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HLA haplotypes in children with adrenogenital syndrome and their parents

by

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HLA-A and B antigen typing was carried out by the standard NIH lymphocytotoxicity test in children with androgenital syndrome (AGS) from 11 families, further in 3 healthy siblings and 20 obligatory gene carrier parents. Of the children 7 had the salt-losing form. The AGS homozygote group was examined for the geno- and haplotypes of the HLA antigen and the heterozygote group for HLA antigen genotypes. Two AGS-affected children of the same family proved to be HLA-identical, while in other families the haplotypes of the healthy siblings were different from those of the affected children.

As compared with the data of 222 healthy blood donors, the antigens of the HLA-A and B loci in the homo- and heterozygote groups showed no significant difference.

The close connection between 21 hydroxylase deficiency and the HLA system in autosomal recessive hereditary congenital adrenal hyperplasia (AGS, CAH) was demonstrated by Dupont et al [4a, 4b]. The connection between HLA-B and CAH has been confirmed by several authors [16, 19], but contradictory interpretations have also been published. This fact has motivated our study.

MATERIAL AND METHODS

The occurrence of HLA-A and B antigens has been studied in 10 AGS children from 11 families, among them 7 with salt-losing form, and in 3 healthy brothers of these, further in 20 obligate gene-carrier parents. As a control, occurrence of the HLA antigen was studied in 222 healthy blood donors. One AGS child of each of two families was already dead at the time of the present study. In one family 2 children had AGS.

Typing for HLA-A and B antigens was done by means of standard NIH lymphotoxicity test [18] with lymphocytes taken from peripheral blood. Evaluation was made by the χ^2 test.

RESULTS

Table I shows the HLA genotypes and HLA haplotypes in the heterozygotic parents and the children with AGS.

The occurrence of the HLA-A and B antigens in AGS homozygotes is shown in Table II. Most frequent among the antigens of locus A was

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TABLE I

HLA genotypes of AGS families

Family	Parents HLA	AGS-children HLA	HLA Haplotype	Helathy children HLA	Haplotype HLA
1. E. father:	A2,3/B12,18	f.A2,28/B18,40	2–18/28–40	–	–
mother:	A28.32/B27,40				
* 2. Cs. father:	A2.11/B5,21	1.A11,26/B5,7	11–5/26–7	–	–
mother:	A2,26/B7,17	1.A11,26/B5,7	11–5/26–7	–	–
3. Z. father:	A1,2/B17,w50			1.A2w24/B15,17	2–17/24–15
mother:	A3,w24/Bw15,38				
4. K. father:	A2,33/B14,w35,Cw4.	f.A1,33/B5,14	33–14/1–5	1.A2/B27,w35,Cw4	2–35/2–27
mother:	A1,2/B5,27				
* 5. H. father:	A24/B18,w35	f. + A1/B18,35(?)	–	–	–
mother:	A2,11/Bw15,w35				
* 6. K. father:	A3,w24/B14,w35Cw4	f.A2w24/B12,w35,Cw4	24–35/2–12	–	–
mother:	A2,11/B12,w35,Cw4				
* 7. S. father:	A1,3/B8,w35	1.A1,2/B8,12	1–8/2–12	–	–
mother:	A2/B12,38				
8. L. mother:	A3,26/B12,18	1.A26/B12	26–12/26,12	f.A1,3/B18	1–/3–18
father:	–				
* 9. V. father:	A2/B5,27	f.A2,3/B5,40	2–5/3–40	–	–
mother:	A1,3/B8,40				
* 10. N. father:	A2.3/B5,40	f.A28,3/B12,40	3–40/28–12	–	–
mother:	A3,28/B12,40				
* 11. F. father:	–				
mother:	A26,28/B27,w35	1.A11,28/B7,w35	11–7/28–35	–	–

* *salt-loosing form*

TABLE II

Occurrence of HLA antigens in the AGS homozygote group

HLA-A	AGS		Control		X^2	P	HLA-B	AGS		Control		X^2	P
	+	—	+	—				+	—	+	—		
1	2	7	49	173	0.15	0.99	5	3	6	28	194	1.66	0.20
2	4	5	110	112	0.15	0.99	7	2	7	30	192	0.62	0.72
3	2	7	43	179	0.47	0.99	8	1	8	41	181	0.14	0.97
11	2	7	33	189	0.16	0.81	12	3	6	44	178	0.31	0.53
w24	1	8	32	190	0,43	0.99	14	1	8	15	207	0.27	0.96
28	3	6	22	200	2.78	0.12	15	1	8	16	206	0.44	0.99
w33	1	8	6	216	0.20	0.49	18	2	7	34	188	0.83	0.84
							w35	3	6	50	172	0.12	0.68
							40	3	6	31	191	0.27	0.26
							Cw4	1	8				
							B5+35 +18	6	3	112	110	0.37	0.54

