HLA-DR, DRw52.53, AND DQ HOMOZYGOSITY
IN PATIENTS WITH NEURAL TUBE DEFECTS

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Summary   Studies of the possible relationship between malformations
of the neuraxis and the human major histocompatibility complex have been
contributive to the search for a human equivalent of the murine T/t com-
plex.   We conducted a population and family study in 34 Japanese couples
together with their children with neural tube defects.   HLA-DR homo-
zygosity among the affected children was significantly increased as com-
pared with that in controls.   HLA-DR and DQ maternal-paternal sharing
was also significantly higher than the controls.   Significantly higher fre-
quencies of A11 (father), B35 (mother) and A11 (affected children) were
observed.   These data support the hypothesis that a T/t-like complex in or
near the HLA complex might be etiologically related to neural tube defects.

INTRODUCTION

While the aetiology of neural tube defects (N.T.D.) remains inadequately under-
stood, their occurrence may reasonably be explained by the interaction of environ-
mental factors with genetic predisposition (Carter, 1970; Carter and Evans, 1973;
Fraser, 1976).   As to the genetic predisposition, much attention has been paid to a
restricted region on mouse chromosome 17, which contains an important gene com-
plex controlling stages in early embryogenesis.   It has been known for nearly fifty
years that mutations in the so called T/t complex in mice resulted in different kinds
of congenital malformations (Bennett, 1975; Pious, 1975).   Close chromosomal
proximity of the T/t complex and the H-2 complex (15 crossover units) means that

mutations in alleles of the T system are associated with atypical patterns of inherit-
ance of histocompatibility antigens (Bennett, 1975; Klein and Hammerburg, 1977;
Macri *et al.*, 1981).

H-2 is associated with the reproductive performance by a genetic linkage with
the T/t complex. This complex consists of a series of semidominant alleles which
is highly polymorphic and, if homozygous, leads to stage specific failures in early
embryonic development and fetal death (Bennett, 1975). It has been suggested that
some evolutionary biological relationships between the T/t and H-2 complexes are
present: Certain characteristics of the T/t complex may be due to segregation dis-
tortion of certain allels (the mutant t being favored) and T/t complex-induced sup-
pression of recombination may extend into the H-2 region (Klein and Hammerburg,
1977). Considering the analogies between H-2 and HLA, the presence of a human
analogue of the T/t complex linked to HLA might be postulated based on the evo-
lutionary conservation of many features of the major histocompatibility complex
(Snell *et al.*, 1976). Evidence for a reciprocal temporal relationship between F9
(a public antigen of T/t complex) and H-2 expression in early mouse embryos (Jacob,
1977) and F9 and HLA in human teratomas strengthens this argument (Buc-Caron
et al., 1974; Hogan *et al.*, 1977; Holden *et al.*, 1977).

To examine the hypothesis that a putative T/t complex-like region in or near
the HLA complex is related to neural tube defects in man, the sample of 34 families
with children of spina bifida or anencephaly were studied.

MATERIALS AND METHODS

In the present study 34 N.T.D. affected families from Central Japan were ex-
amined. They were 23 couples with children of spina bifida with myelomeningocele
and 11 couples with stillborns of anencephaly. The children examined were 18
patients with myelomeningocele and five patients with anencephaly. No consan-
guinity was observed. Unaffected sibs of these patients were not available for tissue
typing. All mothers were in their twenties when they gave birth to the patients
during 1978–1984. Obstetrical and gynaecological abnormalities were not observed
among the mothers, and chromosomal and sperm analysis were normal on the fa-
ther's side.

The control consisted of the following; 206 unrelated healthy individuals were
typed on HLA-A and -B antigens, from which 180 were also typed on HLA-DR,
DRw52.53, and DQ antigens. The control families for the comparison of children
homozygosity and maternal-paternal sharing at HLA-A, -B, -DR, -DRw52.53, and
DQ loci were 90 unrelated healthy families (one family included both parents and
two children). The criteria for selection of these 90 control families were that they
were unrelated and each family had at least two healthy children delivered normally
and without a history of involuntary fetal wastage or offspring with neural tube
defects. All control members were from Gifu and Aichi prefectures, Central Japan.

