CASE REPORT

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Four Consecutive False Negative Newborn Screens in a Patient with Classical Congenital Adrenal Hyperplasia: A Case Report

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What is already known on this topic?

Classical congenital adrenal hyperplasia (CAH) presents early in life and requires lifelong treatment. It is marked by life-threatening "adrenal crises" due to critical steroid hormone deficiency. Newborn screening (NBS) offers protection against such crises by prompting diagnosis and treatment of CAH soon after birth, when patients are still generally asymptomatic. It is imperative that NBS has high sensitivity to minimize false negatives.

What this study adds?

NBS for CAH, including classical CAH, is subject to false negative results, even using high-sensitivity assays. Possible sources of false negative results on NBS for CAH include antenatal steroids, decreased 11HSD2 activity in pregnancies with intrauterine growth restriction, and sodium supplementation prior to sample collection. Persistent hyponatremia without an alternate explanation may indicate classical CAH in spite of normal NBS results. It should be remembered that NBS is screening and not diagnostic; appropriate diagnostic tests should be performed if clinical suspicion remains despite unremarkable NBS results.

ABSTRACT

21-hydroxylase deficiency is the most common cause of congenital adrenal hyperplasia (CAH). Salt-wasting CAH can present with life-threatening salt-wasting crises, underscoring the importance of universal newborn screening. We present a patient diagnosed with classical CAH despite four negative newborn screening (NBS). A male infant was born at 35 weeks gestation with birthweight 1470 grams following signs of placental insufficiency. While hospitalized in the neonatal intensive care unit (NICU), four NBS samples from days of life 2 to 38 were all within normal range, including on repeat analysis using fully integrated fluoroimmunoassay. After initially normal biochemical testing, hyponatremia and hyperkalemia developed by day of life (DOL) 26, responsive to sodium chloride supplementation. Following recurrent hyponatremia after a trial off supplementation after DOL 50, 17-hydroxyprogesterone measured by liquid chromatography-tandem mass spectrometry were reported by

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two different labs as 10,900 ng/dL and 11,200 ng/dL (normal range at DOL 50<2 ng/dL). Subsequent testing identified deletion of one *CYP21A2* allele and a mutation, *I172N*, in the second. This report illustrates the importance of maintaining a high index of suspicion for classical forms of CAH in infants with persistent electrolyte disturbances despite negative NBS results.

Keywords: Congenital adrenal hyperplasia, newborn screening, hyponatremia, 17-hydroxyprogesterone

Introduction

Steroid 21-hydroxylase deficiency (21-OHD) is the most common cause of congenital adrenal hyperplasia (CAH), resulting from deficiency of the 21-hydroxylase enzyme encoded by *CYP21A2* that plays an essential role in cortisol and aldosterone synthesis in the adrenal cortex. The most common classical forms consist of simple virilizing (SV) type which accounts for 25% of classical 21-OHD; and the salt-wasting type (SW), which accounts for more than 75% of affected individuals (1).

In all 50 of the United States, including New Jersey, newborn screening obtained in the neonatal period includes 17-hydroxyprogesterone (17-OHP) to identify and begin treatment of CAH before the life-threatening salt-wasting crises that occur in SW CAH without hormone replacement are likely to develop. In New Jersey, state law requires this newborn screen to be obtained in all babies 24 to 48 hours after birth. 17-OHP is measured using the Revvity DELFIA assay. The thresholds for elevated values vary depending on birthweight, increasing with lower weight. These cutoffs are included in Table 1.

Given the high sensitivity of newborn screening, providers may conclude in error there is no possibility of CAH in babies with a negative screening result. However, false negative newborn screens for CAH have been reported. One identified risk factor for a false negative CAH screen is antenatal steroid administration, such as to induce fetal lung maturity. Even in the absence of antenatal exogenous steroids, the possibility remains of a false negative CAH screen. Given the life-threatening nature of a salt-wasting crisis, failure to investigate the possibility of CAH and to institute appropriate treatment carries high morbidity and mortality.

In this case report, we present a patient diagnosed with the classical form of 21-OHD CAH despite four negative newborn screening (NBS) results. We identify pertinent features of his presentation that may have led to the false negative screens, as well as aspects that ultimately assisted in making the diagnosis. The parents of the patient consented to the publication of this case report.

