Molecular Genetics and Metabolism Reports 1 (2014) 312–323

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

# Novel method to characterize CYP21A2 in Florida patients with congenital adrenal hyperplasia and commercially available cell lines

Christopher N. Greene [a,*], Suzanne K. Cordovado [a], Daniel P. Turner [a], Lisa M. Keong [a], Dorothy Shulman [b,c], Patricia W. Mueller [a]

[a] U.S. Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Newborn Screening and Molecular Biology Branch, Atlanta, GA, United States
[b] University of South Florida, Department of Pediatrics, Tampa, FL, United States
[c] All Children's Hospital, St. Petersburg, FL, United States

ARTICLE INFO

*Article history:*
Received 14 May 2014
Received in revised form 8 July 2014
Accepted 8 July 2014
Available online 8 August 2014

*Keywords:*
Congenital adrenal hyperplasia
CYP21A2
DNA sequence
Mutation analysis

ABSTRACT

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder and affects approximately 1 in 15,000 births in the United States. CAH is one of the disorders included on the Newborn Screening (NBS) Recommended Uniform Screening Panel. The commonly used immunological NBS test is associated with a high false positive rate and there is interest in developing second-tier assays to increase screening specificity. Approximately 90% of the classic forms of CAH, salt-wasting and simple virilizing, are due to mutations in the CYP21A2 gene. These include single nucleotide changes, insertions, deletions, as well as chimeric genes involving CYP21A2 and its highly homologous pseudogene CYP21A1P. A novel loci-specific PCR approach was developed to individually amplify the CYP21A2 gene, the nearby CYP21A1P pseudogene, as well as any 30 kb deletion and gene conversion mutations, if present, as single separate amplicons. Using commercially available CAH positive specimens and 14 families with an affected CAH proband, the single long-range amplicon approach demonstrated higher specificity as compared to previously published methods.



* Corresponding author at: Centers for Disease Control and Prevention, National Center for Environmental Health, 4770 Buford Highway, MS F-24, Atlanta, GA 30047, United States. Fax: +1 770 488 4521.
*E-mail address:* crg0@cdc.gov (C.N. Greene).

http://dx.doi.org/10.1016/j.ymgmr.2014.07.002
2214-4269/

## 1. Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroid biosynthesis. In most populations, nearly 90% of CAH cases are due to mutations in 21-hydroxlase (21OH), a key enzyme required for the conversion of progesterone and 17-hydroxyprogesterone (17OHP) to deoxycorticosterone and 11-deoxycortisol, respectively, in the cortisol and aldosterone biosynthesis pathways [1–3]. The clinical disease severity of CAH 21OH deficiency (21OHD) is dependent upon the levels of 21OH enzyme activity. Classic CAH can be defined as either salt wasting (SW) or simple virilizing (SV) [4]. In SW-CAH, there is complete loss of 21OH activity which results in the concurrent loss of cortisol and aldosterone synthesis, causing an elevated stress response and loss of electrolyte homeostasis in affected individuals. The absence of 21OH activity can lead to an adrenal crisis and cardiac arrest in an untreated individual. In SV-CAH, there is sufficient partial 21-OH activity (1–2%) to retain normal sodium balance while excess 17OHP is shunted into the androgen synthetic pathway, resulting in masculinization of external female genitalia and early onset of puberty for both sexes. In non-classical (NC) CAH, up to 50% of enzyme activity is retained and individuals present with milder late-onset phenotypes [2].

The gene encoding 21OH, CYP21A2, and a non-functional pseudogene, CYP21A1P, are located in the HLA class III region of chromosome 6. The CYP21A2 gene and the corresponding pseudogene each consist of 10 exons and are highly homologous, sharing approximately 98% nucleotide sequence identity [5]. The chromosomal region containing CYP21A2 and CYP21A1P is part of a 30 kb tandem duplication of four genes (serine/threonine Kinase RP, Complement C4, 21-hydroxylase CYP21 and Tenascin TNX) known as the RCCX module. The high degree of similarity between the gene repeats has been shown to result in unequal non-homologous recombination and gene conversion events between CYP21A2 and CYP21A1P [6–8]. Greater than 95% of inactivating CYP21A2 mutations associated with both SW- and SV-CAH are due to the transfer of CYP21A1P pseudogene sequence segments to the CYP21A2 functional gene through short intragenic conversion events, while large-scale conversions and the commonly occurring 30 kb-gene deletion alleles result from recombination between the CYP21A2 and CYP21A1P loci [9].

All newborns in the United States are tested for CAH as part of the recommended universal screening panel of conditions for newborn screening (NBS). The primary goals of NBS programs are to identify cases of CAH that might lead to early death due to an unrecognized salt wasting crisis as well as undetected cases of severe virilization that might result in erroneous sex assignments in females. The primary method for CAH detection is a fluoroimmunoassay assay that measures 17OHP from dried blood spot (DBS) specimens on filter paper card taken between 24 and 48 h of life. This method, however, is known to generate a great number of false-positive results due to a lack of antibody specificity, cross-reactivity with other steroid compounds in the DBS matrix, and elevated 17OHP levels in stressed, premature or sick newborns [2]. In order to reduce the overall number of false-positive results, states have implemented strategies to reduce the false positive rate for CAH screening. The first is the use of second-tier liquid chromatography tandem mass spectrophotometry (LC-MS/MS) methods to compare steroid profile measurements in the DBS specimen to increase testing accuracy [10–13]. However, LC-MS/MS second-tier assay has not yet been widely adapted by state screening programs. A recent study in Minnesota documented that while second-tier steroid profile assay reduced the rate of repeat testing of individuals due to equivocal test values, and improved the overall false positive screening rates, there was still a significant false negative rate and concern of the efficacy of the LC-MS/MS method. This result may be due to the use of the same DBS specimen utilized for the primary immune-assay and not a repeat DBS sample which would have increasing levels of 17-OHP for a true positive that accumulate with age in CAH [14,15]. The false positive rate for CAH screening has also been reduced by the collection and testing of a second DBS in some state newborn screening programs. Currently, nine states have a mandated second screen DBS collected from every infant at 8–14 days of age. Two states, Colorado and Texas, have published retrospective analyses of infants diagnosed with CAH on the basis of if they were detected with the single 17-OHP screen collected at 24–48 h or with the second DBS collected at 8–14 days. In both studies, the second DBS collected at 8–14 days identified cases of salt-wasting CAH that would have been missed with just a single screen (add Chan 2013, Therrell, 1998). While this second specimen increased the sensitivity of CAH screening, there is concern that the results of the second test may not be available prior to the onset of salt-wasting crises in affected infants [16].