

The First-year Outcomes of the Nationwide Neonatal CAH Screening in Türkiye: High Rate of False Positives for 21-hydroxylase Deficiency and a Higher Detection Rate of Non-classical Cases

Guran T et al. Nationwide Neonatal Screening for CAH in Türkiye

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What is already known on this topic?

Salt-wasting 21-hydroxylase deficiency CAH is a recessive disorder that can lead to high mortality if treatment is not initiated early. Neonatal screening, particularly in males and in groups with limited access to healthcare, reduces mortality and morbidity associated with 21OHD CAH. In Türkiye, neonatal CAH screening began with pilot studies in 2017 and gradually expanded to become nationwide in 2022.

What this study adds?

The prevalence of classical 21OHD CAH in the nationwide CAH screening in Türkiye is 1:12,044. Implementing updated cutoffs will help reduce false positives, lower neonatal screening costs, and prevent the detection of non-classical CAH cases.

Abstract

Objective: Neonatal screening for congenital adrenal hyperplasia (CAH) was implemented nationwide in Türkiye in 2022. We assessed the performance of this screening program during its first year.

Method: This retrospective, descriptive study included neonates born in Türkiye between January 1 and December 31, 2022, with gestational age ≥ 32 weeks and birth weight ≥ 1500 grams. The screening protocol used a two-tier approach. In the first step, 17 α -hydroxyprogesterone (17OHP) levels were measured using fluoroimmunoassay (FIA) in dried blood spots (DBS) collected at 3-5 days of life. Infants with positive results underwent second-tier testing using liquid chromatography-tandem mass spectrometry to measure 17OHP, 21-deoxycortisol (21DF), cortisol (F), and 11-deoxycortisol (S) in DBS. Those with a steroid ratio (21DF+17OHP)/F ≥ 1 were referred to pediatric endocrinology clinics for diagnostic evaluation.

Results: Of 1,096,069 neonates screened (including 149,652 refugees), second-tier tests were performed on 70,455 (6.88%) infants, and 3,429 (0.27%) were referred to clinics, resulting in 91 confirmed cases of classic 21-hydroxylase deficiency (21OHD) CAH (77; salt-wasting, 14; simple virilizing). Twenty two patients diagnosed with non-classical 21OHD CAH. The frequency of classic 21OHD was 1 in 12,044. The first-tier FIA-17OHP values were below 17.5 ng/mL in 99.8% of healthy neonates with ≥ 36 weeks gestation or ≥ 2500 grams and below 50 ng/mL in those with 32-36 weeks or 1500-2500 grams. Using refined cut-offs could reduce referrals by 6 times and eliminate second-tier testing for 95% of infants.

Conclusion: Neonatal CAH screening facilitates early diagnosis of 21OHD and improved patient care. Ongoing evaluation can enhance the efficiency and cost-effectiveness of the screening protocol.

Keywords: Neonatal screening; congenital adrenal hyperplasia; second-tier; steroid profiling; LC-MS/MS

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Introduction

Neonatal screening (NS) for congenital adrenal hyperplasia (CAH) reduces mortality and morbidity associated with classical 21-hydroxylase deficiency (21OHD). Therefore, it is included in the neonatal screening programs of many developed countries and is recommended by international guidelines (1).

Although novel molecular-based CAH screening methods, such as long-read sequencing, show promise, hormone measurement-based screenings, particularly those utilizing mass spectrometric steroid profiling, remain highly sensitive in early diagnosis of the condition (2, 3). Consequently, current practices favor two-tiered screening programs, with the second-tier employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) (1).

