (Bhatia 1977). The enzyme (papain-cysteine) technique was used to detect the Rh antibodies. Detection of fetal cell leakage on stained EDTA blood smear was done by Nierhaus Betke technique (Dacie and Lewis 1967). The amount of fetal cell leakage was calculated using the standard graph published by Mehta *et al* (1976).

### 2.3 HLA typing

Ten to fifteen millilitres of venous blood (in heparin 50 IU/ML) was collected in a sterile tube from each pregnant mother. The lymphocytes were isolated by density gradient centrifugation on Histopaque (Boyum 1968). HLA A, B, and C locus antigens were identified by NIH two stage microlymphocytotoxicity assay (Terasaki and McClland 1964). HLA DR and DQ locus typing were performed on B-lymphocytes isolated using a miniature nylon wool column (Manikasundari *et al* 1984) and a long incubation method. A total of 238 HLA antisera were used for defining 16 specificities of HLA A locus, 22 for HLA B locus, 5 for HLA C locus, 10 for HLA DR locus and 3 for HLA DQ locus antigens. These antisera were commercial (Biotest, Germany; Pelfreez, USA); gifted (NIH, Bethesda) as well as indigenous (Shankarkumar *et al* 1998) in origin. The typing tray included a minimum of 3 antisera for each serotype specificity.

### 2.4 Statistical analysis

The phenotype frequency (PF); genotype frequency (GF); two locus haplotype frequency (HF); coefficient of linkage disequilibrium ( $\delta$ ) and 't' value were calculated following the methods described by Baur and Daniloves (1980).

## 3. Results

### 3.1 HLA gene frequencies in Rh negative women

The percentage gene frequencies of HLA A, B, C, DR and DQ loci antigens among the 76 immunized

(responders) and the 54 non-immunized (nonresponders) pregnant women and normal controls are presented in table 1. Phenotype frequencies of HLA A1, A9, A11, A19, A24, B5, B40, CW3, CW4, DR2, DR5, DR7 and DQ1 were increased while that of HLA A28, B8, B13, B16, B18, CW1, CW2, DR6 and DR9 were decreased in both responders and non-responders women. An increased genotype frequency for HLA A2, A3, A28, B13, B17, B35, B52, B60, Cw2, Cw6, DR4 and DQ3 while a decreased frequency for HLA A11, A29, A31, B7, B37, B51, Cw1 and DR9 was observed in the immunized (responders) women. HLA A30 (19) split antigen was not identified in immunized women while HLA A23 (9) split antigen was not identified in non-immunized women. HLA antigens HLA A25, A33, A34, A36, B14, B38 (16), B39 (16), B45 (12), B49 (21), B50 (21), B54 (22), B56 (22), Cw5, Cw7, Cw8, DR8 and DR12 (5) were not identified in both the responders and non-responders women. HLA A3, B17, Cw2 and DR4 showed a significant relative risk among the immunized women.

Our preliminary molecular typing using the PCR-SSP technique for HLA DRB and DQB gene on 45 samples from the same ethnic origin revealed that HLA DRB1\*02,

