ORIGINAL ARTICLE

Two-year pilot study of newborn screening for congenital adrenal hyperlasia in New South Wales compared with nationwide case surveillance in Australia

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Aim: To assess the benefits and practicalities of setting up a newborn screening (NBS) program in Australia for congenital adrenal hyperplasia (CAH) through a 2 year pilot screening in ACT/NSW and comparing with case surveillance in other states.

Methods: The pilot newborn screening occurred between 1/10/95 and 30/9/97 in NSW/ACT. Concurrently, case reporting for all new CAH cases occurred through the Australian Paediatric Surveillance Unit (APSU) across Australia. Details of clinical presentation, re-sampling and laboratory performance were assessed.

Results: 185,854 newborn infants were screened for CAH in NSW/ACT. Concurrently, 30 cases of CAH were reported to APSU, twelve of which were from NSW/ACT. CAH incidence was 1 in 15 488 (screened population) vs 1 in 18,034 births (unscreened) (difference not significant). Median age of initial notification was day 8 with confirmed diagnosis at 13(5–23) days in the screened population vs 16(7–37) days in the unscreened population (not significant). Of the 5 clinically unsuspected males in the screened population, one had mild salt-wasting by the time of notification, compared with salt-wasting crisis in all 6 males from the unscreened population. 96% of results were reported by day 10. Resampling was requested in 637 (0.4%) and median re-sampling delay was 11(0–28) days with higher resample rates in males (p < 0.0001). The within-laboratory cost per case of clinically unsuspected cases was A$42 717.

Conclusion: There seems good justification for NBS for CAH based on clear prevention of salt-wasting crises and their potential long-term consequences. Also, prospects exist for enhancing screening performance.

Key words: Newborn screening; Congenital adrenal hyperplasia; Australian Paediatric Surveillance Unit; Adrenal crisis.

Newborn screening (NBS) for congenital adrenal hyperplasia (CAH, 21-hydroxylase deficiency) was first introduced in the late 1970s.[1] A pilot NBS programme in Alaska demonstrated the feasibility of NBS in CAH,[2] and it is now performed in a number of countries, including New Zealand.[2–11]

Some of the evidence supporting the efficacy of NBS in CAH is based on the differences observed in incidence, sex ratio and disease spectrum in screened versus unscreened populations. Male babies with CAH are likely to go completely undiagnosed until clinically significant salt-wasting adrenal crisis occurs.

Key Points

1 Newborn screening (NBS) programs in Australia currently do not include CAH.
2 Newborn males with CAH are usually undiagnosed until salt-wasting adrenal crisis occurs.
3 NBS for CAH prevents salt-wasting adrenal crises prior to diagnosis.

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Female babies frequently present with ambiguous genitalia; rarely, male gender may be incorrectly assigned and salt-wasting adrenal crisis follows. In addition to the risk of death from adrenal crisis, there is also evidence of intellectual deficit and learning difficulties.[12]

Despite this evidence, NBS is not universal, and debate is ongoing as to its true benefit. In Australia, NBS programmes do not include CAH. To further explore this question, a two-year pilot study of NBS was performed in New South Wales/Australian Capital Territory (NSW/ACT), and in conjunction, CAH was included as a study condition in the Australian Paediatric Surveillance Unit (APSU) nationwide.