In Türkiye, CAH has been included in the NS program since 2017. The second-tier of the program involves measuring 17 α -hydroxyprogesterone (17OHP), 21-deoxycortisol (21DF), cortisol (F), and 11-deoxycortisol (S) in dried blood spots (DBS) using LC-MS/MS as one of the first countries in the world to adopt this approach. Of note, the inclusion of 21DF and S in the second-tier testing not only reduces the false positivity of the screening but also facilitates the differentiation of rare CAH forms that may present with elevated 17OHP levels (4). This feature is particularly important in countries, like in Türkiye, where consanguineous marriages are common, to effectively identify the targeted patient for screening. However, in contrast to many other countries that use dissociation-enhanced lanthanide fluoroimmunoassay (DELFLIA) method to measure 17OHP in the first step of screening, the fluoroimmunoassay (FIA) method was employed due to higher costs. FIA-17OHP cut-off values of 10 ng/mL and 15 ng/mL were used in the first step of screening for newborns ≥ 36 weeks and/or ≥ 2500 g, and for those 32–36 weeks and/or 1500–2500 g, respectively. The cut-off values used in the screening were determined based on examples from previous screening programs (3, 5). Audits of the pilot NS for CAH conducted in Türkiye between 2017 and 2022 were used to analyze false-positive rates and overall effectiveness (6, 7). The aim of this study was to evaluate the outcomes of the first year of the nationwide neonatal CAH screening program, which was expanded in 2022, and to develop strategies for improvement based on the identified pitfalls.

Method

A retrospective cross-sectional study was conducted based on reports generated from the screening program database including all newborns screened under the authority of Turkish Directorate of Public Health (TDPH) from 1 January to December 31, 2022.

DBS samples were collected on filter paper (“Guthrie” cards) between the 3rd and 5th days of life by heel prick, at public health units, general hospitals, and maternity hospitals. The CAH screening algorithm was presented in **Figure 1**.

Initial CAH screening was based on the measurement of 17OHP in DBS on filter paper by fluoroimmunoassay (FIA) (LabSystems Diagnostics, Finland). If the 17OHP level exceeded the cut-off in the first-tier immunoassay, the filter paper was analyzed using LC-MS/MS for a steroid profile of 17OHP, 21DF, F, and S (6, 7). Normal values were: for neonates 32–36 weeks and/or 1500–2500 g, 17OHP < 8 ng/mL, 21DF < 1.5 ng/mL, F > 50 ng/mL, S < 1.8 ng/mL; for neonates ≥ 36 weeks and/or ≥ 2500 g, 17OHP < 1.5 ng/mL, 21DF < 1.5 ng/mL, F > 50 ng/mL, S < 1.8 ng/mL. A (21DF+17OHP)/F ratio ≥ 1 and/or S > 10 ng/mL were the main criteria for referral for further clinical evaluation for 21OHD and 11 β -hydroxylase deficiency (11 β -OHD) CAH, respectively.

Classical CAH was defined as an elevated 17OHP level confirmed by retest and/or clinical evaluation, followed by genotyping. False positives were characterized by the absence of genital abnormalities and/or salt-wasting, with normal 17OHP levels on retest. The clinical, laboratory, and genetic investigation results of infants diagnosed with CAH were collected from the centers where they were followed after referral to clinics. We also collected information from the centers on which type of 21OHD (SW, SV or NC) the patients diagnosed with CAH had. SW CAH is the most severe form, caused by complete enzyme deficiency, leading to low cortisol and aldosterone, and high androgen levels, resulting in prenatal virilization of females. Without treatment, infants may die within weeks due to salt-wasting crisis. SV CAH, with mild residual 21-OH activity (1–5%), has sufficient aldosterone to prevent salt loss but still requires glucocorticoid treatment for cortisol deficiency and androgen excess. NC CAH, with 30–50% enzyme activity, maintains normal cortisol and aldosterone but has elevated androgens, typically diagnosed in childhood or young adulthood due to symptoms of androgen excess (8).

Final calculations of true-positive (TP), false-positive (FP), true-negative (TN) and false-negative (FN) screening results were recorded.

Efficiency of screening protocol was assessed with positive predictive value (PPV), sensitivity and specificity calculated by following formulas: $PPV = TP/(TP+FP)$, $sensitivity = TP/(TP+FN)$, $specificity = TN/(TN+FP)$. Although it is not always possible to reach babies born in the country, especially refugee babies, data from the Turkish Directorate of Public Health were compared with data from pediatric endocrine centers, and data from the Turkish Directorate of Public Health were reviewed by a member of a neonatal screening scientific advisory committee (TG) to reduce FN and TN rate.

