Congenital adrenal hyperplasia — a clinical and genetic survey

Are we detecting male salt-losers?

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SUMMARY In a genetic and clinical study in Wales of congenital adrenal hyperplasia due to 21-hydroxylase deficiency, 26 cases were found in the period 1966 to 1977. No one was known to have died from the condition in the period. There were 14 female and 5 male salt-losers, and 5 female and 2 male nonsalt-losers. The discrepancy between the sexes suggests that some cases of congenital adrenal hyperplasia in males are not being detected. This is of concern as salt-losing cases may be dying without a diagnosis being established. The carrier incidence of the condition in females, assuming complete ascertainment, is 1:55. Salt-losers in Wales appear to be more than twice as common as nonsalt-losers. HLA typing of certain affected families confirmed a genetic linkage between the congenital adrenal hyperplasia gene and HLA. This linkage appears to exist in both forms of congenital adrenal hyperplasia and suggests that the genes for salt-losing and nonsalt-losing forms of congenital adrenal hyperplasia are alleles. The linkage between congenital adrenal hyperplasia and HLA provides a potential method for antenatal detection. However, only a few parents would wish to take advantage of this method if it were readily available.

There have been few population studies on congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency and none in the UK. Estimates of the carrier frequency vary considerably from as low as one in 128¹ to as high as one in 11.² There has been recent interest in the genetics of the condition with the discovery of the genetic linkage between the HLA locus and CAH,³ leading to the possibility of detecting CAH antenatally and elucidating the genetic basis for the salt-losing and nonsalt-losing forms of the condition. These aspects of CAH were investigated in a study of the disease in Wales.

Methods

All patients with CAH due to 21-hydroxylase deficiency in Wales from 1966 to 1977 were identified by writing to paediatricians through the Welsh Paediatric Society. The thoroughness of the detection was confirmed by the Hospital Activity Analysis of the Welsh Office. Paediatricians in neighbouring areas were also contacted. Each patient was visited and hospital records were examined. Full family pedigree and clinical information were recorded. The Registrar General was consulted

about deaths during the period. In families with more than one child, HLA typing was done on each member using standard NIH microlymphocytotoxicity technique.⁴ Certain other families with affected children born before 1966 were studied also. Population figures were obtained from the Welsh Office. Parents were asked about their attitudes to antenatal detection for CAH.

Results

Numbers of cases. In the 12 years from 1966 to 1977 14 female and 5 male salt-losing cases, and 5 female and 2 male nonsalt-losing cases were detected (Table 1). No one was known to have died in the period. The difference between the male and female cases was significant ($P < 0.05$). The ratio of salt-losers to nonsalt-losers was 19 to 7 (or 2.7 : 1). This discrepancy suggests that some male cases of CAH had not been detected; this was probably because some male salt-losers had died before the diagnosis had been established.

Gene and carrier frequency calculations. In view of the difference in the number of male and female

Table 1 *Details of the CAH families*

Family	Year of birth	Sex	Type	Area of birth	Family members
3	1966	F	Nonsalt	South Glamorgan	● ○ □ ○ ○
4	1972	F	Salt	Mid Glamorgan	● □
5	1966	F	Salt	Gwent	□ ■ ● ●
	1970	F	Salt	Gwent	
6	1972	F	Salt	South Glamorgan	○ ●
7	1976	F	Nonsalt	Gwent	●
8	1976	F	Nonsalt	South Glamorgan	○ ●
9	1966	F	Nonsalt	South Glamorgan	○ ○ ●
10	1968	F	Salt	West Glamorgan	■ ○ ○ ○ ○ ●
12	1972	F	Salt	Clwyd	●
13	1976	M	Salt	Clwyd	■
14	1977	M	Salt	Clwyd	□ □ ■
15	1969	M	Salt	Clwyd	■ □
16	1970	F	Nonsalt	Gwynedd	○ ●
17	1971	F	Salt	Gwynedd	□ ● □
18	1972	M	Nonsalt	Powys	○ □ ■
19	1973	M	Salt	Mid Glamorgan	■
20	1974	F	Salt	Mid Glamorgan	●
22	1970	M	Salt	South Glamorgan	■
23	1975	F	Salt	Mid Glamorgan	● □
24	1974	F	Salt	Dyfed	●
25	1971	F	Salt	West Glamorgan	●
26	1966	F	Salt	Gwynedd	○ ●
27	1976	F	Salt	West Glamorgan	●
28	1977	F	Salt	Gwynedd	○ ● □
29	1969	M	Nonsalt	South Glamorgan	■ ○

□ Unaffected male, ■ affected male, ○ unaffected female, ● affected female.

Table 2 *HLA haplotypes of certain affected families*

Family		Parents (haplotype)	HLA haplotype of parents	Affected		Nonaffected siblings		
				1st child	2nd child			
CAH 2	F	A2 B27/A3 B7	a/b	b/d		a/c		
Salt	M	A31/33 B5/A31/33 B39	c/d					
CAH 3	F	A2 B12/— B35	a/b	a/d		a/c	a/c	a/c
Nonsalt	M	A2 B5/A11 B22	c/d					
CAH 4	F	A2 B27/A1 B40	a/b	b/d		b/c		
Salt	M	A2 B5/A3 B7	c/d					
CAH 5	F	A2 B5/A11 B22	a/b	b/c	b/c			
Salt	M	A26 B38/A2 —	c/d					
CAH 6	F	A1 B39/A2 B7	a/b	a/d		b/c		
Salt	M	A11 B35/A3 B47	c/d					
CAH 9	F	A3 B18/A26 B12	a/b	a/c		b/d		
Nonsalt	M	A3 B18/A3 B18	c/d					
CAH 10	F	A3 B13/A11 B27	a/l	a/d		a/c	a/c	b/c
Salt	M	A2 B12/A26 B16	c/d					
CAH 11	F	A3 B7/A2 B40	a/b	a/d		b/c		
Nonsalt	M	A1 B8/A29 B12	c/d					
CAH 15	F	A3 B47/— B12	a/b	a/c		a/d		
Salt	M	— B15/A25 B18	c/d					
CAH 16	F	A2 B40/(not tested)	a/b	a/c		a/d		
Nonsalt	M	A2 B12/A24 B12	c/d					
CAH 17	F	A3 B15/—	a/b	a/c		a/d		
Salt	M	A2 B7/ B12	c/d					
CAH 18	F	A1 B35/A25 B8	a/b	b/d		b/d*	b/c	
Nonsalt	M	A2 B12/A28 B8	c/d					
CAH 28	F	A2 B12/A3 B7	a/b	a/d		b/c		
Salt	M	A2 B16/A2 B12	c/d					