Heparinized blood, taken by venopuncture, provided a mononuclear cell suspension prepared by Ficoll-Hypaque density centrifugation. T and B lymphocytes were isolated according to the thrombin-nylon wool column method (Danilovs et al., 1978). The purities of T and B lymphocytes were checked by sheep erythrocyte rosetting method and reactions with rabbit anti-B antisera. Only B specimens of purity more than 80% were utilized for HLA-DR, DRw52.53, and DQ typing.

All T lymphocytes were tested for eight HLA-A and 19 HLA-B specificities using about two monospecific antisera defining each of the following HLA antigens: A1, A2, A3, A9, A11, A26, A31, and Aw33; and B7, B8, B12, B13, B14, B15, B17, B27, B35, B37, B39, B40, Bw46, Bw48, B51, Bw52, Bw54, Bw55, Bw59, Bw4, and Bw6. HLA-DR, DRw52.53, and DQ typing was performed with B lymphocytes using a panel of 60 platelet-absorbed antisera for the following ten HLA-DR and three HLA-DQ specificities: DR1, DR2, DR3, DR4, DR5, DRw6, DRw7, DRw8, DRw9, and DRw10; and DQw1, DQw2, and DQw3. DP antigens were not included in this study. Tissue-typing was done by the National Institute of Health standard lymphocyte microcytotoxicity test (Terasaki et al., 1978). All antisera were produced and supplied by Tissue Typing Laboratory, Nagoya II Red Cross Hospital, Central Japan.

Statistical significance of differences in gene frequency was subjected to a 2×2 chi-square test with Yates' correction for association. P values were corrected for the number of HLA specificities tested to account for the chance significance. Expression of HLA-A, B, DR, DRw52.53 and DQ antigens of the parents and children of the 34 affected families were compared to those of the 206 healthy individuals. HLA-A, B, DR and DQ maternal-paternal sharing were compared between the 90 control couples and parents of the patients. HLA-A, B, DR, DRw52.53 and DQ homozygosity were compared between the patients and the children of the control familes.

RESULTS

Haplotype segregation in 23 out of 34 N.T.D. affected families are shown in Table 1 and Table 2. Parental phenotypes of the remaining 11 families are shown in Table 3. There was a higher gene frequency of HLA-A11 among the father of the patients than the control of 206 unrelated healthy individuals ($\chi^2 = 16.62$, $p_c < 0.008$, relative risk = 4.50). This was also true for the expression of HLA-B 35 among the mother of the patients ($\chi^2 = 7.55$, $p < 0.01$, relative risk = 3.04). The patients showed a higher gene frequency of HLA-A11 ($\chi^2 = 4.62$, $p < 0.05$, relative risk = 2.70). No significant deviations were found with the antigens on the B locus. There was neither significant difference in the expression of HLA-DR nor -DQ antigens.

Frequencies of antigen sharing at HLA-A and HLA-B loci in N.T.D. families were higher but not significant as compared to those of the 90 control couples. Seventeen couples (50%) shared at least one antigen at A-locus in the 34 N.T.D.

Table 1. HLA haplotype segregation in N.T.D. families affected with DR homozygous children.

