CONGENITAL ADRENAL HYPERPLASIA: SHOULD NATIONWIDE SCREENING BE IMPLEMENTED IN THAILAND?

Noppavan Janejai, Piamnukul Krasao, Jaruwan Phansang, Rojanaphan Pankarnjanato, Wiyada Charoensiriwatana

National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand

Abstract. A project to establish the Thailand National Neonatal Screening Program was started in 1996 with the objective of screening every newborn for congenital hypothyroidism and phenylketonuria. Over a million newborns were screened and over 430 abnormal cases were detected. A study was also conducted to determine the feasibility of including CAH screening in the program. The incidence of this disease has not yet been clearly determined. Since 1999, 58,563 newborns have been screened for CAH and 144 newborns with serum 17-OHP higher than 40 ng/mL were recalled for confirmatory tests. Of those, 68 were retested and 6 were found to have elevated 17-OHP levels. Two were confirmed with salt wasting CAH one month after birth, two others were diagnosed with another disease that caused electrolyte imbalance, one patient died, and the sixth required further clinical diagnosis. Five other babies were reported dead before the second specimens could be collected for confirmation. It appears that CAH may be one of the underlying causes of death among Thai newborns and the incidence may be higher than thus far shown due to incomplete confirmation of positive screens and deaths to some infants.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a treatable group of inherited disorders with severe manifestations that results from a deficiency in one of the enzymes of cortisol biosynthesis. In about 95% of cases, 21-hydroxylation is impaired so that 17-hydroxyprogesterone (17-OHP) is not converted to 11-deoxycortisol (New, 1992). Because of defective cortisol synthesis, ACTH levels increase resulting in overproduction and accumulation of cortisol precursors, particularly 17-OHP, proximal to the block. This causes excessive production of androgens, resulting in virilization of girls and rapid somatic growth with early epiphyseal fusion in both sexes. The inadequate synthesis of aldosterone due to severely impaired 21-hydroxylation of progesterone results in salt wasting, which is life threatening. This form of disease can be found among three-fourths of the classic CAH (Funder, 1993).

Steroid 21-hydoxylase (P450c21, CYP21) is a microsomal P-450 enzyme. The structural gene encoding human CYP21 (CYP21, CYP21A2 or CYP21B) and a pseudogene (CYP21P, CYP21A1P, or CYP21A) are located on the short arm of chromosome 6, closely linked to the gene encoding HLA-B and HLA-DR. CYP21 and CYP21P each contain 10 exons spaced over 3.1 kb. Their nucleotide sequences are 98% identical and approximately 96% identical in intron. Most mutations causing 21-hydroxylase deficiency are apparently the results of either of 2 types of recombinations between CYP21 and the CYP21P. These two mechanisms are unequal crossing over during miosis, resulting in a complete deletion of C4B and a net deletion of CYP21, or apparent gene conversion events that transfer deleterious mutations normally present in CYP21P to CYP21.

In Thailand, the national newborn screening program was implemented in 1996 to screen for congenital hypothyroidism (CHT) and phenylketonuria (PKU), 2 treatable diseases that cause mental retardation. To date, over a million babies have been screened with more than 430 positive cases confirmed and referred for treatment. Besides CHT and PKU, congenital adrenal hyperplasia is another inherited disorder that can be found in newborns in every part of the country. Most of the cases diagnosed without screening are the simple virilizing form (personal communication). There are no actual figures of the incidence of CAH in Thailand. This study was conducted to determine whether CAH should be added to the newborn screening program along with suitability of the methodology and ascertainment of the difficulties of screening for CAH.

MATERIALS AND METHODS

Dried blood spot-filter papers collected from 5,863 newborns after 48 hours of life for routine screening were randomly sampled, following completion of specimen preparation for routine newborn screening. Screening for CAH was performed by determining 17-OHP levels using RIA technique (ICN Biomedicals, USA). The cut off value was 40 ng/ml. When the 17-OHP level was higher than the cut off value, the patient was immediately recalled by fax and/or phone for confirmation. The recalling process was done with the aid of provincial health officers and the hospital staff. Recalled patients were examined and electrolyte levels were determined so that treatment could be instituted immediately if salt-wasting was evident.

