Location of the gene for 21-hydroxylase deficiency

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SUMMARY HLA typing of 33 families with one or more children suffering from congenital adrenal hyperplasia confirmed that the gene for 21-hydroxylase deficiency is closely associated with the HLA region. Analysis of two families in which recombination of chromosome 6 had occurred indicated that the gene locus is between the *A* and *D* loci of the HLA region. The rare allele *Bw47* was observed in 18 parents and was always associated with the carrier state for 21-hydroxylase deficiency.

Congenital adrenal hyperplasia (CAH) is a recessively inherited condition most commonly the result of a deficiency of 21-hydroxylase.¹ There is evidence suggesting that the gene locus for 21-hydroxylase deficiency may be situated on the short arm of chromosome 6 close to the HLA region.² Further work indicates that the gene lies between the *HLA* locus and the *glyoxylate* locus, which is the nearest identifiable locus between the HLA region and the centromere.³ From the present study it would appear that the gene for 21-hydroxylase deficiency lies within the HLA region and its position is likely to be close to the *HLA-B* locus.

Patients and methods

Thirty-three families with one or more children suffering from CAH have been studied by measurement of HLA types. The families resided in South Yorkshire, Nottinghamshire, and Leicestershire. In nine families there were two affected children, 11 families had two or more children one of whom was affected, and in 13 families there was a single affected child. In total there were 69 children of whom 42 had CAH. There were 24 affected girls, 18 affected boys, 17 normal girls, and 10 normal boys.

Tissue typing was carried out at the Sheffield Regional Blood Transfusion Centre. A standard two stage microlymphocytotoxic test with 120 well characterised typing sera was used to define HLA-A, -B, and -C antigens. The antigen *Bw47* was identified by the operationally monospecific serum CAS 12551-8W 1160. HLA-DR typing was performed by a two stage microlymphocytotoxic test on B cell

enriched lymphocyte suspensions, T cell depletion having been obtained by rosetting with sheep red blood cells and separation on Ficoll Triosil. Sixty sera, including several characterised in the 7th and 8th International Histocompatibility Workshops, were used to define the DRw antigens, DRw1 to DRw8.

Results

The haplotypes of all families studied (tables 1, 2, 3) agreed with the original observation of Dupont *et al*² and this has subsequently been confirmed by other workers.⁴⁻⁷ In any family the affected children carried identical haplotypes, but the HLA alleles forming the haplotypes differed between families.

In two families recombination of chromosome 6 occurred in the HLA region. In the first family the father had died, but it was possible to reconstruct his probable haplotypes from those of his parents, children, and sister (fig 1). The elder affected daughter carried haplotype *a* from her father and *c* from her mother. The second affected daughter carried haplotype *c* from her mother, but the haplotype from her father suggested that recombination had occurred between the loci *C* and *A* of the paternal haplotypes *a* and *b*. As this girl has CAH it would appear that the locus for 21-hydroxylase deficiency is situated on the HLA segment carrying the *C*, *B*, and *D* loci.

In the second recombinant family there were also two affected daughters (fig 2). The elder daughter carried haplotypes *a* from her father and *c* from her mother. The younger daughter carried haplotype *a* from her father. The haplotype from her mother

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TABLE 1 *Families with two affected children*