Case Report

A Caucasian male was born at an outside institution at 35 weeks gestation by Cesarean section for non-reassuring fetal heart tracing and intrauterine growth restriction (IUGR) to a 35-year-old mother. At 30 weeks, the mother was hospitalized due to absent end diastolic flow on umbilical Doppler, consistent with placental insufficiency. To promote fetal lung maturity, standard doses of glucocorticoids (2 doses of betamethasone at 12 mg/dose 24 hours apart) were administered at 30 and at 33 weeks gestation.

Both birth weight (BW) of 1470 g and length of 40.5 cm were consistent with small for gestational age (SGA). The baby was not in distress at birth. Examination revealed bilaterally descended testes palpable in a normally developed scrotum, and a normal sized phallus. He was admitted to the neonatal intensive care unit (NICU) for monitoring due to prematurity and to gradually introduce enteral feeds.

Initial routine biochemical testing showed normal sodium and potassium levels for postnatal age. After day of life (DOL) 26, hyponatremia and hyperkalemia developed with sodium of 127 mEq/L (various reference ranges have been proposed) (2) and potassium of 6.2 mEq/L (Harriet Lane newborn range: 3.7-5.9 mEq/L). Hyponatremia persisted with a nadir of 124 mEq/L; peak potassium was 8.0 mEq/L. These lab values are displayed in Table 2. Additional testing did not reveal hypoglycemia or metabolic acidosis. There was no hypotension, tachycardia, or other clinical evidence of dehydration.

Table 1. 17-hydroxyprogesterone (17-OHP) thresholds used for newborn screening in the state of New Jersey, USA. As birthweight decreases, the thresholds for elevated values increase. That table was provided by the Newborn Screening Laboratory of the New Jersey Department of Health

Birth weight (grams)	Within acceptable limits (ng/mL)	Borderline range (ng/mL)	Presumptive/critical range (ng/mL)
<1500	<100	100-<145	≥145
1500-<2500	<60	60-<80	≥80
2500-<300	<40	40-<48	≥48
≥3000	<35	35-<55	≥55

The NICU team attributed both electrolyte disturbances to prematurity and started sodium chloride supplementation at 4 mEq/kg/day on DOL 30. The hyponatremia resolved, leading to discontinuation of sodium supplementation. After the supplement was stopped, hyponatremia then recurred within one day, and again resolved with reintroduction of sodium supplementation. Due to incomplete records, it is unclear if additional specific treatment was provided for the hyperkalemia, which normalized during the first course of sodium supplementation and did not recur at any point. On DOL 39, with appropriate sodium and potassium levels for postnatal age and on full enteral feeds, he was discharged home on sodium supplementation of 3 mEq/kg/day. While in the NICU, neither Pediatric Endocrinology nor Nephrology were consulted.

Eleven days after discharge, he was admitted to our institution on DOL 50 for additional evaluation due to the pediatrician's concern about the prior electrolyte abnormalities. His examination was unchanged. His weight of 2.78 kg represented an average daily weight gain since birth of approximately 30 g.

During admission, at our request, all four NBS samples for CAH drawn on DOL 2, 6, 13 and 38 were re-analyzed in duplicate by the New Jersey Department of Health using fully integrated fluoroimmunoassay (3) and again were within the normal range.

During this second admission, electrolytes were normal on sodium supplementation. Hyponatremia again developed within one day after supplementation was discontinued; hyperkalemia did not develop. Additional diagnostic testing off sodium at that time included plasma renin activity (PRA) of 120 ng/mL/hr (normal range for 0-2 yrs: 1.4-7.8 ng/mL/hr; Mayo Medical Laboratories, Rochester, MN, USA), and an aldosterone level of 24 ng/dL (normal for 31 days-11 months of age: 6.5-86 ng/dL; Mayo Medical Laboratories). Sodium supplementation was restarted followed by normalization of sodium within one day.

Table 2. Laboratory values obtained during first hospitalization, with respective normal ranges for given age

Analytes	Day of life (DOL) 26	Between DOL 26 and DOL 30 (prior to sodium supplementation)
Sodium (mEq/L)	127 (various)	124 (various)
Potassium (mEq/L)	6.2 (3.7-5.9)	8.0 (3.7-5.9)

Table 3. Laboratory values obtained during second hospitalization, with respective normal range for given age

Analytes	Day of life (DOL) 51	DOL 56 (after beginning hydrocortisone)
Plasma renin activity (ng/mL/hr)	120 (1.4-7.8)	
Aldosterone (ng/dL)	24 (6.5-86)	
Mayo medical laboratories 17-hydroxyprogesterone (ng/dL)	10,900 (<2)	2,850 (<2)
Esoterix laboratory services 17-hydroxyprogesterone (ng/dL)	11,200 (<2)	2,250 (<2)