Patients and Methods

Case surveillance across Australia

Reporting of all new cases of CAH under the age of 16 years was undertaken by the APSU from 1 April 1995 to 31 December 1997 to overlap with a period of NBS in NSW/ACT from 1 October 1995 to 31 September 1997. Notifications through the APSU initiated a questionnaire to gather details on clinical and biochemical features at presentation and management. CAH incidence was calculated by using data from the Australian Bureau of Statistics. Clinical presentation, management,
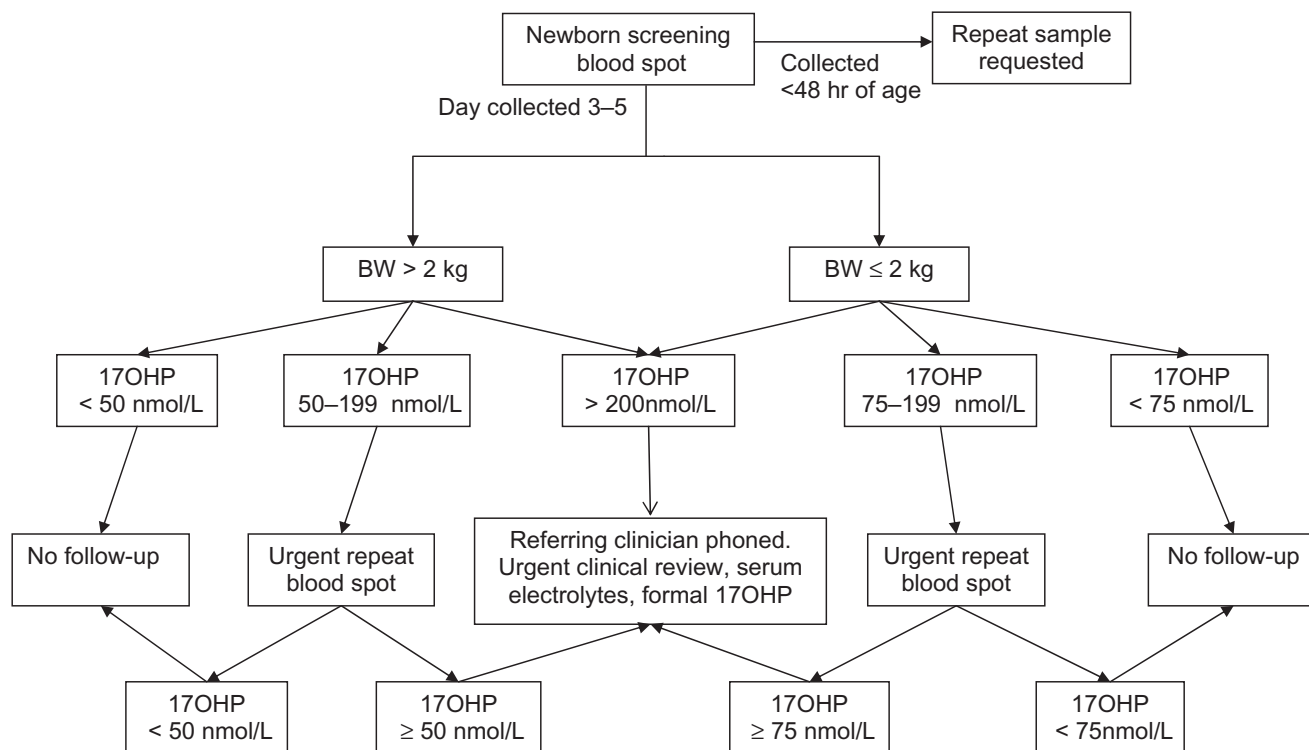
Fig. 1 Flow diagram of follow-up procedures for congenital adrenal hyperlasia newborn screening. BW, birthweight; 17OHP, 17-hydroxyprogesterone.

incidence and gender ratios were compared between screened (NSW/ACT) and unscreened (other Australian states and territories) populations. Classical CAH was defined as patients presenting in the neonatal period or early infancy (<6 months old), and non-classical CAH was defined as patients presenting after this period.

NBS for CAH in NSW/ACT

The NBS programme screens all babies born in NSW/ACT. At the time of this study, routine screening was performed for hypothyroidism, galactosaemia, phenylketonuria and cystic fibrosis at between 3 and 5 days of life. For a pilot period of 2 years between 1 October 1995 and 30 September 1997, the NBS was extended to include CAH.