*Recombinant.

cases we performed gene and carrier frequency calculations on the female cases and on the female population in Wales. We presumed that not all males had been ascertained, but that all females had in view of their obvious genital abnormality.

There were 19 female cases and 229 875 female births between 1966 and 1977. This gives an incidence of one in 12 099 (0.0000826). The gene frequency is thus one in 110 ($\sqrt{1/12099}$ or 0.00909, and the carrier or heterozygote frequency one in 55.

Segregation analysis confirmed the recessive inheritance of the condition. Using the priori method⁵ there were 15 cases compared with 15 675 expected cases (standard error 1.795). There was no known consanguinity.

HLA studies. HLA groups on families with more than one boy are shown in Table 2. Some families with affected children born before 1966 are included.

There was only one recombinant family (Case 18) where a presumed unaffected boy had the same typing as his affected brother. The unaffected brother showed no clinical abnormality. The baseline and 60-minute post-ACTH stimulated 17OH-progesterone concentration were normal. This excluded CAH due to 21-hydroxylase deficiency and indicated that he was a genuine recombinant rather than a subclinical case of CAH.

Antenatal detection. Only 2 of the 26 parents would have wished to take advantage of antenatal detection and abortion had a reliable method of detecting an affected fetus been available.

Discussion

Numbers of cases. It was expected that not all boys with nonsalt-losing CAH would be detected because in them the clinical presentation in this form of the disease is delayed. There was a surprising difference however, between the numbers of male and female salt-losers. This suggests that some male salt-losing CAH cases had died without an established diagnosis. In the study there were 2 brothers of index patients who had died in early life; in retrospect the circumstances surrounding both deaths suggest a salt-losing crisis due to CAH.

There are two possible approaches to the early diagnosis of CAH and to the prevention of death in salt-losing boys. Firstly, it is now well established that the measurement of plasma 17OH-progesterone is a reliable test for the early diagnosis of CAH due to 21-hydroxylase deficiency.⁶ More widespread use and easy availability of this assay in cases of unexplained vomiting and electrolyte disturbances in newborn boys should reduce the numbers of missed salt-losing

CAH cases. In addition, the use of assays of plasma renin activity can be useful in documenting salt depletion before any electrolyte disturbance or clinical sign of salt-wasting occurs.⁷ Secondly, it may be possible to screen newborn boys. A microfilter paper method for plasma 17OH-progesterone has been described⁸ which could, if necessary, be incorporated in the present newborn screening programme for inborn errors of metabolism. However, this would not be cost effective, as, unlike cases of phenylketonuria, such infants die and do not become long-term financial burdens to the community.

Carrier frequency. The carrier frequency (1/55) calculated for CAH in this study makes this the second most common recognisable recessive gene in Wales. It is less common only than cystic fibrosis and is more common than phenylketonuria which has a carrier frequency of 1/59.⁹ Our results show that CAH is less common in Wales than in Switzerland,¹⁰ but more common than in the USA.¹

HLA studies. It is clear from our study and from several others^{3 11-12} that the linkage of HLA and the CAH locus is very close, with a recombination fraction less than 0.05. There are difficulties in calculating exact Lod scores and recombination fractions for a recessive gene with a locus such as HLA, without loss of information with such a close linkage.¹³ It also appears that the linkage applies equally for both salt-losing and nonsalt-losing forms of CAH.

The linkage between HLA and CAH is of considerable interest. However, it is likely to have only limited clinical application. Only a few parents would wish to take advantage of antenatal detection and abortion of a fetus affected by a condition which can adequately be treated postnatally. It has been suggested that HLA typing could be used to test siblings of affected cases at birth.⁸ However, the measurement of plasma 17OH-progesterone concentrations is a rapid, accurate, and reliable test for the diagnosis of this condition within the first few days of life.¹⁴ Siblings of affected cases could have heterozygote detection using HLA typing, but the risks of an affected child in a nonconsanguineous marriage are low (1/165). Heterozygote detection would generally not be justified and would cause unnecessary anxiety for the family.

Genetic basis for salt-losing and non-salt-losing cases. In Wales, unlike Switzerland,¹⁰ salt-losing cases are more than twice as common as nonsalt-losing ones. The genetic basis for the two forms of the condition is unclear. However, we now know that the HLA linkage applies equally in both forms. The most likely explanation for this is that there are two

genes for CAH which are alleles. If these genes are represented by S for salt-losing CAH and N for nonsalt-losing CAH, cases would have 3 genotypes: nonsalt-losers being homozygous NN and salt-losers being of two types, homozygous SS and heterozygous NS.

There is some evidence that salt-losing CAH cases may be phenotypically divisible insofar as they appear to vary in their tolerance to a sodium-restricted diet.¹³ If the two-allele hypothesis is correct, the gene frequencies for the salt-losing and N genes are 1/219 and 1/214 respectively.

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