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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Short title: Screening for congenital adrenal hyperplasia

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Number of words in the abstract: 250
Number of words in the manuscript: 3398
Number of figures: 3
Number of tables: 4 + 1 supplementary table

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Abstract

Objective. Neonatal screening for congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) 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 virilised genitalia at birth. The aim of this study was to assess the efficiency of the national screening programme for 21OHD.

Design. Population-based study.

Setting. National neonatal screening program, paediatric endocrinologists nationwide and reference centre for genotyping.

Participants. All newborns screened for 21OHD in mainland France between January 1st, 1996 and December 31st 2003.

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

Results. 6,012,798 newborns were screened, 15,407 were considered positive for 21OHD and 383 cases were identified, giving a prevalence of 1/15,699 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%. The false-positive rate was particularly high in preterm infants, for which the positive predictive value was 0.4% (0.2-0.5). Screening allowed clinical diagnosis in 162 of 383 cases (42%), the others being detected clinically or through family history. There was a trend towards declining neonatal mortality due to 21OHD.

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

Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroid biosynthesis [1-3]. The commonest form (≈95%) is due to 21-hydroxylase deficiency (21OHD). It affects about one child in 15,000 and results in clinical symptoms that vary with the severity of the enzymatic defect. Classical forms include salt-wasting forms (SW), for which there is a high risk of life-threatening adrenal insufficiency during the first month of life, and simple virilising forms (SV). In both cases, female neonates present with markedly virilised external genitalia. Non-classical forms can manifest with hyperandrogenism later in life and do not warrant early recognition through neonatal screening. 3β-hydroxysteroid dehydrogenase deficiency (3ß-HSD) is a rare form of CAH that results in the undervirilisation of external genitalia and adrenal insufficiency; it can be detected by 21OHD screening [4].

21OHD screening is carried out to prevent neonatal death from acute adrenal insufficiency, inaccurate sex assignment in females with complete virilisation and irreversible childhood hyperandrogenism, which may result from incorrect or late diagnosis [3, 5]. 21OHD fulfils 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 USA and some European countries [3, 7-9]. However, it remains controversial with three main arguments against routine screening: i) the test has a low positive predictive value, with frequent false-positive results in preterm neonates due to cross-reactions with steroids other than 17-OHP [10], ii) the proportion of cases for which screening really contributes to diagnosis is unclear, as most female cases are easy to detect clinically and salt-wasting is often detected before the screening results are obtained and iii) there is a lack of consensus concerning the 17-OHP threshold to be used, due to changes in 17-OHP distribution with gestational age at birth.

In France, 21OHD screening was introduced for all newborns as part of the national screening programme in 1996, after a short pilot feasibility study [11]. However, as in many other countries, routine 21OHD screening was never evaluated. The main objective of this study was to evaluate the efficiency of the national French screening program for 21OHD. 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 organisation, 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 1st, 1996 and December 31st, 2003. Screening was carried out at 21 regional centres, under the auspices of the *Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant* (AFDPHE) [12]. Blood was collected from three-day-old infants on filter paper and 17-OHP concentration was determined by automated time-resolved fluoroimmunoassay (DELFIA®) or RIA. Infants with 17OHP levels above the threshold applied for screening purposes were evaluated further for the diagnosis of 21OHD. We collected data from the regional centres on all newborns for whom 21OHD 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 final conclusions concerning the status of the child: *affected* with CAH (true positive), *unaffected* (false positive) or *deceased*. 17-OHP concentrations are expressed in nmol/l 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, based on 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 classical salt wasting (SW), classical simple virilising (SV) or non classical forms [13, 14]. If genotyping results were not available or not informative (n = 2) due to the detection of mutations with unknown functional repercussions, patients were classified as a function of the clinical data, leaving only one 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 true negatives was derived from reference values published annually in France (DRESS 2001).