21-OHD CAH was considered despite the negative screening. Serum 17-OHP measurements on DOL 51 off sodium supplementation for one day performed at both Mayo Medical Laboratories (Rochester, MN) and Esoterix Laboratory Services (Calabasas Hills, CA, USA) by liquid chromatography-tandem mass spectrometry were reported at 10,900 ng/dL and 11,200 ng/dL (normal range<2 ng/dL at 50 DOL), respectively. Repeat levels five days after sodium supplementation was restarted had decreased to 2,850 and 2,250 ng/dL, respectively. The above lab values from the second hospitalization are displayed in Table 3.

A diagnosis of SW CAH was then made based on the history of hyponatremia and 17-OHP levels>10,000 ng/dL on two assays. Hydrocortisone (15 mg/m^2/day) and fludrocortisone (0.1 mg/day) were then started with sodium supplementation at 3 mEq/kg/day. Subsequent genetic testing for 21-OHD CAH at Mayo Medical Laboratories (Rochester, MN) with full Sanger gene sequencing and multiple ligation-dependent probe amplification identified deletion of one *CYP21A2* allele and the *I172N* mutation in the second allele.

Discussion

NBS for CAH using 17-OHP measurements was first introduced to the USA in the late 1970's, and since then has been adopted by all 50 states, as well as 52 other countries. In healthy, unaffected newborns, physiological 17-OHP levels are elevated at birth and then decline over the next 2-3 weeks (4). In contrast, levels in classical CAH typically increase postnatally (5). The main purpose of screening is to identify newborns with classical CAH, particularly the salt wasting form in males, to avoid potential life-threatening salt wasting crises. It is also utilized to prevent incorrect sex assignment in virilized females with classical CAH.

Certain studies have shown that the sensitivity of the NBS for CAH is lower than expected. Sarafoglou et al. (6) reviewed 838,241 NBS results in Minnesota reported over a 12-year period, and reported that of 67 patients with classical CAH, 15 were missed on initial NBS (ten with SV CAH and five with SW CAH; six males, nine females). This was a false negative rate (FNR) of 22%. The diagnosis in five of the female patients was based on presentation of atypical genitalia at birth. Although three others also presented with atypical genitalia at birth, their diagnoses were delayed until as early as three months and up to six years of age. The remaining female patient had vaginoplasty six years prior to diagnosis. Despite these numbers, the authors suggested that the FNR was likely even higher due to the likelihood of undiagnosed patients with CAH, patients

moving out of state, and/or infants that may have died with unidentified CAH (6).

A review by Varness et al. (7) of all NBS results in Wisconsin over a 12-year period identified eight patients diagnosed with classical CAH whose NBS results were negative. Overall sensitivity was 86%; although constrained by small numbers, sensitivity was further determined by sex to be 67% in females and 97% in males. The higher FNR for females could be partly explained by significantly lower 17-OHP levels reported in females on NBS (7). However, as most female patients with classical CAH are expected to be virilized, detection prior to reporting of the NBS result is expected to occur, making the reduced sensitivity less problematic for female patients. However, even significantly virilized females have unfortunately been missed as atypical genitalia may not be noted on physical examination (6).

Schreiner et al. (8) reported that in a questionnaire-based study sent to 24 medical center members of the German Working Group of Pediatric Endocrinology, five out of 214 children with classical CAH were not detected by NBS, including two with the salt-wasting form.

Based on the reported FNR using single screen protocols and the rise in 17-OHP after birth in classical CAH, 13 states have instituted a two-screen protocol to improve sensitivity (3). Chan et al. (9) reported that 11 out of 39 (28%) cases of classical CAH in Colorado were detected by the second NBS test at age 8-14 days of life. A five-year retrospective study by Held et al. (4) demonstrated that 6.5% of all SW CAH cases missed on the first screen were identified by the second screen. Similarly in Texas, 14% of classical CAH cases were detected on a second screen (10).

This patient's genotype is typically associated with SV CAH (1) although, as in the presented case, may also result in the SW phenotype (11). Certain studies (6,10) have found that the majority of CAH cases missed by NBS are SV CAH, due to less dramatic elevations in 17-OHP. This is an important distinction, as the motivation for NBS hinges on rapid detection prior to life-threatening crises developing, which are unlikely in the milder defects associated with SV CAH. It is notable, however, that this patient had significant enough disease to develop hyponatremia and hyperkalemia, and may have been spared clinical dehydration primarily due to being in a NICU for other reasons. Furthermore, the final 17-OHP levels that were obtained prior to diagnosis were robustly elevated, and well into the range associated with classical CAH. Therefore, while the FNR is likely lower in the population of patients with SW CAH whose identification is the primary goal of newborn screening, our case does demonstrate a possibility of false negative screening in patients who may potentially suffer clinical complications as a consequence.