	Father	Mother	Affected children
	A, B, C, DR, DQ	A, B, C, DR, DQ	A, B, C, DR, DQ
1.	11,54, 1, 9, 3 / 9, -, -, 4, -	2,54, -, 9, 3 / 9,51, -, 4, -	11,54, 1, 9, 3 / 2,54, -, 9, 3 (male)
2.	9,40, 3, 2, 1 / 2,46, -, 8, -	11,52, 3, 2, - / 33,12, -, 6, 1	9,40, 3, 2, 1 / 11,52, 3, 2, - (male)
3.	9,54, 3, 4, 3 / 2,15, -, 2, 1	9,15, 1, 4, 3 / 11,54, 4, 5, -	9,54, 3, 4, 3 / 9,15, 1, 4, 3 (male)
4.	2,40, 1, 9, 3 / 11, -,-, 5, -	11,40, -, 9, 3 / -, -, -, 4, -	2,40, 1, 9, 3 / 11,40, -, 9, 3 (female)
5.	1,37,-, 4, 3 / 11,39, 3, 2,1	31,40, -, 4, 3 / 9,52, 7, 2, 1	1,37, -, 4, 3 / 31,40, -, 4, 3 (male)
6.	11,51,1, 9, 3 / 31, -, -,-, -	9,40, -, 9, 3 / -,35, -, 4, -	11,51, 1, 9, 3 / 9,40, -, 9, 3 (male)
7.	11,39, 7, 9, 3 / 9, 7, -, 1, 1	31,-, -, 9, 3 / 9,59,-, 4, -	11,39, 7, 9, 3 / 31, -, -, 9, 3 (female)
8.	2,51, -, 2, 1 / 11,54, 1, 6, -	11,39, 7, 2, 1 / 9, 7, -, 1, -	2,51, -, 2, 1 / 11,39, 7, 2, 1 (male)
9.	9,15, 3, 8, 1 / -,48, -, 4, 3	10,35, -, 8, 1 / -,15, 3, 2, -	9,15, 3, 8, 1 / 10,35, -, 8, 1 (female)
10.	2,40, 3, 8, 1 / 10,15, -, 9, 3	2,15, 3, 8, 1 / 10,35, -, 5, 3	2,40, 3, 8, 1 / 2,15, 3, 8, 1 (male)
11.	2,54, 1, 9, 3 / 9,40, 3, 4, -	2,54, 3, 4, 3 / 9, -, 1, 5, -	9,40, 3, 4, - / 2,54, 3, 4, 3 (female)
12.	11,51, -, 9, 3 / 31,40, -, 4, -	2,40, -, 9, 3 / 31, -, 3, -, -	11,51, -, 9, 3 / 2,40, -, 9, 3 (female)

affected families, while the 41 couples (45.6%) did in the 90 controls. At B-locus, sharing of one or two antigens occurred in 11 couples (32.4%) as compared to 17 control couples (18.9%). As are shown in Fig. 1, sharing of one or two antigens on DR-locus was significantly increased: DR sharing was 61.8% among the N.T.D. parents and 30% among the control couples. Twenty-one out of 34 couples with affected children shared at least one antigen ($\chi^2 = 10.49$, $p < 0.001$). DQ sharing was also increased ($\chi^2 = 5.47$, $p < 0.025$). DRw52.53 sharing was not significant (Fig. 1).

As are shown in Table 4, there was a significant increase in HLA-DR homozygosity among the patients when compared to the 180 normal children of the 90

Table 2. HLA haplotype segregation in N.T.D. families affected with DR heterozygous children.

