Newborn Screening for Congenital Adrenal Hyperplasia in New Zealand, 1994–2013

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Objective: The objective of the study was to evaluate the efficacy of national newborn screening for severe congenital adrenal hyperplasia (CAH) in New Zealand over the past 20 years.

Methods: Newborn screening for CAH is performed through the estimation of 17-hydroxyprogesterone by a Delfia immunoassay. CAH cases diagnosed in the newborn period from 1994 to 2013 were identified from Newborn Metabolic Screening Programme records.

Results: Between 1994 and 2013, 44 neonates (28 females, 16 males) were diagnosed with CAH, giving an incidence of 1:26 727. Almost half (n = 21) of the newborns with CAH were detected solely via screening (not clinically suspected), including 21% of all affected females. Among the group solely ascertained by screening, 17-hydroxyprogesterone sampling occurred at a mean age of 3.3 days (range 2–8 d), the duration from sampling to notification was 5.2 days (0–12 d), and treatment was initiated at 12.0 days (6–122 d). Vomiting was present in 14% of those ascertained by screening, but none had hypotension or collapse at diagnosis. Increasing age at treatment was correlated with a progressive decrease in serum sodium ($r = -0.56$; $P < .0001$) and an increase in serum potassium concentrations ($r = 0.38$; $P = .017$). Compared with newborns diagnosed by screening alone, those clinically diagnosed were predominantly female (96% vs 29%; $P < .0001$), notification occurred earlier (4.8 vs 8.5 d; $P = .002$), and had higher serum sodium (136.8 vs 130.8 mmol/L; $P < .0001$) and lower serum potassium (5.3 vs 6.0 mmol/L; $P = .011$) concentrations.

Conclusions: Screening alone accounted for nearly 50% cases of CAH detected in the newborn period, including a fifth of affected females, indicating that clinical diagnosis is unreliable in both genders. Symptoms were mild at diagnosis and there were no adrenal crises. This study confirms the benefits of newborn CAH screening. (***J Clin Endocrinol Metab*** **100: 1002–1008, 2015)**

Congenital adrenal hyperplasia (CAH), due to 21-hydroxylase deficiency, is an inherited metabolic disorder with a wide spectrum of severity. It is characterized by a reduced ability to synthesize cortisol and aldosterone, coupled with the overproduction of adrenal androgens. Severe or salt-wasting CAH is a rapidly evolving and life-threatening disorder with a worldwide incidence of approximately 1:18 850 (1). Babies with untreated, severe CAH typically present in the first month of life with vomiting, weight loss, dehydration, and shock, (2) and may die if the diagnosis and treatment are delayed (3).

Although the first pilot study of newborn screening for CAH occurred nearly 3 decades ago (4), the value of CAH screening remains controversial (5, 6). Whereas many countries have adopted newborn screening for CAH, other countries with well-established newborn screening

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Abbreviations: CAH, congenital adrenal hyperplasia; LC-MS/MS, liquid chromatography followed by tandem mass spectrometry; NMSP, Newborn Metabolic Screening Programme; 17-OHP, 17-hydroxyprogesterone; PPV, positive predictive value.

programs such as the United Kingdom (7) and Australia (8) do not include CAH. The lack of uptake partly reflects concern around the rate of false positive tests, especially in unwell and preterm neonates (1, 9). Others argue that CAH can be diagnosed clinically, especially in girls, because in utero androgen exposure leads to virilization of the external genitalia. However, there is increasing evidence that the diagnosis of severe CAH is missed frequently in both genders (1, 10, 11).

Much of the controversy reflects the philosophy behind CAH screening. If the aim is to detect all subjects with CAH, including the milder variants, then screening will be inefficient with a high rate of false-positive and false-negative results. However, in New Zealand, the aim of screening has been to detect only the life-threatening form of disease, ie, salt-wasting CAH. Screening aims to detect treatable conditions in a presymptomatic stage (12). The early diagnosis and treatment of severe CAH can prevent salt-wasting adrenal crises and possible neonatal death. In addition, screening reduces the time taken to correct gender assignment in severely virilized girls (1, 10).