We searched for false negatives (FN) detected before March 2010, which is at least six years after the birth of the last child studied, using five data sources: 1) regional screening centres notified of FN cases by physicians; 2) mail and e-mail surveys of all paediatric endocrinologists registered with the national society or treating children with CAH; 3) the French reference centre for CAH genotyping in Lyon and another molecular biology laboratory performing CAH genotyping and 4) the Centre for Epidemiology Medical Causes of Death database (CépiDc, INSERM), in which we looked for children dying from causes corresponding to International Classification of Diseases (ICD) 9 and 10 codes 255.2, 255.4, E25, 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% confidence intervals (CI) for preterm neonates born before 37 weeks of gestational age, for term newborns, 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 classical 21OHD (SW or SV forms) or 3ß-HSD deficiency that was not suspected clinically because there were no symptoms or because the symptoms and signs (genital abnormalities, dehydration) had not been recognised; screening was considered *not useful* if CAH was diagnosed before the results of screening became available (on the basis of family history, prenatal diagnosis or neonatal systematic examination). Screening was also considered *not useful* for false negative cases of classical CAH and for children with positive screening results diagnosed with non-classical 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 amenorrhoea 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 under the age of one year were calculated between 1979 and 2007, from CepiDc data. Changes in mortality rate over time were assessed by Poisson regression analysis. We looked for a possible change in slope after 1996 (the year in which the screening programme was generalised), 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 with SAS 9.2 software (SAS Institute, Cary, NC, USA). The study was approved by the CCTIRS and CNIL and was conducted in accordance with French legislation.

Results

During the eight-year study period, 6,012,798 screening tests for 21OHD were performed on children born in mainland France (Figure 1, Table 1 and supplementary Table 1). The laboratory methods for 17-OHP determinations and their thresholds are shown in supplementary Table 1. Neonatal screening tests were positive for 15,407 newborns, with 370 considered *affected*, 11,324 considered *unaffected* and no conclusion reached by the screening centres for 3,132. For 1,814 infants, the conclusion was discordant with the last recorded 17-OHP concentration (n = 338 considered *unaffected* with a last 17-OHP determination considered positive and n = 1476 considered *unaffected* without the recorded monitoring of 17-OHP concentrations). 581 children were identified as *deceased* : most of these children were preterm and, in all cases, the death of the child was considered by clinical centres to be unrelated to 21OHD (Figure 1). Most of the newborns with positive results for 21OHD screening were born before term (91% 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 newborns considered to be affected, 358 had a classical form of 21OHD (n = 354) or 3βHSD (n = 4) deficiency and 12 had a non-classical form of CAH.

The median age at diagnosis of CAH was seven days (Table 2). Weight loss was severe (>10% of expected body weight) in 19% of those for whom data were available, and plasma sodium concentration was below 130 mmol/l in 18% of the infants. Screening was useful for diagnosis in 162 of the 358 children with classical CAH and positive screening results, mostly males with the SW form (n = 106/162). Screening results were positive but not useful for diagnosis in 74 children with a family history of 21OHD and in 96 girls with genital abnormalities detected during neonatal examination. In addition, screening results were positive in 13 boys with classical 21OHD who were diagnosed clinically before the screening results became available. Of interest, among the 38 premature babies with positive

screens, screening was useful to the diagnosis in only 13 among whom only 6 had a SW form. We identified 25 children as false negative for 21OHD screening: 23 were reported by genotyping laboratories, and 20 of these cases were also reported by the screening centres, with two reported by the CépiDC. Most of the false negatives (16/25) had SV forms (Figure 1).

Altogether, the incidence of classical 21OHD (SW and SV forms) and 3ß-HSD deficiency in France between 0 and 1 year was $0.78/10^5$ births/year, with a 95% CI of [0.70 – 0.86] and the prevalence was 1/15699 births (95%CI, 1/17445 - 1/14269) (including false negative subjects in their birth cohort). The sensitivity of screening was 93.5% with an overall positive predictive value of 2.3% (Table 3). Sensitivity was higher for SW 21OHD (96.9%, 95%CI, 94.8-98.9) than for SV 21OHD (82.8%, 95%CI, 75.1-90.5). Most false-positive screening test results were obtained for preterm newborns, for which the positive predictive value of screening was only 0.36%, whereas that for term newborns was 30.4%. We investigated whether adjustment of the 17-OHP threshold would have improved screening efficiency in preterm newborns, by calculating linear regression models of (positive) 17-OHP levels on filter paper. Gestational age accounted for 9.5% and weight accounted for 7.4% of the variance (R^2) of 17-OHP concentration. Adding polynomials and assay techniques increased the R^2 to 10%. Figure 2 illustrates the difficulty of establishing threshold values based on gestational age.

