UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

Hiperplasia Adrenal Congênita no Brasil: Incidência, Custos da Triagem Neonatal e Aplicação Clínica da Biologia Molecular

TESE DE DOUTORADO

ELIZABETH LEMOS SILVEIRA

Porto Alegre, dezembro de 2008

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Orientadores: Prof.ª Dr.ª Regina Helena Elnecave

Co-Orientadora: Prof.ª Dr.ª Tânia Aparecida Sartori Sanchez Bachega

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito parcial para obtenção do título de Doutor em Endocrinologia.

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LISTA DE ABREVIATURAS, SÍMBOLOS E CONVENÇÕES

11OH:	11-hidroxilase
17OHP:	17-hidroxiprogesterona
21OH:	21-hidroxilase
Grupo A1:	mutações que conferem total comprometimento da atividade da 21-hidroxilase
Grupo A2:	mutações que conferem quase total comprometimento da atividade da 21-hidroxilase
Grupo B:	mutações que conferem grave comprometimento da atividade da 21-hidroxilase
Grupo C:	mutações que conferem moderado comprometimento da atividade da 21-hidroxilase
Composto S:	11-desoxicortisol
Composto F:	Cortisol
Δ4:	Androstenediona
ACTH:	hormônio adrenocorticotrófico
APAE:	Associação dos Pais e Amigos dos Excepcionais
CAH:	congenital adrenal hyperplasia
CYP21:	21-hidroxilase esteróide
CYP21A1P:	pseudogene da 21-hidroxilase
CYP21A2:	gene codificador da 21-hidroxilase
DNA:	deoxyribonucleic acid
EDTA:	ácido etileno-diaminotetracético
GUTHRIE	nome do cientista que desenvolveu a técnica de coleta de sangue capilar para triagem neonatal da fenilcetonúria
HAC:	hiperplasia adrenal congênita
HAC-21OH:	hiperplasia adrenal congênita por deficiência da 21-hidroxilase
HLA:	antígeno leucocitário humano
LCR:	locus-control-region
NC:	forma não clássica da deficiência da 21-hidroxilase

NSRC:	neonatal screening referral center
NSSP:	neonatal screening state program
PCR:	reação de polimerização em cadeia
PETN:	Programa Estadual de Triagem Neonatal
POR:	P450 óxido-redutase
Primer:	oligonucleotídeo inicializador
PS:	forma perdedora de sal da deficiência de 21-hidroxilase
RCCX:	módulo composto pelos genes RP, C4, CYP21 e TNX
SRTN:	Serviço de Referência em Triagem Neonatal
STAR:	proteína reguladora aguda da esteroidogênese
T:	Testosterona
TNXA:	cópia truncada do gene da tenascina-X
TNXB:	gene da tenascina-X
VS:	forma virilizante simples da deficiência de 21-hidroxilase
kB:	quilopares de bases
kg:	Quilograma
mg/dL:	miligrama por decilitro
ng/dL:	nanograma por decilitro
pb:	pares de bases
pg/mL:	picograma por mililitro
rpm:	rotações por minuto

RESUMO

A triagem neonatal no Brasil é regulamentada pelo Ministério da Saúde desde 1991 (http://portal.saude.gov.br/portal/sas/mac/area.cfm?id_area=830) através do Programa Nacional de Triagem Neonatal (PNTN).

Os programas municipais e estaduais de triagem neonatal recebem incentivos do PNTN para realizar a triagem para fenilcetonúria, hipotireioidismo congênito, hemoglobinopatias e fibrose cística.

Dessa forma, atualmente, a triagem neonatal para hiperplasia adrenal congênita (HAC) no Brasil, ou é realizada em laboratório privado ou em programas públicos que recebem incentivos municipais e/ou estaduais. Em Goiás, uma lei estadual que tornou obrigatória a realização dessa triagem para nascidos vivos daquele estado desde 1997, além das outras triagens contempladas pelo PNTN, também regulamentou o destino de verbas estaduais para essa expansão do rol das doenças triadas.

O laboratório da Associação de Pais e Amigos dos Excepcionais – APAE de Anápolis é o laboratório credenciado pelo Ministério da Saúde para realizar a triagem neonatal dos recém nascidos do estado de Goiás. Além de realizar os testes de triagem, os laboratórios credenciados devem concluir sobre os testes, ou seja, realizar a confirmação da presença ou da ausência da doença, além de acompanhar o tratamento das crianças diagnosticadas.

Um dos principais objetivos do PNTN é aumentar o número de doenças triadas em todo o território nacional. Como uma das doenças candidatas ao painel nacional é a HAC, os objetivos dessa tese de doutorado foram estudar a incidência brasileira da doença, os custos dessa prática e os benefícios da inclusão de biologia molecular no seguimento dos pacientes com triagem alterada.

Elizabeth Lemos Silveira

Dezembro de 2008

CAPÍTULO 1

ARTIGO ORIGINAL 1

THE ACTUAL INCIDENCE OF CONGENITAL ADRENAL HYPERPLASIA IN BRAZIL MAY BE NOT AS HIGH AS INFERRED – AN ESTIMATE BASED ON A PUBLIC NEONATAL SCREENING PROGRAM IN THE STATE OF GOIÁS.

Elizabeth Lemos Silveira; Eliane Pereira dos Santos; Tânia A.S. Bachega; Ivana van der Linden Nader; Jorge Luiz Gross and Regina Helena Elnecave.

ABSTRACT

The incidence of 21-OH CAH in Brazil is purportedly one of the highest in the world (1/7,533). However, this information is not based on official data. The aim of this study was to determine the incidence of 21-OH CAH in the state of Goiás, Brazil, based on the 2005 results of government-funded mandatory screening. Of the live births during this period, 92.95% were screened by heel-prick capillary 17-OHP. Of these, 82,343 were normal, 28 were at high risk for CAH and 232, at low risk for CAH. Eight cases, all from the high risk group were confirmed. Eight asymptomatic children at 6 to 18 months of age, still have high 17-OHP levels and await diagnostic definition. Based on the number of confirmed 21-OH CAH cases among the 82,603 screened, the estimated annual incidence of the disease was 1/10,325, lower than the previously reported rate in Brazil.

Keys words: Congenital Adrenal Hyperplasia; CYP21 deficiency; Incidence: Neonatal Screening, Brazil

1 INTRODUCTION

Approximately 95% of the cases of congenital adrenal hyperplasia (CAH) are due to steroid 21-hydroxylase (CYP21) deficiency, with failure to synthesize cortisol, secondary to the autosomal recessive inheritance of mutated CYP21A2 genes[1,2,3]. Phenotype is determined by the resultant enzyme activity remaining[5].

New cases of the classical form of CAH are diagnosed in the neonatal period in girls because of ambiguous genitalia caused by antenatal androgen exposure, and in 2/3 of the cases, in both sexes, because of life-threatening salt loss due to mineralocorticoid deficiency[5,6]. In boys, the absence of striking physical hallmarks may cause excessive preventable deaths and neonatal screening for the disorder has reduced them[6].

The added benefit of neonatal screening of 21OH CAH is the determination of its actual incidence in the population. With this information, a more realistic approach to implementing widespread, universal screening programs can be planned.

The estimated worldwide CAH incidence of 1:14,199 live births was calculated from data of neonatal screening in different countries, some nationwide and others, of regional reach[7]. In Brazil, the informed incidence of 1/7,533, one of the highest in the world, derives from data on voluntary screening[3,8]

The aim of this study was to determine the clinical outcome of the newborns (NB) with abnormal neonatal screening tests for 21-OH CAH in the state of Goiás-Brazil, where government-funded neonatal screening is mandatory statewide and thus to establish the incidence of the disease.

2 METHODS

The results of the CAH neonatal screening program of the state of Goiás, Brazil, from January 1 to December 31, 2005 were retrospectively analyzed.

The neonatal screening referral center of the state of Goiás (SRTN-Goiás) is the laboratory of the Associação de Pais e Amigos dos Excepcionais - Lab-APAE (Parents and Friends of Exceptional People Association) of the city of Anápolis-Goiás, since 1994, when it was certified by the Brazilian Ministry of Health. Neonatal screening for CAH was added to the existing panel, in 1997.