New Zealand was one of the first countries in the world to introduce a national newborn screening program for CAH (1), which was incorporated into the ongoing Newborn Metabolic Screening Programme (NMSP) in 1984. We have previously published outcomes from the first 10 years of newborn screening for CAH (13). We now describe the efficacy and efficiency of the subsequent 20 years of CAH screening in New Zealand, from 1994 to 2013.

Materials and Methods

Newborn screening for CAH was commenced in New Zealand in 1984, initially based on estimation of 17-hydroxyprogesterone (17-OHP) by an in-house RIA and changed to the Delfia immunoassay in 1998. Assays are carried out on whole-blood samples collected on Guthrie cards after 48 hours of life, usually obtained in the community by the lead maternity caregiver. Samples are subsequently posted to the laboratory, located in Auckland, which is the single laboratory conducting all tests in New Zealand.

After the measurement of 17-OHP, samples with values more than 2 SD above the assay mean are reassayed after diethyl ether extraction. A positive screening result is indicated by 17-OHP concentrations above 23 nmol/L for babies with a birth weight above 1500 g, and 32 nmol/L for those less than 1500 g. These cutoffs are used for request of a second whole-blood sample. In addition, for values of greater than 50 and 100 nmol/L in babies with birth weights of greater than 1500 g and less than 1500 g, respectively, the laboratory directly informs a pediatrician or pediatric endocrinologist, who arranges for same-day review of the baby. The intraassay and interassay coefficients of variation for a whole-blood 17-OHP value of 23 nmol/L are 10%. The only change to the protocol over the past 2 decades has been the incorporation of a neonatal intensive care unit protocol, whereby babies of a birth weight less than 1500 g have a further routine screening sample at 2 weeks of age, and those less than 1000 g another at 4 weeks. Preterm babies with high 17-OHP values have this checked at the next routine sample, unless symptoms suggestive of CAH are present. An additional sample is requested if the 17-OHP level remains elevated in the final scheduled sample.

Data on newborn screening for CAH were obtained from NMSP records for the time period 1994–2013. In addition to screening results, the NMSP collects clinical data on babies diagnosed with CAH in the neonatal period by means of standardized forms sent out to pediatric endocrinologists and pediatricians. Cases were defined as clinically detected if the diagnosis was suspected prior to screening results being available. CAH was classified as simple virilizing if pretreatment serum electrolytes from day 7 onward did not reveal hyponatremia.

Statistical analyses were performed in Minitab version 16 (Pennsylvania State University, State College, Pennsylvania) and SAS version 9.3 (SAS Institute Inc). The significance level was set at 5% (two sided) for all tests. Descriptive summaries are provided for those cases detected via clinical and screening diagnosis separately as well as overall. Continuous variables are presented as mean, SD, median, and range as appropriate. Categorical variables are presented as frequency and percentage. Comparisons between two diagnostic groups were carried out using two-sample parametric (Student's t) and nonparametric (Wilcoxon rank sum) tests for continuous variables, depending on the distribution of data. A standard χ^2 test was used for categorical variables or, alternatively, the Fisher's exact test with small cells in frequency. Correlations between serum sodium and potassium concentrations with treatment age were assessed using Pearson correlation coefficients.

Results

Between 1994 and 2013, 1 175 973 newborns were screened for CAH. Over this period, 44 cases of CAH were diagnosed (28 girls and 16 boys; Table 1), giving an incidence of 1:26 727, at an annual rate of 2.2 cases per year.

Descriptive statistics are shown in Table 1. Most newborns diagnosed with CAH were New Zealand Europeans (73%), with the remainder of the cases being of Pacific Islander (16%), Maori (7%), and other (2%) ethnicities (Table 1). Most children diagnosed with CAH were born at term (37–41 wk gestation), but six were born preterm (<37 wk gestation) and one postterm (≥42 wk gestation). Although the proportion of CAH children born preterm (14%) appeared to be higher than the background rate for preterm birth in New Zealand (7%), this was not statistically significant ($P = .13$). All preterm infants were confirmed to have CAH that required ongoing treatment.