A possible explanation for this patient's initial two negative NBS results could be antenatal administration of glucocorticoids to promote lung maturity. Gatelais et al. (12) demonstrated that multiple courses of antenatal glucocorticoids lowered 17-OHP levels in the newborns by about 30% compared to controls. The reason is transplacental transfer of glucocorticoids with suppression of fetal adrenal steroidogenesis. Inhaled and intranasal corticosteroids have also been reported to produce the same effect (13). However, this postnatal suppression is believed to last only about one week (14), so would not likely explain the negative third and fourth NBS results. Even though in this case repeat NBSs did not detect CAH after the suppression is expected to have faded, repeating the NBS for infants born following antenatal glucocorticoid exposure, or alternatively checking a serum 17-OHP level, 1-2 weeks after delivery will lead to detection of some cases missed on the first screen.

Sodium supplementation may explain the 4th negative NBS result, and the decrease in 17-OHP during the patient's readmission. As 21-hydroxylase enzyme plays a role in the production of both cortisol and aldosterone in the adrenal cortex, mutations in the *CYP21A2* gene in the SV type are expected to also affect aldosterone synthesis. In SV CAH, this can lead to a relative state of hypovolemia, which in turn stimulates production of renin, angiotensin II, and possibly vasopressin (15). Angiotensin II can directly stimulate adrenal steroidogenesis independently of adrenocorticotropic hormone (ACTH), while vasopressin can increase pituitary ACTH secretion. As sodium supplementation corrects hypovolemia, angiotensin II and vasopressin production decreases, in turn reducing adrenal steroidogenesis and lowering 17-OHP levels. Therefore, if repeating the NBS or measuring 17-OHP directly, it is important to do so prior to sodium supplementation in babies with hyponatremia, or if supplements have already been started to discontinue them prior to further evaluation, as in this case.

An additional factor could be decreased activity of placental 11-hydroxysteroid dehydrogenase type 2 (11HSD2) reported in pregnancies complicated by IUGR (16). 11HSD2 inactivates cortisol by conversion to cortisone (16), thus protecting the fetus from potentially harmful effects of endogenous maternal glucocorticoids. We hypothesize that decreased 11HSD2 activity increases maternal cortisol levels that cross the placenta and induce suppression of fetal adrenal steroidogenesis, including 17-OHP production. It is possible this contributed to the false negative in our particular case in a manner similar to the antenatal glucocorticoids.

A significant limitation to this case report is the lack of complete records. Since hyperkalemia was noted with the initial hyponatremia, but not subsequent episodes of it, additional information regarding any treatment for the hyperkalemia itself

would have been valuable. Once a diagnosis of CAH has been established, the standard of care is to provide mineralocorticoid replacement with fludrocortisone in addition to hydrocortisone and sodium supplementation (15), as our patient ultimately received after diagnosis. It is also unclear if there were any investigations, such as renin and aldosterone measurements, during the first admission exploring an adrenal pathology for the electrolyte abnormalities. Additional insights into the treating team's evaluation of the electrolyte disturbances, and why they were ultimately attributed to prematurity, would have allowed a more comprehensive analysis of this case.

Conclusion

Despite efforts to improve the sensitivity of NBS for CAH, challenges persist, with potentially devastating consequences. This report emphasizes the importance of maintaining a high index of suspicion for classical forms of CAH in all newborns presenting with similar electrolyte disturbances despite negative NBS results.

Ethics

Informed Consent: The parents of the patient consented to the publication of this case report.

Footnotes

Authorship Contributions

Concept: Patrick Rizzuto, Mariam Gangat, Ahmed Khattab, Ian Marshall, Design: Patrick Rizzuto, Mariam Gangat, Ahmed Khattab, Ian Marshall, Analysis or Interpretation: Patrick Rizzuto, Mariam Gangat, Ahmed Khattab, Ian Marshall, Literature Search: Patrick Rizzuto, Mariam Gangat, Ahmed Khattab, Ian Marshall, Writing: Patrick Rizzuto, Mariam Gangat, Ahmed Khattab, Ian Marshall.

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