	Father	Mother	Affected children
	A, B, C, DR, DQ	A, B, C, DR, DQ	A, B, C, DR, DQ
1.	$\frac{9, 7, 7, 1, 1}{31,54, 1, 4, 3}$	$\frac{9,52, -, 2, 1}{2,40, -, 9, 3}$	$\frac{9, 7, 7, 1, 1}{9,52, -, 2, 1}$ (male)
2.	$\frac{9, 7, 7, 1, 1}{-,40, -, 9, 3}$	$\frac{2,39, 7, 8, 1}{-,35, -, -, -}$	$\frac{9, 7, 7, 1, 1}{2,39, 7, 8, 1}$ (male)
3.	$\frac{33,12, 1, 6, 1}{31, -, 3, -, -}$	$\frac{9,59, -, 2, 1}{11,54, 1, -, -}$	$\frac{33,12, 1, 6, 1}{9,59, -, 2, 1}$ (male)
4.	$\frac{9,54, 1, 8, 3}{-,35, -, 5, -}$	$\frac{9,40, -, 9, 1}{-,52, -, 2, 3}$	$\frac{9,54, 1, 8, 3}{9,40, -, 9, 1}$ (male)
5.	$\frac{33,12, -, 6, 1}{10,40, 3, 2, -}$	$\frac{2,59, -, 4, 3}{9, 7, -, 1, 1}$	$\frac{33,12, -, 6, 1}{2,59, -, 4, 3}$ (male)
6.	$\frac{9,52, 3, 2, 1}{2,40, -, 9, 3}$	$\frac{10,35, -, 4, 3}{33,12, 3, 6, 1}$	$\frac{9,52, 3, 2, 1}{10,35, -, 4, 3}$ (female)
7.	$\frac{11,15, 4, 4, 3}{9,37, -, -, -}$	$\frac{2,35, 3, 8, 1}{10,40, -, 2, -}$	$\frac{11,15, 4, 4, 3}{2,35, 3, 8, 1}$ (male)
8.	$\frac{9,54, 1, 4, 3}{2,35, -, 8, 1}$	$\frac{33,12, -, 6, 1}{10,15, -, 9, 3}$	$\frac{9,54, 1, 4, 3}{33,12, -, 6, 1}$ (female)
9.	$\frac{2,51, 3, 6, 1}{11,35, -, 5, 3}$	$\frac{2,35, 4, 4, 3}{11,54, 1, 6, 1}$	$\frac{2,51, 3, 6, 1}{2,35, 4, 4, 3}$ (female)
10.	$\frac{-,13, -, 7, 2}{9,40, 3, 6, 1}$	$\frac{1,37, 6, 10, 1}{10,13, 3, 6, -}$	$\frac{-,13, -, 7, 2}{1,37, 6, 10, 1}$ (female)
11.	$\frac{9,35, 3, 4, 3}{11,40, -, 6, 1}$	$\frac{33,17, 3, 6, -}{2,46, 1, -, -}$	$\frac{9,35, 3, 4, 3}{33,17, 3, 6, 1}$ (female)

control families ($\chi^2 = 25.57$, $p < 0.0001$). Twelve out of 23 typed patients were DR homozygotes whereas only 20 in 180 normal children were homozygous for DR. HLA-DQ homozygosity was also increased but the difference was not significant. Among the 11 deceased patients, parental DR sharing occurred in seven families (63.3%) and DQ sharing in all families (100%) (Table 3).

DISCUSSION

The HLA disease associations have been summarized by Dausset (1977), Dausset and Svejgaard (1977), Pietrzyk and Turowski (1976), and Naito (1986). An association, whether strong or weak, implies a genetic contribution to etiology of the particular disease. Earlier investigators studying the genetics of N.T.D. suggested

Table 3. Maternal-paternal DR, DQ sharing in N.T.D. families with affected deceased children (not-typed) N=11/34.

