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HLA and congenital adrenal hyperplasia

by

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From 25 families, 27 patients with congenital adrenogenital syndrome (AGS) and their 20 healthy siblings were examined for HLA-A, B, C and DR antigens. A statistically significant relationship was found with the HLA phenotype.

The frequency of HLA Bw47 which was found only in the patients, suggests a certain disposition for the disease. The presence of HLA B8 and DR3 which were detected only in the healthy siblings, probably implies a resistance to the disease.

Among the patients, cases of HLA recombination and homozygous individuals were frequent. Assessment of risk haplotypes involved in hereditary transmission of the condition and the finding of positive and negative associations with HLA antigens might offer significant help in prenatal diagnosis of the disease.

Congenital adrenogenital syndrome (AGS) is a disorder caused by inborn 21-hydroxylase deficiency. Depending on the severity of the enzyme deficiency, either the simple or the salt-losing type of the syndrome develops. AGS diagnosed in time and properly treated ensures to the affected subject normal somatic development, fertility and enables him to play a normal role in society. Some of our female patients have reached adult age, married and delivered children. We are frequently faced with the problem of the incidence of the disease in the offspring of affected mothers, and also in the offspring of clinically healthy siblings of patients with AGS or other children in families where one child is already affected.

AGS is a disease with autosomal recesive heredity. Recent investigations have revealed that 21-hydroxylase deficiency is linked with the HLA system. We have therefore investigated the possible relationship between the distribution of HLA antigens and the incidence of the disease.

Material and Methods

We examined 25 families with 27 affected children and 20 healthy siblings. All affected children displayed the salt-losing type of AGS. The children were followed up and treated from neonatal or early infant age. In the girls, masculinization grade 1—5 according to Prader's classification was observed after birth. The impaired salt metabolism manifested itself with various degrees of dehydration and

impaired mineral balance in every patient during the first weeks of life. The patients were treated continuously with hydrocortisone, during the first years of life with mineral corticoids and a supplement of salt mixture (NaCl, $NaHCO_3$). An acute adrenal crisis developed repeatedly during common infections of the respiratory tract.

Somatic development of the children displayed a slight acceleration of growth and bone maturity, especially between 4—7 years of age. Early growth of pubic hair occurred at the age of 8—9 years.

In girls in preschool age, a plastic operation was performed on the genital organ. The intelligence of these children corresponded to that of the healthy population, the behaviour of the girls was of feminine type.

For typization of HLA antigens we used the standard NIH microcytotoxic test by 180—240 antisera; 79 HLA antigens were assessed.

For the assessment of B lymphocytic HLA DR antigens, the method of the 7th International HLA Workshop, Oxford 1977, was used. B lymphocytes were isolated by the rosette technique, using sheep erythrocytes. By means of 120—180 typing sera, provided mostly by the organizers of the 8th International HLA Workshop in 1980 in Los Angeles, HLA DR 1—7 antigens were assessed.

Results

The frequency of HLA-A, B, C, DR antigens in patients was compared with a control group of non-related healthy subjects [3]. In the families we determined the haplotype transfer of individual HLA specificities. The significance of differences as regards the frequency of HLA antigens in non-related patients and controls was evaluated by means of the X^2 test. The value of p was corrected by multiplying with the number of assessed antigens. The number of patients and healthy children in different families is given in Table I. The assessment of risk haplotypes in different families is illustrated in Figs. 1 and 2. We assume that healthy children should have identical HLA (agreement of both haplotypes) and different from those of sick children (difference in one or both haplotypes). In the first family there were two healthy children, the third child, a boy, and the fourth child a girl, suffer from adrenogenital syndrome with impaired salt metabo-

Table I

Number of families and the incidence of AGS

Families(n)	children	
	sick (n)	healthy (n)
1	2(2)← identical	2(2)←identical
3	2(2)← recombinant	0
1	1(1)←semiidentical →	2(2)
16	1(16)	1(1)6
	← 11 semiidentical →	
	← 5 different →	
6	1(6)	0
25	(27)	(20)

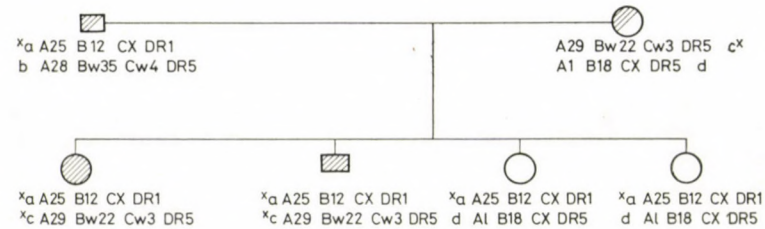

FIG. 1. HLA haplotypes for HLA- A, B, C and DR in family D. The paternal haplotypes are a and b and the maternal haplotypes c and d. The parents are obligate heterozygous carriers for the 21-hydroxylase deficiency gene denoted by light black symbols. The affected children are denoted by black symbols. Two affected siblings are HLA genotypically identical and shared one haplotype with the healthy children