Genotyping was carried out in specimens that screened positive. Three dried blood spots (3 mm dia) were punched into a 1.5 ml plastic tube. Genomic DNA was extracted by using a QIAamp DNA blood minikit (Qiagen, USA) following the manufacturer's procedure. The mutation screening was performed by the amplification created restriction site technique (ACRS) (Oriola *et al*, 1997). Genomic DNA was selectively amplified by PCR using 2 pairs of primers thus resulting in 2 fragments of CYP21; a 1339 bp fragment from exons 1-6 and a 2.2 kb fragment from the 8 bp-deletion in exon 3 to the sequence beyond exon 10. Another PCR was performed using appropriate primers as described by (Oriola *et al*, 1997). The first fragment was specifically amplified to detect the P30L, (A,C→G) in intron 2 and 8 bp deletions in exon 3. The second fragment was used to detect the I172N, Q318X, R356W and P453S mutations.

RESULTS AND DISCUSSION

Since 1999, a total of 58,563 newborns have been screened for CAH. The results are summarized in Tables 1 and 2. The 144 newborns with abnormal 17-OHP levels were recalled for confirmatory tests. Only 68 specimens were returned by approximately one month after birth. The results showed that six of these retests had elevated 17-OHP levels. Two of the babies were confirmed to have salt wasting CAH approximately one month after birth by both laboratory and clinical diagnoses (crude incidence 1:29,281). Two cases were diagnosed with another disease that also causes elevated 17-OHP and abnormal electrolyte concentrations. One patient was reported dead. The sixth patient required further clinical diagnosis by a pediatric endocrinologist. If this case were confirmed positive for CAH, the crude incidence would be 1:19,521. Since five babies were reported dead before the second specimens could be collected, the incidence might be even higher since these deaths could have been caused by the severe form of CAH. Further studies must occur and recall must improve. All positive cases thus far are males, which is inconsistent with the expected equal distribution of the sex ratio.

Genetic analysis was performed to study the genetic variability of the patients. The 2 positives and the 1 expected positive case were found to have intron 2 (A,C→G) mutations, as shown in Fig 2. However, the work on genetic analysis has just started and further experiments must be completed. In the near future, the genetic test may be useful as a confirmatory test, using a primary blood spot specimen.

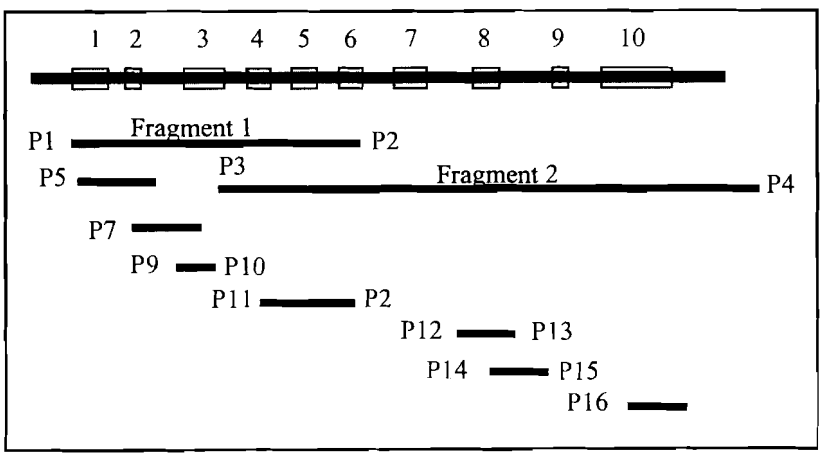

Fig 1. Approximate location of the PCR primers used to detect the mutations: P30L (P5/P6), intron 2 (A,C→G) (P7/P8), and exon 3 (8 bp deletion) (P9/P10), V281 and R339H (P3/P4), I172N (P11/P2), Q318X (P12/P13), R356W (P14/P15), and P453S (P16/P17) 3.