Ethics

The parents were informed about NBS. Heel-prick blood samples were collected from live-born babies after written consent from the parents was obtained. The study was carried out with the written permission of the Scientific Committee of the TDPH.

Statistical analysis

Statistical evaluation was performed using GraphPad Prism® V9.0 software (GraphPad Software Inc., San Diego, California, USA). The results for each steroid are reported as mean, SD or as median (IQR) in the text. We performed a *t*-test for the comparison of the means of two independent samples. Values were considered statistically significant when *P* value is less than 0.05.

Results

The total number of newborns that underwent CAH screening was 1,096,069 (including 149,652 refugees). Of those babies, 1,015,200 (92.6%) were ≥ 36 gestational weeks and/or ≥ 2500 gr birth weight. Out of these infants, 70,455 (6.9%) underwent second-tier testing because their FIA-17OHP levels were above the first-tier cut-off values.

For healthy infants without a final diagnosis of CAH, the 99.8th percentile values for first-tier FIA-17OHP measurements were found to be below 50 ng/mL for those with a gestational age of 32–36 weeks and/or a birth weight of 1500–2500 g, and below 17.5 ng/mL for those with a gestational age of ≥ 36 weeks and/or a birth weight of ≥ 2500 g.

Neonates, who failed to pass second-tier testing (3,429 of 70,455; 4.8%); were referred to paediatric endocrinology clinics for further evaluation, which corresponds to an overall recall rate of 0.31%. The average number of days for referral of neonates to the clinic was 11.75 \pm 6.05 days.

Consequently, 113 neonates were diagnosed with CAH (55 females, 58 males). Seventy seven were diagnosed as classical salt-wasting (SW) 21OHD CAH, while 14 were simple virilizing (SV) and 22 were non-classical (NC) 21OHD CAH. Second-tier testing results of SW, SV and NC-21OHD CAH patients diagnosed by neonatal screening are presented in **Supplemental Table 1a, b and c**, respectively.

The distribution of 21OHD CAH forms by sex is shown in **Table 1**, and no differences in prevalence of forms were observed between the sexes.

The incidence of classical 21OHD in the screened population was 1:12,044. None of these babies was premature nor had low birth weight. Out of the 91 neonates diagnosed with classical CAH through screening, 17 (15%) were refugees, and all of these cases were of the classical salt-wasting type.

In all infants diagnosed with classic 21-hydroxylase deficiency (21OHD), the (21DF+17OHP)/F ratio was above 2. Overall; PPV, sensitivity and specificity of the current screening protocol for classical 21OHD CAH was calculated as 2.5, 100 and 99.6%, respectively. There was no false-negative case.

Out of 113 patients diagnosed with CAH, a molecular diagnosis was achieved in 64 patients. Forty-five (70%) patients were homozygous, 14 (22%) were compound heterozygous carriers of *CYP21A2* pathogenic variants, and 5 (8%) patients had a single pathogenic allele identified. The distribution of a total of 149 detected alleles, according to their frequency and severity, is shown in **Figures 2A and 2B**.

We were unable to collect the number of patients with biochemically/genetically confirmed 11 β -OHD from the centers.

Discussion

This audit presents the first evaluation of the nationwide neonatal CAH screening in Türkiye. The most significant finding of the analysis, as we previously recommended in our pilot studies, is the need to increase the cut-off values for FIA-17OHP used in the first-tier screening. This approach will particularly reduce the number of false-positive cases referred to the clinic and decrease the number of cases subjected to second-tier testing, thereby lowering the screening costs.

In line with our previous findings (6, 7), if the cut-off values of 50 ng/mL for neonates with a gestational age of 32-36 weeks and/or a birth weight of 1500-2500 g, and 17.5 ng/mL for those with a gestational age of ≥ 36 weeks and/or a birth weight of ≥ 2500 g, had been used for first-tier FIA-17OHP measurements, the number of infants subjected to second-tier screening would have been 3,596 instead of 70,455. Even with this proposed approach, 95.1% of the samples subjected to second-tier screening would not have needed this step. This will result in significant savings in both workload and costs.