Family	Parental haplotypes and symbols				Affected children: haplotypes and sex	Unaffected children: haplotypes and sex
1	Father	A2; Cw4; Bw35; DRw1	a	a	$\frac{a}{c}$ F	
		A9; Cw3; Bw40; DRw2	b	c		
	Mother	A2; C—; B17; DRw7	c	a	$\frac{a}{c}$ M	
		A3; Cw6; B7; DRw4	d	c		
2	Father	A3; Cw4; Bw35; DRw2	a	a	$\frac{a}{c}$ M	
		Aw30; C—; B13; DRw7	b	c		
	Mother	A25; Cw5; B12; DRw4	c	a	$\frac{a}{c}$ F	
		Aw32; C—; B—; DRw2	d	c		
3	Father	A3; Cw4; Bw35; DRw1	a	a	$\frac{a}{c}$ F	$\frac{b}{c}$ M
		A11; Cw3; B22; DRw4	b	c		
	Mother	A9; C—; B18; DRw1	c	a	$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A26; Cw3; B22; DRw4	d	c		
4	Father	Aw31; C—; Bw47; DRw7	a	a	$\frac{a}{c}$ F	$\frac{b}{c}$ F
		Aw32; Cw6; B12; DRw1	b	c		
	Mother	A9; C—; B7; DRw2	c	a	$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; C—; B8; DRw3	d	c		
5	Father	A2; C—; B18; DRw1	a	a	$\frac{a}{c}$ M	$\frac{b}{c}$ M
		A29; C—; B12; DRw7	b	c		
	Mother	A1; C—; B12; DRw1	c	a	$\frac{a}{c}$ M	$\frac{b}{d}$ M
		A2; C—; B7; DRw2	d	c		
6	Father	A3; C—; B7; DRw—	a	a	$\frac{a}{c}$ M	
		A9; Cw4; B12; DRw—	b	c		
	Mother	A11; Cw3; B15; DRw—	c	a	$\frac{a}{c}$ M	
		Aw31; C—; B5; DRw—	d	c		
7	Father	A3; C—; B7; DRw2	a	a	$\frac{a}{c}$ M	$\frac{b}{c}$ F
		A2; Cw5; B12; DRw1	b	c		
	Mother	A3; C—; Bw47; DRw7	c	a	$\frac{a}{c}$ M	
		A2; Cw5; B12; DRw4 × 5	d	c		

TABLE 2 *Families with a single affected child*

Family	Parental haplotypes and symbols				Affected children: haplotypes and sex	Unaffected children: haplotypes and sex
8	Father	A3; C—; B39; DRw4	a	a	$\frac{a}{c}$ F	$\frac{a}{d}$ F
		Aw32; Cw4; Bw35; DRw4	b	c		
	Mother	A1; Cw1; IM2; DRw1	c	a	$\frac{a}{c}$ M	$\frac{b}{d}$ M
		A2; Cw3; B40; DRw6	d	c		
9	Father	Aw33; C—; B14; DRw1	a	a	$\frac{a}{c}$ M	$\frac{b}{d}$ F $\frac{b}{d}$ M
		A11; Cw4; B12; DRw4	b	c		
	Mother	A29; C—; B12; DRw1	c	a	$\frac{a}{c}$ F	$\frac{a}{d}$ M $\frac{b}{d}$ M $\frac{a}{d}$ F
		A3; Cw3; B15; DRw6	d	c		
10	Father	A26; Cw6; B12; DRw7	a	a	$\frac{a}{c}$ F	$\frac{b}{d}$ M $\frac{a}{d}$ M
		A1; C—; B17; DRw7	b	c		
	Mother	A3; C—; B7; DRw7	c	a	$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; C—; B8; DRw7	d	c		
11	Father	A2; C—; B7; DRw5	a	a	$\frac{a}{c}$ F	$\frac{b}{c}$ F
		A3; C—; B7; DRw2	b	c		
	Mother	A3; C—; Bw47; DRw7	c	a	$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A11; C—; B40; DRw3	d	c		