As the primary objective of 21OHD screening is to prevent the death of newborns, we analysed 21OHD-related mortality in France from 1979 to 2007, a 29-year period including the year in which 21OHD screening was introduced. Twenty-one children under the age of one year were classified with an underlying cause of death due to adrenogenital disorders, other and unspecified adrenocortical insufficiency (Figure 3). There was a significant (p=0.002) trend towards a decrease in specific mortality rate during this period, with most of

206 this decrease occurring in 1991 to 1995, before the generalisation of screening. Thus, neither
207 screening itself (yes/no) nor the interaction of screening and time was associated with specific
208 mortality rate (p=0.31 and 0.31, respectively).
209

Discussion

With the inclusion of 6,012,798 newborns screened in mainland France between 1996 and 2003, this study is by far the largest to date to assess neonatal screening for 21OHD with particular emphasis on its contribution to early diagnosis. We found that sensitivity was good (93%), but that the positive predictive value of screening was low (2.3%), although it improved markedly if we considered only term newborns (30.4%). Screening results contributed to diagnosis in 42% of the cases. Moreover, the large number of infants for which no conclusion was drawn raises questions about the practical organisation of 21OHD screening, due to the large number of false positives.

Table 4 summarises 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 (positive predictive value of 50%), 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 we found that screening efficiency differed considerably between term and preterm newborns. Among preterm newborns, there were almost 277 false positives for each case of 21OHD discovered, whereas there were only two to three false positives for each case for term newborns. These difficulties arise from the low specificity of immunological assay techniques for determining levels of 17-OHP in preterm newborns due to high plasma concentrations of steroids other than 17-OHP that cross-react in the assays (with sulphated metabolites), generating false-positive results [16-18]. Some countries have adopted variable threshold values based on gestational age (Table 4), 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 particular population would result in a loss of sensitivity. One possible alternative is the use of tandem mass spectrometry as a second line

235 test to improve the positive predictive value of screening [10, 19]. These techniques were recently
236 recommended in the Endocrine Society guidelines [3], but they are costly, not widely available
237 for population screening and require thorough evaluation, including cost-benefit analyses.

238 Although 21OHD screening correctly identified 93% of cases, its impact on diagnosis
239 was much smaller, as it contributed to early diagnosis in 45 to 50% of the children identified,
240 corresponding to about 20 children per year in France or an incidence of $2.66/10^5$/year. The
241 main reasons for this minor contribution are that girls with classical 21OHD are readily
242 identified during neonatal paediatric examination and CAH is an autosomal recessive
243 disorder, making prenatal or neonatal diagnosis more likely in families with an index case. In
244 addition, in a small proportion of boys (9/153) with SW forms, adrenal crisis occurred before
245 screening results became available and the children were correctly managed based on their
246 clinical presentation.

247 Screening for 21OHD is designed principally to decrease neonatal disease-specific
248 mortality. A decrease in specific mortality has been observed over the last three decades, but
249 the timing of this decrease suggests that it was due to improvements in paediatric care rather
250 than to the introduction of screening. The probability of death due to neonatal adrenal crisis
251 in the absence of screening is widely debated and has been reported to vary from 0 to 4% of
252 patients with SW 21OHD in populations with high standards of clinical awareness and care
253 for 21OHD [7]. In our study population of 285 children with salt-wasting 21OHD born
254 between 1996 and 2003 (276 true positives and 9 false negatives), using 4% as an estimate
255 suggests that 11.5 neonatal deaths would have been expected in the absence of screening, a
256 figure to compare to 3 deaths observed during the first year of life. In addition to preventing
257 mortality, screening for 21OHD can prevent inaccurate gender assignment and irreversible
258 childhood hyperandrogenism. In our study, inaccurate gender assignment was not made in
259 the 5 fully virilised females (Prader stage V) but screening allowed the identification of 47/77

patients with a SV form confirming the value of screening to detect the 21OHD before the appearance of severe hyperandrogenism.

Our findings also show that the organisation of screening, as currently conducted in France (and possibly elsewhere), was not satisfactory. Screening centres encountered major operational difficulties with follow-up of the large number of positive tests 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 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, although 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 due to insufficient statistical power, given the limited numbers in the mortality analysis (21 deaths in 29 years), the unmeasured effect of pilot 21OHD screening programs during the 1990-1995 period and more importantly undiagnosed 21OHD-related deaths in the period before screening as documented in central Europe [20]. 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 (17-OHP concentration below the threshold, positive test not taken into account correctly, other reason). 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 paediatric endocrinologists or subjected to molecular analyses. We were also unable to determine the number of unnecessary visits and laboratory investigations for children eventually found not to have 21OHD, or their impact on 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 1996-2003 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 in order to be able to identify FN SV forms since the diagnosis is made as late as 5 or 6 years in some cases. Indeed, we searched for FN 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 in the absence of follow-up (US$ 1.7), corresponding to approximately €924,500/year. As ≈ 20 cases of 21OHD are identified by screening each year, this budget corresponds to approximately €50,000 (US$ 70,000) per case. In the US, the cost per screened infant without follow-up has been estimated at US$ 2.3 to 6.0 and applying these figures to our data would result in estimates of US$ 95,000 to 245,000 per case [21]. 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 [21].

