

Prenatal diagnosis of congenital adrenal hyperplasia: reliability of amniotic fluid steroid analysis

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SUMMARY The concentration of 170H-progesterone was measured in amniotic fluid samples collected from 55 mothers who had previously had a child with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. In eight pregnancies the levels of 170H-progesterone were raised; the parents elected to terminate in four and examinations of the fetus confirmed the diagnosis of congenital adrenal hyperplasia. In each case, the affected sib was a salt loser. The remaining four affected pregnancies proceeded to term and each infant had salt losing 21-hydroxylase deficiency. All 47 infants predicted to be unaffected were normal at birth. However, an increased plasma concentration of 170H-progesterone was documented in a male non-salt loser at three months of age.

Prenatal diagnosis of congenital adrenal hyperplasia by amniotic fluid steroid analysis is reliable only for the salt losing variant of 21-hydroxylase deficiency. Of the affected sibs in this study, 20% died during infancy in a salt losing crisis. This simple and rapid prenatal test is sufficiently reliable to predict the group of infants most at risk in early infancy.

Congenital adrenal hyperplasia is an autosomal recessive disorder of steroid biosynthesis due to deficiency of the enzyme 21-hydroxylase in more than 90% of cases.¹ The increased concentration of 170H-progesterone in the blood provides a rapid and reliable test for the disorder at birth.^{2,3} The fetal adrenal gland synthesises steroids from early gestation⁴; consequently the prenatal diagnosis of congenital adrenal hyperplasia can be established in pregnancies at risk by measurement of increased concentrations of 170H-progesterone in amniotic fluid.⁵⁻⁹

The majority of patients with congenital adrenal hyperplasia are salt losers due to a deficiency in both glucocorticoid and mineralocorticoid biosynthesis. The remainder are classified as either simple virilisers, late onset, and cryptic or non-classical cases.¹⁰ A recent review of published cases detected prenatally suggested that only the salt losing variant was identified by an increased concentration of 170H-progesterone in amniotic fluid.¹¹ We report our experience with the use of this test in a large number of pregnancies at risk of fetal 21-hydroxylase deficiency. The results are reviewed in relation to

details of the affected sibs (index cases) and the outcome of the pregnancies at risk.

Patients

A total of 55 pregnancies in 52 mothers who already had children with congenital adrenal hyperplasia was studied. One mother was tested during two and another during three consecutive pregnancies. Amniocentesis was performed between 14 and 19 weeks' gestation. A further 96 amniotic fluid samples collected from pregnancies at risk for fetal chromosomal abnormalities or neural tube defects but with a normal outcome were analysed to determine a concentration range for 170H-progesterone. Levels of this steroid remain constant between 14 and 20 weeks in maternal serum¹² and amniotic fluid.⁶ Furthermore, there is no correlation between the concentration of 170H-progesterone in samples of maternal serum and amniotic fluid collected simultaneously.⁶

Gestational age was calculated from the date of the last menstrual period and confirmed by measurement of the fetal biparietal diameter. Each family received genetic counselling before amniocentesis. Details were recorded about the outcome of pregnancies tested and about the affected sibs. These included sex, the appearance of

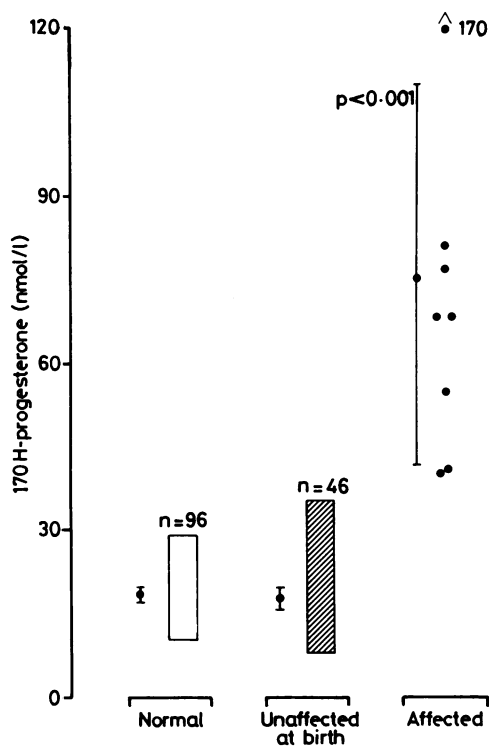


FIG 1 Mean concentration of 170H-progesterone (and 95% confidence intervals) in amniotic fluid from normal pregnancies and from pregnancies at risk for fetal 21-hydroxylase deficiency. The amniotic fluid was collected during the second trimester.

the genitalia, and whether the infant or child was a salt loser.