The following data, filed at the SRTN-Goiás, were recorded by the investigator in Excel®: birth weight, sex, date of birth and date of sampling; the neonatal screening 17-OHP results; confirmatory filter paper 17-OHP in the low risk NB and peripheral blood 17-OHP in the high risk ones.

Data regarding the high risk for CAH NB were complemented by direct phone contact with the family and in person, with the physician.

According to the SRTN-Goiás guidelines, heel-prick capillary blood is collected between 3-7 days of age, in 617 locations in 243 municipalities. The samples of dried capillary blood in S&S 903 filter paper are shipped daily to Lab-APAE.

Hormone Assays

For the filter paper hormone assays, the time-resolved, solid-phase fluoroimunoassay (DELFIA - Wallac Oy, Turku, Finland) 17-OHP neonatal kit was used and the concentration of 17-OHP was expressed as serum-equivalence. The conversion

calculation was: 1 nmol/L = 0.33 ng/mL in whole blood = 0.73 ng/mL in serum-equivalence

Commercially available radioimmunoassay (RIA) kits were utilized for the serum measurement of 17-OHP in peripheral blood.

Categorization of CAH screening results

The SRTN-Goiás utilizes the European Society of Pediatric Endocrinology criteria for ranking the risk of being affected with CAH[9], as depicted below, using birth weight adjusted cut-off points, establishing two groups: children weighing less than 2,500 g and children weighing 2,500 g or more.

Normal NB: Term NB or those weighing 2,500 g or more are considered normal if 17-OHP < 41 nmol/L (<30 ng/mL); those weighing less than 2,500 g are considered normal when 17-OHP < 55 nmol/L (<40 ng/mL).

NB at high risk of being affected with CAH: Regardless of birth weight, NB with 17-OHP levels > 110 nmol/L (>80 ng/mL) at screening are considered at high risk of being affected with CAH and are immediately referred for venous blood sampling and endocrine consultation. Those with no immediate diagnostic confirmation and persistently high 17-OHP levels are followed until outcome definition, that is, until 17-OHP normalizes or a definitive diagnosis is established.

NB at low risk of being affected with CAH: NB weighing ≥ 2,500 g with 17-OHP values between 41 – 110 nmol/L (30-80 ng/mL) and those weighing less than 2,500 g weighing less than 2,500 g, 55-110 nmol/L (40-80 ng/mL) are considered as low risk for the diagnosis of CAH. Initial phone interviews establish whether they are asymptomatic, and a second filter paper blood specimen is obtained; when symptoms of CAH (weight loss > 10% and/or dehydration\) are suspected, the high risk protocol is followed.

The present study was approved by the Ethics in Research Committee of the Universidade Federal do Rio Grande do Sul.

The incidence of CAH was estimated as the number of NB with confirmed CAH, divided by the total number of screened NB.

3 RESULTS

The 17-OHP levels of 82,603 NB screened between January and December 2005 at the SRTN-Goiás were analyzed. During this period, screening included over 92.95% of the NB with civil registry in the state[10].

The weight of 918 NB (1.1% of the sample) was not recorded at the local health centers; since all these children had 17-OHP levels of < 55 nmol/L (40 ng/mL), which is considered normal irrespective of their weight, the inclusion of this information was not necessary in the present study.

Of the 82,603 screened NB, 28 (0.03%) were considered as of high risk of being affected with CAH and 232 (0.28%), as of low risk.

All the low risk NB had normal 17-OHP levels in the second filter paper specimen, except one girl, who is being followed by the endocrinologist until the outcome is defined; she remained asymptomatic at the age of 16 months.

Of the 28 high risk cases, 13 were considered as false positive, because peripheral blood serum 17-OHP levels were normal at about 30 days of age; in eight of these (61. 5 %), birth weight was < 2,500 g.

None of the confirmed CAH cases were from the low risk group.

Of the eight patients with confirmed diagnosis of CAH, three were male and treatment was begun early, between days 14 and 28 of life; the information on who had the salt-losing or the simple virilizing forms was not made available. In one of the five girls, diagnosed because of ambiguous genitalia, the screening 17-OHP levels were normal (false negative case).

One boy belonging to the high risk group died on the day the screening capillary blood was collected, that is, day 5. Neonatal tetanus was recorded as the cause of death.

Eight children with 6 to 18 months of age and persistently elevated 17-OHP levels remained asymptomatic: one was from the low risk group and seven, from the high risk one; half of them were female and have normal genitalia.

The results are summarized in Figure 1.

The sensitivity, and specificity of the CAH neonatal screening were 87.5 % and, 99.98 %, respectively.

From the above data, the estimated incidence CAH in the state of Goiás, Brazil, was of 1 / 10,325 live births.

4 DISCUSSION

Government-funded mandatory neonatal screening for CAH in the state of Goiás, Brazil, has made possible to estimate the actual incidence of the disorder in a defined, non-isolated population.

The incidence (1:10,325) found in the state of Goiás, with surface area of 340,086 km^2 and 5,619,917 inhabitants in 2005 resembles that of some European countries, Sweden (1:10,749) [11] and Switzerland (1:9,800) [12], also calculated from mandatory, universal

CAH neonatal screening; however, it is higher than that found in the state of Texas, USA, of 1: 16,008[13].

The CAH neonatal screening in Goiás identified a higher number of confirmed cases in females although it can not be ruled out that the four asymptomatic boys with persistently high 17-OHP levels will later reveal clinical manifestations of the classical simple virilizing form of the disease[6]; also, this could have occurred because the incidence was calculated from the data of a single year.

The boy who died at the age of 5 days, when the heel-prick specimen was collected, would have been considered as high risk, with the screening 17-OHP level of 128.4 nmol/L (93.7 ng/mL); as the disease was not confirmed, a false-positive test can not be ruled out, especially because the 17-OHP levels are increased in severely ill newborns[14]. In addition, the neonatal screening 17-OHP levels of severe salt-wasting CAH patients are reported as much higher than those found[2]. In the present study, the lowest 17-OHP level found in a confirmed case of simple virilizing CAH was 139.7 nmol/L (102 ng/mL); the 17-OHP levels of all confirmed cases of salt-wasting CAH ranged from 398.6 - 695.9 nmol/L (291 – 508 ng/mL). These results suggest the "low risk" group might be extinct and that the screening 17-OP values below 110 nmol/L (80 ng/mL) should be considered "normal".

The girl with ambiguous genitalia, normal capillary screening but markedly increased peripheral 17-OHP levels, might be a case of CAH due to the deficiency of another enzyme, since other steroids cross-react in the17-OHP radioimmunoassay used in peripheral blood[15]. Likewise, it can not be ruled out that a boy screened as normal has CAH.

Incidence data attempts to measure the number of people who become affected with a condition each year. Two incidence rates are not necessarily comparable. Some incidence

data uses government notifications, as in the present study; others are based on physician or hospital diagnoses, and various other methods.

The quoted incidence of CAH in Brazil is based on data obtained from test results of voluntary, self-paid testing, with unreported clinical outcome[3,8]. Thus, the reported high incidence of CAH in Brazil (1:7,533) can not be assumed as a true population incidence.

As Brazil is a country of large territorial size (8,500,000 Km^2), with several migratory movements and the Brazilian population derives from large miscegenation[16], it is likely that the incidence of CAH found in Goiás is different from that in other states with no official, mandatory screening. On the other hand, the figure obtained differs from the purported high incidence in Brazil. Despite calculated from a single year appraisal, and the yet inconclusive diagnosis of the asymptomatic children with persistently high 17-OHP levels, the incidence of CAH in the state of Goiás may resemble the actual incidence in the Brazilian population.

Non-classical CAH is a diagnostic possibility in these asymptomatic children with persistently high 17-OHP levels, which it is not part of the goals of neonatal screening programs[5,11].