Overall, we found that neonatal 21OHD screening was efficient in term newborns, with a variable impact 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 positives, flooding the system and leading to its dysfunction and leading to the identification of 6 cases of potentially lethal salt-wasting 21OHD among more than 10,000 positives in 8 years. Improved organisation might have prevented this dysfunction and allowed a comprehensive follow-up of all positive cases. However, a decrease of the false positive cases is necessary to improve efficiency.

So, what recommendations should be made based on our results? We recommend that newborn screening for 21OHD is continued for term newborns in areas in which it is already performed and that careful consideration is given to its implementation in areas in which this is not the case. By contrast, we recommend that 21OHD screening, as performed in this study, should not be carried out for preterm newborns since the positive predictive value of the test is very low and most preterm newborns are subject to careful paediatric care that should ensure that incipient salt wasting adrenal crises are readily recognised. In France, the national neonatal screening organisation and representatives of professional organisations in neonatology, paediatric 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.

Acknowledgment

The authors wish to acknowledge the role of Unité de Recherche Clinique Paris-Cochin and in particular Christelle Auger and Sandra Colas. Cécile Klochendler, Sandrine Borderie and Pr Jean-Marc Treluyer from Unité de Recherche Clinique Paris-Cochin are also acknowledged. Louise Montagne, Claire Jeandel, Kasia Marchal, Candace Bensignor, Chaza Fallouh and the Centres of Clinical Investigation of Lyon and Bordeaux also participated in data collection.

Authors' contributions statement

Jean-Claude Carel, Joel Coste and Yves Morel conceived and conducted the study, and analysed the data. Emmanuel Ecosse established the database and some of the statistical analyses. Bénédicte Coulm participated in the conduction of the study analyzed the data. Bénédicte Coulm, Jean-Claude Carel and Joel Coste had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis and wrote the paper. Yves Morel and Véronique Tardy performed the molecular analysis and the classification of patients. Michel Roussey participated in the conduction of the study and contributed to data analysis. All authors participated in the elaboration of the manuscript and commented on it. The members of DHCSF study group are to be considered as co-authors of the manuscript given their involvement in the elaboration of the protocol, data collection or follow-up of patients. The final version of the English text was edited by Julie Sappa (Alex Edelman & Associates, Scientific Editors).

Source of funding statement

The study was funded by the French Ministry of Health "PHRC, Programme Hospitalier de Recherche Clinique" grant number AOM03048, by the French National Health Insurance "CNAM, Caisse Nationale d'Assurance Maladie" and by the French National Neonatal Screening Programme Organisation (AFDPHE, Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant). The PHRC and CNAM did not participate in the study or its interpretation. AFDPHE was involved in the conduction of the study and its president (Michel Roussey) is co-author of the paper.

Conflict of interest statement

No conflict of interest to disclose.

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Legends for figures

Figure 1. Results of neonatal screening for 21-hydroxylase deficiency in France 1996-2003

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

Individual values for affected children are shown as red dots ◆ and those for false positives are shown as green dots ◆.

Figure 3. Specific mortality rates due to adrenogenital disorders, other and unspecified adrenocortical insufficiency during the first year of life in France, 1979-2007.

Table 1 – Principal characteristics of newborns with positive screening results for CAH in mainland France between 1996 and 2003

	N = 15,407
Sex	
Male	9,031 (59)
Female	6,218 (41)
Gestational age (WA)†*	31 (28 – 34)
Preterm (<37 WA)	10,563 (68)
Term (≥37 WA)	1,058 (7)
Missing data	3,786 (25)
Birth weight (g)†*	1,490 (1,005 – 2,090)
Age at screening (days)†	4 (3-5)
17-OHP screening result (nmol/l)†	80 (65-109)
17-OHP measurement method used	
Radioimmunoassay	8,457 (55)
DELFIA®	6,945 (45)

Data are presented as numbers (percentages) unless otherwise stated; †, median, interquartile range; WA: weeks of amenorrhoea; * imputations of gestational age from sex and birth weight: n = 998, imputations of birth weight from sex and gestational age: n = 2653; † missing data: sex n = 158; age at screening n = 1117; 17-OHP screening result n = 72; 17-OHP measurement method n = 5.

