ARTICLE

Efficiency of Neonatal Screening for Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency in Children Born in Mainland France Between 1996 and 2003

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Objective: To assess the efficiency of the French national screening program for 21-hydroxylase deficiency (21-OHD). Neonatal screening for congenital adrenal hyperplasia due to 21-OHD is mainly intended to prevent death due to salt wasting but remains controversial because of the number of false-positive results and the ease with which most female cases can be identified by virilized genitalia at birth.

Design: Population-based study.

Setting: National neonatal screening program, pediatric endocrinologists nationwide, and reference center for genotyping.

Participants: All neonates screened for 21-OHD in mainland France between January 1, 1996, and December 31, 2003.

Outcome Measures: Screening efficiency indicators, disease severity, contribution of screening to early diagnosis, and disease-specific mortality before and during the study period.

Results: A total of 6 012 798 neonates were screened; results in 15 407 were considered positive for 21-OHD. Three hundred eighty-three cases were identified, giving a prevalence of 1 for every 15 699 births. The positive predictive value of screening was 2.3% (95% CI, 2.1%-2.6%), with a sensitivity of 93.5% (90.9%-95.9%) and a specificity of 99.7% (99.7%-99.7%). The false-positive rate was particularly high in preterm infants, for which the positive predictive value was 0.4% (95% CI, 0.2%-0.5%). Screening allowed clinical diagnosis in 162 of 383 cases (42.3%), with the others being detected clinically or through family history. There was a trend toward declining neonatal mortality due to 21-OHD.

Conclusions: In this large population-based study, the efficiency of routine 21-OHD screening was moderate in neonates born at term and very low in preterm neonates. We recommend the discontinuation of screening, as currently performed in France, in preterm neonates.

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CONGENITAL ADRENAL HYperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroid biosynthesis.[1-3] The most common form (approximately 95%) is due to 21-hydroxylase deficiency (21-OHD). It affects about 1 child in 15 000 and results in symptoms that vary with the severity of the enzymatic defect. Classic forms include salt-wasting (SW) forms, for which there is a high risk of life-threatening adrenal insufficiency during the first month of life, and simple-virilizing (SV) forms. In both cases, female neonates present with markedly virilized external genitalia. Nonclassic forms can manifest with hyperandrogenism later in life and do not warrant early recognition through neonatal screening. 3β-Hydroxysteroid dehydrogenase (3β-HSD) deficiency is a rare form of CAH that results in the undervirilization of external genitalia and adrenal insufficiency; it can be detected by 21-OHD screening.[4]

Screening for 21-OHD is carried out to prevent neonatal death from acute adrenal insufficiency, inaccurate sex assignment in females with complete virilization, and irreversible childhood hyperandrogenism, which may result from incorrect or late diagnosis.[3,5] 21-Hydroxylase deficiency fulfills the usual criteria[6] for neonatal screening, with its low cost and the availability of a widely applicable test (17-hydroxyprogesterone [17-OHP] determination) and has been implemented in many Western countries, including the United States and some European countries.[3,7-9] However, screening remains controversial, with 3 main arguments against its routine use: (1) the test has a low positive predictive value, with frequent false-positive results in preterm neonates resulting from cross-

reactions with corticosteroids other than 17-OHP[10]; (2) the proportion of cases for which screening contributes to diagnosis is unclear, as most cases in females are easy to detect clinically and salt wasting can occur before the screening results are obtained; and (3) there is a lack of consensus concerning the 17-OHP threshold to be used because of changes in 17-OHP distribution with gestational age at birth.

In France, 21-OHD screening was introduced for all neonates as part of the national screening program in 1996 after a short pilot feasibility study.[11] However, as in many other countries, routine 21-OHD screening was never evaluated. The main objective of this study was to evaluate the efficiency of the national French screening program for 21-OHD. We retrospectively collected real-life screening data and clinical data for affected neonates to determine whether screening by the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant (AFDPHE), a national organization, had facilitated the identification of cases before clinical diagnosis.