Blood samples were collected onto filter paper between day 3 and 5 days of life, air dried and sent to the NBS laboratory by courier or by post. Levels of 17-hydroxyprogesterone (17OHP) were estimated on day of receipt, and the clinician was informed of elevated 17OHP levels the same day. The criteria for the follow-up of elevated 17OHP results were as follows (summarised in Fig. 1):

1 17OHP $\geq$200 nmol/L – the referring clinician was advised by telephone of strong suspicions of CAH, and an urgent clinical review, serum electrolytes and formal 17OHP were recommended.
2 17OHP 50–199 nmol/L (birthweight (BW) >2.0 kg) or 75–199 nmol/L (BW $\leq$2.0 kg) – a repeat dried blood spot sample was urgently requested.

3 If 17OHP on a repeat sample was $\geq$50 nmol/L (BW >2.0 kg) or $\geq$75 nmol/L (BW $\leq$2.0 kg), notification as in number 1 was undertaken.
4 For babies weighing <1.25 kg, a repeat sample was requested at 4 weeks of age.
5 A repeat dried blood spot sample was also requested if the initial sample was collected at <48 h of age.

Laboratory assay

Measurement of 17OHP in dried filter paper was made using the AutoDELFIA Neonatal 17OHP assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). This is a solid-phase time-resolved fluoroimmunoassay based on the competition between europium-labelled 17OHP and sample 17OHP for a limited number of binding sites on 17OHP-specific polyclonal antibodies (derived from rabbit). A second antibody is directed against rabbit IgG and was coated to the solid phase, giving convenient separation of the antibody-bound and free antigen. The intra-assay variance was 3.2–5.9% (at high and low concentrations), and the inter-assay variance was 9–10.5%. Results are expressed as nmol/L whole blood.

Analysis of NBS data

The NBS database included information on gender, date of birth, BW, 17OHP level and collection date. Analysis included only those with the following data available: date of birth 1 October 1995 to 30 September 1997, BW 0.5–5 kg and date of collection between 0 and 30 days after birth.

Table 1 CAH cases and incidence in Australia (by state) during newborn screening time period

State	Total	Males	Females	Live births	Incidence (1 in)
NSW/ACT	12	6	6	185 854	(Screened)15 488
Victoria	8	4	4	122 933	15 367
Western Australia	5	0	5	49 721	9 944
South Australia	3	3	0	37 904	12 635
Queensland	1	0	1	94 065	94 065
Northern Territory	1	1	0	7 346	7 346
Tasmania	0	0	0	12 638	–
Total (excluding NSW/ACT)				324 607	(Unscreened)18 034
Total	30	14	16		

CAH, congenital adrenal hyperlasia; NSW/ACT, New South Wales/Australian Capital Territory.

Results

Case detection and incidence

Case surveillance by the APSU across Australia

The APSU received 131 notifications of a potential diagnosis of CAH from 1 April 1995 to 31 December 1997, and questionnaires on 114 were returned (87%). From these, 30 cases of confirmed classical CAH (including 10 cases detected in NSW/ACT by NBS) and 15 cases of non-classical CAH occurred within the NBS period. Two cases had 3-beta-hydroxysteroid dehydrogenase deficiency (1 classical, 1 non-classical); all other cases were 21-hydroxylase deficiency.

Case detection by NSW/ACT NBS programme

In the two-year study period, 185 854 newborn infants were screened for CAH. Twelve cases (6 males) of CAH were diagnosed during this time, 10 of which were detected by NBS. The two cases that were not detected by NBS were a female who had commenced treatment prior to sampling and a male with a mild enzyme block who had a normal NBS result and was detected because of a family history of CAH. Six cases (5 males, 1 female) were clinically unsuspected and detection was attributable entirely to NBS.

In NSW/ACT during NBS, the incidence (Table 1) was 1 in 15 488 births (95% confidence intervals 1:9891–1:35 668), with a male to female (M : F) ratio of 1:1. In the remaining Australian states without NBS, 18 cases of classical CAH were diagnosed during the same time period, giving an incidence of 1 in 18 034 births (95% confidence intervals 1:12 335–1:33 517), which was not significantly different from the incidence in NSW and ACT. The M : F ratio was 1:1.25 (also not significantly different).