Table 2 – Characteristics of affected newborns with CAH due to classical 21OHD or 3ß-HSD detected by screening in mainland France between 1996 and 2003

	True positives (n = 358)
Sex	
Boys	205 (57)
Girls	153 (43)
Gestational age (WA)†	39 (38-40)
Preterm (<37 WA)	38 (10.7)
Term (≥37 WA)	318 (89.3)
Birth weight (g)†	3,370 (2,980 – 3,680)
Age at screening (days)†	3 (3-4)
Age at diagnosis (days)†	7 (1-10)
Contribution of screening to the diagnosis of CAH, n (M/F)	
Useful	162 (137/25)
Salt-wasting 21OHD	114 (106/8)
Simple virilising 21OHD	47 (30/17)
3ß-HSD	1 (1/0)
Not useful*	196 (68/128)
Clinical diagnosis before screening results	109 (13/96)
Salt-wasting 21OHD	99 (9/90)
Simple virilising 21OHD	8 (2/6)
3ß-HSD	2 (2/0)
Prenatal diagnosis or family history	74 (46/28)
Salt-wasting 21OHD	54 (33/21)
Simple virilising 21OHD	20 (13/7)
3ß-HSD	0 (0/0)
Information on usefulness unavailable	13 (9/4)
Salt-wasting 21OHD	10 (6/4)
Simple virilising 21OHD	2 (2/0)
3ß-HSD	1(1/0)
Plasma sodium concentration at diagnosis (nmol/l)†	
≥135	177 (78M/99F)
130-135	80 (61M/19F)
<130	56 (48M/8F)
Relative weight change at diagnosis (% of expected)†	
≥0%	42 (21/21)
[0; -5%]	62 (34/28)
[-5; -10%]	76 (60/16)
<-10%	42 (39/3)

Data are presented as numbers (percentages) unless otherwise stated; †, median, interquartile range; WA: weeks of amenorrhoea; SD: Standard deviation; missing data: gestational age, n=2; age at screening, n = 28; age at diagnosis, n = 38; plasma sodium concentration, n = 47; relative weight change at diagnosis, n = 138. The 25 false-negative cases (11 boys and 14 girls), for whom screening was not useful, are not included in this table.

Table 3. Efficiency of 21OHD screening as a function of gestational age at birth

Table 3a: Raw data

	Screening	Affected	Unaffected	Total
All newborns	Positive	358	15,049	**15,407**
	Negative	25	5,997,366	**5,997,391**
	Total	**383**	**6,012,415**	**6,012,798**
Term newborns (≥37 WA)	Positive	318	740	**1058**
	Negative	21	5,578,196	**5,578,217**
	Total	**339**	**5,578,936**	**5,579,275**
Preterm newborns (<37 WA)	Positive	38	10,524	**10,562**
	Negative	2	422,959	**422,961**
	Total	**40**	**433,383**	**433,523**

Table 3b: Efficiency calculations

All newborns	Positive predictive value	2.3 [2.1-2.6]	Specificity	99.7 [99.7 - 99.7]
	Negative predictive value	99.9 [99.9 - 99.9]	Sensitivity	93.5 [90.9 – 95.9]
Term newborns (≥37 WA)	Positive predictive value	30.1 [27.3-32.8]	Specificity	99.9 [99.9-99.9]
	Negative predictive value	99.9 [99.9 ; 99.9]	Sensitivity	93.8 [91.2-96.4]
Preterm newborns (<37 WA)	Positive predictive value	0.4 [0.2-0.5]	Specificity	97.6 [99.5-97.6]
	Negative predictive value	99.9 [99.9 ; 99.9]	Sensitivity	95 [83.1 -99.4]

Data are expressed in % [95% confidence interval]; efficiency in term and preterm newborns was calculated for those without missing data for gestational age at birth (11,620/15,407).