**Table 1.** HLA A, B, C, DR and DQ gene frequencies of Rh negative women compared to controls from Mumbai, India.

HLA antigens	Controls %GF N = 392	Responder %GF N = 76	Non responder %GF N = 54	Relative risk
A1	13.70	16.50	19.50	0.80
A2	15.60	15.70	9.70	1.75
A3	6.70	9.70	3.80	2.60
A9	18.60	12.60	12.90	0.97
A10	4.00	6.80	9.70	0.67
A11	13.30	14.20	20.70	0.61
A19	12.40	11.10	13.90	0.76
A23	0.90	0.70	0.00	0.00
A24	17.50	11.90	11.80	1.00
A25	0.00	0.00	0.00	0.00
A26	4.00	2.70	6.70	0.39
A28	4.70	8.20	4.70	1.74
A29	2.50	2.70	6.70	0.39
A30	3.40	0.00	0.90	0.00
A31	0.30	2.00	3.80	0.53
A32	2.20	1.30	0.90	1.20
A33	3.50	0.00	0.00	0.00
A34	0.00	0.00	0.00	0.00
A36	0.00	0.00	0.00	0.00
A-	18.70	11.90	12.90	-
B5	16.90	17.30	18.40	0.92
B7	14.90	5.40	10.80	0.47
B8	2.70	2.00	1.90	1.00
B12	8.10	10.40	8.70	1.21
B13	4.00	3.30	1.90	1.62
B14	0.50	0.00	0.00	0.00
B15	5.00	5.40	4.70	1.12
B16	0.80	0.70	0.90	0.71
B17	9.10	10.40	4.70	2.27
B18	1.20	1.30	0.90	1.20
B21	1.30	1.30	4.70	0.30
B22	2.80	2.00	2.80	0.70
B27	2.10	4.70	2.80	1.59
B35	11.60	12.60	8.70	1.51
B37	0.60	2.00	3.80	0.53
B38	0.50	0.00	0.00	0.00
B39	0.30	0.00	0.00	0.00
B40	13.50	19.70	17.20	1.19
B44	8.10	9.70	8.70	1.11
B45	0.00	0.00	0.00	0.00
B49	1.20	0.00	0.00	0.00
B50	0.10	0.00	0.00	0.00
B51	8.10	6.10	12.90	0.43
B52	1.80	5.40	2.80	1.83
B53	0.00	0.00	0.00	0.00
B54	0.00	0.00	0.00	0.00
B55	1.00	1.30	1.90	0.70
B56	0.00	0.00	0.00	0.00
B60	3.70	6.80	3.80	1.77
B61	9.80	9.00	12.90	0.65
B62	0.00	0.00	0.00	0.00
B63	0.00	0.00	0.00	0.00
B73	0.10	0.70	0.00	0.00
B-	10.60	6.80	12.90	-
Cw1 <sup>a</sup>	4.00	2.00	4.70	0.43
Cw2	1.40	3.30	0.90	2.74
Cw3	19.00	13.40	18.40	0.67
Cw4	15.00	14.20	13.90	1.01
Cw5	0.00	0.00	0.00	0.00
Cw6	10.60	14.20	8.70	1.74
Cw7	0.00	0.00	0.00	0.00
Cw8	0.00	0.00	0.00	0.00
Cw-	56.50	58.60	57.00	-
DR1 <sup>b</sup>	8.60	10.40	12.90	0.77
DR2	26.90	24.80	33.30	0.62
DR3	6.20	7.50	5.70	1.31
DR4	5.90	5.40	1.90	2.61
DR5	15.10	19.70	19.50	1.01
DR6	7.10	2.00	0.90	1.70
DR7	13.70	15.70	17.20	0.88
DR8	0.00	0.00	0.00	0.00
DR9	2.00	2.00	3.80	0.53
DR10	6.50	7.50	4.70	1.58
DR11	4.70	7.50	6.70	1.11
DR12	0.00	0.00	0.00	0.00
DR-	15.70	12.60	9.70	-
DQ1	46.90	41.10	45.60	0.80
DQ2	16.70	18.40	17.20	1.08
DQ3	13.90	12.80	8.70	1.54
DQ-	38.20	37.80	42.30	-

%GF, Percentage genotype frequency; RR, Relative risk (> 2 is significant); <sup>a</sup>N = 180, <sup>b</sup>N = 176.

DRB1\*16, DRB1\*04, DRB1\*0701, DRB1\*11, DRB1\*1105, DRB1\*14, DQB1\*02, DQB1\*0203, DQB1\*03 and DQB1\*0301 were increased while DRB1\*0103, DRB1\*1106, DRB1\*1001, DQB1\*05 and DQB1\*06 were decreased in their genotype frequencies.

**Table 2.** Phenotype frequencies (in percentage) of Indian Rh immunized (responders) women compared with other Rh immunized women in the world.