Genotyping could help to elucidate the diagnosis in asymptomatic cases with persistently elevated levels of 17-OHP, because a strong correlation between genotype and phenotype.[2,17,18]

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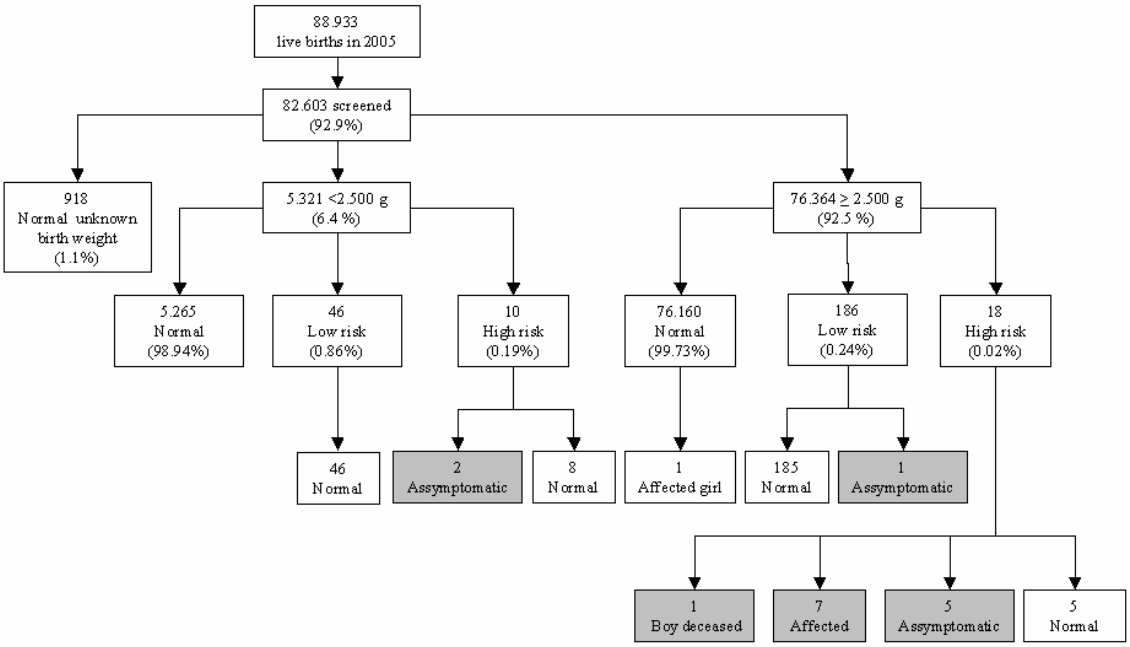

Fig. 1. Flow chart of NB screening for CAH in the state of Goiás, Brazil, in the year 2005. The cutoff points are decribed in text.

CAPÍTULO II

ARTIGO ORIGINAL 2

ALTERNATIVE MODEL FOR CAH NEWBORN SCREENING: COST-MINIMIZATION BASED ON THE PATHOPHYSIOLOGY OF THE DISORDER AND THE COSTS OF THE SCREENING

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ABSTRACT

Background The main goal of newborn screening for Congenital Adrenal Hyperplasia (CAH) is to prevent infant death due to adrenal insufficiency. All girls affected by the classical form of CAH have signs at birth; boys, however, do not show physical marks. This study determined the economic impact of newborn screening for CAH in the state of Goiás-Brazil and proposed an alternative model based on 17-hydroxyprogesterone screening for boys and clinical examination for girls.

Methods The cost of universal newborn screening for CAH in the state Goiás-Brazil in 2005 was calculated. The findings were extrapolated to the entire country (Brazil). Based on the concept that all affected girls should undergo full diagnostic workup for ambiguous genitalia after birth, an alternative model (selective for boys) was constructed, aiming to compare its economic impact with that of the traditional model, for inserting CAH screening in public health programs. The software TreeAge (Williamstown, Mass) was used and sensitivity analysis calculated.

Subjects: A database of 82,603 newborns in the year 2005 in the state of Goiás-Brazil was used to extrapolate to Brazil's newborns in the same year.

Results: The cost of universal complete CAH screening of all newborns, in 2005, in the state of Goiás amounted US$609,211.00. When extrapolating to Brazilian population, the estimated annual cost ranges from US$22,542,257.00 to

US$22,997,121.00. The use of selective models allows 50% savings with maintenance of efficacy of screening.

Conclusion: The proposed alternative model is the best choice because it detects the patients with risk of dying unrecognized and it represents expressive savings.

INTRODUCTION

Congenital Adrenal Hyperplasia (CAH) is the generic denomination for a large group of inborn errors of the synthesis of cortisol from cholesterol, [1] some of which are fatal. Over 90 % of them are caused by defective 21-hydroxylase (CYP21), and severe forms in girls are the major cause of genital ambiguity. [2]

The first newborn screening program for CAH was developed in Alaska, in the late 1970's, [3] justified by the high incidence of the disease among the native population (1/490) [3] and because the screening method was very simple, which consisted in the assay of 17-hydroxyprogesterone on a filter paper dried capillary blood sample.[4]

Early treatment is able to prevent death in the most severe forms of the disease, what makes newborn screening for CAH quite desirable not only in Alaska, but also in many other regions and countries.[5]

Although worldwide incidence based on newborn screening of CAH (1/15,000 to 1/16,000)[6] is much lower than that in Alaska, several programs added it to their panel of screened disorders.[5]

Its incidence in Brazil was believed to be one of the highest in the world (1/7,533).[7]A recent population study with follow up of the outcome of positive screened cases showed a more realistic incidence of (1/10,325) in the state of Goiás.[8]

High 17-hydroxyprogesterone in neonatal screening may comprise false-positive cases [9], or may even occur in individuals with the less severe, non-fatal, non-classical form of the disease, that is to say, cases that are not a goal for newborn screening.[10]

In the most severe form of the disease, also known as classical form, the hyperandrogenism causes variable degrees of virilization with troubled differentiation of the external genitalia of affected girls.[2, 11]

All girls can be diagnosed routinely during the first exam of the external genitalia, [1] however boys do not display evident clinical signs and usually remain undiagnosed until an adrenal crisis ensues in the salt wasting form, or until they present signs of sexual precocity in the simple virilizing form.[1]

There is not a worldwide consensus about the recommendation of newborn screening for CAH in public health policies. [12,13] The newborn screening program of the state of Goiás, Brazil, in the year 2005, was used as a pilot for the study of the economic impact of the inclusion of this disease in the panel of screened disorders of the Neonatal Screening State Program (Programa Estadual de Triagem Neonatal – PETN) - Goiás and proposed an alternative model, according to the criteria proposed by Wilson and Jungner, [14] based on 17-hydroxyprogesterone screening for boys and clinical examination for girls.

METHODS

The PETN - Goiás granted the access to all data needed for writing this article, although it did not take part in the design, analysis, and interpretation or reporting of this study. The use of the data was approved by the Ethics in Research Committee of the Universidade Federal do Rio Grande do Sul.[8]

Study design

The cost analysis of newborn screening for CAH in the state of Goiás, Brazil, and, based on it, the extrapolation to the entire country were performed with the development of a decision model using the TreeAge 2007 Software, (Williamstown, Mass).

Decision model structure and assumptions

We constructed a decision analysis model (Figure 1) for the newborn screening for CAH not assuming equal sex ratios.

Traditionally, neonatal screening for CAH is targeted for both sexes. The adjustments of 17-hydroxyprogesterone cutoffs were made according to birth weight in order to improve the predictive value.[14]

In the previous study, children were categorized either as non-affected by CAH, or as bearing low risk, or else with high risk of being affected by CAH, according to the levels of 17-hydroxyprogesterone in the first heel prick blood sample and to the birth weight. [8]

In the present study, we re-categorized the children according to the costs involved after initial screening. The analytical horizon for calculating the total cost per child was set as up to the age of one year (Table 1).

Based on the review of traditional criteria of newborn screening, proposed by Jungner and Wilson and recently revised by WHO, which argues that only asymptomatic children must be screened. Thus, we assumed that the presence of genital ambiguity in girls must prompt expert medical attention,[1,2,10-15] and we created a hypothetical model of selective screening for only boys.