Methods

Amniotic fluid supernatants were stored at -20°C until analysed. The 170H-progesterone concentration was determined by radioimmunoassay using an ^{125}I -radioligand and a magnetisable solid phase antiserum.¹³ Progesterone showed 6% cross reactivity with a 170H-progesterone antiserum. The sensitivity of the assay was 0.8 nmol/l; the within and between assay coefficients of variation of high, medium, and low quality control samples did not exceed 10%. The data were calculated as means and standard deviation (SD) and expressed with 95% confidence intervals. Group results were compared by the unpaired Student's *t* test.

Results

AMNIOTIC FLUID STEROID ANALYSIS

The concentration of 170H-progesterone in all amniotic fluid samples analysed is shown in fig 1. The results from pregnancies at risk for fetal 21-hydroxylase deficiency were classified according to known pregnancy outcome.

The mean concentration of 170H-progesterone in second trimester amniotic fluid from normal pregnancies was 18.5 nmol/l (5.2 SD). In 47 term pregnancies at risk with normal infants at birth, there was no significant difference in mean amniotic fluid 170H-progesterone concentration (17.1 nmol/l, 6.3 SD, $p>0.1$). There was a significant increase ($p<0.001$) in mean 170H-progesterone concentration in amniotic fluid from known affected pregnancies

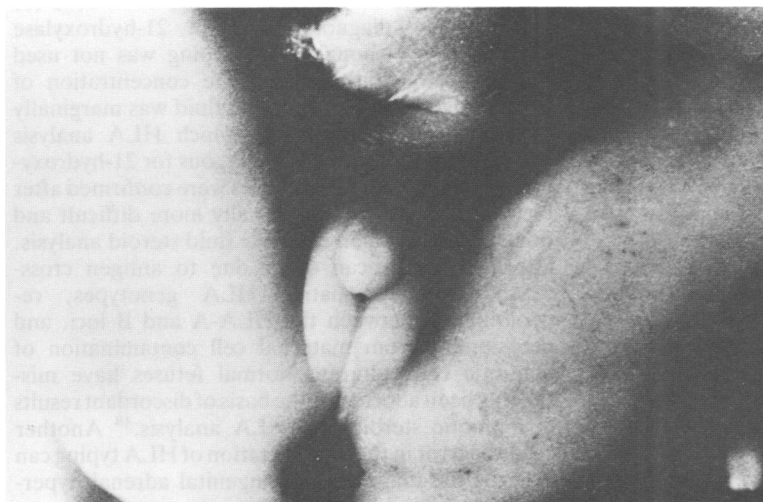


FIG 2 Appearance of the external genitalia of a female fetus with 21-hydroxylase deficiency. There is clitoromegaly and fusion of the labia.

(76.0 nmol/l, 44.5 SD). Individual values ranged from 40 to 170 nmol/l.

OUTCOME OF PREGNANCIES TESTED

Four of the eight affected pregnancies were terminated before 20 weeks' gestation at the request of the parents. All three female fetuses showed evidence of virilisation with fused labia and a single urogenital opening at the base of an enlarged clitoris (fig 2). The external genitalia of the affected male fetus were normal but the combined weight of the adrenal glands (2.4 g) was increased for gestational age (in normals, the mean combined weight is 1.2 g with 95% confidence intervals of 0.6 to 1.7 g).¹⁴ HLA typing of amniotic cells was identical to the haplotype in the affected sib and concurred with a raised 170H-progesterone concentration (55 nmol/l).

The remaining four pregnancies proceeded to term. A male infant was delivered in each case and 21-hydroxylase deficiency was confirmed by a raised plasma 170H-progesterone concentration. The pattern of plasma electrolyte concentrations was consistent with each infant being a salt loser. A male infant, one of the 47 infants normal at birth, was investigated at three months of age because of scrotal pigmentation. The diagnosis of congenital adrenal hyperplasia was confirmed by increased plasma concentrations of 170H-progesterone and testosterone. He was a non-salt loser, as was his affected sib.

AFFECTED SIBS

Details were available on 44 affected sibs (index cases) with congenital adrenal hyperplasia, as summarised in the table. The majority were female and 77% of the total were salt losers. Nine of these (20% of all index cases in this series) had died in infancy during an episode of acute adrenal insufficiency. There were no reported deaths in non-salt losers.