HLA	Present study (N = 76)	Rh women USA <sup>a</sup> (N = 38)	Rh women UK <sup>b</sup> (N = 288)	Rh women Hungary <sup>c</sup> (N = 128)
A1	30.3	17.0	38.8	28.1
A2	28.9	21.0	41.6	50.0
A3	18.4	14.0	35.7	17.2
A9	23.7	NT	15.3	22.7
A23	1.3	NT	NT	21.9
A24	22.4	13.0	NT	0.8
A10	13.2	NT	7.6	16.4
A25	0.0	NT	NT	10.9
A26	5.3	NT	NT	5.5
A34	NT	NT	NT	NT
A66	NT	NT	NT	NT
A11	26.3	7.0	12.5	14.5
A19	21.1	NT	NT	NT
A29	5.3	NT	8.2	7.8
A30	0.0	7.0	NT	10.2
A31	3.9	NT	NT	0.0
A32	2.6	NT	NT	7.0
A33	0.0	NT	NT	NT
A74	NT	NT	NT	NT
A28	15.8	NT	9.4	8.6
A68	NT	NT	NT	NT
A69	NT	NT	NT	NT
A36	NT	NT	NT	NT
A43	NT	NT	NT	NT
A80/A-	22.4	0.0	0.0	0.0
B5	31.6	NT	4.2	12.5
B51	11.8	NT	NT	NT
B52	10.5	NT	NT	NT
B7	10.5	18.0	32.3	18.8
B8	3.9	17.0	32.6	11.7
B12	19.7	8.0	28.5	25.0
B44	18.4	NT	NT	NT
B45	NT	NT	NT	NT
B13	6.6	4.0	6.3	7.8
B14	0.0	8.0	11.1	3.9
B64	NT	NT	NT	NT
B65	NT	NT	NT	NT
B15	10.5	17.0	13.5	8.6
B62	NT	NT	NT	NT
B63	NT	NT	NT	NT
B75	NT	NT	NT	NT
B76	NT	NT	NT	NT
B77	NT	NT	NT	NT
B16	1.3	0.0	NT	4.7
B38	NT	NT	NT	NT
B39	NT	NT	NT	NT
B17	19.7	NT	7.3	3.9
B57	0.0	NT	NT	NT
B58	0.0	NT	NT	NT

HLA	Present study (N = 76)	Rh women USA <sup>a</sup> (N = 38)	Rh women UK <sup>b</sup> (N = 288)	Rh women Hungary <sup>c</sup> (N = 128)
B18	2.6	17.0	7.3	16.4
B21	2.6	NT	3.5	5.5
B49	NT	NT	NT	NT
B50	NT	NT	NT	NT
B22	3.9	NT	NT	1.6
B54	0.0	NT	NT	NT
B55	2.6	NT	NT	NT
B56	0.0	NT	NT	NT
B27	9.2	NT	7.3	8.6
B35	23.7	NT	14.9	29.7
B37	0.0	NT	NT	NT
B40	35.5	NT	11.5	7.8
B60	13.2	NT	NT	NT
B61	17.1	NT	NT	NT
B41	NT	NT	NT	NT
B42	NT	NT	NT	NT
B46	NT	NT	NT	NT
B47	NT	NT	NT	NT
B48	NT	NT	NT	NT
B53	NT	NT	NT	NT
B59	NT	NT	NT	NT
B67	NT	NT	NT	NT
B70	NT	NT	NT	NT
B71	NT	NT	NT	NT
B72	NT	NT	NT	NT
B73	1.3	NT	NT	NT
B78	NT	NT	NT	NT
B81	NT	NT	NT	NT
B-	13.2	0.0	0.0	0.0
Cw1	3.9	NT	NT	NT
Cw2	6.6	NT	NT	NT
Cw3	25.0	NT	NT	NT
Cw4	26.3	NT	NT	NT
Cw5	NT	NT	NT	NT
Cw6	26.3	NT	NT	NT
Cw7	NT	NT	NT	NT
Cw8	NT	NT	NT	NT
Cw-	82.9	0.0	0.0	0.0
DR1	19.7	8.0	NT	NT
DR2	43.4	26.0	NT	NT
DR3	14.5	19.0	NT	NT
DR4	10.5	17.0	NT	NT
DR5	35.5	4.0	NT	NT
DR6	3.9	5.0	NT	NT
DR7	28.9	16.0	NT	NT
DR8	0.0	NT	NT	NT
DR9	3.9	NT	NT	NT
DR10	14.5	NT	NT	NT
DR11	14.5	NT	NT	NT
DR12	0.0	NT	NT	NT
DR-	23.7	0.0	NT	NT
DQ1	65.3	NT	NT	NT
DQ2	33.3	NT	NT	NT
DQ3	24.0	NT	NT	NT
DQ-	61.3	0.0	NT	NT

<sup>a</sup>Kruskall et al 1990; <sup>b</sup>Murray et al 1976; <sup>c</sup>Petryni et al 1974; NT, Not tested.