Model parameters

The robustness of the model and of the estimated parameters was tested by applying a multi-factorial sensitivity analysis, which involved the prevalence associated to the predicted value of the test. The range for such variables were defined arbitrarily in a way to warrant prevalence values much higher and positive predictive values very different from the ones found in the previous study (Table 2). [8]

The sensitivity analysis involved eight variables, simultaneously (Table 2), allowing the estimate for the upper and lower limits of the total cost of newborn screening for CAH in Brazil. For the selective model, the sensitivity analysis was performed only with the pertinent variables. In Table 2 the positive predictive values found, based on the same data, are displayed.

For the analysis, the North American currency (US$) was used as the monetary unit. The conversion of Real (Brazilian currency) to the US dollar was done by fixing the average

exchange rate available at the Banco Central do Brasil (Brazilian Federal Reserve), in the last days of December, 2005. Therefore, in the present study, one US dollar (US$1.00) corresponded to two reais and two hundred and eighty four cents (R$ 2.28).

The cost of 17-hydroxyprogesterone assay in filter paper informed by the PETN–Goiás was US$7.41. Such value includes all additional costs, both direct and indirect, for measuring the 17-hydroxyprogesterone value from the same filter paper collected for the other screening tests performed by PETN (phenylketonuria, hypothyroidism and hemoglobinopathies).

Children with a positive screen make a complete endocrine evaluation including the urgent medical examination and confirmatory lab tests. The values of these procedures are set by the Ministry of Health and totalize US$28.06 per visit to PETN-Goias, being US$10.95 [15] referring to the medical assistance and US$17.11 of lab tests costs. Furthermore, all children with a positive screen are followed by the endocrinologist of PETN until the outcome is defined. Besides the follow-up, the PETN undertakes the treatment of affected children (the treatment costs, as well as routine pediatric visits, are not considered in the present study). We counted four annual visits to PETN, which is the maximum number of attendance to the newborn screening reference service established by the Brazilian Ministry of Health. [16] The costs by category of children were showed in Table 1.

To estimate the economic impact of the inclusion of CAH in the national panel and modeling these results to Brazil, the total number of newborn and sex ratio was used

from the official database (DataSUS) from the Ministry of Health for the year 2005, [17] when the preliminary study was done in Goiás.[8]

RESULTS

In 2005, PETN-Goiás used the universal complete model when they identified seven children (four girls and three boys) and another others eight asymptomatic children with persistently elevated levels of 17-hydroxyprogesterone and inconclusive diagnosis. [8] At this period the cost of newborn screening for CAH for that state was US$609,281, therefore, for every child identified one can attribute a cost of US$87,049.

Modeling of the cost of newborn screening for Brazil is depicted in Table 3, where minimum and maximal values for the models of newborn screening studied for CAH, according to the sensitivity analysis. Extrapolation was based in a total of 3,035,096 living newborns in Brazil in 2005. Of these, 1,551,805 (51.1%) were boys, 1,479,872 (48.7%) were girls and 3,419 (1.1%) with error of notification.[17] (not include in selective model).

The total expected cost of universal newborn screening for CAH in Brazil may vary from US$22,542,257.00 to US$22,997,121.00. The cost of newborn screening is directly related to the incidence of high risk cases (Table 2), once only 0,5 % low risk retested children keep elevated values in the second filter paper sample and, thus, require follow-up.

Another determining factor in the assessment of the cost is how long cases remain inconclusive after screening, because they require the same follow-up as the cases with confirmed diagnosis.[8]

The results of the assessed models suggest that from the economic point of view, the best choice is to screen boys, because this reduces the cost by 50% with maintenance of efficacy of screening the classical forms of CAH (Table 3).

COMMENT

The critical analysis of the economic impact of including new diseases in the panel of the National Program of Neonatal Screening is fundamental not only in Brazil, but in other countries as well.

Due to the great scientific development seen in the past 40 years which allow, among others, the performance of predictive genetic tests, it is essential for public health, the review of newborn screening programs,[18] re-editing the Wilson e Jungner as recently proposed by the WHO.[14]

The classical form of CAH in boys is a relevant health problem, as they do not have an evident clinical sign at birth and their diagnosis is done at the first adrenal crisis. Non-specific symptoms of adrenal insufficiency are responsible for delaying the diagnosis, which may cause important neurological sequels or death.[19]

The girls, on the other hand, show variable degrees of external genital malformation at birth, except for those diagnosed and treated intra-uterus, and having this diagnostic hypothesis is mandatory.

In short, newborn screening for CAH is a relevant health problem, which can only be diagnosed in its pre-clinical state in boys, which meets the three first criteria for screening, determining that the best choice is screening for boys instead of for both sexes.

Also complying to the Wilson and Jungner criteria, [14] boys with the classical form of CAH are the target population for neonatal screening, especially those with the salt wasting form (100% of these are identified in screening); newborn screening has a low sensitivity (70%) for simple virilizing classical CAH. [11, 21, 22]

Up to the present moment, a universal model for newborn screening of CAH has been used worldwide. Although not considered a priority in many centers and thus not included in many public health programs, [21] this study proposes a cost saving model based on the rationale that girls should be clinically suspected and thoroughly assessed for CAH bypassing the screening routine (see Box 1).

By applying a sensitivity analysis in the model studied, the total cost of newborn screening for CAH could be estimated in Brazil irrespective of the incidence of the disease in different states.

If, on the one hand, the proposed model of screening males exclusively suggests that it may miss girls not properly examined at birth, on the other hand evidences have

shown that 75% girls are clinically diagnosed[5, 24] while the Delfia 17-hydroxyprogesterone assay is only sensitive for 60% of girls [5, 23, 24]

Although the inclusion of new technology in newborn screening has the goal of improving the health of children, [24, 26] an analysis of economical impact is mandatory in order not to overburden the public health system.[18] Newborn screening may seem to have a low cost if only the test costs are assessed, in the case of the test for CAH, a little over US$7.00.

A more profound analysis should assess all the additional costs, such as the diagnostic elucidation costs (laboratory and clinical) and the follow up of confirmed cases and those not defined. Therefore the direct costs per diagnosis was of almost US$90,000.00 in the year 2005 for Goiás; a life is priceless but reality shows that resources are not infinite and it is necessary that public programs have sustainability and have been proven effective. [19]

CONCLUSION

Establishing that the objective of newborn screening for CAH is to avoid infant death by the salt wasting classical form, [10, 25] it is suggested that newborn screening based on 17-hydroxyprogesterone level should be directed for boys because this reduces significantly the costs and it was ethically sound alternatives methods for the entire country.

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Authors Contributions: E L Silveira and R H Elnecave designed the study. Data collection was done by E L Silveira. Data were analysed by S. Camey, I Prestes, E L Silveira, and R H Elnecave and were interpreted by E L Silveira and R H Elnecave. The manuscript was written by R H Elnecave, E L Silveira, S, Camey and I Prestes. All authors were involved in the decision to submit the manuscript for publication.

Conflict of interest statement We declare that we have no conflict of interest.

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Table 1 Costs for different categories after CAH screening until the age of one year

Categories	Risk at screening	Cost Description	Cost per child (US$)/year
Not affected by CAH	-	(A)	7.41
Not affected by CAH, retested*	Low	2 (A)	14.82
False positive	High	(A) + [(B) + (C)]	35.46
	Low	2 (A) + [(B) + (C)]	42.87
Affected by CAH or inconclusive cases	High	A + 4 [(B)+(C)]	119.65
	Low	2 (A) + 4 [(B)+(C)]	127.06

* Children retested not affected were not considered false positive at PETN-Goiás; (A) 17-hydroxyprogesterone in filter paper; (B) medical consultation in PETN; (C) laboratory test in PETN (17-hydroxyprogesterone, androstenedione, testosterone, sodium and potassium).