Of the 44 cases of CAH detected in the newborn period, 21 were diagnosed by screening and 23 clinically (Table 1). When compared with cases identified by the screening program, clinically identified cases had screening 17-OHP

Table 1. Demography, Clinical Characteristics, and Blood Biochemistry of All New Cases of CAH Diagnosed by Clinical Examination or Through the Newborn Screening Programme in New Zealand in 1994–2013

	All Cases	Clinical Diagnosis	Screening Diagnosis	*P* Value
n	44	23	21	
Demography				
Sex ratio (males:females)	16:28	1:22	15:6	<.0001
Family history (yes)	5 (11%)	5 (22%)	0	.048
Birth weight, kg	3.48 ± 0.55	3.47 ± 0.58	3.49 ± 0.54	.99
Gestational age, wk	39.0 ± 2.3	38.6 ± 2.2	39.3 ± 2.5	.21
Age at screening assessment, d	2.4 ± 1.6	1.6 ± 1.3	3.3 ± 1.4	<.0001
Time from sample to test result, d	4.2 ± 3.3	3.2 ± 3.4	5.2 ± 3.0	.018
Age at test result, d	6.6 ± 4.2	4.8 ± 4.3	8.5 ± 3.2	.002
Age at treatment, d[a]	7.7 ± 6.3	3.9 ± 2.2	12.0 ± 6.7	<.0001
Region of diagnosis				.14
Auckland, New Zealand	18 (41%)	12 (52%)	6 (29%)	
Ethnicity				.32
New Zealand European	32 (73%)	15 (65%)	17 (81%)	
Maori	3 (7%)	2 (9%)	1 (5%)	
Pacific Islander	7 (16%)	5 (22%)	2 (10%)	
Other	2 (5%)	1 (4%)	1 (5%)	
Clinical characteristics				
Abnormal virilization	30 (68%)	23 (100%)	7 (33%)	<.0001
Gender misidentification	1 (2%)	0	1 (5%)	.48
Vomiting	3 (7%)	0	3 (14%)	.11
Hypotension/collapse	0	0	0	
Blood biochemistry				
Whole-blood 17-OHP, nmol/L[b]	183 ± 148	191 ± 171	173 ± 123	.68
Serum sodium at diagnosis, mmol/L	133.8 ± 5.5	136.8 ± 4.1	130.8 ± 5.2	<.0001
Serum potassium at diagnosis, mmol/L	5.64 ± 0.94	5.29 ± 0.95	6.02 ± 0.78	.011

Where appropriate, data are means ± SD. *P* values correspond to comparisons between clinical and screening diagnosis.

[a] One outlier (a girl) treated at 122 days has been excluded.

[b] 17-OHP concentration in final extraction assay.

test results that were notified 3.7 days earlier ($P = .002$) and treatment initiated 8.1 days earlier ($P < .0001$) (Table 1). Among the group identified by screening, samples were collected at a mean age of 3.3 days (median 3.0 d, range 2.0–8.0 d), and the duration from sampling to notification of results was 5.2 days (median 5.0 d, range 1.0–12.0 d). Notification of the diagnosis of CAH by the National Testing Centre was made at 8.5 days (median 8.0 d, range 3.0–16.0 d), and treatment initiated at 12.0 days (median 9.5 d, range 6.0–30.0 d) (Table 1). Treatment was commenced within the first 2 weeks of life in 89% of cases (39 of 44) and within 10 days in 77% of cases (34 of 44). This demonstrates an improvement as compared with our previously reported experience from 1983 to 1993 (13), in which the mean age at notification was 11 ± 3 days ($P = .003$ for the difference) and treatment commenced 3 days later (range of 0–26 d).