TABLE 2—continued

Family	Parental haplotypes and symbols				Affected children: haplotypes and sex	Unaffected children: haplotypes and sex
12	Father	A26; Cw6; Bw36; DRw2	a		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A1; C—; B7; DRw2	b			
	Mother	A2; Cw5; B12; DRw4	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; C—; B8; DRw3	d			
13	Father	A3; C—; B7; DRw4	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; Cw6; Bw50; DRw7	b			
	Mother	A2; C—; B12; DRw4	c		$\frac{a}{c}$ M	$\frac{b}{c}$ F
		A1; C—; B8; DRw3	d			
14	Father	A3; Cw4; Bw35; DRw1	a		$\frac{a}{c}$ F	$\frac{a}{d}$ M
		A9; Cw3; Bw22; DRw2	b			
	Mother	A29; C—; Bw47; DRw7	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A2; Cw6; B12; DRw4	d			
15	Father	A3; Cw4; Bw35; DRw4	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A11; 1M2; Cw1; DRw5	b			
	Mother	A1; C—; B8; DRw3	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A3; Cw3; B40; DRw2	d			
16	Father	Aw31; C—; Bw47; DRw7	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		Aw32; C—; Bw44; DRw1	b			
	Mother	Aw24; C—; B7; DRw2	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; C—; B8; DRw3	d			
17	Father	A2; Cw6; Bw47; DRw7	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A2; C—; Bw44; DRw4 × 7	b			
	Mother	A3; Cw6; Bw47; DRw7	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A1; C—; B8; DRw3	d			
18	Father	A3; C—; B7; DRw—	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A25; C—; B7; DRw—	b			
	Mother	A2; C—; B18; DRw—	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A11; Cw6; B17; DRw—	d			
21	Father	A3; C—; Bw47; DRw7	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		Aw31; C—; Bw38; DRw6	b			
	Mother	A3; C—; Bw47; DRw7	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A3; C—; B7; DRw7	d			
22	Father	A29; C—; B12; DRw7	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A9; C—; B7; DRw2	b			
	Mother	A3; C—; Bw47; DRw7	c		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A9; C—; B12; DRw—	d			
23	Father	A2; C—; B5; DRw5	a		$\frac{a}{c}$ F	$\frac{b}{d}$ F
		A2; Cw4; B12; DRw2	b			
	Mother	A28; C—; B12; DRw2	c		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A1; C—; B8; DRw3	d			
24	Father	A3; C—; Bw47; DRw7	a		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A3; C—; B7; DRw7	b			
	Mother	A3; C—; Bw47; DRw7	c		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A9; C—; B5; DRw7	d			
25	Father	A1; C—; B8; DRw—	a		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A2; C—; B7; DRw—	b			
	Mother	A3; C—; Bw47; DRw—	c		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		Aw36; C—; Bw45; DRw—	d			
26	Father	A9; C—; B7; DRw4	a		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		A2; C—; B8; DRw3	b			
	Mother	A29; C—; B12; DRw1	c		$\frac{a}{c}$ M	$\frac{b}{d}$ F
		Aw32; Cw6; B14; DRw6	d			

TABLE 2—continued

Family	Parental haplotypes and symbols				Affected children: haplotypes and sex	Unaffected children: haplotypes and sex
27	Father	A29; C—; B12; DRw—	a		$\frac{a}{c}$ M	
		A1; C—; B8; DRw—	b			
	Mother	A3; C—; Bw47; DRw—	c			
		A2; Cw3; Bw22; DRw—	d			
28	Father	A3; C—; Bw47; DRw—	a		$\frac{a}{c}$ M	
		A2; C—; B27; DRw—	b			
	Mother	A1; C—; B8; DRw—	c			
		A9; C—; B—; DRw—	d			
29	Father	A2; C—; Bw50; DRw1	a		$\frac{a}{c}$ F	
		A29; C—; B12; DRw7	b			
	Mother	A2; Cw6; B17; DRw7	c			
		A2; C—; B8; DRw3	d			

