A virilised female infant with congenital adrenal hyperplasia: could this have been prevented?

Congenital adrenal hyperplasia (CAH) is caused by a defect in adrenal steroid biosynthesis, causing reduced glucocorticoid production and increased androgen production. This report presents the case of a severely virilised newborn female infant with presumed classic salt-wasting CAH. The role of family education, genetic screening, prenatal diagnosis and the dilemmas of therapeutic options are discussed.

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Key points

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1. A severe case of virilisation in a newborn female infant is presented.
2. It is possible that virilisation could have been prevented by prenatal diagnosis and treatment of the at-risk fetus but such therapy is not standard and remains controversial.

The case

A one-day-old term infant presented with ambiguous genitalia with fusion of the labioscrotal folds and hypospadias (**FIGURE 1**). The infant was born to a 33-year-old woman at 39^{+1} weeks' gestation. The mother had presented for prenatal care at 15^{+2} weeks after her last menstrual period. A prenatal ultrasound scan at 19^{+6} weeks had raised concern about ambiguous genitalia.

It was noted that the first child of the patient's parents, a son, was being followed by the paediatric endocrinology team for classic salt-wasting caused by 21-hydroxylase deficiency. He was found to have bi-allelic mutations (In2G and Q318X) in the *CYP21A2* gene, associated with a phenotype of classic salt-wasting congenital adrenal hyperplasia (CAH). By report, the parents never followed-up with the genetic service to discuss the risk of female virilisation and adrenal insufficiency for future pregnancies nor the testing options based on the previously identified pathogenic variants of the *CYP21A2* gene.

At the time of the patient's prenatal ultrasound scan, the mother was referred to genetic counsellors who, given the family history of CAH, were concerned that the fetus was a virilised female. Non-invasive prenatal testing confirmed that this fetus had two X chromosomes. In addition to ambiguous genitalia, the pregnancy was complicated by gestational diabetes treated with glyburide.

The infant was born by scheduled caesarean section with artificial rupture of the membranes at delivery. Apgar scores were 8 at one and five minutes; the birth weight was 3,480g. The initial examination confirmed ambiguous genitalia. The initial electrolyte tests measured:

- normal sodium levels of 140mEq/L
- normal potassium levels of 5mEq/L
- glucose levels before feeds ranging from 60 to 88mg/dL

A physical examination on day 1 of life revealed:

- blood pressure of 68/36mmHg
- pulse of 137bpm
- respiratory rate of 44bpm.

The infant did not demonstrate acute distress and, aside from the genitourinary aspects, there were no other concerns in the physical examination. The external genitalia (**FIGURE 1**) were noted to be rated four to five on the Prader scale with complete fusion of the labioscrotal folds, which were hyperpigmented and with rugae. There was also a urethral opening at approximately 0.25-0.5cm from the end of the clitorophallus, which had a stretched length of just over 2cm. No gonads could be palpated in the labial area. A pelvic ultrasound scan identified a uterus measuring 1.3 x 1 x 1.2cm but no gonads. The paediatric endocrinology team was consulted for an evaluation of the newborn infant. The presence of two X chromosomes and no Y chromosome was confirmed by *in situ* hybridisation. The infant was monitored closely and remained clinically stable.

After 48 hours of life, a cosyntropin stimulation test was performed to evaluate the ability of the adrenal cortex to produce cortisol after stimulation by synthetic

adrenocorticotropic hormone (ACTH). The baseline laboratory and post-stimulation test results are shown in **TABLE 1**. Immediately after the stimulation test the infant was commenced on hydrocortisone (1mg three times a day; 14.5mg/m^2 surface area/day) and fludrocortisone (0.05mg daily), while awaiting the laboratory test results for presumed 21-hydroxylase (21-OHD) deficiency.

In the future, the girl will be evaluated by the genetics department as an outpatient for confirmation of the diagnosis and for mutation analysis.

Discussion

CAH is the most common disorder of sexual differentiation with a global prevalence of around 1:14,500.[1] The most common form of CAH is 21-OHD deficiency, an autosomal recessive disorder due to bi-allelic homozygous or compound heterozygous mutations in the *CYP21A2* gene. The enzyme 21-OHD helps to make cortisol and aldosterone in the adrenal glands. Cortisol regulates glucose levels and aldosterone has important effects on blood pressure regulation and electrolyte haemostasis. CAH is characterised by increased androgen levels and decreased cortisol and aldosterone.

CAH manifests in a variety of phenotypic severities, which are broadly classified as classic and non-classic disease. Classic disease is more severe and includes salt-wasting and simple virilising forms. The salt-wasting form is associated with hyponatraemia (low sodium in the blood) and hyperkalaemia (high potassium in the blood) in both males and females, with aldosterone deficiency.