Table 4. Efficiency of 21OHD screening in published studies

Reference	Country	Number of newborns	17-OHP threshold (nmol/l)	Variable 17-OHP threshold with term	Sensitivity (%)	Positive predictive value (%)
[22]	USA (Texas)	1,936,998	123	yes	86	NA
[11]	France	408,138	36 to 60	no	89	2.1
[23]	Switzerland	333,221	30 to 90	yes	97	50.0
[24]	Netherlands	176,684	60	yes	100	5.9
[25]	Italy	128,330	36	yes	NA	1.9
This study	France	6,012,798	40 to 100	no	93	2.3

Population-based studies with a sample size >100 000 were selected

Supplementary Table 1: Thresholds and laboratory methods used for 17-OHP determination

Methods and thresholds	Number of newborns who screened positive , n (%)
Delfia®*	6,945 (45.1)
40 nmol/l	595
50 nmol/l	3,209
60 nmol/l	1,416
70 nmol/l	1,725
RIA*	8,457 (54.9)
50 nmol/l	1,572
60 nmol/l	4,050
70 nmol/l	1,209
80 nmol/l	87
100 nmol/l	78
50 pg/spot	1,124
60 pg/spot	34
70 pg/spot	303

*Delfia®, dissociation-enhanced lanthanide fluorescence immunoassay; RIA, radioimmunoassay; missing data: Laboratory methods, n = 5, thresholds n = 5.

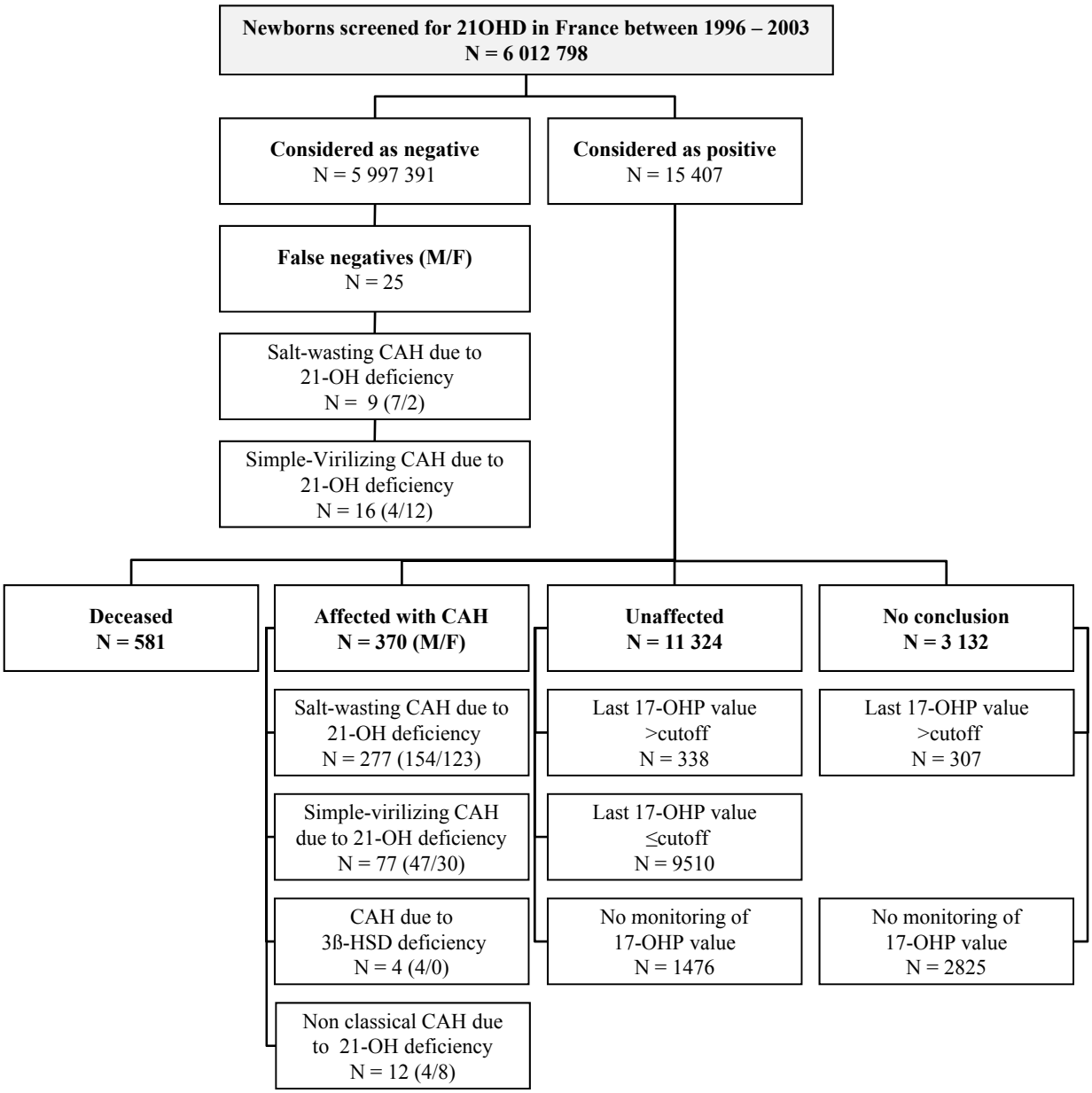
Newborns screened for 21OHD in France between 1996 – 2003
N = 6 012 798
Considered as negative
N = 5 997 391
Considered as positive
N = 15 407
False negatives (M/F)
N = 25
Salt-wasting CAH due to 21-OH deficiency
N = 9 (7/2)
Simple-Virilizing CAH due to 21-OH deficiency
N = 16 (4/12)
Deceased
N = 581
Affected with CAH
N = 370 (M/F)
Unaffected
N = 11 324
No conclusion
N = 3 132
Salt-wasting CAH due to 21-OH deficiency
N = 277 (154/123)
Simple-virilizing CAH due to 21-OH deficiency
N = 77 (47/30)
CAH due to 3ß-HSD deficiency
N = 4 (4/0)
Non classical CAH due to 21-OH deficiency
N = 12 (4/8)
Last 17-OHP value >cutoff
N = 338
Last 17-OHP value ≤cutoff
N = 9510
No monitoring of 17-OHP value
N = 1476
Last 17-OHP value >cutoff
N = 307
No monitoring of 17-OHP value
N = 2825