METHODS

POPULATION STUDIED AND DATA COLLECTED

We carried out a retrospective study on all children born in mainland France between January 1, 1996, and December 31, 2003. Screening was conducted at 21 regional centers under the auspices of the AFDPHE.[12] Blood was collected from 3-day-old infants on filter paper, and 17-OHP concentration was determined by automated time-resolved fluoroimmunoassay (DELFIA; PerkinElmer) or radioimmunoassay. Infants with 17-OHP levels above the threshold applied for screening purposes were evaluated further for the diagnosis of 21-OHD. We collected data from the regional centers on all neonates for whom 21-OHD screening results were considered positive. The data collected included date of birth, gestational age and birth weight, screening and repeat determinations of 17-OHP, assay and threshold used, and conclusions concerning the status of the child: *affected* with CAH (true-positive), *unaffected* (false-positive), or *deceased*. 17-OHP concentrations are expressed in nanomoles per liter of blood and were converted if necessary (65 pg/spot = 80 nmol/L of blood). The threshold applied was that recommended nationally by the AFDPHE but was modified slightly at different times and in different regions on the basis of the local distribution of 17-OHP levels. We collected additional data from the medical records of affected children concerning sex, date, weight, and plasma sodium concentration at diagnosis; genital abnormalities classified as described by Prader[1]; and *CYP21A2* genotyping results, classified as classic SW, classic SV, or nonclassic forms.[13,14] If genotyping results were not available or not informative (n = 2) resulting from the detection of mutations with unknown functional repercussions, patients were classified as a function of the clinical data, leaving only 1 unclassified patient, who was then arbitrarily classified as affected with SW CAH. Weight at diagnosis was expressed as a percentage of expected weight at a given age, based on birth weight and the expected 1% gain in weight per day after day 8.[15] The distribution of the gestational ages of neonates with true-negative results was derived from reference values reported in the National Perinatal Survey in 1998 and 2003.[16]

We searched for false-negative cases detected before March 2010, which is at least 6 years after the birth of the last child studied, using 5 data sources: (1) regional screening centers notified of false-negative cases by physicians; (2) mail and e-mail surveys of all pediatric endocrinologists registered with the national society or treating children with CAH; (3 and 4) the French reference center for CAH genotyping in Lyon and another molecular biology laboratory performing CAH genotyping; and (5) the Centre for Epidemiology Medical Causes of Death database (CépiDc, INSERM [Institut National de la Santé et de la Recherche Médicale]), in which we looked for children dying from causes corresponding to *International Classification of Diseases, 9th Revision* and *10th Revision* codes 255.2, 255.4, E25, and E27.4 (adrenogenital disorders, other and unspecified adrenocortical insufficiency).

STATISTICAL ANALYSIS

We calculated the sensitivity, specificity, and predictive values of the screening test, with 95% CIs for preterm neonates born before 37 weeks of gestational age, for term neonates, and for both considered together. We classified the contribution of CAH screening to the diagnosis of true-positives as follows: screening was considered useful if it led to the diagnosis of classic 21-OHD (SW or SV forms) or 3β-HSD deficiency that was not suspected clinically because there were no symptoms or because the symptoms and signs (genital abnormalities, dehydration) had not been recognized; screening was considered not useful if CAH was diagnosed before the results of screening became available (on the basis of family history, prenatal diagnosis, neonatal systematic examination, or salt-wasting adrenal crisis). Screening was also considered not useful for false-negative cases of classic CAH and for children with positive screening results diagnosed with nonclassic forms of CAH.

The relationship between gestational age at birth and 17-OHP concentration was studied by linear regression analysis in a sample of 10 523 preterm neonates born before 37 weeks of amenorrhea selected from the infants testing positive. The values for 17-OHP concentration were not normally distributed, and a natural logarithm transformation was therefore applied. Goodness of fit (R^2) was calculated for various linear regression models to identify the factor best predicting 17-OHP concentration: gestational age or birth weight. Linear regression models were constructed for the imputation of missing data for term or birth weight.