Currently, in the second-tier of the screening programme, neonates with an LC/MS-MS-based (21DF+17OHP)/F ≥ 1 are referred to clinics for assessment for classical CAH. This cut-off value was determined based on the results of a previous study that retrospectively examined DBS samples (9). However, both in this current nationwide screening analysis and in previous pilot CAH screenings in Türkiye, all patients diagnosed with classical CAH had a second-tier (21DF+17OHP)/F ratio greater than 2 (6, 7). If a (21DF+17OHP)/F ratio of ≥ 2 had been used instead of ≥ 1 as the referral criterion in the current screening, there would have been 563 referrals (about 1/6 of all referrals) instead of 3,429. This means that the recall rate in the screening would have been 0.051% instead of 0.31%. Therefore, the PPV will increase by up to 16%.

An additional concern is the detection of non-classical CAH cases through neonatal screening. The detection of some non-classical CAH cases can be explained by the low cut-off values used in first-tier 17OHP measurements. Non-classical CAH cases identified through screening do not reflect the true prevalence of NC-CAH and these neonates should be monitored without treatment unless a clear indication for treatment arises.

One area that requires improvement is the recall time. The success of screening depends on detecting the patient before symptoms develop. In salt-wasting CAH, symptoms are expected to begin after the first week of life. DBS samples in the current screening are collected after 72 hours postnatally. If the neonate is discharged before 72 hours, a heel-prick blood sample is taken by contacting the family through their affiliated primary healthcare provider. Delays in processes, such as reaching the family or the family's attendance at the healthcare facility, can prolong the recall time in some cases. This situation particularly creates challenges in reaching the babies of migrant families living in Türkiye, who constitute approximately 1/7 of all screened neonates. Since the second-tier screening uses LC-MS/MS with very low hormone interference, we recommend a pilot study to analyse false positives and false negatives in DBS samples collected before discharge. If there is no negative impact on screening results, moving the DBS collection to before discharge may shorten the recall time. As we suggested in previous pilot studies, referring neonates with very high first-tier test results (i.e., FIA-17OHP > 90 ng/mL) to clinical evaluation without undergoing second-tier testing would also shorten the time to diagnosis, especially in salt-wasting cases.

Patients were diagnosed with classic CAH based on clinical findings (ambiguous genitalia and/or salt loss) in addition to a positive screening test, and the diagnosis was confirmed by biochemistry and further hormone testing. Inadequacies in accessible genetic testing, such as the inability to perform MLPA testing in all patients as molecular evidence, or the fact that only the 10 most common mutations leading to 21OHD were examined, may have led to the failure to find the pathogenic second *CYP21A2* allele in some patients.

One limitation of this audit is the inability to reliably determine steroid hormone cut-off levels that define subgroups of 21OHD in diagnosed patients. This is due to the differences in the variants carried by the patients and the relatively small number of diagnosed cases. Furthermore, although second-tier CAH screening strategy, would facilitate to identify cases with 11 β -OHD we were unable to collect the number of patients with biochemically/genetically confirmed 11 β -OHD from the centers. Therefore, we could not include data on the prevalence of 11 β -OHD in this study.

In conclusion, as the Neonatal CAH Screening Scientific Committee, we believe that implementing the aforementioned changes during the early phase of the nationwide screening will enhance its long-term effectiveness and cost-efficiency, while also reducing laboratory and clinical workload. Furthermore, we consider that such audits in newborn screening programs are crucial for improving preventive healthcare services.