Table 2 Data used in Decision Tree

Sex	Birth Weight (g)	High risk of being affected by CAH		Low risk of being affected by CAH		PPV*	Sensitivity analysis (range)
		Prevalence (in 10,000)	Sensitivity analysis (range)	Prevalence (in 10,000)	Sensitivity analysis (range)		
female	≥ 2,500	1.9	[0; 10]	28.7	ND	0.86	[0.65; 1.00]
	< 2,500	15.4	[0; 100]	104.2	ND	0.25	[0.00; 0.50]
male	≥ 2,500	2.8	[0; 10]	20.3	ND	0.54	[0.35; 0.75]
	< 2,500	20.8	[0; 100]	65.8	ND	0.25	[0.00; 0.50]

* Positive Predictive Value; ND not determinate

Table 3 Costs of newborn screening for CAH according to the model studied for Brazil

Models	Minimum cost (US$)	Maximum cost (US$)
Universal	22 542 257	22 997 121
Selective (only boys)	11 529 874	11 741 257

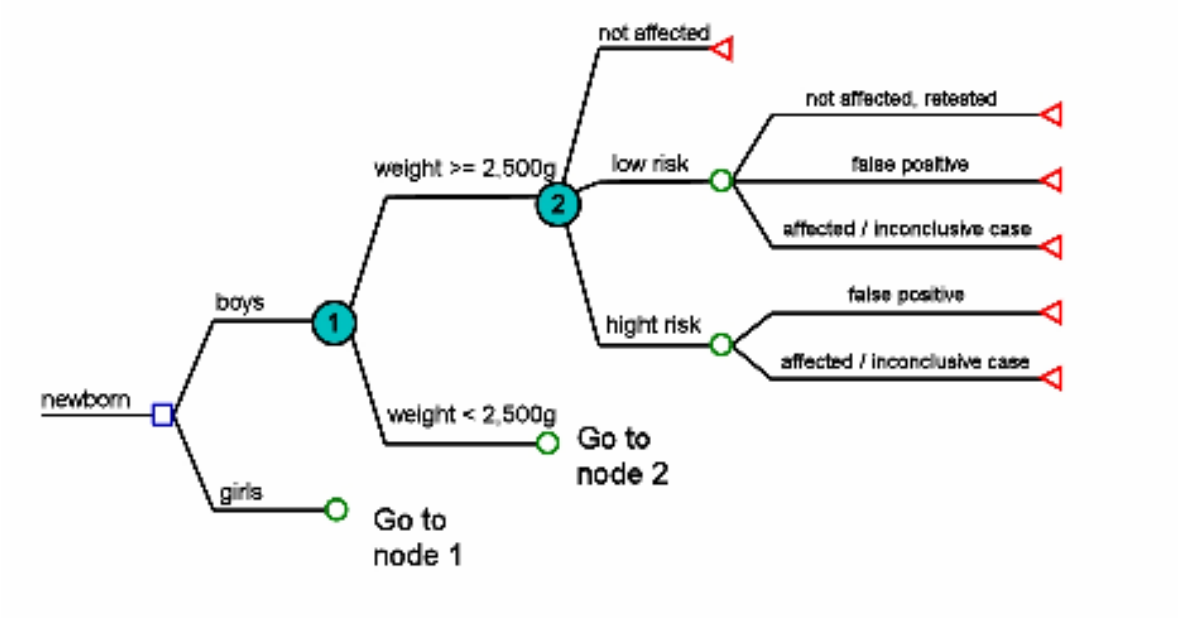

Figure 1. Decision Tree for detecting CAH representing universal model

CAPÍTULO III

ARTIGO ORIGINAL 3

THE ROLE OF GENOTYPING IN NEWBORN SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA

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Short Title: CYP21A2 analysis in newborn screening.

Key words: congenital adrenal hyperplasia, newborn screening, CYP21A2 mutations, confirmatory tests, 21-hydroxylase deficiency, 17OHP level.

Abbreviations: CAH, congenital adrenal hyperplasia; 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; CYP21, 21-hydroxylase; CYP21A2, active gene CYP21; CYP21A1, inactive gene CYP21; CF, classical form; SW, salt-wasting; SV, simple virilizing; NC, nonclassical; NSSP, Newborn Screening State Program

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ABSTRACT

Neonatal screening for congenital adrenal hyperplasia (CAH) is useful in diagnosing salt wasting form (SW). However, there are difficulties in interpreting positive results in asymptomatic newborns. Objectives: to analyze the genotyping, as a confirmatory test, in children with neonatal positive results. Patients: 23 CAH children and 19 asymptomatic with persistently elevated 17OHP levels. Methods: CYP21A2 gene was sequenced and genotypes grouped according to the less severe allele: A1 null, A2 < 2%, B >3%, C >20% of enzymatic activity. Results: 21 children with neonatal symptoms and/or 17OHP levels > 80 ng/mL carried A genotypes, except 2 virilized girls (17OHP < 50 ng/mL) without CAH genotypes. Patients carrying SW genotypes (A1 and A2) and low sodium levels presented 17OHP > 200 ng/mL. Three asymptomatic boys carried simple virilizing genotypes (A2 and B), in two, the symptoms began at 18 months; another two asymptomatic boys had nonclassical genotypes (C). The remaining 14 patients did not present CAH genotypes, and their 17OHP levels normalized by 14 months of age. Conclusions: molecular analysis is useful as a confirmatory test of CAH, mainly in boys. It can predict the clinical course and perform the differential diagnosis between clinical forms; additionally, it identifies the false-positive cases.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is the most frequent cause of congenital adrenal hyperplasia, presenting an autosomal recessive inheritance.[1,2] The disease is caused by CYP21A2 gene mutations, which result in a continuous spectrum of enzymatic deficiency.

There is a wide range of clinical manifestations, varying from prenatal external genitalia virilization in females and postnatal virilization in both sexes (simple virilizing form) to late onset symptoms (nonclassical form).[3,4] Nearly 75% of the newborns also present an important mineralocorticoid deficiency, which leads to dehydration with hyponatremia and shock in the first weeks of life.[1,2,5] Males presenting the most severe clinical form, the salt wasting form, have a high rate of neonatal mortality, due to the absence of evident signs of adrenal insufficiency.[5]

Characteristics of classical forms, such as high risk of morbidity and mortality, the existence of an accessible treatment and the possibility of its screening in the same samples obtained for those for other diseases, qualify the CAH for neonatal screening programs. The objectives of CAH screening programs are to identify newborns with a risk of being affected by the classical forms, and to prevent adrenal crisis. However, precocious initiation of the treatment creates some difficulties, such as, the salt wasting form possibly not being distinguished from the simple virilizing form, which has therapeutic implications.[6,7] Moreover, it may be

equally difficult to differentiate between the simple virilizing and the nonclassical forms in boys, and even from those with false-positive results.[7-9]

One of the major problems of screening programs is the false-positive results that can occur with preterm or low birth weight newborns or with neonatal diseases.[2, 10-13] These infants should be followed up until there is diagnostic definition or a normalization of serum 17OH-progesterone (17OHP) levels. These situations, besides increasing the cost of screening programs, also cause great anxiety to the parents.

In order to minimize the abovementioned problems, screening programs have established cutoffs for 17OHP levels according to birth weight or gestational age. However, despite these approaches, the rate of false-positive results is still around 1%,[14] and a second sample collection has been observed to elucidate the diagnosis of only 10% of these cases. It has been suggested that CYP21A2 genotyping may improve the specificity of newborn screening for detection of classical forms. This methodology could aid in delineating the follow-up of asymptomatic infants with persistently increased 17OHP levels and also in defining a differential diagnosis among clinical forms, especially in males.[7-9]

Our objective was to evaluate the benefits of CYP21A2 molecular analysis, as a confirmatory test, in children with positive neonatal results, who are still undergoing follow-up by the Goias-NSSP. In order to make this analysis, we compared the molecular data genotype categories with neonatal 17OHP levels.

SUBJECTS

This study was approved by the Research Ethics Committee of the Universidade Federal do Rio Grande do Sul, and all parents gave their written informed consent.