Mortality rates for children younger than 1 year were calculated between 1979 and 2007 from CépiDc data (http://www.cepidc.vesinet.inserm.fr/). Changes in mortality rates over time were assessed by Poisson regression analysis. We looked for a possible change in slope after 1996 (the year when the screening program was generalized) by looking for an interaction between *year* (considered as a continuous variable) and *before/after screening introduction* (considered as a dichotomous variable).

All analyses were performed using commercial software (SAS 9.2; SAS Institute, Inc). The study was approved by the Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé and Commission nationale de l'informatique et des libertés and was conducted in accordance with French legislation.

RESULTS

During the 8-year study period, 6 012 798 screening tests for 21-OHD were performed on children born in mainland France (**Figure 1**, **Table 1**, and the eTable [http://www.archpediatrics.com]). The laboratory methods for 17-OHP determinations and their thresholds are shown in the eTable. Neonatal screening tests were positive for 15 407 neonates, with 370 considered affected and 11 324

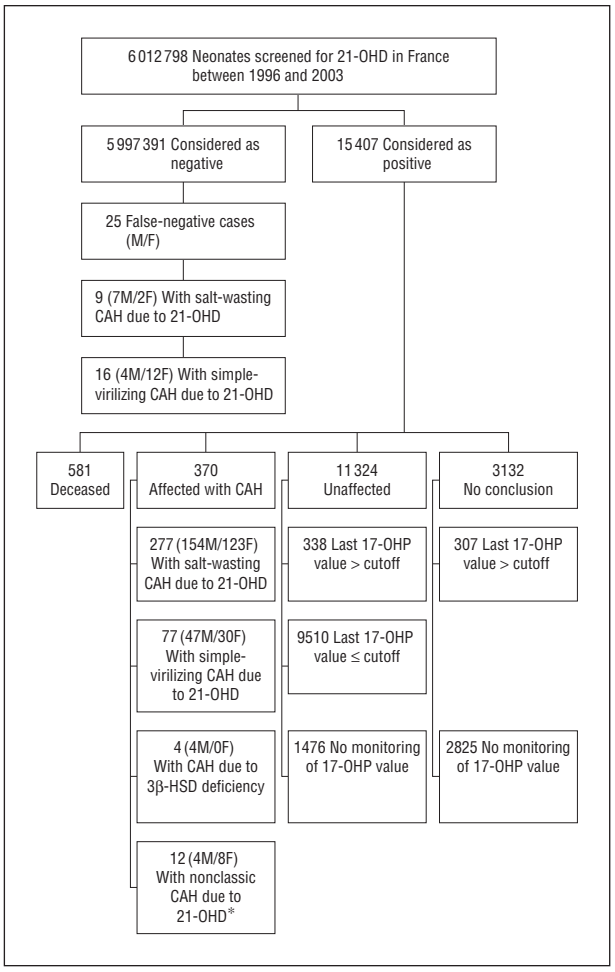

Figure 1. Results of neonatal screening for 21-hydroxylase deficiency (21-OHD) in France, 1996-2003. CAH indicates congenital adrenal hyperplasia; 3β-HSD, 3β-hydroxysteroid dehydrogenase; and 17-OHP, 17-hydroxyprogesterone. *These 12 patients were not counted as cases, since identification of nonclassic CAH is not an aim of screening.

considered unaffected; no conclusion was reached by the screening centers for 3132 infants. For 1814 infants, the conclusion was discordant with the last recorded 17-OHP concentration (338 were considered unaffected with a last 17-OHP determination considered positive and 1476 were considered unaffected without the recorded monitoring of 17-OHP concentrations). Five hundred eighty-one children were identified as deceased: most of these children were preterm and, in all cases, the death was considered by clinical centers to be unrelated to 21-OHD (Figure 1). Most of the neonates with positive results for 21-OHD screening were born before term (90.9% of those for whom data were available). The median day for filter paper sampling was day 4, although the screening protocol called for sampling on day 3. Of the 370 neonates considered to be affected, 358 had a classic form of 21-OHD (n=354) or 3β-HSD (n=4) deficiency and 12 had a nonclassic form of CAH.