Table 1. Summary of the pilot CAH screening results.

Screening	
No. of babies screened	58,563
Positive screening	144
Died	5
2nd Specimen	
2nd specimen received	68
2nd specimen with elevated 17-OHP level	6
Confirmed salt wasting & hospitalize	2
Died	1

Table 2. Details of positive CAH cases.

Specimen	Birth weight (g)	Sex	17-OHP (ng/ml)	Age	Remark
UB00642	2850	F	137	1 month	false
UB11678	1300	M	395	1 month	died
UB02138	3100	M	251	2 month	treated
NK1518	3200	M	182	2 month	treated
SR5452	3050	M	246	1 month	waiting for result
NK5319	2700	M	183	1 month	false

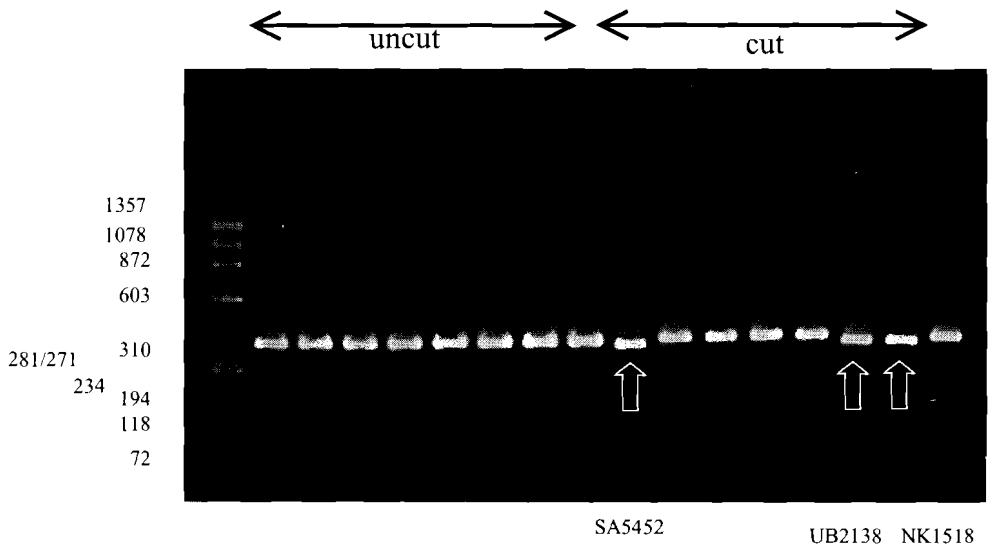

Fig 2. Agarose gel eletrophoresis of the P7/P8 PCR products cut with Hhal. The normal PCR product was 378 bp while the mutant yielded 24 and 354 bp fragments.

Problems occurred during the recall process, resulting in delayed confirmation. These delays may be due to a lack of understanding and awareness of the severity of CAH among members of the health community. CAH is considered a rare disease in Thailand. Thus, the health staff, with the exception of specialists, does not yet understand the critical nature of a potential positive testing result. The situation for CAH is unlike that for CHT and PKU for which education and advocacy among health staff and parents were already established. Clinical diagnosis and appropriate treatment for CAH usually requires a specialist, and this, along with the necessary education for health professionals and parents, must be taken into account if CAH screening is to be implemented nationwide.

CONCLUSIONS AND RECOMMENDATIONS

The results of this study show that CAH may be one of the underlying causes of death among Thai newborns. A delay in screening can be fatal. The crude incidence reported in this study is probably lower than the actual incidence because some babies died before the proper diagnosis could be made and some did not return for follow-up testing. In order to implement CAH screening for every newborn, activities to promote the education of health personnel and parents are necessary. The incidence and type of disease must be clearly determined so that the proper screening methodology can be selected. At this stage, genetic testing is being developed to assist in determining whether or not nationwide screening will have a beneficial impact on Thai newborns.

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