Mode of presentation

Females

In NSW/ACT where there was NBS, five of the six females had ambiguous genitalia and therefore would have been identified with CAH even without NBS. Four were positive on NBS and one started treatment prior to NBS. The remaining female without ambiguous genitalia detected by NBS had a mild (non-

Table 2 Mode of diagnosis in CAH cases during newborn screening period

Presentation	NSW/ACT		Other states	
	Males	Females	Males	Females
Genital abnormalities	0	5†	1	10
Salt losing/adrenal crisis	0	0	6	0
Prenatal diagnosis	0	0	1	0
Newborn screening	5	1	0	0
Family history	1‡	0	0	0

†Four were positive on newborn screening, one was treated prior to newborn screening sample; ‡Mild enzyme block, normal newborn screening. CAH, congenital adrenal hyperlasia; NSW/ACT, New South Wales/Australian Capital Territory.

salt-losing) 21-hydroxylase deficiency. In the remaining Australian states without NBS, all 10 females had either ambiguous genitalia or evidence of virilisation and were identified early without NBS. There were no recorded cases of incorrect gender assignment (Tables 2 and 3).

Males

In NSW/ACT where there was NBS, there were no cases of salt-losing crises; however, one of five males diagnosed with CAH by NBS, initially notified on day 10, had failure to thrive and low plasma sodium level (128 mmol/L) at diagnosis on day 23 (case 28, Table 3). In contrast, in the non-screened states, all six of the males with unsuspected CAH had evidence of adrenal failure with clinically significant salt loss, and one had a hypoglycaemic seizure. All required parenteral glucocorticoids and intravenous fluid. Sodium levels were lower and potassium levels were higher in the cases not detected by NBS (Tables 2–4).

In NSW/ACT, in addition to the five male cases of CAH diagnosed by NBS, one case with a mild form of CAH (non salt-losing) secondary to 21-hydroxylase deficiency was subsequently diagnosed on day 16 of life because of a family history of CAH (he had a false negative 17OHP result of 13 nmol/L on day 3). Of the other two male cases identified in the remaining Australian states without NBS, one case had ambiguous

Table 3 Details of CAH cases in NSW/ACT – 17OHP (nmol/L) levels and age

Case number	Gender	Sample 1		Sample 2		Age (days)	
		Age (days)	17OHP	Age	17OHP	Notification	Treatment
11	F	4	225	–	–	8	2
13	F	1	505	–	–	8	2
14	F	4	495	–	–	6	2
17	M	2	225	–	–	8	9
19	F	4	648	–	–	9	7
21	F	4	88	14	323	20	22
26	F	4	26	–	–	†	2
37	M	4	443	–	–	5	6
28	M	3	101	17	341	23	23
32	M	3	124	15	204	18	18
35	M	3	125	10	243	13	17
36	M	3	13	10	13	‡	–

†Started treatment prior to NBS; ‡Family history. –, no resample recorded on database. CAH, congenital adrenal hyperlasia; NBS, newborn screening; NSW/ACT, New South Wales and the Australian Capital Territory; F, female; M, male; 17OHP, 17-hydroxyprogesterone.

Table 4 Clinical data of unsuspected male CAH cases diagnosed during newborn screening period – median (range)

	Screening	No screening
Number of patients	5	6
Day of diagnosis	13 (5–23)	16 (7–37)
Serum sodium (mmol/L)	132 (128–134)	113 (110–128)**
Serum potassium (mmol/L)	5.8 (4.6–6.6)	7.8 (5.0–9.8)*
Number of patients requiring IVT or IV/IM glucocorticoids	2	6

*$P = 0.07$; **$P < 0.001$. CAH, congenital adrenal hyperlasia; IVT, intravenous fluid; IV/IM = parenteral.

genitalia secondary to 3-beta-hydroxysteroid dehydrogenase deficiency, and one case was diagnosed prenatally.

In the screened population in NSW/ACT, the median age of initial notification was day 8, with confirmed diagnosis at a median of 13 days (5–23) compared with a median of 16 days (7–37) in Australian states without NBS (not significant). Three of the five cases diagnosed by NBS required a repeat blood spot sample. The time from initial sampling to report was 5, 6 and 7 days, but there was an additional delay of 5, 9 and 13 days, respectively, between the request for a repeat sample and the repeat blood spot sample being received and tested.