Of note, there were two infants who received markedly delayed diagnoses and treatment. In the first case, the lead maternity caregiver was notified of a high 17-OHP on day 12 but had difficulty in contacting the family because they had moved from the area. The infant was subsequently assessed and commenced treatment on day 30, at which point serum sodium was 118 mmol/L and potassium 6.4 mmol/L. In the second case, the initial 17-OHP was mildly elevated and a second sample was requested. Unfortunately, this was performed on the wrong infant, so that the affected infant (with simple virilizing CAH and normal pretreatment serum electrolytes) did not receive a definitive diagnosis and treatment until 122 days of age. The data from this second case were not included in the analysis of days to treatment.

Importantly, all clinically identified CAH cases were noted to have abnormal virilization and were therefore predominantly female (96%; Table 1). Only one male was detected clinically, on the basis of an affected sibling and subtle excess virilization. All but two girls with CAH (93%) displayed abnormal virilization (including one female initially assigned male gender). Overall, five affected females were not identified clinically: one was severely virilized (Prader 4) and misassigned male gender, two had mild virilization (Prader 1 clitoromegaly) that was not detected initially, and the final two were initially assessed as not abnormally virilized. After a screening diagnosis and subsequent review, 20% of males were noted to have subtle signs of excess virilization (stretched penile length > 2

SD above mean and scrotal hyperpigmentation). Apart from abnormal virilization, clinical symptoms were uncommon (Table 1), with three boys experiencing vomiting prior to treatment, and no infants presented with hypotension or collapse.

There were no differences in 17-OHP concentrations between cases ascertained clinically or by screening (Table 1). However, in keeping with the shorter time period to diagnosis, clinically identified cases had serum sodium concentrations that were 6.0 mmol/L higher ($P < .0001$) and potassium concentrations 0.72 mmol/L lower ($P = .011$) than those identified by screening (Table 1).

After the exclusion of those children considered to have a clinical diagnosis of simple virilizing CAH (n = 4) and a further case in whom abnormal serum electrolytes on day 1 reflected maternal levels, increasing age at treatment was associated with a progressive decrease in serum sodium concentrations ($r = -0.56$; $P < .0001$) (Figure 1). Conversely, increasing age at treatment was associated with increasing serum potassium concentrations ($r = 0.38$; $P = .017$) (Figure 1). Both hyponatremia and hyperkalemia were uncommon prior to day 7.

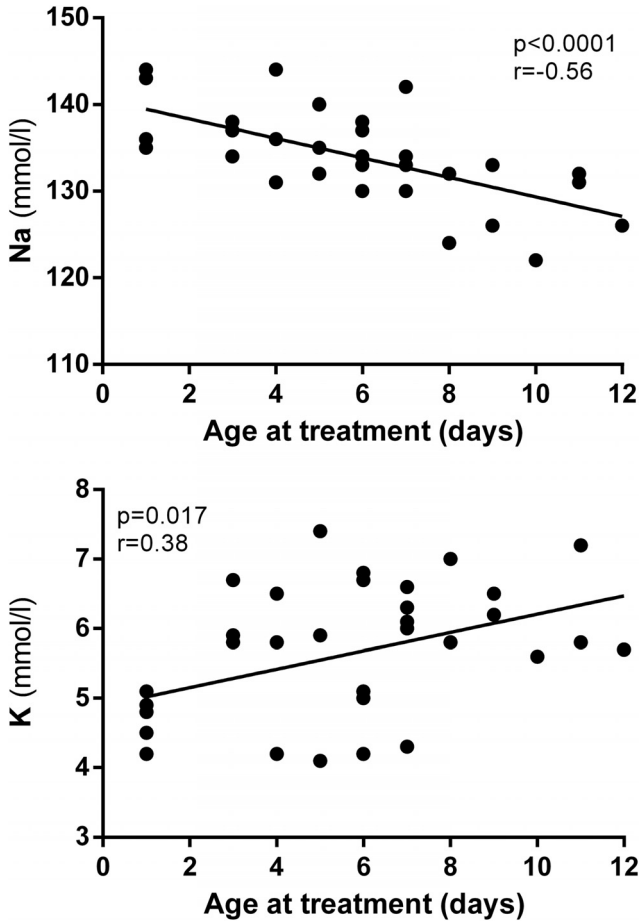

Figure 1. Association between age at treatment and serum sodium and potassium concentrations. Note that four subjects considered to have simple virilizing congenital adrenal hyperplasia and a further case with abnormal serum electrolytes on day 1 reflecting maternal levels were excluded.