Clinical and laboratory data were provided by NSSP (Newborn Screening State Program), located at APAE (Association of Parents and Friends of Exceptional Persons), in the city of Anapolis of Goias state, Brazil. The CAH screening program is public, government-funded and certified by the Health Ministry. It has been mandatory statewide since 1997; and approximately 900,000 newborns had been screened until 2007, comprising 90% live births. This program established different 17OHP cutoffs, as follows: for normal (≥ 2,500 g) and low birth weight (< 2,500 g) newborns with low risk to being affected by CAH the cutoffs are 30-80ng/mL and 40-80 ng/mL, respectively; and a second sample filter paper is requested.[9] All children with some CAH clinical features or with neonatal 17OHP levels ≥ 80 ng/mL, independent of birth weight, are considered at high risk to being affected by CAH, and follow-up includes: urgent notification of basic health services and immediate pediatric-endocrinologic evaluation at Goias-NSSP.

Although the recommended age for sampling by the Health Ministry was set at 3-7 days after birth, in this series, the sample collection varied from 1 to 46 days

of life (median = 7 days). All hormonal confirmatory tests, serum 17OHP levels, were performed by a private laboratory, not belonging to the Goias-NSSP.

The Goias-NSSP, between January 1997 and December 2007, diagnosed 96 CAH newborns. Of those children still being followed-up by the pediatric-endocrinologist of Goias-NSSP, the caretakers of 23 of them agreed to participate in this study. Nineteen other children (16 boys, 3 girls, 5 of them preterm) with positive neonatal 17OHP results (from 31.5 to 215 ng/mL) also participated and are still being followed-up due to absence of symptoms. These children persistently presented elevated 17OHP levels and were classified as having uncertain diagnoses. Data from all subjects are shown in Table 1.

METHODS

For the filter paper hormone assays, the time-resolved, solid-phase fluoroimmunoassay (DELFIA - Wallac Oy, Turku, Finland) 17OHP neonatal kit was used and the concentration of 17OHP was expressed as serum-equivalence. The conversion calculation was: 1 nmol/l = 0.33 ng/mL in whole blood and 0.73 ng/mL in serum-equivalence.

CYP21A2 genotyping

DNA samples from the patients were extracted from peripheral blood leukocytes by salting-out procedures. We recruited at least one parent from each family (77 subjects) to perform mutation segregation analysis. The entire CYP21A2

gene, including the promoter region, was PCR amplified with specific primers, and the products were submitted to direct sequencing, as previously described. [10, 15] The CYP21A2 numbering was according to Higashi et al.[16]

SALSA MLPA P050B CAH kit (LOT 0107, MRC-Holland, Amsterdam, Netherlands) was used to confirm the presence of a large gene rearrangement, such as CYP21A2 deletion or large gene conversion, in subjects carrying a point mutation in homozygous state, which was found in heterozygous state in only one of the parents.

The aforementioned methodologies in the Brazilian CAH cohort detected mutations in 100% of the classical alleles and in approximately 85% of the nonclassical alleles.[17]

Genotypes were classified according to the number of alleles with identified mutations, as follows: undefined (at least one allele without identified mutation) or defined genotypes (mutations identified in both alleles). Defined genotypes were sub-classified according to the impairment of enzymatic activity predicted by the less severe allele: A1 (null), A2 (< 2%), B (3-7%) and C (> 20%), according to previous studies.[18-20]

Statistical Analysis

The Kruskal-Wallis and Mann-Whitney tests were used to compare genotype categories with neonatal 17OHP levels. For this analysis, only the neonatal 17OHP results from samples collected up to 10 days after birth were selected. $P < 0.05$ was considered as being statically significant. The program SPSS (Statistical Package for the Social Sciences) version 13.0 was used.

RESULTS

Out of 23 infants who had the neonatal diagnosis of classical form by the Goias-NSSP, 21 presented neonatal 17OHP levels > 80 ng/mL. We identified mutations in the CYP21A2 gene, predicting total or severe impairment of enzymatic activity in both alleles in all 21 patients. In the two remaining patients, two females with prenatal external genitalia virilization and lower neonatal 17OHP levels (case 33 = 50.5 ng/mL and case 34 = 9 ng/mL), we did not identify mutations in both CYP21A2 alleles.

Among the 19 asymptomatic infants with positive neonatal 17OHP tests (from 31.5 to 215 ng/mL), we identified a molecular diagnosis of 21OHD in five males. The first boy (patient 16) carried mutations predicting total impairment of enzymatic activity in both alleles (A2 genotype), having never had salt wasting crisis and being asymptomatic until 1 year old, when he presented an accelerated growth velocity. Simple virilizing form of CAH was diagnosed after the molecular studies. The second boy (patient 20) developed symptoms of pseudoprecocious puberty at 3 years old, and carried a B genotype, which defined the diagnosis of simple virilizing form. The third boy (patient 15) is still asymptomatic at 6 months old, at the time of this study, and molecular analysis revealed a B genotype, also predicting simple virilizing form. The remaining two boys (patient 14 is 8.7 years old and patient 21 is 6.8 years old) are still asymptomatic and carry C genotypes, predicting the nonclassical form. For further clinical details, see Table 1. Among the

remaining 14 patients with uncertain diagnoses, we found mutations in heterozygous state in five children, two girls and three boys. The female (patient 27), presented mild clitoromegaly, probably due to an extreme premature birth age. She received glucocorticoid treatment during the first year of life, after that, it was stopped and she remained asymptomatic at 3.3 years of age. The male (patient 37), presented pubarche at 4 years old and the nonclassical form of CAH was ruled out after the molecular analysis. He was considered as having idiopathic precocious pubarche. The remaining three heterozygous cases, one girl and two boys (ages varying from 1 y to 7.1 years), are still asymptomatic.

No mutations were found in the last 9 children, 5 of them were preterms (3 girls and 2 boys). Boy n° 39 had very high 17OHP levels (205 ng/mL) and received treatment before conclusion of endocrinologic evaluation. After withdrawing medication at 1.1 years old, he remained asymptomatic despite the presence of persistently high 17OHP levels.

We considered the neonatal 17OHP levels only from the patients in whom the filter paper samples were collected up to 10 days of birth (n = 31). In this group, 6 patients carried the A1 genotypes, 9 the A2 genotypes, 1 the B genotype, 1 the C genotype, 3 were heterozygotes and 11 did not carry mutations (Fig. 1). Medians of neonatal 17OHP levels of newborns carrying the A1 and A2 genotypes, which predict the salt wasting form, were significantly higher than the remaining groups. The median of the A1 genotype was 471.0 ng/mL, the 25^{th} and 75^{th} percentiles were 375.0 and 584.5, respectively, that of the A2 genotype was 416.0 ng/mL (330.0 and

496.5, respectively) and that of the B and C genotypes, heterozygotes and the false-positive test for 21OHD was 43.5 ng/mL (37.5 and 131.5, respectively) ($P < 0.001$). In isolated analyses, although the number of cases was not sufficient enough, there was no difference in neonatal 17OHP levels among patients carrying B, C genotypes, heterozygotes and false-positives results, neonatal 17OHP levels were 91.0 ng/mL, 45.0 ng/mL, 35.0 ng/mL and 42.0 ng/mL, respectively ($P=0.109$). Girls carrying genotypes A1 and A2 presented variable degrees of external genitalia virilization (from Prader III to V), and two of them were assigned as boys at birth. Both patients were the third sibling, in both families the first sibling died before 2 years old.

In order to define a panel of mutations for diagnostic purposes, we analyzed the mutation frequencies of patients with defined genotypes. We identified large rearrangements in 8 (17.7%) alleles, in which MLPA methodology revealed a CYP21A2 deletion in 5 alleles and a large gene conversion in 3 alleles. The remaining alleles carried point mutations and the most frequent were those derived from gene conversion events involving the pseudogene, such as: I2 splice (33.3% of alleles), Q318X (15.5%), R356W (15.5%), Ins T (4.4%), I172N (8.9%) and V281L (6.7%). Mutations P30L, Del 8nt and Cluster were not identified. Moreover, 15% of alleles harbored 2 or more point mutations. We also identified mutations not derived from the pseudogene; [10] the IVS 2-2 A>G mutation was found in four non-related children, and the R408C in one case. Two non-related children presented a new substitution in heterozygous state, R483L, in cis with the I2 splice mutation.