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Table 1. The distribution of 21OHD CAH forms by sex

	Female	Male	Total
Salt-wasting	38 (69%)	39 (67%)	77
Simple virilizing	5 (9%)	9 (16%)	14
Non-classical	12 (22%)	10 (17%)	22
Total	55	58	113

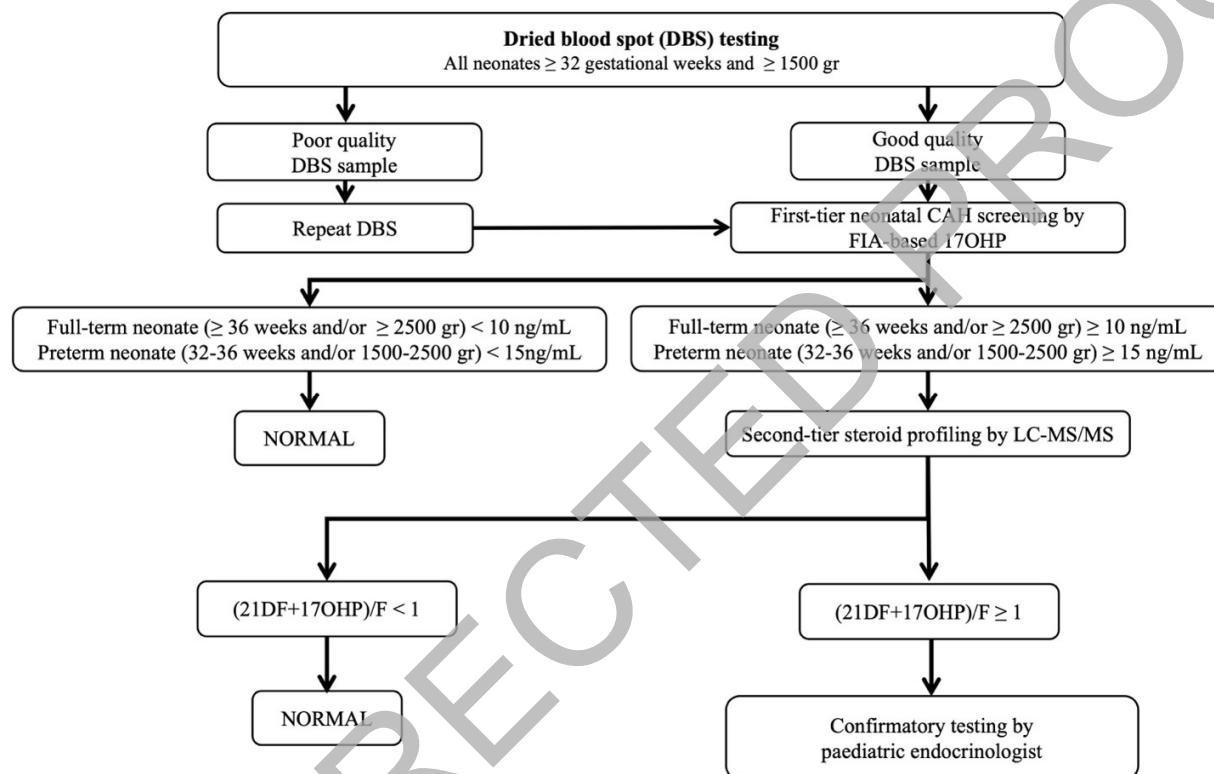
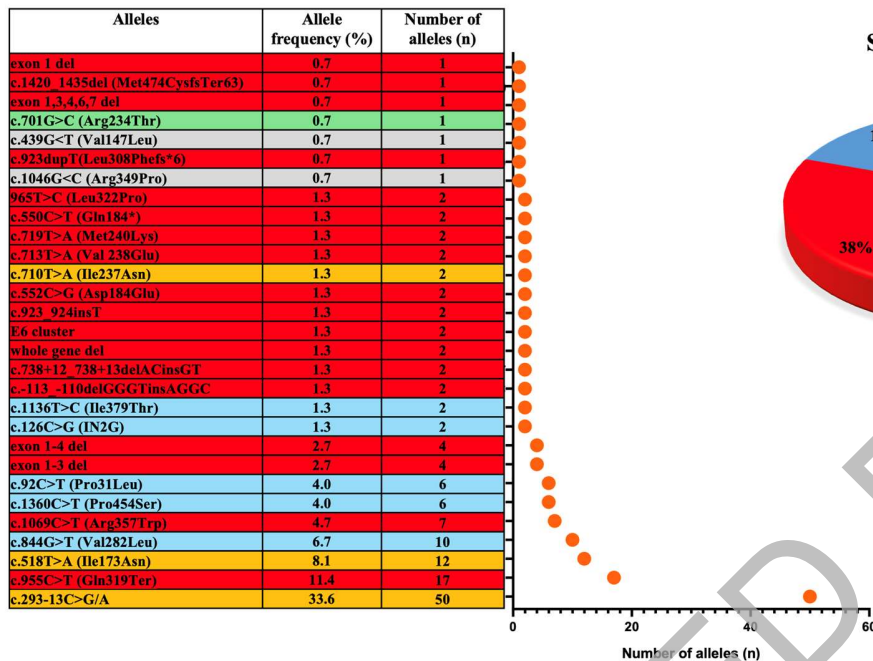