TABLE III

Occurrence of HLA antigens in obligatory heterozygote AGS gene carriers

HLA-A	AGS		Control		X^2	P	HLA-B	AGS		Control		X^2	P
	+	—	+	—				+	—	+	—		
1	4	16	49	173	0.45	0.99	5	4	16	28	194	0.34	0.52
2	10	10	110	112	0.37	0.99	7	1	19	30	192	0.55	0.48
3	8	12	43	179	3.53	0.07	8	2	18	41	181	0.41	0.54
9	3	17	55	167	0.50	0.49	12	5	15	4	178	0.68	0.75
10	3	17	43	179	0.32	0.89	14	2	18	15	207	0.75	0.84
11	3	17	33	189	0,97	0,99	15	2	18	16	206	0.12	0.90
w24	3	17	32	190	0.67	0.99	16	2	18	20	202	0.66	0.99
w26	3	17	24	198	0.39	0.78	17	1	19	18	204	0.37	0.99
w28	3	17	22	200	0.11	0.68	18	3	17	34	188	0.82	0.99
w32	1	19	13	209	0.11	0.99	W21	2	18	15	207	0.75	0.84
w33	1	19	6	216	0.11	0.91	27	4	16	29	193	0.27	0.56
							w35	7	13	50	172	0.96	0.32
							38	2	18	10	212	0.29	0.51
							40	4	16	31	191	0.16	0.64
							49	1	19	10	212	0.21	0.99
							50	1	19	5	217	0,38	0,81

the type A 28; among the antigens of locus B, most frequent were the types B5, B 12, and B 40. Compared with the population figures, however, the differences were not significant statistically. The related antigens of the B group (B 5, B 35, B 18) showed no significant correlation.

Table III shows the HLA antigen distribution of the obligatory AGS heterozygotic gene carriers. Mathematical evaluation of the data showed no significant difference.

The two children with AGS in one family proved HLA-identical (Table I, family 2); none of the healthy siblings had the same haplotype as the affected child.

Discussion

The gene frequency of AGS in the USA is 1:150, and its incidence 1:80.000—100.000, while in Switzerland the incidence of the disease is much higher: 1:5.000 [8]. The way of inheritance is autosomal recessive.

The underlying defect is a lack of 21-hydroxylase, the enzyme which catalyses the formation of 17.20 di-OH progesterone from 17-OH progesterone. In the case of the non-salt-losing form, increased plasma 11-deoxy-corticol (= S-compound) and increased 17 KS, TSH, and TH-DOC in the urine are of diagnostic importance. Plasma 17-alpha—OH progesterone and urinary pregnanetriol may be slightly increased [14].

As to the salt-losing form of AGS, Hesse et al [7] summarized the enzyme defects and the clinical picture of the disease, underlining the 21-hydroxylase, 18-hydroxylase, 18-dehydrogenase and the 3-beta-OH-steroid-dehydrogenase defect as well as the increase in blood testosterone, 17-OH progesterone and plasma renin activity. Diagnostic among the laboratory findigs are the increased urinary excretion of 17 KS and pregnanetriol, and a rise of the 11-oxygenization index.

Dupont et al [4b] found a close connection between the CAH and the HLA complexes in the course of the family investigation of 32 CAH patients with 21-OH defect and a genetic study of the HLA-B complement factor and the polymorphy of the HLA-B complement factor and glyoxolyase-1. Levine et al [10] too found a close connection between HLA and the 21-OH defect in a study of 32 New York and Zurich families. All their CAH patients differed in HLA genotype from their healthy siblings, and among the identical siblings, two or more of the affected were HLA-identical. According to these authors analysis of the HLA genotype can be used for finding the heterozygotic gene carriers of the 21-OH defect among brothers and sisters and other relatives in high-risk families, in which HLA typing of amnial cell cultures may help to diagnose the disease. The mentioned authors underline that CAH is the first steroid biosynthetic enzyme defect in which a close connection with the HLA system can be demonstrated. Lieberman et al [11], on the other hand, could

not demonstrate a close connection between the C-11-OH defect and the HLA-A-B-C- loci.

Grosse-Wilde et al [6] found a good correlation between the segregation of HLA haplotypes and the result of the above mentioned heterozygote test in the course of HLA-A-B-C typing for CAH heterozygosity demonstrated on the basis of the elevated plasma 17-OH-P level measured after ACTH stimulation.

According to Lorenzen et al [12], HLA typing was helpful in the further differentiation of the group suspected of carrying the gene on the basis of the ACTH stimulation test. Pucholt et al [17] confirmed the strong linkage to the HLA locus on the basis of 34 families with CAH. According to Levine [9], the gene responsible for the 21-OH defect is located between the HLA-A and GLO (glyoxolyase) loci in the great histocompatibility region of the 6th chromosome, between the A and DR loci. Gelsthorpe et al [5] found a connection with the Bw47 antigen in the 21-OH deficiency gene-carriers, a finding subsequently confirmed in the CAH homozygote patient population by the Brkljacic team of Zagreb [2]. In a large patient population with 21-OH defect of the late type, Pollack et al [15] found a significant positive connection with B5, Bw35, DRw1, DRw7 and a negative association with B8 and DRw3. In our own material, the B5 antigen occurred in 3 out of 9 in the AGS homozygote group, and Bw35 occurred with a similar frequency; but the differences were not significant in comparison with the control group. In our AGS obligatory gene carrier group the above mentioned antigens were not significantly more frequent; B4 occurred in 4 subjects, and Bw35 in 7 out of 20, while the studies seemed to confirm the negative association with B8. Coullin et al [3], too, found a decreased frequency of the B8 antigen on the basis of the investigation of 67 CAH families. Our own studies support this observation, as in the AGS homozygote group the B8 occurred in one out of 9 as against 41 out of 222 in the control group, while in the gene carrier group it occurred only in 2 out of 20, as against 41 positive cases in the control group of 222 subjects. Mayer et al [13] also obtained negative results, although in a small patient population.

Betuel et al [1] found a higher frequency of DRw2 in 43 CAH-affected children.

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