DISCUSSION

Goias was the first state in Brazil that added the CAH screening in the public neonatal screening program, differently from the southeastern regions, where the CAH screening is a paid test and not mandatory, because it is not included in the public health programs. As the capacity of neonatal CAH screening to save lives is unquestionable around the world, it also holds true in the state of Goias. In spite of the presence of social and educational problems in Goias state, which contribute in the delaying of sampling, CAH screening diagnosed more cases with the salt wasting form and the ratio of affected males to females increased in comparison with the southeastern region cohort.[17] However, due to the great amount of late sampling in our community, it is apparent that there is a great need for new initiatives to be taken, such as educational programs for the population as well for physicians.[13]

One of the greatest benefits of screening is avoiding the salt wasting crisis. However, precocious treatment creates some doubts: as to how to differentiate the salt wasting from the simple virilizing form before symptoms appear, as well as, the simple virilizing from the nonclassical form in boys with moderate increases in neonatal 17OHP levels. Molecular analysis confirmed the CAH diagnosis in all cases with neonatal symptoms and neonatal 17OHP levels > 80 ng/mL, except in two virilized females with lower levels. Our results reinforce data from a previous study, which identified CYP21A2 mutations in both alleles in 95% of the symptomatic newborns.[21]

However, we did not confirm the CAH molecular diagnosis in the two aforementioned virilized girls, one with a normal (9 ng/mL) and the other with a moderate increase (50.5 ng/mL) in neonatal 17OHP levels. The first girl was initially suspected to have a false-negative result. The absence of CYP21A2 mutations in these girls evidenced that they must have another adrenal enzymatic defect, such as, 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase or P450 oxidoreductase, in which the phenotypes could be mistaken for the ones of 21OHD. Moreover, these enzymatic defects could also present moderate increases in neonatal 17OHP levels.[9, 22-26] As the girls are undergoing glucocorticoid treatment, we are sequencing the CYP11B1 gene, in order to investigate the presence of CAH due to 11-hydroxylase deficiency.

Nineteen patients, asymptomatic in the neonatal period, carried classical genotypes A1 and A2, mainly associated with the salt wasting form, and 63% of them presented low sodium levels, indicating aldosterone deficiency. In the remaining CAH patients, it is still not possible to make a differential diagnosis between classical forms due to precocious treatment initiation or even due to the presence of the I2 splice mutation in the less severe allele, which could be also associated with the simple virilizing form.[2] The lowest neonatal 17OHP level of patients carrying a genotype that predicted the salt wasting form was 200 ng/mL. Few studies have made the comparison of neonatal 17OHP levels with genotypes predicting the salt wasting, and it seems most of them present neonatal levels higher than 166 ng/mL. Data from the literature demonstrated that the employment of 30

ng/mL as a cutoff has 100% sensitivity in detecting the salt wasting form and 70% in detecting the simple virilizing form.[27,28]

Another common difficulty of CAH screening is the high rate of false-positive results due to preterm, low birth weight or stressed newborns. Generally, these cases present moderate increases in neonatal 17OHP levels, which could also include newborns affected with simple virilizing and nonclassical forms.[14] These problems are not totally elucidated even after the adjustments of neonatal 17OHP levels according to birth weight, gestational age, the employment of extraction procedure or mass spectrometry methodologies.[29]

One of the most significant contributions of this study was the elucidation of inconclusive cases with molecular analysis. Among the 19 children, 5 presented a genotype confirming the 21OHD diagnosis. In three, the genotype predicted the simple virilizing form, and in two the nonclassical form. By the end of the molecular analysis, two boys with simple virilizing genotypes manifested acceleration of growth velocity and glucocorticoid therapy was started; the remaining cases are still asymptomatic. Moreover, in our series, no child classified as low risk due to moderate increases in neonatal 17OHP levels (30-80 ng/mL) had a genotype consistent with the salt wasting form. The use of genotyping, as a confirmatory test, has the advantage of providing the means of designing the follow up of simple virilizing form in males and of nonclassical form in both sexes. Some similar cases are described in the literature, including the neonatal diagnosis of nonclassical patients, in whom the diagnosis is not the aim of the screening programs.[8] Because there is a great overlap of neonatal 17OHP levels of patients

presenting simple virilizing and nonclassical forms and with those with false-positive results, it is not possible to create cutoffs to diagnose all cases with classical form and none with nonclassical form.[7, 30-31] Additionally, nonclassical form presents late onset symptoms, and nowadays, it is not clear whether these patients should undergo treatment, at the neonatal diagnosis or at the beginning of symptoms.

The addition of molecular analysis excluded the diagnosis of 21OHD in our patients with neonatal false-positive results, presenting an advantage in comparison with hormonal confirmatory tests, considering that second samples might not be conclusive in 10.3% of full term infants with false-positive results.[27]

As in other populations, the most frequent mutations in patients from Goias state were those derived from the pseudogene, but we also identified a higher frequency of mutations with founder effect than those from the southeastern region.[10] We also identified a new substitution in cis with the I2 splice mutation, R483L, in two non-related patients; but in vitro studies are necessary to verify if it affects the enzymatic activity. In the same codon, R483P and R483frameshift mutations were reported, both related to the salt wasting form.[32]

To create a panel of mutations for a screening program, it is important to determine the mutation distribution in each ethnic group, in order to detect all possible disease-causing mutations. Additionally, it is very important to evaluate the cost of the employment of molecular methodology as well as that of the follow up of false-positive cases. The cost of molecular study was previously evaluated at

USD $ 500 [31] and the cost of the follow-up of a false-positive case is around USD 848.[29] It was suggested that molecular analysis could reduce the number of recalls and could eliminate the necessity of a retest in 88%.[21]

In summary, in this series we observed that molecular analysis is useful in concluding the diagnosis of classical form of 21OHD in all cases and that the genotypes' determination could predict the disease severity of less severe clinical forms, which have therapeutic implications. In addition, molecular analysis elucidated false-positive cases, shortening follow-up time and decreasing family stress, in which the cost cannot be measured.

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Table 1. Clinical, hormonal, and molecular data of children.

Case nº	Birth weight (g)	GA	Sex	Prader (score)	Age (days)	17OHP (ng/mL)	Electrolytes Na	Electrolytes K	Clinical Features	Age (yrs)	Genotype
1	3700	Term	F	III-IV	1	508	134	4.9	Genital ambiguity	0.3	Del 21A2/ I2 sp,R483L‡
2	3700	Term	M	-	5	622	124	7.8	Salt losing crisis	1.9	Del21A2/ Q318X,R356W
3	2400	Preterm	M	-	7	572	128	4.6	Salt losing crisis	2.1	I2sp,Q318X /I2sp, Ins T, R356W
4	2900	Term	F	IV	9	416	141	5.9	Genital ambiguity	0.5	LGC/ I2 splice
5	2840	Term	M	-	10	375	-	-	Screening test	2.9	LGC/ IVS2 -A>G
6	3400	Term	F	V	7	347	134	6.7	Genital ambiguity	0.3	IVS2-A>G/ InsT,Q318X
7	2630	Preterm	F	III	5	136	134	7.1	Genital ambiguity	2.4	LR/I2 sp
8	2000	Preterm	F	III	7	330	125	7.0	Genital ambiguity	3.9	I2 sp/R408C
9	3350	Term	F	IV	10	102	-	-	Sex assignment error	0.8	I2 sp/ I2sp,R356W
10	3420	Term	M	-	4	515	126	6.1	Salt losing crisis	7.4	LGC/ IVS2 -2A>G
11	3950	Term	F	IV	2	427	120	5.9	Salt losing crisis	3.3	LGC/ IVS2 -2A>G,
12	3300	Term	M	-	18	449	130	6.2	Screening test	0.7	Q318X/ I2 sp, R483L‡
13	3750	Term	M	-	11	291	136	6.3	Screening test	2	Q318X/ R356W
14	3500	Term	M	-	3	44.6	-	-	undefined	8.7	V281L/ V281L
15	3100	Term	M	-	5	91.1	136	4.3	undefined	0.2	I172N/ R356W
16	3750	Term	M	-	30	215	135	4.9	undefined	2.5	I2 sp/I2 sp
17	3200	Term	F	IV	6	541	122	6.6	Salt losing	7.1	I2 sp/