Classic forms of 21-OHD cause affected females to experience virilisation physically and possibly psychologically.[2] In classic CAH, virilisation of the external genitalia is noticeable at birth in a newborn with 46,XX chromosomes and presents as ambiguous genitalia due to excess adrenal androgen production *in utero*. Virilisation of the external genitalia is variable but extreme cases can present with almost normal male external genitalia with cryptorchidism, as displayed in the patient in this case report.

Infants with 46,XY classic CAH generally present with normal or slight over-virilisation (slightly larger penis, perineal hyperpigmentation and smaller fontanelles). If undiagnosed in the newborn period, 46,XY individuals with markedly impaired 21-OHD activity may present with a salt-wasting episode. If some enzyme activity is present, as in simple-virilising forms, presentation may involve progressive virilisation and increased growth, or salt-wasting crises during periods of stress.

The case presented here highlights current dilemmas and controversies in the prenatal treatment of classic virilising forms of CAH. Appropriate professional genetic counseling is recommended to at-risk families, not only to identify virilisation in female fetuses but to evaluate for genotype-phenotype correlations and to educate about the expectations of the disease.[3] At-risk families have a one-in-four chance of an affected fetus and a one-in-eight chance of an affected female with some degree of external genitalia virilisation. If a previous male is affected with a salt-wasting form or a simple virilising form, future daughters of affected families will be at risk for severe virilisation, as demonstrated in this case.

Molecular genetic analysis for delineation of the type of CAH is available and this information could be used for targeted analyses of fetal DNA (obtained by chorionic villus sampling) for early prenatal diagnosis of 21-OHD, as early as 14 weeks' gestation.[4,5] In addition, fetal sex determination is available for clinical use as early as nine weeks' gestation;[6] virilisation of external genitalia in affected females occurs after week nine.

Prenatal treatment of pregnant mothers with dexamethasone has been offered to at-risk families to prevent virilisation in affected 46,XX fetuses.[2,4,7] At-risk families include families with previous children affected with classic forms of virilising CAH or couples of susceptible populations known for an increased carrier frequency (eg Yupik Eskimos of Alaska, Native Americans, populations from northeast Brazil and east India). Prenatal dexameth-

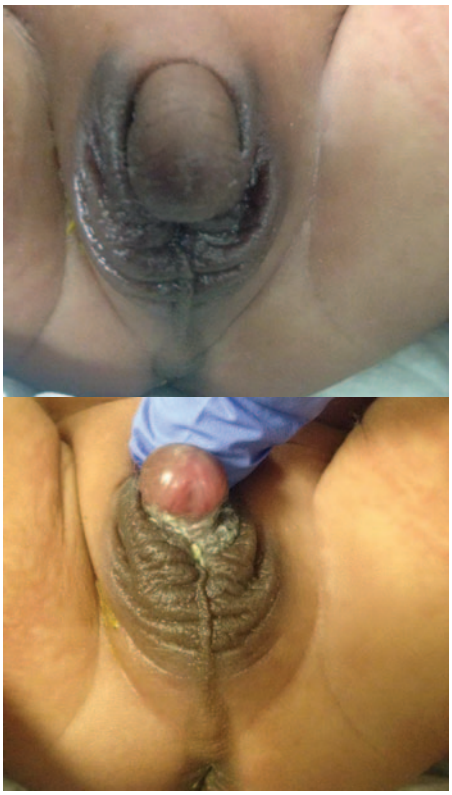

FIGURE 1 The infant presented with ambiguous genitalia. There is fusion of the labioscrotal folds, with hyperpigmentation and rugae. A urethral opening can be seen at approximately 0.25-0.5cm from the end of the clitorophallus. No gonads could be palpated in the labial area.

Baseline laboratory tests	Level	Reference range	Interpretation
Adrenocorticotropic hormone (ACTH, pmol/L)	34.1	1.6-13.9	Elevated
Cortisol (nmol/L)	372.5	94.9-619.4	Normal
Renin (ng/L/sec)	3.72	0.6-10.3	Normal
Aldosterone (nmol/L)	<0.028	0.2-5.1 (day 3)	Very low
Post-cosyntropin stimulation (125µg)			
Cortisol at 30 minutes (nmol/L)	430.4	94.9-619.4	Not passing
Cortisol at 60 minutes (nmol/L)	474.5	94.9-619.4	Not passing
17-hydroxyprogesterone at 60 minutes (nmol/L)	265.9	0-2.1	Elevated

TABLE 1 A summary of the laboratory results. The cosyntropin stimulation test confirmed 21-OHD deficiency.

asone therapy can reduce virilisation in the female fetus when started very early in pregnancy.[7,8] However, currently there are many opposing opinions about prenatal dexamethasone therapy due to an association with orofacial clefts, decreased birth weight, poor verbal memory and poor social competence,[9] along with the possibility of hypertension and oedema in treated mothers.[7]

In order to prevent virilisation, dexamethasone treatment has to be started as soon as pregnancy is confirmed, before the gender and the status of the fetus (affected *vs* carrier *vs* non-carrier) is known. If, at 14 weeks' gestation, prenatal diagnosis determines that the fetus is either 46,XY or an unaffected female, dexamethasone therapy should be stopped immediately.[4,10] For an affected female fetus, treatment should be continued until the end of the pregnancy.