The median age at diagnosis of CAH was 7 days (**Table 2**). Weight loss was severe (>10% of expected body weight) in 18.9% of those for whom data were available, and plasma sodium concentration was below 130 nmol/L in 17.9% of the infants. Screening was useful for

Table 1. Principal Characteristics of Neonates With Positive Screening Results for Congenital Adrenal Hyperplasia in Mainland France Between 1996 and 2003

Characteristic	Data (N=15 407)[a]
Sex	
Male	9031 (58.6)
Female	6218 (40.4)
Gestational age, median (IQR), WA[b]	31 (28-34)
Preterm (<37 WA)	10 563 (68.6)
Term (≥37 WA)	1058 (6.9)
Missing data	3786 (24.6)
Birth weight, median (IQR), g[b]	1490 (1005-2090)
Age at screening, median (IQR), d	4 (3-5)
17-OHP screening result, median (IQR), nmol/L[c]	80 (65-109)
17-OHP measurement method	
Radioimmunoassay	8457 (54.9)
Time-resolved fluoroimmunoassay	6945 (45.1)

Abbreviations: 17-OHP, 17-hydroxyprogesterone; IQR, interquartile range; WA, weeks of amenorrhea.
[a] Data are presented as number (percentage) unless otherwise stated. Missing data: sex, 158 neonates; age at screening, 1117; 17-OHP screening result, 72; and 17-OHP measurement method, 5.
[b] Imputations of gestational age from sex and birth weight, 998 neonates; imputations of birth weight from sex and gestational age, 2653.
[c] Expressed as nanomoles per liter of blood and converted if necessary (65 pg/spot = 80 nmol/L of blood).

diagnosis in 162 of the 358 children (45.3%) with classic CAH and positive screening results, mostly males with the SW form (106 of162). Screening results were positive but not useful for diagnosis in 74 children with a family history of 21-OHD and in 96 girls with genital abnormalities detected during neonatal examination. In addition, screening results were positive in 13 boys with classic 21-OHD who were diagnosed clinically before the screening results became available. Of interest, among the 38 premature neonates with positive screening results, screening was useful to the diagnosis in only 13, among whom only 6 had an SW form. We identified 25 children as having false-negative results for 21-OHD screening: 23 were reported by genotyping laboratories, and 20 of these cases were also reported by the screening centers, with 2 reported by the CépiDC. Most of the false-negatives (16 of 25) had SV forms (Figure 1).

Altogether, the incidence of classic 21-OHD (SW and SV forms) and 3β-HSD deficiency in France between 0 and 1 year was 0.78 per 100 000 births per year (95% CI, 0.70-0.86), and the prevalence was 1 for every 15 699 births (95% CI, 1:17 445 to 1:14 269) (including infants with false-negative results in their birth cohort). The sensitivity of screening was 93.5%, with an overall positive predictive value of 2.3% (**Table 3** and **Table 4**). Sensitivity was higher for SW 21-OHD (96.9%; 95% CI, 94.8%-98.9%) than for SV 21-OHD (82.8%; 75.1%-90.5%). Most false-positive screening test results were obtained for preterm neonates, for which the positive predictive value of screening was only 0.4%, whereas that for term neonates was 30.1%. We investigated whether adjustment of the 17-OHP threshold would have improved screening efficiency in preterm neonates by calculating linear regression models of (positive) 17-OHP levels on filter paper. Gestational age accounted for 9.5%

Table 2. Characteristics of Affected Neonates With CAH Due to Classic 21-OHD or 3β-HSD Deficiency Detected by Screening in Mainland France Between 1996 and 2003