Program costs and assessment of screening

The cost of each 17-OHP test performed as an additional test to an established newborn screening program was NZ $2.60 (~US $2.25), which includes labor, reagents, and confirmatory testing. This equates to NZ $69 489 (~US $60 249) for each case of severe CAH identified and NZ $145 597 (~US $126 237) for each case identified by the screening program alone.

We analyzed screening test performance after the introduction of the neonatal intensive care unit protocol. From 2011 through 2013, there were a total of 372 abnormal screening samples with 17-OHP above the cutoff. Using the above criteria, eight babies were directly referred to a pediatrician, there were 202 requests for second samples in babies born weighing greater than 1500 g, and 158 routine second samples in babies born weighing less than 1500 g. Over this time period, there were four true cases of severe CAH and no known cases of severe CAH incorrectly identified as normal. Therefore, the sensitivity and specificity of screening for severe CAH were 100% and 99.80%, respectively. The positive predictive value (PPV) of a true case given an abnormal initial screen result was 1.08% overall and 50% after a direct pediatrician referral.

Discussion

Nearly 50% of infants with CAH were detected by screening alone. This included a fifth of all affected girls, indicating that, contrary to popular belief, the clinical diagnosis of CAH is unreliable in both genders. Treatment was commenced early, generally within the first 10 days of life, and symptoms were consequently uncommon. Conversely, in the absence of screening, it is likely that half of the infants would have presented with salt-wasting adrenal crises.

Some skepticism against CAH screening arises from the fact that girls with more severe disease are virilized at birth and therefore assumed to be easily detected (12). However, like others (1, 10), we have shown that the clinical diagnosis of severe CAH is unreliable in females. Within our cohort, approximately one in five girls with severe CAH would have been missed: either not recognized as virilized or misassigned male gender. Furthermore, Swedish population data suggest that, prior to screening, the diagnosis was in fact missed equally in both genders because the proportion of salt-wasting CAH increased equally in both genders after the introduction of screening (11).

Of importance, newborn screening for CAH reduces the time to correct gender assignment in severely virilized girls who are initially misidentified as boys (1, 10). Interestingly, there was just one case of erroneous gender assignment among 25 girls diagnosed with severe CAH over the last 18 years, as compared with two of nine girls diagnosed in New Zealand from 1984 through 1994. This suggests an increased awareness of disorders of sexual differentiation, of which CAH is a common diagnosis, and that gender assignment of babies with ambiguous genitalia is more likely to be deferred until assessment is complete. Conversely, clinical signs in boys (macrogenitalia and scrotal hyperpigmentation) are subtle and expected to be missed at birth. Family history is the most likely means of early clinical detection in males.

It is well established that newborn screening decreases the time to the diagnosis of CAH (6, 10, 14). Although symptoms of salt wasting are uncommon within the first 10 days of life, vomiting, severe hyponatremia, hyperkalemia, and clinical deterioration can then develop rapidly. In unscreened populations, male infants with severe CAH typically present with salt-wasting adrenal crisis (6, 15). In addition to being life threatening, salt-wasting episodes in infancy are associated with reduced intelligence (16, 17). The timing of diagnosis of CAH is therefore critical to the success of newborn screening programs. We found that electrolyte abnormalities were related to age of treatment and were uncommon prior to day 7. Symptoms were also uncommon within our cohort, reflecting early diagnosis and treatment because nearly 80% commenced treatment within 10 days of life.