### 3.2 Comparison of phenotype frequencies of Rh immunized women

When compared with Rh immunized women reported from USA, England and Hungary the phenotype frequencies of HLA A11, A24, A28, B5, B17, B40, DR2 and DR5 were increased while HLA A23, B8, B18 and DR6 were decreased in the Rh immunized pregnant women from Mumbai (table 2). However, most of the newly identified and defined WHO nomenclature for HLA antigen 1998 (Bodmer *et al* 1999) have not been tested in these studies.

### 3.3 Significant haplotype frequencies and linkage disequilibrium identified

Two-locus haplotype frequency analysis among the immunized pregnant mothers and significant linkage disequilibrium identified are presented in table 3. It was

interesting to observe that haplotypes A2–B5, B7–Cw1 and DR2–DQ1 showed the highest significant “*t*” value among the positive linkage disequilibrium haplotypes identified. Haplotypes A1–B5, A1–B7, A2–B8, B5–Cw2, and B7–Cw3, and DR1–DQ2 and DR2–DQ3 had significant negative linkage disequilibrium. When these observed significant haplotypes identified were compared with the normal control population haplotypes where the Rh immunized women were selected the frequencies of these haplotypes was less than 1%. The difference was highly significant ( $P < 0.001$ ).

## 4. Discussion

The involvement of HLA antigen in immune response has been established. It is now believed that there is a large difference in HLA antigen repertoire distribution among various populations, which determines their

**Table 3.** Haplotype frequencies and significant linkage disequilibrium identified in Rh (D) immunized Indian women compared with controls.

Haplotypes	Immunized women				Controls	
	HF	Delta ( $\Delta$ )	<i>t</i> value	$Ki^2$	HF	Delta ( $\Delta$ )
Positive LD						
A2–B5	96.2	59.1	6.71	46.17		*
A19–B7	35.0	23.3	3.70	18.89		*
A26–B8	19.9	16.2	3.41	26.95	5.3	4.2
A30–B12	34.4	26.9	2.88	14.69	7.2	4.5
A1–B17	18.7	13.1	2.80	11.24	31.2	18.5
A29–B7	11.2	9.2	2.57	14.69	13.7	9.9
A1–B37	19.9	16.6	2.52	11.16	4.1	3.0
A11–B35	46.0	28.1	2.50	6.82	29.3	13.8
A10–B8	22.6	13.3	2.42	7.10	21.3	18.2
A24–B52	23.7	17.3	2.10	6.18	4.1	3.7
B7–Cw1	109.3	69.4	6.97	48.48		*
B12–Cw6	56.8	42.1	2.60	8.88		*
B35–Cw4	71.0	53.1	3.06	12.47	24.6	20.0
DR1–DQ9	16.0	11.9	2.14	6.92	19.4	12.3
DR2–DQ1	147.8	79.4	6.27	30.45		*
DR7–DQ2	53.7	34.0	3.52	14.66		*
DR5–DQ3	33.6	19.7	2.40	6.36		*
DR11–DQ3	32.1	26.8	3.57	30.52	6.3	4.5
Negative LD						
A1–B5	75.0	–23.4	–2.83	8.49		*
A1–B7	10.0	–12.7	–2.46	5.65	13.6	–17.0
A2–B8	24.0	–11.7	–2.20	4.79	1.2	–57.0
B5–Cw2	3.0	–14.4	–2.41	5.49		*
B7–Cw3	14.0	–15.8	–2.40	5.68		*
DR1–DQ2	16.0	–15.0	–2.02	4.15		*
DR2–DQ3	10.0	–18.3	–2.22	5.29	2.3	–75.0

HF, Haplotype frequency/1000 samples; Delta ( $\Delta$ ), linkage disequilibrium/1000 samples; *t* value > 2 indicates positive linkage disequilibrium.