Characteristic[a]	True-Positive Results (n=358)[b]
Sex	
Male	205 (57.3)
Female	153 (42.7)
Gestational age, median (IQR), WA	39 (38-40)
Preterm, <37 WA	38 (10.7)
Term, ≥37 WA	318 (89.3)
Birth weight, median (IQR), g	3370 (2980-3680)
Age at screening, median (IQR), d	3 (3-4)
Age at diagnosis, median (IQR), d	7 (1-10)
Contribution of screening to the diagnosis of CAH, No. (%) [No. M:F]	
Useful	162 (45.3) [137:25]
Salt-wasting 21-OHD	114 (31.8) [106:8]
Simple virilizing 21-OHD	47 (13.1) [30:17]
3β-HSD	1 (0.3) [1:0]
Not useful	196 (54.7) [68:128]
Clinical diagnosis before screening results	109 (30.4) [13:96]
Salt-wasting 21-OHD	99 (27.7) [9:90]
Simple virilizing 21-OHD	8 (2.2) [2:6]
3β-HSD	2 (0.6) [2:0]
Prenatal diagnosis or family history	74 (20.7) [46:28]
Salt-wasting 21-OHD	54 (15.1) [33:21]
Simple virilizing 21-OHD	20 (5.6) [13:7]
3β-HSD	0 [0:0]
Information on usefulness unavailable	13 (3.6) [9:4]
Salt-wasting 21-OHD	10 (2.8) [6:4]
Simple virilizing 21-OHD	2 (0.6) [2:0]
3β-HSD	1 (0.3) [1:0]
Plasma sodium concentration at diagnosis, nmol/L, No. (%) [No. M:F]	
≥135	177 (49.4) [78:99]
130 to 135	80 (22.3) [61:19]
<130	56 (15.6) [48:8]
Relative weight change at diagnosis, % of expected, No. (%) [No. M:F]	
≥0	42 (11.7) [21:21]
0 to –5	62 (17.3) [34:28]
–5 to –10	76 (21.2) [60:16]
<–10	42 (11.7) [39:3]

Abbreviations: CAH, congenital adrenal hyperplasia; 3β-HSD, 3β-hydroxysteroid dehydrogenase; IQR, interquartile range; 21-OHD, 21-hydroxylase deficiency; WA, weeks of amenorrhea.

[a] Missing data: gestational age, 2; age at screening, 28; age at diagnosis, 38; plasma sodium concentration, 45; relative weight change at diagnosis, 136. The 25 false-negative cases (11 boys and 14 girls), for whom screening was not useful, are not included in this Table.

[b] Data are presented as number (percentage) unless otherwise stated.

and weight accounted for 7.4% of the variance (R^2) of 17-OHP concentration. Adding polynomials and assay techniques increased the R^2 value to 10%. **Figure 2** illustrates the difficulty of establishing threshold values based on gestational age.

Because the primary objective of 21-OHD screening is to prevent the death of neonates, we analyzed 21-OHD–related mortality in France from 1979 to 2007, a 29-year period, including the years in which 21-OHD screening was introduced. Twenty-one children younger than 1 year were classified with an underlying cause of death due to adrenogenital disorders, as well as other and unspecified adrenocortical insufficiency (**Figure 3**). There was a significant (P=.002) trend toward a decrease in specific mortality rate during this period, with most of this decrease occurring in 1991-1995, before the generalization of screening. Thus, neither screening (yes/no) nor the interaction of screening and time was associated with a specific mortality rate (P=.31 and .31, respectively).

Table 3. Efficiency of 21-OHD Screening as a Function of Gestational Age at Birth: Raw Data[a]

Screening	No.		
	Affected	Unaffected	Total
All neonates			
Positive	358	15 049	15 407
Negative	25	5 997 366	5 997 391
Total	**383**	**6 012 415**	**6 012 798**
Term neonates (≥37 WA)			
Positive	318	740	1058
Negative	21	5 578 196	5 578 217
Total	**339**	**5 578 936**	**5 579 275**
Preterm neonates (<37 WA)			
Positive	38	10 524	10 562
Negative	2	422 959	422 961
Total	**40**	**433 483**	**433 523**

Abbreviations: 21-OHD, 21-hydroxylase deficiency; WA, weeks of amenorrhea.

[a] Gestational age at birth was missing for 3787 neonates with a positive screening result.