Figure 1. The flowchart of neonatal CAH screening, which became nationwide in Türkiye in 2022. Screening is carried out in Türkiye in all newborns above 32 weeks of gestation and 1500 g birth weight with a single sample two-tier testing strategy.

A



B

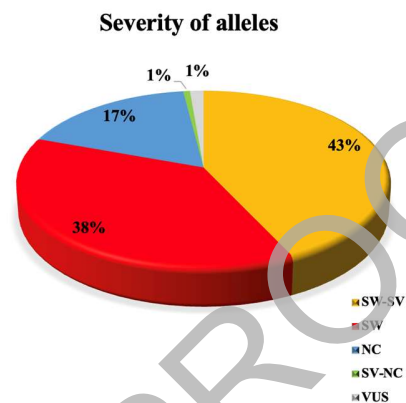


Figure 2. Distribution of the severity and frequency of *CYP21A2* variants detected in patients diagnosed in the first year of the nationwide neonatal CAH screening in Türkiye. A. Specific *CYP21A2* variants are indicated by allele frequency and allele numbers and highlighted in red, orange, blue, yellow and grey according to clinical severity representing salt-wasting, salt-wasting-simple virilising, non-classical, simple virilising-non-classical and variant of unknown significance, respectively. Prevalence of the variants are shown in the frequency dot plot graph on the right. B. A pie chart shows the frequency of all alleles detected in diagnosed CAH patients according to their clinical effects. Abbreviations: SW: salt-wasting, SV: simple virilising, NC: non-classical, VUS: variant of unknown significance.

Supplemental Material

Second-tier screening results of 21OHD CAH patients diagnosed by neonatal screening

Supplemental Table 1a. Screening results of SW-21OHD patients diagnosed by neonatal screening.

Number	17OHP (1st tier)	LC-MS/MS 17OHP	LC-MS/MS 11-DEOXYCORTISOL	LC-MS/MS 21-DEOXYCORTISOL	LC-MS/MS ANDROSTENEDIONE	LC-MS/MS CORTISOL	(21S+17OHP)/F
1	56,5	45,782	0,318	12,808	1,482	1,65	35,51
2	67	44,297	1,107	2,051	3,022	2,711	17,10
3	36,1	53,554	0,582	59,765	4,34	17,825	6,36
4	47,09	56,576	2,282	31,177	4,068	8,59	10,22
5*	48,6	10,2	1,73	0,2	2,95	69,19	0,14
6	49,08	14,913	0,16	10,12	3,689	2,943	8,51
7*	51,07	23,326	0,225	11,798	3,488	48,426	0,47
8	54,93	51,979	0,984	46,131	5,871	14,389	6,82
9	57,15	26,46	0,738	7,291	10,105	11,278	2,99
10	58,15	125,232	0,729	9,345	15,99	6,775	19,86
11	58,61	24,312	1,269	3,624	5,595	5,755	4,85
12	60,8	262,18	4,828	12,507	56,834	12,093	22,71