Since prenatal dexamethasone therapy has to be started in early pregnancy, before the gender of the fetus is known and before a diagnosis of classic CAH is established, it may result in the unnecessary treatment of unaffected female fetuses (a probability of three in every eight) or male fetuses (a probability of four in eight). Only one in eight treated at-risk fetuses will benefit from prenatal treatment. Despite the effectiveness in preventing female virilisation, the long-term effect of treating unaffected fetuses poses a dilemma with conflicting opinions.[9] Consequently the procedure is not included as a standard of care in many practice guidelines.[11]

The quality of life and mental health aspects of affected virilised females, who may require multiple urological surgical procedures, should always be a part of CAH care.[11] The medical and psychological care needs to be individualised for each affected patient.[12] It is very important to recognise at-risk families and to counsel them about the possibility of prenatal therapy, with its potential risks and benefits,[13] and to consider the physical and psychological effects of virilisation of affected females if prenatal therapy is not given. Prenatal dexamethasone therapy is still considered experimental and is only advised in specialised centres after ethical and legal board approval.[14]

Conclusion

With no global consensus about the use of prenatal dexamethasone it is difficult to draw up guidelines for the standard of care when dealing with a potential diagnosis and prenatal therapy of CAH. Parents and healthcare providers face a medical and ethical dilemma when aiming to reduce the virilisation of affected females with 21-OHD by prenatal dexamethasone therapy. The potential risks to the fetus and mother will need to be considered and measured against the medical and psychological consequences for affected virilised females.

Parental consent

The authors received written consent to publish this report from the patient's parents.

References

1. **Krege S.** Congenital adrenal hyperplasia in girls/young women. *Urologe A* 2014;53:206-12.
2. **Nimkarn S., New M.I.** Prenatal diagnosis and treatment of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Mol Cell Endocrinol* 2009; 300:192-96.
3. **New M.I., Abraham M., Gonzalez B. et al.** Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *PNAS* 2013;110:2611-616.
4. **Nimkarn S., New M.I.** Congenital adrenal hyperplasia due to 21-hydroxylase deficiency: a paradigm for prenatal diagnosis and treatment. *Ann NY Acad Sci* 2010;1192:5-11.
5. **New M.I., Tong Y.K., Yuen T. et al.** Noninvasive prenatal diagnosis of congenital adrenal hyperplasia using cell-free fetal DNA in maternal plasma. *J Clin Endocrinol Metab* 2014;99:e1022-30.
6. **Committee Opinion No. 640.** Cell-free DNA screening for fetal aneuploidy. *Obstet Gynecol* 2015;126:e31-37.
7. **Mercè Fernandez-Balsells M., Muthusamy K., Smushkin G. et al.** Prenatal dexamethasone use for the prevention of virilization in pregnancies at risk for classical congenital adrenal hyperplasia because of 21-hydroxylase (CYP21A2) deficiency: a systematic review and meta-analyses. *Clin Endocrinol* 2010;73:436-44.
8. **Gorduza D., Tardy-Guidollet V., Robert E. et al.** Late prenatal dexamethasone and phenotype variations in 46,XX CAH: concerns about current protocols and benefits for surgical procedures. *J Pediatr Urol* 2014;10:941-47.
9. **Miller W.L.** Fetal endocrine therapy for congenital adrenal hyperplasia should not be done. *Best Pract Res Clin Endocrinol Metab* 2015;29:469-83.
10. **Maryniak A., Ginalska-Malinowska M., Bielawska A., Ondruch A.** Cognitive and social function in girls with congenital adrenal hyperplasia: influence of prenatally administered dexamethasone. *Child Neuropsychol* 2014;20:60-70.
11. **Speiser P.W., Azziz R., Baskin L.S. et al.** Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010;95:4133-60.
12. **Nordenström A.** Adult women with 21-hydroxylase deficient congenital adrenal hyperplasia, surgical and psychological aspects. *Curr Opin Pediatr* 2011 23:436-42.
13. **Lajic S., Nordenström A., Hirvikoski T.** Long-term outcome of prenatal dexamethasone treatment of 21-hydroxylase deficiency. *Endocr Dev* 2011;20:96-105.
14. **Witchel S.F., Miller W.L.** Prenatal treatment of congenital adrenal hyperplasia – not standard of care. *J Genet Couns* 2012;21:615-24.