Figure 1

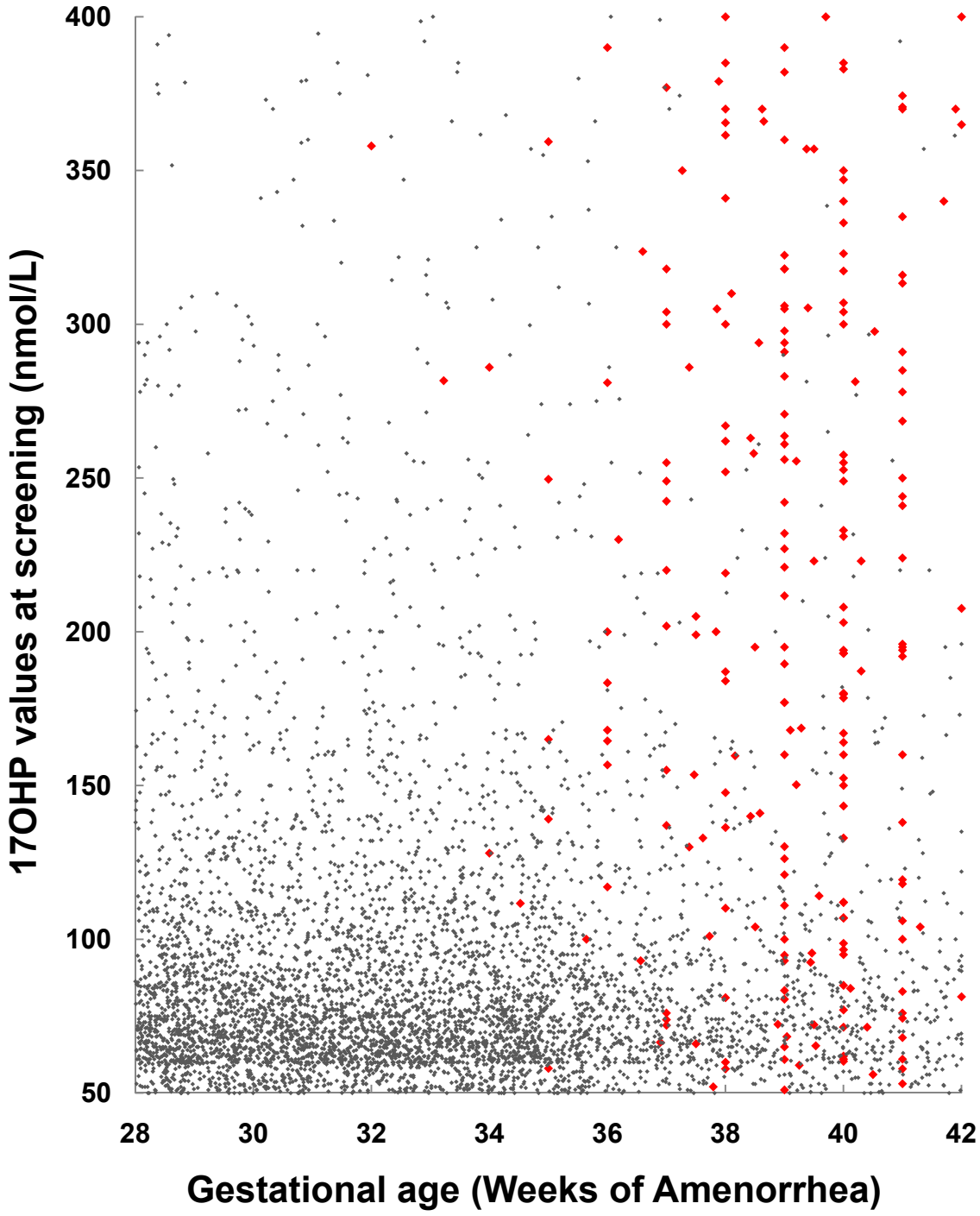
400
350
300
250
200
150
100
50
17OHP values at screening (nmol/L)
28
30
32
34
36
38
40
42
Gestational age (Weeks of Amenorrhea)