Table 4. Efficiency of 21-OHD Screening as a Function of Gestational Age at Birth: Efficiency Calculations[a]

Characteristic	Data, % (95% CI)
All neonates	
Positive predictive value	2.3 (2.1-2.6)
Negative predictive value	99.9 (99.9-99.9)
Specificity	99.7 (99.7-99.7)
Sensitivity	93.5 (90.9-95.9)
Term neonates (≥37 WA)	
Positive predictive value	30.1 (27.3-32.8)
Negative predictive value	99.9 (99.9-99.9)
Specificity	99.9 (99.9-99.9)
Sensitivity	93.8 (91.2-96.4)
Preterm neonates (<37 WA)	
Positive predictive value	0.4 (0.2-0.5)
Negative predictive value	99.9 (99.9-99.9)
Specificity	97.6 (97.5-97.6)
Sensitivity	95.0 (83.1-99.4)

Abbreviations: 21-OHD, 21-hydroxylase deficiency; WA, weeks of amenorrhea.

[a] Efficiency in term and preterm neonates was calculated for those without missing data for gestational age at birth (11 620 of 15 407).

COMMENT

With the inclusion of 6 012 798 neonates screened in mainland France between 1996 and 2003, this study is, to our knowledge, by far the largest to date to assess neonatal screening for 21-OHD with particular emphasis on its contribution to early diagnosis. We found that sen-

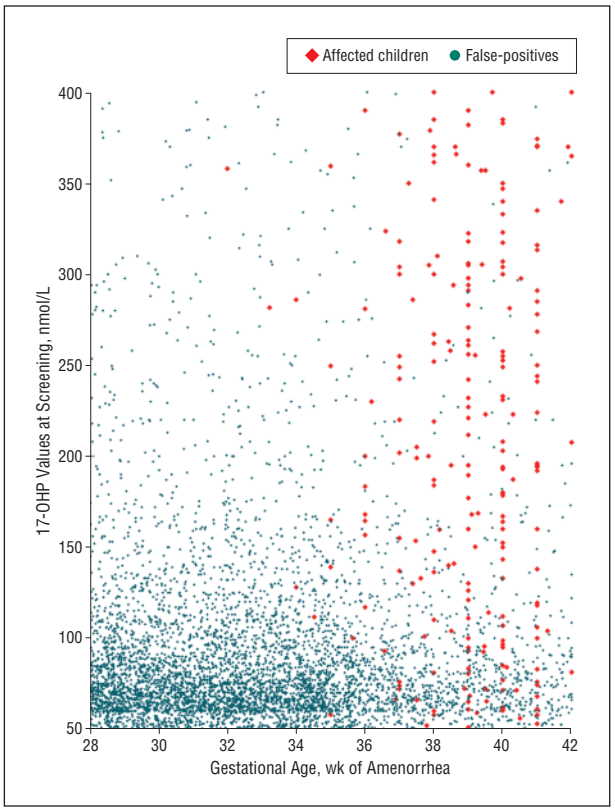

Figure 2. 17-Hydroxyprogesterone (17-OHP) concentration at neonatal screening in affected and unaffected (false-positive) children as a function of gestational age.

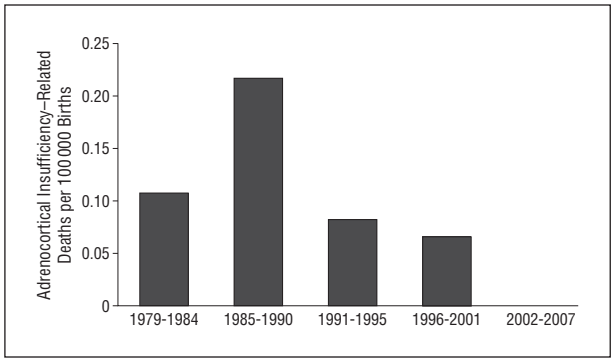

Figure 3. Specific mortality rates due to adrenogenital disorders, as well as other and unspecified adrenocortical insufficiency during the first year of life in France, 1979-2007. There were no deaths in 2002-2007.

sitivity was good (93.5%) but that the positive predictive value of screening was low (2.3%), although it improved markedly if we considered only term neonates (30.1%). Screening results contributed to diagnosis in 162 of the 383 cases (42.3%). Moreover, the large number of infants for whom no conclusion was drawn raises questions about the practical organization of 21-OHD screening owing to the large number of false-positive results.