									crisis		Q318X
18	3100	Term	M	-	7	470	127	8.1	Salt losing crisis	2.8	I2 sp/Q318X
19	3380	Term	M	-	20	564	112	9.3	Salt losing crisis	2.2	Q318X/ Q318X
20	3840	Term	M	-	46	183	-	-	undefined	2.7	I2 sp/I172N
21	3780	Term	M	-	-	50	142	5.5	undefined	6.9	V281L/I2 sp
22	2800	Term	F	III	1	349	121	7.9	Salt losing crisis	5.1	R356W/ R356W
23	4280	Term	M	-	17	93.8	136	4.6	Newborn screening	8.3	I172N/I172 N
24	3470	Term	M	-	-	-	-	-	Family study	11	I172N/I172 N
25	3800	Term	F	IV	5	485	141	3.9	Genital ambiguity	4.8	LR/I2 sp
26	2785	Preterm	F	normal	9	195	136	4.3	undefined	0.3	Normal
27	840	Preterm	F	I	26	135	-	-	undefined	2.2	L198P/Nor mal
28	2280	Preterm	M	-	3	133	-	-	undefined	0.8	Normal
29	2100	Preterm	M	-	25	52.5	-	-	undefined	10	Normal
30	3320	Term	M	-	9	41.5	-	-	Undefined	1.7	Normal
31	2750	Preterm	M	-	9	130	136	5.6	undefined	1.1	Normal
32	2280	Preterm	F	normal	5	159	-	-	undefined	0.7	Normal
33	3600	Term	F	IV	5	50.5	143	5.1	Genital ambiguity	3.8	Del 21A2/ Normal
34	3400	Term	F	IV	7	9	143	5.5	Genital ambiguity	1.5	Normal
35	4170	Term	M	-	35	121	-	-	undefined	0.3	-126CT/ Normal
36	3250	Term	F	normal	5	42.2	-	-	Family stress	0.1	Normal
37	3000	Term	M	-	10	8.6	-	-	Premature pubarche	7.1	V281L/ Normal
38	3800	Term	M	-	4	39.7	-	-	Undefined	0.4	Normal
39	3480	Term	M	-	7	205	-	-	Neonatal screening	1.1	Normal
40	3300	Term	M	-	6	35	142	6.0	Undefined	1.0	V281L/ Normal
41	3300	Term	M	-	9	39.9	140	4.8	Family stress	0.1	Normal
42	2705	Term	M	-	10	31.5	-	-	Family history	0.2	Normal

Case nº 18 and 24: affected sibling of case nº 17 and 23, respectively; 17OHP (serum equivalent values); * first sample ; ‡ new substitution identified in Brazilian cohort, functional study was not available;

LR: large gene rearrangement; LGC: large gene conversion; Del: deletion, Dup: duplication, sp: splice.

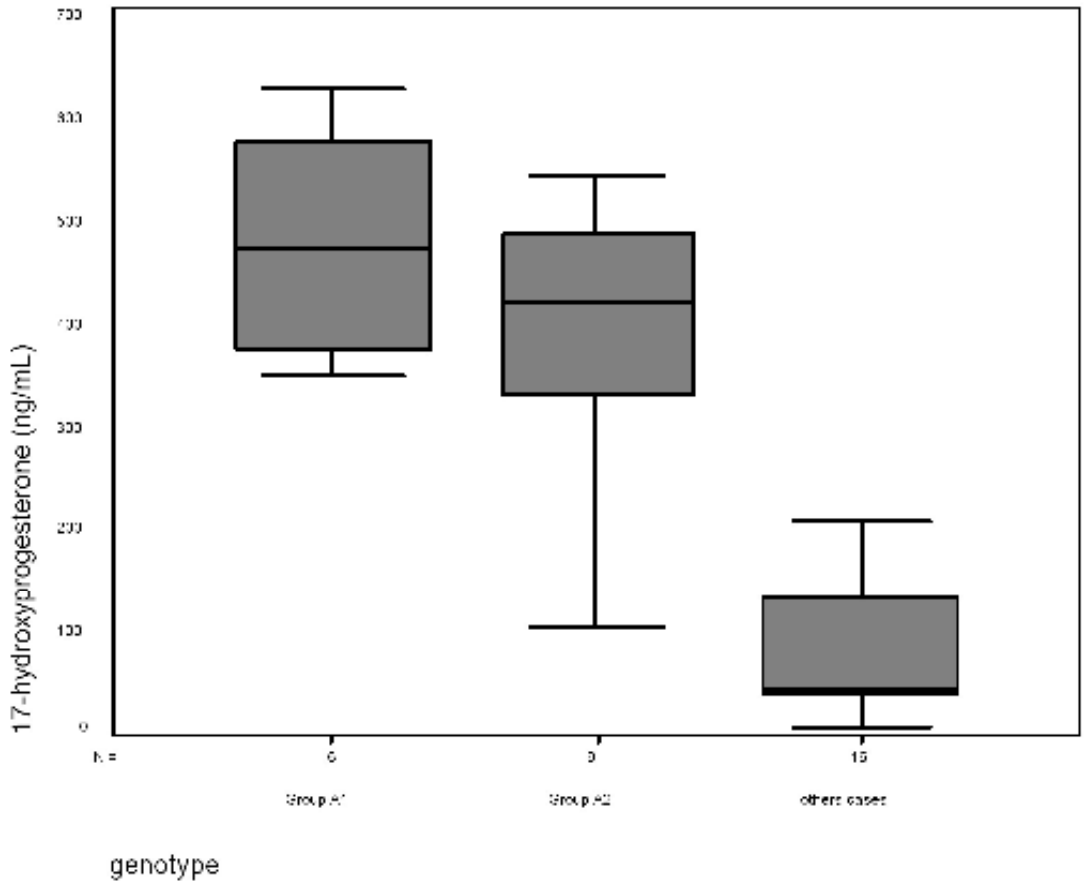

Figure 1: Neonatal screening values of 17OHP, first sample, in relation to A1 and A2 genotypes and remaining cases (B and C genotypes, heterozygotes and false positive test for 21OHD). p < 0,001

CONSIDERAÇÕES FINAIS

A triagem neonatal para a forma clássica da hiperplasia adrenal congênita (HAC) é o único meio de diagnosticarmos precocemente meninos afetados pela doença, pois eles não apresentam sinais evidentes da doença nas primeiras semanas de vida; enquanto todas as meninas apresentam genitália externa virilizada em função da exposição a níveis elevados de andrógenos na vida intra-uterina. Elas devem ser diagnósticas nas primeira horas de vida e submetidas a uma avaliação endocrinológica completa de urgência.

O ponto de corte da 17OHP adequado para a triagem neonatal da deficiência da 21-hidroxilase, que é a causa mais comum de HAC é aquele que identifica crianças com a forma clássica da doença, no caso de Goiás, foram as crianças com valores superiores a 200 ng/mL em 2005.

Com base nas evidências encontradas nessa tese, elaborou-se uma síntese de critérios recomendados para programas de triagem neonatal para HAC (Quadro 1).

Box 1. Synthesis of emerging screening criteria proposed by Wilson and Jungner *, adapted by the authors for CAH.

1. Neonatal screening boys is a relevant one;
2. The goal for newborn screening for CAH is to diagnose classical forms;
3. 100 % of the girls are born with ambiguous genitalia, therefore they may be diagnosed clinically with no newborn screening; thus the total population is composed by boys;
4. Newborn screening for classical CAH identifies 100% of the affected children with the salt wasting form and 70% of those with the simple virilizing form; [10,20]
5. The program must include, aside from the screening, continuing education, clinical services and should be responsible for the treatment.
6. The program must have mechanisms to minimize risks, for instance, to avoid unnecessary treatments and stress;
7. The program must be made available for all boys born in Brazil (access equity);
8. The program should have informed consent forms and warrant confidentiality and respect family autonomy;
9. The program must be planned from the beginning: it should collect the first blood spot between the 3rd and 7th day of life; it should also be responsible for investigating suspicious cases before the second week of life;
10. The program should have more benefits than harm.

* Andermann A, Blancquaert I, Beauchamp S, Déry V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ. 2008;86(4):317-319.

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