As compared with our previous report of CAH screening in New Zealand from 1984 through 1994, it is reassuring to note the shorter duration to diagnosis and treatment. However, over the time period studied, there were two cases in which treatment was markedly delayed, as a result of a family being lost to follow-up, and 17-OHP resampling occurring in the wrong infant. New Zealand has a national newborn advisory panel that reviews all missed cases as part of continuous quality improvement. Most missed or delayed cases reflect lead maternity caregiver issues. The two most common problems are not realizing the importance of a second sample being performed in a timely fashion and a delay in posting the results. Efforts made to address these barriers have included a change in recommended heel prick sampling time (from 3–5 d to 48–72 h in the late 1980s), ongoing lead maternity caregiver/midwife education, the provision of fast-post prepaid envelopes, and discouraging the practice of mailing blood spot samples to the laboratory in batches. Most recently the Newborn Screening Service has begun to directly contact the lead maternity caregiver by telephone if the newborn is older than 8 days and/or if the second sample is more than 15 days late in reaching the laboratory. Once the samples reach the laboratory, 17-OHP is typically analyzed within 1 day, with high levels notified immediately.

The low PPV of newborn screening for CAH is a major challenge for screening programs (12). Our program aims to detect only cases of severe CAH, as compared with cases of milder, simple virilizing CAH. Consistent with this, we detected a relatively low incidence of CAH (1:26 727) as compared with international reports (1, 6). The major advantage of this approach is to retain a relatively high PPV and minimize screening costs. Cost-effectiveness is a major barrier to the international uptake of newborn screening for CAH, and analyses are complicated by the uncertain public health impact of missed or delayed diagnoses (18). Although a few cases of simple virilizing disease were also detected by our screening program, we would expect the majority to be diagnosed later in childhood. Although early diagnosis of simple virilizing CAH can minimize advancement in bone age and protect final height (19, 20), the 17-OHP cutoff levels required to detect these milder cases lead to an unacceptably low PPV (12). Therefore, it is important that physicians remain vigilant to clinical signs of CAH, even in instances in which there has been a normal report from newborn screening (21, 22).

Importantly, half of all babies who met criteria for direct referral to a pediatrician were true cases of severe CAH. Conversely, despite high test specificity (99.80%), the likelihood of an initial abnormal screening sample representing CAH was just 1.08%. One of the major problems in newborn screening for CAH is the high rate of false-positive tests in stressed premature babies, who have elevated 17-OHP levels (1, 9). Our calculations were made based on data from 2011 to 2013, after the introduction of 17-OHP cutoff levels stratified by birth weight and are comparable with other programs that have incorporated multitiered thresholds based on birth weight or gestational age (23, 24). To date, we have used organic solvent extraction as a second-tier test to increase immunoassay specificity. However, liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) addresses many of the limitations of immunoassays, particularly in instances in which steroid ratios are used (25, 26) to further reduce the rates of false-positive tests and improve PPV (27–29). There is likely to be an increasing use of LC-MS/MS methodology in newborn screening for CAH, either as first- or second-tier testing. However, data from Minnesota using second-tier steroid profiling has continued to show a relatively low PPV (30). Therefore, attempting to identify all individuals with simple virilizing CAH may not be cost effective, even with LC-MS/MS, and fo-

cusing on infants who are at risk of salt-wasting crises may remain the best approach.

Despite the relatively low incidence of CAH, it is unlikely that there were further missed cases. The New Zealand newborn screening program has an estimated coverage of greater than 98% live births. As far as we are aware, there were no missed cases of severe CAH, either after autopsy or presentation with adrenal crisis during infancy, which we would expect to be reported to either the NMSP or pediatric endocrinologists. Clearly missed diagnoses and subsequent deaths from CAH carry an enormous cost to both families and society. However, despite concern about the mortality of undiagnosed severe CAH in unscreened populations (7, 31), a recent UK postmortem study of 1198 infants who died aged 0–6 months detected no cases of CAH, indicating that undiagnosed CAH is a rare cause of infant death in the developed world (32).

In summary, national newborn screening for severe CAH effectively identifies all affected infants before serious sequelae occur. Screening conclusively demonstrates that the clinical diagnosis of CAH cannot be made reliably in either gender so that, in the absence of screening, affected infants of both genders will present in a life-threatening salt-wasting crisis. This study adds further argument for the international uptake of newborn screening for CAH.

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