Abnormal screening results and resampling

Complete data including BW were available for 177 349 babies born during the NBS period. Data are summarised in Table 5. In 99.7% of babies with a BW >2 kg and in 94.1% of babies with a BW ≤2 kg, CAH was excluded on the initial sample. In 24 babies >1.25 kg, urgent review was recommended after the initial blood sample, and five of these babies were diagnosed with CAH. Based on the need for any intervention (i.e. urgent review or repeat sample), the positive predictive value was 1.5% for all babies screened and 2.2% for babies >2 kg. For urgent review only, the positive predictive value was 52.6% for babies >2 kg or more. There was only one false negative case identified.

Most (98.5%) of all initial samples were collected before day 6, and 95.9% of the results were reported by day 10. Median resampling delay was 11 days (0–28). Only 6.8% (12 063) of babies had 17OHP levels measured earlier than recommended by the protocol (i.e. within the first 2 days of life). Of those babies, only 164 had elevated 17OHP levels as per protocol for samples taken after day 2 of life, equating to 0.1% of the total number of babies screened and 25% of all resample requests.

Gender

For the 164 495 patients who had an initial blood sample taken between day 3 and day 30 of life, both BW and gender were significant predictors of 17OHP concentration, with levels of 17OHP testing higher in males ($P < 0.0001$). The relationship of BW and gender for males was $\log(17OHP) = 3.02 − 0.21(BW) + 0.09$ ($r^2 = 3.6$, $P < 0.0001$). This may explain why there was a preponderance of males requiring a repeat sample with an M:F ratio of 2.2 to 1 ($P < 0.0001$).

Cost of NBS programme for CAH

In 1997, the within-laboratory costs of the overall NBS programme for four disorders (phenylketonuria, congenital hypothyroidism, cystic fibrosis and galactosaemia) was A\$11.60 per sample screened. The incremental cost (additional cost to screen for an additional disorder in an established NBS programme) of each test for CAH was A\$1.44. This compares with an incremental cost of A\$0.40 for phenylketonuria and A\$1.97 for congenital hypothyroidism. The within-laboratory cost per case (including resampling costs) of classic CAH detected was A\$25 630. The cost per case of clinically unsuspected cases was A\$42 717.

Table 5 Outcome of newborn screening for CAH protocol

Birthweight		<1.25 kg	1.25–2 kg	>2 kg	Total
Babies tested		847	2509	173 993	177 349
CAH excluded on first sample (% of total)		798	2347	173 543	176 688
		(94.2)	(93.5)	(99.7)	(99.6)
Second sample requested (% of total)		49	149	439	637
		(5.8)	(5.9)	(0.3)	(0.4)
Urgent follow-up (First + Second)		0 + 6 = 6	13 + 4 = 17	11 + 8 = 19	24 + 18 = 42
CAH confirmed (First + Second)		0	0	5 + 5 = 10	5 + 5 = 10
False negative results		0	0	1	1
Sensitivity %		0	0	90.9	90.9
Any intervention†	Specificity (%)	94.2	93.5	99.5	99.6
	Positive predictive value (%)	0	0	2.2	1.5
Urgent review only	Specificity (%)	99.3	99.3	99.99	99.98
	Positive predictive value (%)	0	0	52.6	23.8

†any intervention – repeat sample or urgent review. CAH, congenital adrenal hyperlasia.

Discussion

It was reassuring in this pilot study that the incidence of CAH did not differ between the screened and the unscreened populations, and incidence was similar to composite world-wide data.[13,14] However, there was a clear benefit in the avoidance of salt-wasting adrenal crises in the screened population compared with this being universal in unscreened males. This is in agreement with previous studies.[14,15] Nevertheless, a number of practical issues exist around resampling delays and appropriate screening cut-off levels, particularly for low BW babies.