Discussion

This study extends our previous observations on the value of amniotic fluid 170H-progesterone measurements for the prenatal diagnosis of congenital adrenal hyperplasia.^{7,9} The assay is simple and results are available within two hours of receipt of

the sample.¹³ There is now a question about the reliability of the test for the prenatal diagnosis of certain variants of congenital adrenal hyperplasia.

In the eight pregnancies where the fetus was known to be affected by 21-hydroxylase deficiency, the concentration of 170H-progesterone in amniotic fluid was clearly raised. The salt losing variant of congenital adrenal hyperplasia was a common factor in all these cases; this was confirmed in four infants delivered at term. The affected sibs of all four aborted fetuses were also salt losers. Of the 55 pregnancies at risk for fetal 21-hydroxylase deficiency, 14.5% were affected compared to an expected incidence of 25% for an autosomal recessive disorder. However, in the larger group predicted to be unaffected and with normal infants at birth, there was one male non-salt loser proven to be affected at three months of age. In this instance the proband was also a non-salt loser. However, the accepted dogma that the simple virilising or salt losing variant breeds true within a family is challenged by the recent observation of non-concordance for salt wasting in some HLA identical sibs.¹⁵ It is possible that simple virilising, late onset, and cryptic variants of congenital adrenal hyperplasia may only become recognised with continued follow up. The predominance of salt losers among cases detected prenatally in this and in two recently reported studies^{11,16} emphasises that amniotic fluid 170H-progesterone measurement is reliable only for the salt losing variant of congenital adrenal hyperplasia.

There is close genetic linkage between HLA and congenital adrenal hyperplasia due to 21-hydroxylase deficiency.¹⁰ HLA typing of cultured amniotic cells and comparison with HLA antigens in the affected sib and parents has also been used for the prenatal diagnosis of fetal 21-hydroxylase deficiency.¹⁷ Although HLA typing was not used systematically in this study, the concentration of 170H-progesterone in amniotic fluid was marginally raised in one pregnancy in which HLA analysis showed the fetus to be heterozygous for 21-hydroxylase deficiency. The HLA types were confirmed after birth. HLA typing is technically more difficult and time consuming than amniotic fluid steroid analysis. Spurious results can occur due to antigen cross-reactions, uninformative HLA genotypes, recombination between the HLA-A and B loci, and occasionally from maternal cell contamination of amniotic cell cultures. Normal fetuses have mistakenly been aborted on the basis of discordant results of amniotic steroid and HLA analysis.¹⁸ Another cause of error in the interpretation of HLA typing can occur if the diagnosis of congenital adrenal hyperplasia in the index case is incorrect.

TABLE Details of index cases (sibs) with congenital adrenal hyperplasia.

	Male	Female	Total No	% of total
All index cases	17	27	44	100
Salt losers	15	19	34	77
Deaths	4	5	9	20
Non-salt losers	2	8	10	23
Deaths	0	0	0	0

An alternative approach to prenatal diagnosis is by analysis of DNA isolated from chorionic villi. Using suitable cDNA probes which recognise sequences in the 21-hydroxylase gene complex, a structural gene deletion associated with the HLA-Bw47 haplotype,¹⁹ and conversion of the 21-hydroxylase gene to an inactive or pseudogene,²⁰ have recently been reported in patients with 21-hydroxylase deficiency. This allows the potential for prevention of virilisation in an affected female fetus by maternal administration of glucocorticoids in early gestation. However, present techniques of DNA analysis do not permit recognition of smaller gene deletions or point mutations which give rise to the various clinical phenotypes of congenital adrenal hyperplasia.²¹ It is likely that cDNA probes for the HLA-B and DR genes will be more informative for early prenatal diagnosis of 21-hydroxylase deficiency since these genes are highly polymorphic.²²

It is uncommon for the parents of a child with congenital adrenal hyperplasia to request termination of another affected pregnancy. The unexpected birth of an infant with ambiguous genitalia or an infant ill from acute adrenal insufficiency are the usual reasons for the parents wishing to know the outcome in subsequent pregnancies. Of the index cases in this study, 77% were salt losers, many of whom were acutely ill before the correct diagnosis was made. A significant number (20% of all index cases) had died in infancy, as had been reported in a previous genetic study of congenital adrenal hyperplasia in Wales.²³ Measurement of amniotic fluid 17OH-progesterone concentrations during the second trimester reliably detects the fetus with salt losing 21-hydroxylase deficiency. The test is simple, rapid, and readily available and can predict the group of infants with congenital adrenal hyperplasia who are most at risk in early infancy.

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