	Father					Mother					Affected children
	A	B	C	DR	DQ	A	B	C	DR	DQ	
1.	2, 9	52, –	1, –	*2, 6	1, –	11, 33	12, –	–, –	*6, –	1, –	Spina bifida (male)
2.	2, 11	40, –	7, –	*2, 5	1, 3	9, –	35, –	3, –	*2, 4	1, 3	Spina bifida (male)
3.	2, 9	52, 48	–, –	*2, –	1, –	2, 9	51, 54	1, –	*2, 4	1, 3	Spina bifida (male)
4.	2, 9	51, 35	3, –	*8, 9	1, 3	9, 11	40, –	–, –	*8, 9	1, 3	Anencephaly (female)
5.	10, 11	39, 40	3, 7	*5, 8	1, 3	9, –	51, 46	1, –	*8, 9	1, 3	Anencephaly (male)
6.	2, 11	35, 54	3, –	*4, 9	3, –	31, 33	54, 12	3, –	*4, 8	1, 3	Anencephaly (female)
7.	9, 10	7, 39	7, –	*1, 2	1, –	2, 9	52, 40	3, –	*2, 5	1, 3	Anencephaly (female)
8.	2, –	48, –	1, 3	4, –	3, –	9, –	51, 35	3, –	2, 5	1, 3	Anencephaly (male)
9.	2, 11	59, 55	1, –	4, –	3, –	9, 33	35, 7	3, –	1, 5	1, 3	Anencephaly (female)
10.	2, 11	55, 40	1, –	4, 9	3, –	9, –	51, 55	1, –	2, 5	1, 3	Spina bifida (male)
11.	2, 11	15, 54	1, 4	4, –	3, –	2, –	35, 40	3, –	2, 9	1, 3	Spina bifida (female)

* DR sharing in 7 out of 11 families.

control (N=90)   affected (N=34)

No. of shared HLA-DR antigens

(63/90) 70.0 }
(26/90) 28.9 } 30.0
(1/90) 1.1 }

0
1
2

38.2 (13/34)
*61.8 { 52.9 (18/34)
        8.9 (3/34)

%70   35   35   70%

No. of shared HLA-DRw52·53 antigens

(37/90) 41.2 }
(49/90) 54.4 } 58.8
(4/90) 4.4 }

0
1
2

32.4 (11/34)
**67.6 { 58.8 (20/34)
         8.8 (3/34)

%70   35   35   70%

No. of shared HLA-DQ antigens

(19/90) 21.1 }
(52/90) 57.8 } 78.9
(19/90) 21.1 }

0
1
2

2.9 (1/34)
***97.1 { 67.7 (23/34)
          29.4 (10/34)

%70   35   35   70%

* $x^2 = 10.49$, p<0.001,   ** $x^2 = 0.80$, N.S.,   *** $x^2 = 5.47$, p<0.025
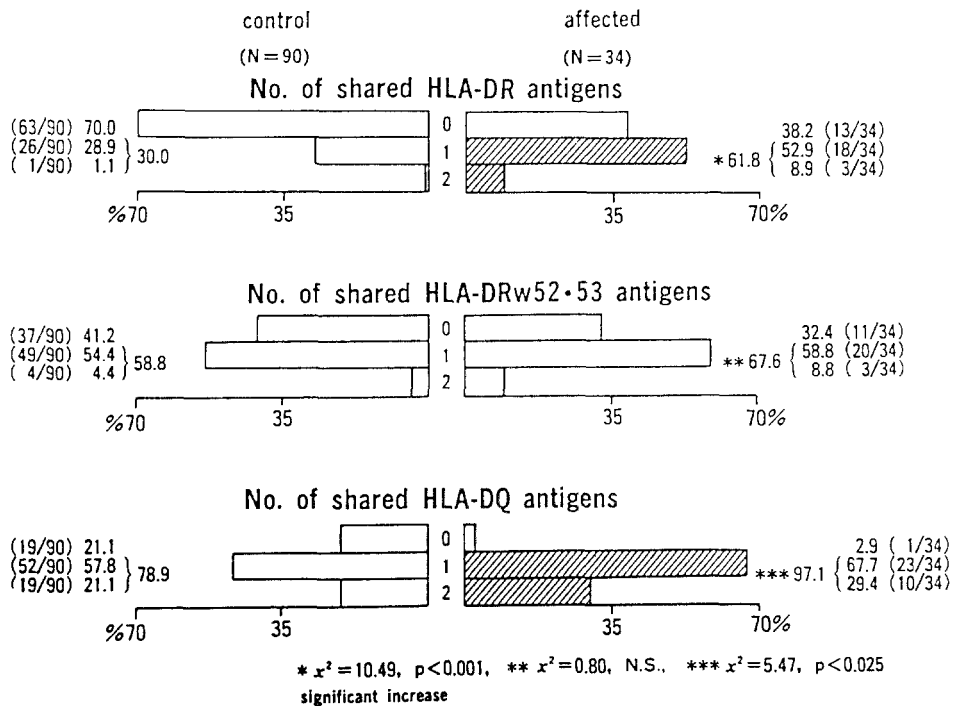significant increase