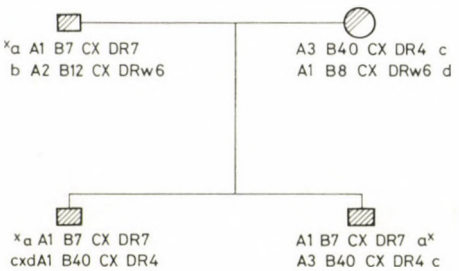

FIG. 2. HLA haplotypes for HLA- A, B, C and DR in family P. The siblings shared one haplotype (a), in the second haplotype they differed in locus A antigens (A3, A1), a recombination of the A/C, B type was involved on the maternal part

lism and are followed up and treated since birth. In the girl there was masculinization of the external genital, type V according to Prader. The healthy children were identical on HLA examination and shared one haplotype with the sick children. Both sick children had identical haplotypes. In another family with two sick children the haplotypes were not completely identical. The siblings shared one haplotype (a), in the second haplotype they differed in the locus A antigen (A3, A1). In this case a recombination of the A/ C, B type from the maternal part was involved. In a further family with two healthy children and one affected child, the healthy children were HLA semi-identical as compared with the sick child. In the remaining 16 families with one sick and one healthy child we found in 11 instances semiidentity and in 5 instances differences in both haplotypes.

We investigated the frequency of HLA antigens of the A, B, C, DR locus in children with AGS, in their healthy siblings and their parents. When there were several sick or healthy children in the family, we included only one in the appropriate group. The frequency of antigens in these groups was compared with the incidence in healthy controls. HLA-A 3, A 25, Bw 22, B 40, Bw 47, Cw 6, DR 5 and DR 7 were present frequently, A 9 and Bw 35 were encountered rarely, and

TABLE II/A

Incidence of HLA-A antigens in the families with AGS and in the healthy controls

HLA-A	frequency, per cent			
	AGS n = 25	healthy n = 18	parents n = 49	controls n = 130—1200
1	16.0	22.22	26.53	28.11
2	32.0	22.22	36.73	48.30
3	56.0	44.44	51.02	26.65
9	8.0	27.77	22.44	22.41
w23	0	0	2.04	2.03
w24	8.0	16.66	20.40	16.12
10	16.0	16.66	14.28	16.48
25	16.0	16.66	12.24	7.28
26	0	0	2.04	5.60
11	8.0	22.22	12.24	11.0
28	4.0	5.55	6.12	3.29
29	12.0	11.11	14.28	4.87
w30	0	0	0	3.31
w31	0	0	0	2.30
w32	0	0	0	2.51

TABLE II/B

Incidence of HLA-B antigens in the families with AGS and in the healthy controls

HLA-B	frequency, per cent			
	AGS n = 25	healthy n = 18	parents n = 49	controls n = 130—1200
5	12.0	16.66	12.24	11.95
7	20.0	27.77	24.48	23.34
8	0	22.22	14.28	16.74
12	24.0	16.66	22.44	22.19
13	0	0	2.04	9.0
14	0	5.55	4.08	2.85
15	12.0	0	6.12	10.92
w16	4.0	0	6.12	9.80
w38	4.0	24.00	6.12	6.39
17	0	5.55	2.04	6.34
18	8.0	22.22	12.24	10.83
w21	4.0	0	6.12	3.12
w22	16.0	5.55	10.20	3.79
27	4.0	5.55	12.24	9.57
w35	4.0	11.11	8.16	16.11
40	32.0	38.88	24.48	9.42
w41	0	0	2.04	3.14
47	28.0	0	16.32	1.52

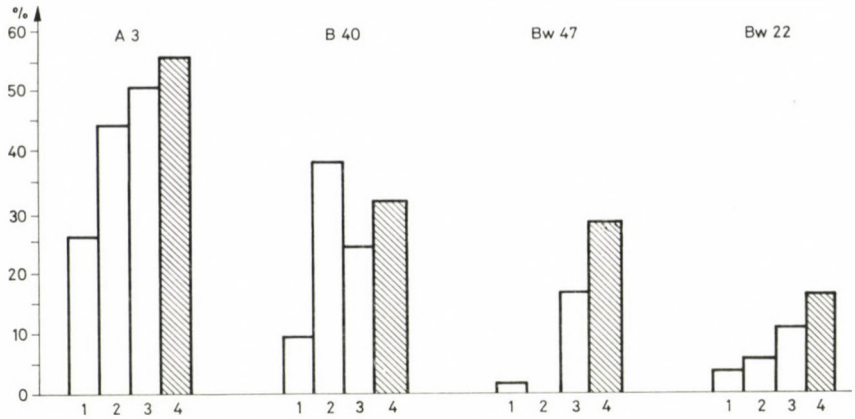

FIG. 3. HLA-A3, B40, Bw57, Bw22 antigens were more frequent in the sick children. 1-controls (n = 130—1200), 2-healthy children (n = 18), 3-parents (n = 49), 4-sick children (n = 25)