13	60,8	156,786	3,049	23,848	54,2	8,293	21,78
14	60,8	236,273	2,325	11,072	34,75	3,267	75,71
15	67	44,297	1,107	2,051	3,022	2,711	17,10
16	67	42,87	0,91	2,054	5,049	5,938	7,57
17	67	129,071	4,086	2,986	72,61	13,565	9,74
18	67	289,375	3,17	34,093	65,5	37,462	8,63
19	67,1	428,274	5,465	14,074	81,95	11,304	39,13
20	67,1	202,755	0,338	45,392	7,546	7,339	33,81
21	67,1	133,151	3,001	3,599	19,63	3,88	35,24
22	67,1	48,639	1,721	22,993	3,576	11,195	6,40
23	67,1	94,287	0,454	10,623	3,051	4,355	24,09
24	67,1	415,506	10,118	30,782	155,89	52,333	8,53
25	67,1	146,328	1,803	31,862	24,48	13,103	13,60
26	67,1	124,131	1,425	18,07	13,017	3,155	45,07
27	68	1686,86	7,579	49,833	132,937	20,2	85,97
28	68	272,769	8,522	5,114	72,693	12,612	22,03
29	68	127,797	1,639	20,378	17,371	5,178	28,62
30	68	165,82	0,873	21,539	16,121	4,783	39,17
31	68	162,558	2,661	15,923	29,655	7,578	23,55
32	68	36,963	1,608	6,638	13,16	4,545	9,59
33	68	25,566	0,647	59,53	2,597	25,662	3,32
34	68	27,576	0,704	14,438	3,162	11,723	3,58
35	68	343,933	7,167	12,064	98,05	11,266	31,60
36	68	53,576	0,586	14,488	6,603	5,091	13,37
37	68	19,417	0,425	3,364	3,064	0,599	38,03
38	70,1	705,13	8,137	15,088	175,458	8,744	82,37
39	70,1	30,529	0,687	16,434	3,002	4,262	11,02
40	70,1	693,908	1,491	33,123	37,402	7,955	91,39
41	70,13	61,969	2,25	16,023	19,373	23,17	3,37
42	70,13	91,355	1,99	29,691	13,76	5,059	23,93
43	70,13	151,221	1,395	10,396	23,143	13,339	12,12
44	70,13	94,764	1,778	8,955	6,746	4,258	24,36
45	70,13	967,571	4,175	23,647	64,903	9,708	102,10
46	70,13	50,049	0,796	16,053	19,507	3,578	18,47
47	76,7	394,795	2,884	165,655	8,827	26,081	21,49
48	76,74	58,225	4,15	37,687	11,6	15,257	6,29
49	77	72,652	3,912	16,629	5,641	2,097	42,58
50	79,6	160,071	2,238	6,564	32,58	5,809	28,69
51	80	18,86	0,904	4,86	1,595	2,339	10,14

52	81,8	326,502	3,591	26,023	47,841	11,424	30,86
53	81,8	249,379	0,954	55,351	4,332	6,324	48,19
54	84,4	165,983	0,657	4,605	35,179	7,534	22,64
55	84,4	88,312	0,922	30,525	9,164	4,103	28,96
56	85,9	1259,83	9,579	12,604	106,4	8,969	141,87
57	85,9	58,812	2,093	4,427	23,11	5,039	12,55
58	85,9	160,446	1,148	129,735	65,36	18,867	15,38
59	86	91,658	0,501	21,653	11,88	4,635	24,45
60	67,000	289,000	3,170	34,090	65,500	37,462	8,6
61	63,23	29,055	0,573	7,796	6,068	2,674	13,781
62	76,72	87,08	1,29	11,75	9,37	9,00	10,90
63	137,30	263,345	7,177	40,656	90,44	29,957	10,14
64	44,29	19,66	0,62	0,052	1,53	1,499	14,28
65	96,00	908,457	27,84	0,492	346,9	41,275	22,14
66	96,00	58,734	3,847	0,020	6,832	12,696	4,83
67	137,30	262,59	0,83	37	23,1	9	32,47
68	90	168,37	0,86	24,56	34,28	6,38	30,23
69	95	287,35	9,32	0,84	43,77	6,8	42,38
70	90	322,55	1,37	41,99	20,9	10,73	33,97
71	95	73,6	1,91	22,94	9,19	9,04	10,67
72	90	234,36	5,56	9,14	44,97	9,04	26,93
73	90,00	321,00	1,37	41,90	20,90	10,70	36,29
74	90,00	104,40	0,28	27,23	7,70	5,44	24,19
75	90,00	220,00	1,18	28,93	9,15	8,10	30,73
76	46,60	41,47	0,25	43,21	3,98	2,33	36,34
77	95,00	73,60	1,91	22,94	9,19	9,04	10,67

*Sampled after hydrocortisone treatment, SW-21OHD: Salt-wasting 21-alpha hydroxylase deficiency

Supplemental Table 1b. Screening results of SV-21OHD patients diagnosed by neonatal screening.