Table 5 summarizes published data from previous population studies, making comparisons with our results possible. The positive predictive values reported in these studies were similar in most cases, with the exception of the Swiss study[18] (positive predictive value of 50.0%), which presented results for a second determination of 17-OHP on filter paper rather than those for the primary screening, as in most studies. Unlike previous studies, we took gestational age into account and found that screening efficiency differed considerably between term and preterm neonates. Among preterm neonates, there were almost 277 false-positive results for each case of 21-OHD discovered, whereas there were only 2 to 3 false-positive results for each case for term neonates. These difficulties arise from the low specificity of immunologic assay techniques for determining levels of 17-OHP in preterm newborns because of high plasma concentrations of corticosteroids, other than 17-OHP, that cross-react in the assays (with sulphated metabolites), generating false-positive results.[21-23] Some countries have adopted variable threshold values based on gestational age (Table 5), but our study shows that there is a large overlap of 17-OHP levels between affected and unaffected preterm newborns and that increasing the threshold level in this population would result in a loss of sensitivity. One possible alternative is the use of tandem mass spectrometry as a second-line test to improve the positive predictive value of screening.[10,24] These techniques were recently recommended in the Endocrine Society guidelines,[3] but they are costly, not widely available for population screening, and require thorough evaluation, including cost-benefit analyses.

Although 21-OHD screening correctly identified 93.5% of cases, its effect on diagnosis was much smaller; it contributed to early diagnosis in 45% to 50% of the children identified, corresponding to about 20 children per year in France or an incidence of 2.66 per 100 000 births per year. The main reasons for this minor contribution are that girls with classic 21-OHD are readily identified during neonatal pediatric examination and CAH is an autosomal recessive disorder, making prenatal or neonatal diagnosis more likely in families with an index case. In addition, in a small proportion of boys (9 of 153) with SW forms, adrenal crisis occurred before screening results became available, and the children were correctly treated based on their clinical presentation.

Screening for 21-OHD is designed principally to decrease neonatal disease-specific mortality. A decrease in specific mortality has been observed during the past 3 decades, but the timing of this decrease suggests that it was the result of improvements in pediatric care rather than of the introduction of screening. The probability of death due to neonatal adrenal crisis in the absence of screening is widely debated and has been reported to vary from none to 4% of patients with SW 21-OHD in populations with high standards of clinical awareness and care for 21-OHD.[7] In our study population of 286 children with SW 21-OHD born between 1996 and 2003 (277 true-positives and 9 false-negatives), using 4% as an estimate suggests that 11.5 neonatal deaths would have been expected in the absence of screening, a figure to compare with 3 deaths observed during the first year of life. In addition to preventing mortality, screening for 21-OHD can prevent inaccurate sex assignment and irreversible childhood hyperandrogenism. In our study, inaccurate sex assignment was not made in the 5 fully virilized females (Prader stage 5), but screening allowed the identifica-

Table 5. Efficiency of 21-OHD Screening in Published Studies[a]

Source	Country	No. of Neonates	17-OHP Threshold, nmol/L	Variable 17-OHP Threshold With Term Neonates	Sensitivity, %	Positive Predictive Value, %
Therrell et al,[17] 1998	United States (Texas)	1 936 998	123	Yes	86.0	NA
Cartigny-Maciejewski et al,[11] 1999	France	408 138	36-60	No	89.2	2.1
Steigert et al,[18] 2002	Switzerland	333 221	30-90	Yes	96.8	50.0
Van der Kamp et al,[19] 2001	The Netherlands	176 684	60	Yes	100.0	5.9
Balsamo et al,[20] 1996	Italy	128 330	36	Yes	NA	1.9
Present study	France	6 012 798	40-100	No	93.5	2.3

Abbreviations: NA, not available; 21-OHD, 21-hydroxylase deficiency; 17-OHP, 17-hydroxyprogesterone.
[a] Population-based studies with a sample size larger than 100 000 were selected.

tion of 47 of 77 patients with an SV form, confirming the value of screening to detect the 21-OHD before the appearance of severe hyperandrogenism.