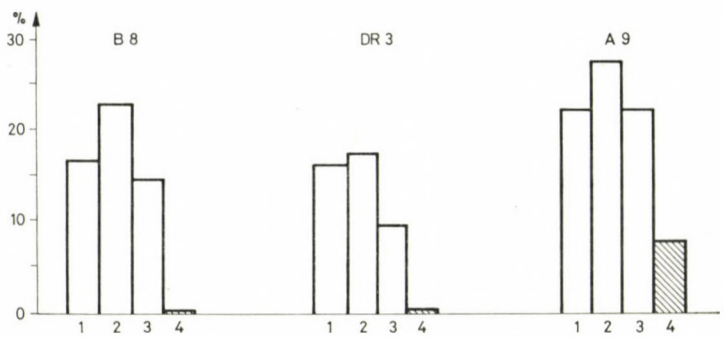

FIG. 4. HlA-A9 was infrequent and B8 and DR 3 were never detected in children with AGS. 1-controls (n = 130—1200), 2-healthy children (n = 18), 3-parents (n = 49), 4-sick children (n = 25)

B 13, B 8 and DR 3 were never detected in the patients. As it is seen in Table II, there were differences in the distribution of HLA antigens in children with AGS, as compared with the controls. More significant differences in the distribution of some HLA antigens in different groups, i.e. a higher incidence (positive association) or a reduced frequency (negative association) are illustrated in Figures 3 and 4. Most marked was the frequent presence in sick children of HLA Bw 47 which was never detected in the healthy siblings, and the absence of HLA B 8 and DR 3 which was only found in healthy children. In four families we found recombinant individuals. Further two possible cases of recombination of the B/DR type could not be proved conclusively as it was not possible to examine the grandparents of the children. In the investigated grown-up parents of children with AGS we found a high number of homozygous individuals, in particular

TABLE III

Incidence of HLA-C and DR antigens in the families with AGS and in the healthy controls

HLA-C	frequency, per cent			
	AGS n = 25	healthy n = 18	parents n = 49	controls n = 130—1200
w1	8.0	0	4.08	6.39
w2	12.0	11.11	16.32	10.34
w3	20.0	5.5	12.24	15.75
w4	8.0	22.22	12.24	15.33
w5	0	0	0	9.83
w6	36.0	11.11	32.65	26.15
HLA-DR				
1	17.39	17.64	9.30	20.76
2	34.78	35.29	26.53	26.79
3	0	17.64	9.30	16.15
4	26.08	23.53	20.93	16.92
5	30.43	11.76	30.23	16.92
6	13.04	0	20.93	27.69
7	52.17	23.52	34.88	30.76

TABLE IV

HLA homozygozity in families with AGS

HLA homozygozity	observed/possible			
	A	B	C	DR
AGS	9/2	6/0	3/2	1/1
healthy	1/2	2/1	0/0	2/0

as regards locus A and B. Table III compares sick and healthy children from this aspect for different HLA loci. Under the heading "possible" are further suspect cases where we were unable to examine the grandparents.

DISCUSSION

The gene causing 21-hydroxylase deficiency is situated in the 6th chromosome within the main histocompatible HLA-complex. The precise localization of the gene in the HLA area is not known. Based on assembled findings, most authors favour the view that it is closer to locus B. The findings of A/C, B recombination in one of the examined families with two sick children, which differed in the antigen of the A locus and were identical in the others, provides evidence for this fact. The number of recombinations in the HLA region

does not exceed 1% as a rule. Evidence of 3 recombinant patients (11%) is high and consistent with the finding of an increased percentage (12.5%) of recombinations in families with juvenile diabetes.

The haplotype transfer of 21-hydroxylase deficiency with the appropriate HLA determinants was proved and by investigation of the parents with the latent disorder and of the sick child it is possible to assess the paternal and maternal "risk" haplotype which transmits the disease. It is assumed that a further sick child should be HLA identical and a healthy child semiidentical or different from the affected child. The fact of complete HLA identity obviously does not apply in a situation when recombination occurs, for instance in our group the above mentioned case where the sick siblings were identical only in HLA-B, C and DR loci. This fact must be taken into consideration in the prenatal diagnosis of AGS based on amniocentesis.

Data on the association of HLA antigens and AGS are not uniform. Levine et al [6] examined 17 families with 20 sick and 22 healthy siblings and, by combination with the results of a Swiss study of 48 patients, 48 healthy siblings and their parents. They did not reveal a significant relationship, depending on the presence of a certain HLA antigen. Some authors found in AGS patients an increased frequency of Aw 24 and Bw 25, while Klouda et al [5, 11] drew attention to the frequent presence of Bw 47 (15% as compared to 0.7% in the controls) and to the absence of B 8 in children with AGS. We were unable to confirm this finding in our group of 25 families. In 27 sick unrelated children Bw 47 was found in 28%, in the controls in 1.53%, while in 20 healthy children it was absent. On the other hand, B 8 was found only in healthy children. The similar finding as regards DR 3 is obviously the consequence of the genetic situation, i.e. the rule of a disbalance of the B 8 and DR 3 links which are frequently found on the same haplotype.

We assume that Bw 47 positive subjects are susceptible to AGS (relative risk, 24.8), while B 8 DR 3 positive individuals are resistant. Confirmation of this assumption will call for a further extensive investigation and could be a guide in the prenatal diagnosis of AGS.

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