Fig. 1. Frequencies of shared HLA-DR, DRw52.53, and DQ antigens in parents of N.T.D. and the control couples.

Table 4. HLA-DR, DRw52.53 and DQ homozygosity in N.T.D. affected children.

	N	DR homozygote		DRw52.53 homozygote		DQ homozygote		Statistical significance
Spina bifida : 18		9	(50.0%)	12	(66.7%)	11	(61.1%)	DR homozygosity $\chi^2 = 19.80$, p<0.001
Anencephaly : 5		3	(60.0%)	4	(80.0%)	3	(60.0%)	DR homozygosity $\chi^2 = 6.68$, p<0.025
N.T.D. : 23 (S.B. & ANEN.)		12	(52.2%)	16	(69.6%)	14	(60.9%)	DR homozygosity $\chi^2 = 25.57$, p<0.0001
Control* : 180		20	(11.1%)	114	(63.3%)	85	(47.2%)	

* Control: 180 children from 90 normal and unrelated familes (2 children from each family).

that a recessive gene was probably responsible for the occurrence of most N.T.D. (Book and Rayner, 1950; Penrose, 1957, 1946; Polman, 1951). Later, multifactorial determination of N.T.D. was postulated based on the fact that environmental and complex genetic factors appear to affect frequency and distribution of these abnormalities (Carter et al., 1968; Carter, 1968, 1976; Richards et al., 1972; Williamson, 1965; Yen and MacMahon, 1968). Linkage between spina bifida and the HLA complex was shown by Amos et al. (1975) and Mendell (1974). No evidence of association, however, was found by Bobrow et al. (1978) and Vannier et al. (1980).

Some similarities are observed between our data and those of Schacter's report (Schacter et al., 1979) in that HLA-A11 was significantly increased among the fathers, HLA-B35 among the mothers and HLA-A11 among the patients. HLA-A, -B maternal-paternal sharing was increased in Schacter's study though the difference was not significant in this study. Increase in parental HLA-DR and DQ sharing was significant in our series. HLA-DR homozygosity among the patients was significantly increased: Of 23 patients typed, 12 carried homozygous alleles at DR-locus (52.8%), as compared to only 20 homozygotes of 180 normal children (11.6%). Among the parents of the 11 patients who deceased and were not typed, there is a high degree of HLA-DR and DQ sharing: Seven out of 11 couples shared at DR-locus (63.6%) and all couples shared at DQ-locus (100%). Feingold et al. (1980) reported an association between spina bifida and anencephaly with HLA-A1, B5, B8 and B35, and suggested a possible genetic background of susceptibility to these malformations. HLA-B35 was found to show a higher gene frequency in the present study among the mothers of the patients.

Although there are similarities between the results in the present study and those of earlier reports, conclusion concerning an association between N.T.D. and HLA is still premature. The present study has been performed on the Japanese population with a lower rate of N.T.D. occurrence as summarized by Imaizumi (1977) compared with Caucasians. Our study did not test directly the hypothesis of a

human T/t-like complex linked to HLA.   However, our data might warrant further investigation on the association of the HLA system with birth defects in man.   Our present study also failed to include HLA typing of unaffected sibs of N.T.D. patients in families.   Further analysis of HLA haplotype segregation in these healthy sibs would certainly be complementary for the present study on the HLA system and N.T.D.   A more extensive study on comparison of segregation and linkage analyses in large N.T.D. pedigrees among different ethnic groups is underway.

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