Our findings also show that the organization of screening, as currently conducted in France (and possibly elsewhere), was not satisfactory. Screening centers encountered major operational difficulties with follow-up of the large number of positive test results, and no conclusion about status was reached in many cases, raising medical, ethical, and responsibility issues. Questions remain concerning the fate of 307 children for whom results of successive assays remained above the threshold value but for whom no further follow-up data were obtained. Data for weight and gestational age were also frequently missing; this information makes it easier to interpret the assay results and should therefore be collected.

Our study was subject to several limitations. The apparent lack of impact of screening on mortality is likely the result of insufficient statistical power given the limited numbers in the mortality analysis (21 deaths in 29 years), the unmeasured effect of pilot 21-OHD screening programs during the 1990-1995 period, and, more important, undiagnosed 21-OHD–related deaths in the period before screening as documented in central Europe.[25] In addition, inclusion in our analysis of misclassified adrenal diseases other than CAH might have obscured the effect of screening on mortality. However, the study had sufficient power (80%) to detect a decrease in specific mortality of 65% or more after screening implementation. We could not trace with precision the reasons for which false-negative cases were missed (eg, 17-OHP concentration below the threshold, positive test not taken into account correctly). False-negative cases may have been underestimated for several reasons, including lack of ascertainment if cases were detected later in life and not followed by pediatric endocrinologists or were subjected to molecular analyses. We were also unable to determine the number of unnecessary visits and laboratory investigations for children in whom 21-OHD was eventually ruled out or their contribution to the anxiety of the parents. Missing data on gestational age may have resulted in an overestimation of the sensitivity of the screening, particularly in subgroup analysis (preterm and term newborns). One further limitation is that data on neonatal screening from 1996 to 2003 are only presented in 2011, at a time when they might be considered less timely. This apparent delay results from long and tedious data collection and monitoring and from the need to wait several years to be able to identify false-negative SV forms, since the diagnosis is made as late as 5 or 6 years of age in some cases. Indeed, we searched for false-negative cases in 2010, at a time when the youngest child in the cohort was older than 6 years, which is an additional strength of our study.

In France, the budget per screened infant (for 21-OHD only) is €1.23 ($1.70), in the absence of follow-up corresponding to approximately €920 000/y. Because approximately 20 cases of 21-OHD are identified by screening each year, this budget corresponds to approximately €50 000 ($70 000) per case. In the United States, the cost per screened infant without follow-up has been estimated at $2.30 to $6.00; applying these figures to our data would result in estimates of $95 000 to $245 000 per case.[26] Assumptions concerning the number of potential deaths among these cases and a complete medical economic analysis of indirect costs might allow a full evaluation of the cost per life-year saved.[26]

Overall, we found that neonatal 21-OHD screening was efficient in term newborns, with a variable effect on clinical management given that most affected female newborns are easy to identify without screening. By contrast, the efficiency of screening was very low in preterm newborns, resulting in large numbers of false-positive cases, flooding the system and leading to its dysfunction and the identification of only 6 cases of potentially lethal SW 21-OHD among more than 10 000 neonates with positive results in 8 years. Improved organization might have prevented this dysfunction and allowed a comprehensive follow-up of all positive cases. However, a decrease in the number of false-positive cases is necessary to improve efficiency.

Therefore, what recommendations should be made based on our results? We recommend that screening for 21-OHD be continued for term neonates in areas where it is already performed and that careful consideration be given to its implementation in areas where this is not the case. By contrast, we recommend that 21-OHD screening, as performed in this study, should not be carried out for preterm neonates, since the positive predictive value of the test is very low and most preterm neonates are subject to careful pediatric care that should ensure that incipient SW adrenal crises are readily recognized. In France, the national neonatal screening organization and representatives of professional organizations in neonatology,

pediatric endocrinology, and rare endocrine diseases are currently discussing how to improve the national screening program. Our improved program, as well as others around the world, will have to be carefully evaluated.

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