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DNA HLA-DRB1 ANALYSIS IN CHILDREN OF POSITIVE MOTHERS AND ESTIMATED RISK OF VERTICAL HIV TRANSMISSION

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SUMMARY

RFLP HLA-DRB1 analysis was performed on a total of 83 children born from HIV-infected mothers, 35 of whom were shown to be HIV-infected, while 48 reverted from seropositivity to seronegativity, indicating that they were not infected. Moreover, 89 healthy children were used as controls. It has been found that DRB1-14a and DRB1-13a.4 alleles were not present in the HIV-infected children, but were found in the sero-reverted (HIV-uninfected) children (in the proportion of 9·6 per cent and 5·3 per cent, respectively), and in the controls (5·6 per cent and 3·9 per cent, respectively). The possible correlation between DR and risk of HIV transmission from mother to baby was analysed considering every single allele, estimated by the ratio between the number of infected children and the number of all children born from seropositive mothers. There was also introduced a statistic G for the control of 'statistical validity' of data.

KEY WORDS HLA-DNA typing Paediatric AIDS Vertical transmission of HIV Risk estimation

INTRODUCTION

The genetic susceptibility to HIV infection and subsequent AIDS disease is still a controversial issue (Enlow *et al.*, 1983; Jeannet *et al.*, 1989), and no strict HLA linkage has been found. In fact most studies have associated HLA specificities with HIV-related diseases. Examples include the association between HLA-DR2 or DR5 and Kaposi's sarcoma (Pollak *et al.*, 1983; Smeraldi *et al.*, 1986); the association between HLA-DR5 and CD8 lymphocytosis and sicca syndrome (Iescu *et al.*, 1989); HLA-A1, -B8, -DR3, and rapid decline in T4 cells coupled with the development (in haemophiliacs) of HIV-related symptoms within a short time after being infected (Steel *et al.*, 1988; Mann *et al.*, 1988; Kaslow *et al.*, 1990). Moreover, more recently the frequency of HLA-DR3 was found to be three times higher in HIV-infected children born from infected mothers compared to HIV-uninfected infants born to infected mothers (Kilpatrick *et al.*, 1991). However, a similar analysis conducted in this study with a larger sample population did not identify any significant difference in HLA-DR3 frequency between the two groups.

Tissue typing for HLA-A, -B and -DR antigens gives a general idea of the haplotype. However, considering AIDS pathogenesis and the involvement of $CD4^+$ T

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cells which express and recognize HIV antigens in the context of HLA class II molecules, molecular typing using RFLP of HLA class II gene variations would be more suitable for investigating the association between HLA and HIV infection.

Thus, in an attempt to determine one of the causes of the 'selectivity' encountered in transplacental transmission, molecular typing was carried out on children born from HIV-infected mothers.

SUBJECTS AND METHODS

Peripheral blood lymphocytes (PBL) from 83 newborns or infants born from HIV-1 infected mothers and 89 healthy children were used for molecular typing by RFLP. The HIV-infected mothers were drug users. During the series of follow-up tests carried out using PCR and viral cultures to identify the presence of HIV-infection, it was found that 35 children were HIV-infected, while the other 48 were HIV-uninfected. It needs to be stressed, however, that in our total cohort the vertical transmission rate is about 15 per cent in these infants (Giaquinto *et al.*, 1992). DNA was extracted from each individual's PBL using standard techniques.

The genomic DNA was subjected to restriction enzyme digestion, electrophoresis, and Southern blotting followed by hybridization with specific cDNA coding for the second exon of the HLA-DRB1 gene (Bidwell *et al.*, 1988). Polymorphic fragments were assigned as described in the 'RFLP Standardization Reports' of the tenth HLA workshop (Marcadet *et al.*, 1989). Statistical analysis was performed using the software package VLTSTAT (Accardi *et al.*, 1992).

RESULTS AND DISCUSSION

Table 1 shows the haplotype distribution of 35 HIV-infected and 48 HIV-uninfected children born from HIV-infected mothers (total 83 children).

Table 2 represents the allele distribution in the 83 children. Table 2 also includes allele distribution in a control population of 89 children (178 alleles) all from the same region. The haplotype distribution is extremely variable because of the very wide number of possible allele combinations. That is why, in order to study possible correlations between DRB1 and the risk of vertical HIV transmission, we analyse the data not for DRB1 in specific combinations, but for every DRB1 allele met in any combination at least once.