Figure 2

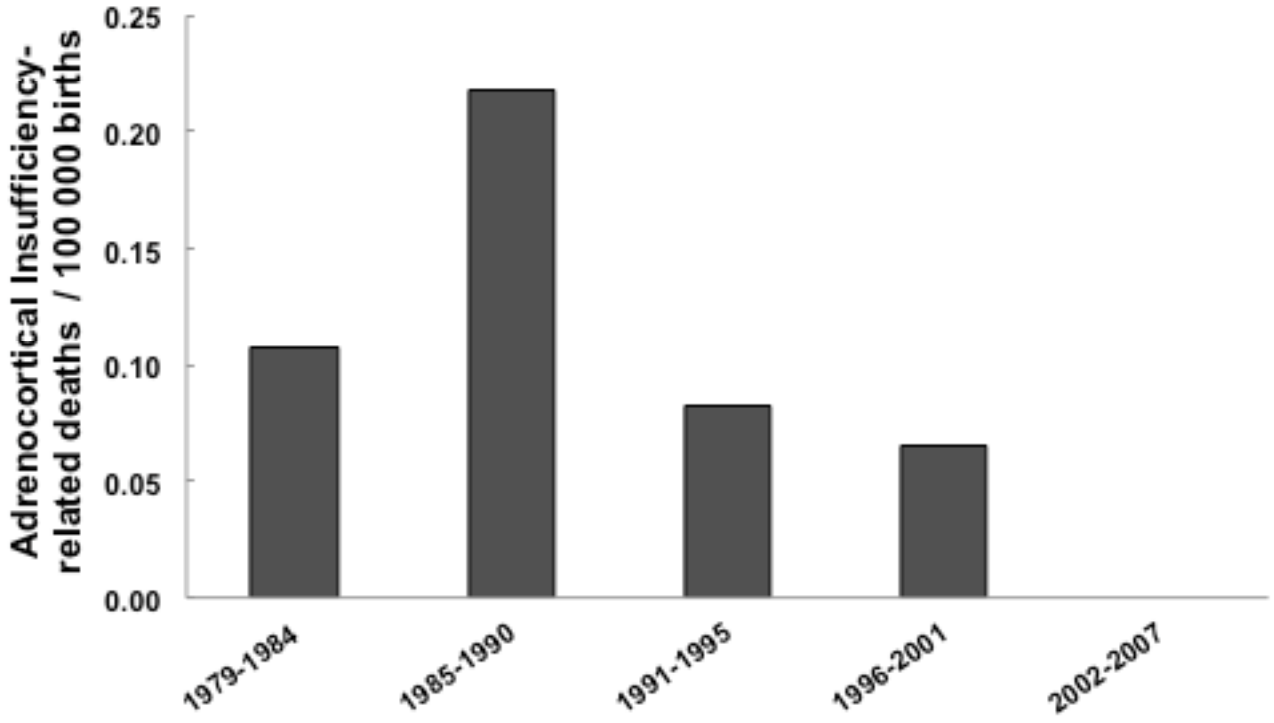
Adrenocortical Insufficiency-related deaths / 100 000 births
0.25
0.20
0.15
0.10
0.05
0.00
1979-1984
1985-1990
1991-1995
1996-2001
2002-2007

Figure 3