Number	17OHP (1st tier)	LC-MS/MS 17OHP	LC-MS/MS 11-DEOXYCORTISOL	LC-MS/MS 21-DEOXYCORTISOL	LC-MS/MS ANDROSTENEDIONE	LC-MS/MS CORTISOL	(21S+17OHP)/F
1	24,22	24,467	0,579	29,446	0,776	14,979	3,60
2	15,32	6,634	0,699	3,822	1,52	4,44	2,35
3	18,71	10,346	0,627	1,543	0,975	7,897	1,51
4	19,83	5,664	0,217	0,307	0,301	1,953	3,06
5	23,96	10,475	0,517	1,717	0,789	3,174	3,84
6	26,66	22,967	0,901	4,23	1,152	46,393	0,59
7	27,55	10,47	1,921	2,001	5,482	52,978	0,24
8	31,45	7,676	0,188	0,95	0,253	1,559	5,53
9	31,74	12,257	1,415	2,986	0,758	26,116	0,58

10	54,98	70,357	4,214	0,001	8,338	63,87	1,10
11	33,81	10,87	0,618	0,604	3,439	2,61	4,76
12	44,29	19,66	0,62	0,052	1,53	14,99	1,33
13	24,22	24,467	0,579	29,446	0,776	14,979	3,60
14	60,09	14,145	4,443	0,06	0,66	8,325	1,71

SV-21OHD: Simple virilizing 21-alpha hydroxylase deficiency

Supplemental Table 1c. Screening results of NC-21OHD patients diagnosed by neonatal screening.

Number	17OHP (1st tier)	LC-MS/MS 17OHP	LC-MS/MS 11-DEOXYCORTISOL	LC-MS/MS 21-DEOXYCORTISOL	LC-MS/MS ANDROSTENEDIONE	LC-MS/MS CORTISOL	(21S+17OHP)/F
1	9,49	2,526	0,655	1,696	0,492	32,621	0,13
2	9,9	7,864	0,406	3,393	0,741	31,551	0,36
3	10	3,64	0,393	0,01	0,255	2,073	1,76
4	10	4,17	0,669	1,906	1,253	48,39	0,13
5	10,84	4,416	0,575	3,356	0,62	35,635	0,22
6	11,54	3,326	0,439	4,615	0,637	45,508	0,17
7	11,56	9,197	0,052	4,548	0,369	44,156	0,31
8	12,31	5,014	0,109	2,673	0,557	39,882	0,19
9	14,92	2,04	0,9	0,314	1,1	1,998	1,18
10	15	3,644	0,372	3,142	0,724	103,175	0,07
11	16,71	5,289	0,805	0,175	0,493	1,058	5,16
12	20,87	11,491	1,231	1,684	1,856	33,811	0,39
13	22,82	7,251	0,525	5,754	0,478	18,99	0,68
14	31,19	15	5,418	0,137	0,801	15	1,01
15	11,59	3,891	0,603	6,439	0,461	6,208	1,66
16	12,16	8,997	0,115	3,679	0,447	5,742	2,21
17	24,93	6,767	0,264	2,208	6,093	25,353	0,35
18	26,74	7,137	0,944	2,636	1,155	4,447	2,20
19	28,22	11,646	2,968	0,19	3,067	7,247	1,63
20	30,53	12,115	0,523	4,889	1,135	9,827	1,73
21	40,23	10,852	0,717	0,799	0,924	5,848	1,99
22	9,49	2,526	0,655	1,696	0,492	32,621	0,13

NC-21OHD: Simple virilizing 21-alpha hydroxylase deficiency