\*HF less than 1 ( $P \leq 0.001$ ).

immune response capability. Rh isoimmunization is an important health problem, which has been successfully prevented in the Western World, by Rh immunoglobulin prophylaxis in antipartum and immediate post partum period. The programmes have been so successful, that it is unlikely that enough numbers of Rh isoimmunized patients would be available to do a similar study in the West.

In India too, the effect of this prophylaxis is quite substantial and it will be extremely difficult if not impossible to carry out a large scale study on effect of HLA antigen repertoire on Rh isoimmunization. Our suspicion was also confirmed by a Medline search on this topic from 1965 to till date using various key words like, Rh isoimmunization, HDN, HLA antigen etc. There was only eight references covering HLA and Rh isoimmunization with no Indian studies.

Since 1957 our Institute has been running an Rh clinic at the Nowrosjee Wadia Maternity Hospital, where Rh typing of all women registered at the antenatal outpatient department is carried out. Our earlier studies (Gupte *et al* 1983; Gupte and Bhatia 1984; Kulkarni *et al* 1987; Gupte and Kulkarni 1994) based on the analysis of data collected from 1969 reported 5% Rh immunized rate until the year 1977, which initially reduced to 3.3% in 1980 and further reduced to 1.7% in 1992. This decline in Rh immunized incidence has been due to efficient family planning procedures and exclusive use of anti-D immunoglobulins. Thus in India only 1.7% of Rh (D) negative women produce Rh antibodies (Gupte and Kulkarni 1994). Although prophylaxis anti-D immunoglobulins is advised postnatally, it is not administered during pregnancy. Since a majority of the patients are covered by health insurance, and anti-D IgG is still inaffordable, Rh alloimmunization in India is still far from eradication.

Studies identifying a possible relationship between HLA phenotype and immune responses to a variety of antigens was of great interest (Tiwari and Terasaki 1985). Studies for the formation of anti-D in women who had been sensitized to Rh antigen as a result of pregnancy have been reported. Further, a number of groups have studied the relationship between MHC alleles and anti-D formation following either transfusion or pregnancy. In 1976 Murray *et al* (1976) studied 288 women sensitized to Rh due to pregnancy and observed a significant increase in HLA A3 antigen. Darke *et al* (1977) also noted a significant increase in HLA A3 among 84 women who were sensitized during pregnancy. Further, in 1983 among 26 transfused immunized men in comparison to non-immunized controls Darke *et al* (1983) found an increase of HLA DR6 antigen. Wojtulewicz-kurkus *et al* (1981) reported that 5 of 21 Rh negative men who made anti-D after transfusion were

HLA DR6 antigen positive, when compared to 15 Rh negative men who did not produce anti-D and were HLA DR6 antigen negative.

By contrast, Hors *et al* (1974) who immunized 93 Rh negative men; Petranyi *et al* (1974) immunized Rh negative men with Rh positive red cells and compared 37 high antibody responders to 39 non responders did not find correlation between HLA. Van Rood *et al* (1975) who studied 37 Rh negative women sensitized after multiple Rh positive pregnancies found no statistical correlation between antibody responders and HLA alleles. Raum *et al* (1984) studied 52 Rh sensitized individuals and reported a possible association between the complement allele BF\*F1 and antibody response. Further, Kruskall *et al* (1990) studying 38 Rh sensitized women found no significant deviations of HLA class I, class II and class III alleles.

Further no conclusive association has been reported between HLA phenotype and immune response to immunogenic Rh (D) antigen although in literature HLA A3 was increased when various studies were pooled (Tewari and Terasaki 1985). The likelihood of finding apparently significant differences between populations in HLA disease association studies is because of the extreme allelic polymorphism and genetic diversity expressed by these HLA antigens in various populations (Shankarkumar *et al* 1999). Thus in the present study, inheritance of HLA A3, B17, Cw2 and DR4 increased the risk of Rh isoimmunization factor by 2.6 (confidence interval: 1.2–3.9).

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