As can be seen from Table 2, the allele distribution in HIV-infected children is comparable to that of the controls. No single allele occurs with significantly different frequency in the first group. It is noteworthy that the alleles DRB1-14b and DRB1-10 are very infrequent in all three groups under study, while alleles DRB1-14a and DRB1-13a.4 are absent in HIV-infected children, but frequent in the others two groups.

To approximate the real risk of transmission of infection, it is possible to introduce the parameter R of risk for every allele as

$$R = \frac{\text{number of infected children}}{\text{number of all children}}$$

Table 1. HLA-DR haplotype distribution in children born from HIV-infected mothers (number of cases typed)

Children HIV-infected			Children HIV-uninfected		
DRBI allele combination		Number of cases (35)	DRBI allele combination		Number of cases (48)
1	1	1	1	15	4
1	13a·1	2	1	12	1
1	4	1	1	17·1	1
1	17·2	1	1	17·2	1
1	11	1	11	4	2
11	11	2	11	13a·4	4
11	8	1	11	14a	4
11	15	1	11	11	3
11	16	1	11	10	1
11	13a·1	1	11	16	2
11	7·1	1	11	7·2	2
11	7·2	1	11	7·1	1
11	17·2	1	11	17·1	2
11	17·1	1	11	8	1
17·2	7·2	1	7·2	7·2	1
17·2	15	1	7·2	17·1	2
17·2	17·2	1	7·2	15	1
17·2	7·1	1	4	13a·4	1
7·1	13a·1	1	4	14a	1
7·1	13b	1	15	17·2	1
7·1	4	1	15	16	2
7·2	13a·1	1	15	14a	1
7·2	15	1	12	14a	1
7·2	12	1	12	13a·1	1
7·2	8	1	17·2	17·2	1
15	15	1	17·2	14a	1
15	17·1	1	17·2	8	1
15	4	2	13a·3	16	1
15	16	1	13a·3	17·1	1
4	13a·3	1	17·1	14a	1
4	17·1	1	7·1	17·1	1
8	13a·1	1			

Applying the software package VLTSTAT, we compute the risk R and its grade of confidence G (Table 3) defined below.

In order to measure how accurately the parameter R estimates the real risk, let us determine a 'confidence interval' for the parameter R in the usual way: fixing a number $\alpha: 0 \leqslant \alpha \leqslant 1$ which will denote some probability termed the 'confidence level', we find the interval $[B, T]$ as follows: the parameter R belongs to the interval $[B, T]$ with the given probability α, that is:

$$\text{Prob.}\{B \leqslant R \leqslant t\} = \alpha$$

For calculation of the parameters R, B, T we shall apply the rules of binomial distribution for random samples and fix the confidence level α to be 0·8 (80 per cent).

Table 2. HLA class II allele distribution in children born from HIV-infected mothers and controls (number of chromosomes examined)

DRBI allele	HIV-infected (70)	HIV-uninfected (96)	Controls (178)
1	7	7	19
15	9	9	16
16	2	5	15
17·1	3	8	8
17·2	7	6	7
4	6	4	8
11	13	25	42
12	1	3	7
13a·3	1	2	6
13a·1	6	1	5
14b	0	0	3
13a·4	0	5	7
14a	0	9	10
13b	1	0	2
7·1	5	2	7
7·2	6	7	9
8	3	2	4
10	0	1	3

Table 3. Values of the parameters *R* and *G* and of some accompanying characteristics

DRBI allele	Number of cases	Statistical equivalence	Statistics HIV+/HIV−	Risk, *R*	Grade of confidence, *G*
11	38	–	13/25	0·34	0·78
14a	9	38	0/9	0	0·77
15	18	–	9/9	0·50	0·66
13a·4	5	16	0/5	0	0·63
1	14	–	7/7	0·50	0·60
17·1	11	13	3/8	0·27	0·59
17·2	13	–	7/6	0·54	0·59
7·2	13	–	6/7	0·46	0·59
13a·1	7	11	6/1	0·86	0·56
4	10	–	6/4	0·60	0·54
16	7	–	2/5	0·29	0·48
7·1	7	–	5/2	0·71	0·48
8	5	–	3/2	0·60	0·36
12	4	–	1/3	0·25	0·35
13a·3	3	–	1/2	0·33	0·23

Now our problem is to calculate the grade of confidence for our estimations. For given confidence level α:

$$L = T - B \text{ (top} - \text{bottom)}$$

the length of confidence level. As the estimate of the grade of confidence of our estimations we take the value

$$G = 1 - L$$

This parameter takes its values in the interval [0, 1] and is equal to 1 when the confidence level has the length 0, i. e. the estimate is precise and vice versa, $G = 0$ if the estimate is dispersed in the whole interval [0, 1]. The computation of G presented in Table 3 has been done for the level of confidence $\alpha = 0{\cdot}8$ (80 per cent). But in fact, we have verified that one can choose any value $\alpha \in [0{\cdot}7, 0{\cdot}98]$ without any change of qualitative picture.