TABLE 3 Families with a single affected child

Family	Paternal haplotypes inferred				Affected children: haplotypes and sex
30	Father	A2; Cw6; B15; DRw4	a		$\frac{a}{c}$ M
		?	b		
	Mother	A2; Cw6; Bw47; DRw7	c		
		A1; C—; B37; DRw6	d		
31	Father	A2; Cw3; B15; DRw—	a		$\frac{a}{c}$ M
		?	b		
	Mother	A3; C—; B7; DRw—	c		
		Aw3; Cw2; B27; DRw—	d		
32	Father	A3; C—; B7; DRw—	a		$\frac{a}{c}$ F
		?	b		
	Mother	A3; C—; Bw47; DRw—	c		
		A9; Cw4; B12; DRw—	d		
33	Father	A1; C—; B17; DRw—	a		$\frac{a}{c}$ F
		?	b		
	Mother	A3; Cw6; Bw47; DRw—	c		
		A2; C—; B27; DRw—	d		

indicated that recombination had occurred between the *B* and *DR* loci of the two maternal haplotypes *c* and *d*. As a confirmatory test of this recombination, a mixed lymphocyte reaction (MLR) test was carried out between the two affected children. HLA-A, B, and C identical sibs are almost invariably non-stimulatory in MLR test. If, however, a different *D/DR* locus has been substituted in the proposed recombination, that is *DRw5* for *DRw1* in the second daughter, stimulation might be expected to occur. The result of MLR test suggests that the two affected sisters do differ in their *DR* loci (table 4). The locus for 21-hydroxylase deficiency would therefore appear to be situated in the HLA segment carrying the *A*, *C*, and *B* loci. The results from these

TABLE 4 MLR in HLA-B/DR recombinant sibs

Stimulator cells	Responder cells		
	Sib 2 (DRw 5/5 recombinant)	Sib 1 DRw 1/5	Unrelated DRw 2/3
Sib 2 'm' DRw 5/5	—	1	52
Sib 1 'm' DRw 1/5	37	—	42
Unrelated 'm' DRw 2/3	60	45	—

'm', mytociin treated cells.

Cells from the second daughter (recombinant DRw5/DRw5) are stimulated by cells from the first daughter (DRw5/DRw1). Predictably, cells from the first daughter (DRw1/DRw5) are not stimulated by the cells from her recombinant sister (DRw5/DRw5). Results of the MLR test are expressed as relative responses.

FIG 1 Haplotypes of family 1. Both daughters are affected. The second daughter carries evidence of recombination between loci A and C of the paternal haplotypes (a;b). (Haplotypes of the father are inferred from those of his relatives.) The thick line represents the chromosome carrying the gene locus responsible for 21-hydroxylase deficiency.

two families indicate that the 21-hydroxylase gene probably lies in the HLA region between the A and D loci.

In our earlier report⁸ we noted an unusually frequent association of a rare HLA allele, Bw47, with the gene responsible for 21-hydroxylase deficiency. In the present series Bw47 was observed in the haplotypes of 18 parents and was also present in the haplotype of all their affected children.

Discussion

The results of this study agree with and extend earlier reports that the gene for 21-hydroxylase deficiency lies within the HLA region.³ The practical implications of this observation are important. Experience from the genetic counselling clinic in Nottingham and elsewhere indicates that many parents with one affected child would welcome a dependable prenatal diagnostic test for this condition in later pregnancies. Recent reports have described

FIG 2 Haplotypes of family 2. Both daughters are affected. The second daughter carries evidence of recombination between B and DR loci of the maternal haplotypes (c;d). The thick line represents the chromosome carrying the gene locus responsible for 21-hydroxylase deficiency. (It is not known if the maternal grandfather was heterozygous for the gene responsible.)

two methods of diagnosing 21-hydroxylase deficiency prenatally. One method uses tissue typing of fetal cells.^{9,10} The other method measures the concentration of 17 α -hydroxyprogesterone in the amniotic fluid which is considerably raised if the fetus is affected.¹¹ Both tests can be carried out on the same sample of amniotic fluid obtained in the second trimester of pregnancy. If confirmed the two tests would make the prenatal diagnosis more reliable.

In addition, HLA typing among relatives of an affected subject may enable heterozygotes to be identified. This could be of importance in countries where the incidence of 21-hydroxylase deficiency is high, for example, Wales and Switzerland.

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