The avoidance of adrenal salt-wasting crisis is paramount in the argument for NBS for CAH. This medical emergency is life-threatening and can result in neurological damage or intellectual disability in later life. Its management entails considerable costs for emergency medical services, hospitals and intensive care, although the total costs are difficult to estimate. Additionally, male babies with CAH may die undiagnosed in unscreened populations, but statistical power to detect these is lacking. As expected, the majority of females presented with ambiguous genitalia or evidence of virilisation and could have been detected without NBS. However, mild genital abnormalities may go undetected clinically or severe virilisation may result in incorrect gender assignment. There were no cases of incorrect gender assignment in this study, although avoiding this is also a potential benefit of NBS.[16]

With any NBS programme, the ability of a test to distinguish those affected from those who are likely normal is paramount. Much of the literature surrounding NBS in CAH scrutinises the number of false positive results and ways of reducing them[17–22] as they increase costs and cause unnecessary anxiety for parents.[23] False positive results are more likely if the sample is taken within the first 2 days of life. In this pilot, 7% of babies had 17OHP levels taken within the first 2 days of life, which contributed to 25% of all repeat sample requests; this was avoidable if the protocol had been followed. False positive results are also more likely in preterm or low BW babies because of neonatal stress, immature adrenal function or increased cross-contamination of fetocortical steroid metabolites. Many

screening programmes have established 17OHP reference ranges that are based on BW and/or gestational age.[11,20,24] Despite a 17OHP reference range based on BW in this pilot, 6% of babies with a BW of <2 kg compared with 0.3% of babies >2 kg required a repeat sample. However, there are no data on which to base cut-off levels in lower BW babies.

We also found male gender was associated with a higher rate of resampling (2.2:1), presumably because healthy male babies have a higher 17OHP level. A gender difference between 17OHP levels has previously been proposed as an explanation for a higher rate of false negatives in female babies; however, introducing a gender-specific 17OHP reference range by lowering the threshold for female babies simply increased the false positive rate.[25] To our knowledge, no previous study has identified gender as a factor in false positive rates. This raises the possibility that gender-specific reference ranges may reduce the false positive rate, but this would increase the complexity of follow-up.

The positive predictive value of this current CAH screening protocol was 1.5% for any intervention (urgent review or repeat sample) and 23.8% for urgent review only, increasing to 2.2% and 52.6%, respectively, excluding babies ≤2 kg. This compares favourably with other published results of CAH NBS programmes[5,6,8,10,11,26] and NBS for other disorders.[27] Employing resampling rather than lowering the threshold for urgent review may reduce the anxiety experienced by parents and the workload of the paediatrician; however, this approach risks diagnostic delay. Three out of five males eventually diagnosed with classic CAH all had initial 17OHP levels >100 but <200 nmol/L, and therefore required resampling, and were diagnosed after 12 days. One of them was showing early signs of salt loss, but the baby's doctor had been notified of the possibility of CAH.

Several changes since this pilot study will address some of these issues. NBS samples are now collected between 48 and 72 h of age, and the efficiency of collecting repeat samples has also increased. Screening methods have improved, and testing using tandem mass spectrometry for steroid profiling[28] greatly improves the specificity of CAH testing. As all Australian NBS laboratories have this technology, the testing could be used as a

second-tier test on samples with a raised 17OHP concentration. Another change is that gestational age is now being recorded and could possibly be used instead of BW to set cut-off levels.

Sensitivity of this NBS pilot study was >90%, similar to other programmes, which vary between 83 and 100%.[4–6,10,11] Importantly, there have been no reports of salt-wasting crises in babies diagnosed with CAH but missed by NBS, and therefore, reassuringly, false negative results may represent less severe forms of the condition.[25] By attempting to improve sensitivity further, the false positive rate increases, thereby increasing economic and psychological burden.

There seems good justification for an NBS programme for CAH in Australia based on the clear prevention of clinical deterioration from salt-wasting adrenal crisis prior to diagnosis that may have long-term consequences. While the CAH NBS programme reported here has a sensitivity and specificity that compares well with other established NBS programmes, there are good prospects for further improving the performance.

Acknowledgements

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