Estimation of the grade of confidence for the risk parameter R by the length of confidence interval (statistic G) is the most appropriate in this case. Other statistical treatments are less adequate; for example, dispersion: if calculated for alleles 13a.4 and 14a, both the risks R and the dispersions D are equal to 0, but the statistics G in this case differ from 0 and have values corresponding to the size of random samples.

The results of evaluation of risks R and their grades of confidence are given in the Table 3. The meaning of the column 'Statistical equivalence' is explained below. For convenience, the lines of Table 3 are ordered by values of the parameter G. Hence, the confidence of results is highest for the first line and worst for the last line. The values reported in the column 'Number of cases', are not in strictly decreasing order, indicating that increasing the number of observations does not always increase confidence. For example, the confidence of risk estimation for DRB1-11 and DRB1-14a are almost the same, despite very different volumes of samples (38 data against 9 data). It can be explained by the fact that in the second case we have 'homogeneity of outcomes': all 9 babies have seroreverted. The probability of such 'coincidence' is:

$$\text{Prob. (0 infected and N seroconverted)} = \beta^N$$

where β is the probability of being seroreverted for a baby with allele 14a; so when β differs markedly from 1, this probability decreases to 0 very rapidly. This fact confirms again the adequacy of the statistic G. Therefore, in the cases of 'homogeneous outcomes', the confidence of risk essentially increases and, as we have mentioned, only 9 data in the 13th case are 'statistically equivalent' to 38 data in the 7th case of 'mixed outcomes'.

Let us consider the first four lines of Table 3, as the most confident results (statistical validity at least 16 observations). We find that for DRB1-14a and for DRB1-13·4 the risk of transmission is minimal while for DRB1-11 and for DRB1-15 it is not so (the empirical probabilities of transmission are correspondingly 1/3 and 1/2).

Moreover, considering the results where the confidence G, is much lower, for example, alleles DRB1-7.1 and DRB1-7.2, we have rather different risk values (0·71 and 0·46). We have tested the hypothesis that these risks are in fact the same with variation in levels of significance. The result is that they do not coincide for any level.

Although several studies have been performed to associate HLA type with HIV susceptibility and progression of the disease in adults (Steel *et al.*, 1988; Pollack

et al., 1983; Mann *et al.*, 1988), only one study has been published so far on the HLA frequencies of children born of HIV-infected mothers (Kilpatrick *et al.*, 1991). In that study, 8 children out of 53 at risk were infected, and the allele distribution showed that DR3 was three times more common in infected children compared to uninfected. A high frequency of DR3 was also found in HIV-infected adults (Steel *et al.*, 1988).

The study reported in this paper analyses 83 children born of infected mothers and of these 35 were HIV-infected. The HLA specificities were determinated by RFLP technique. The value of such technique for analysis of HLA system lies in its ability to demonstrate allelic variations within specificities that do not show variation at the serological level (Peter *et al.*, 1992). The importance of using DNA analysis is exemplified by the above reported association between DR3 and HIV susceptibility (Steel *et al.*, 1988). DR3 is split at the DNA level into 17·1 and 17·2, which are respectively associated, as mentioned above, with different risk of levels, as determined by the parameter *R*. The conclusion is that the association between DR3 and HIV infection may be tested more rigorously by considering the 17·2 split. Moreover, with RFLP analysis it is possible to identify a strong association of the 14a and 13a.4 alleles which are splits of DR6, with protection against infection (high grade of confidence).

Sequencing of the second exon of the DR region of the B1 chain of those HIV-uninfected children and controls who are carriers of alleles DRB1-14a and DRB1-13a.4 is in progress Identification of specific sequences associated with these peptides would substantiate the possibility that given sequences play an important role in the MHC-mediated immune response and hence in protection against HIV.

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