Reverse-hybridization assay for rapid detection of common CYP21A2 mutations in dried blood spots from newborns with elevated 17-OH progesterone

Stefan Németh a,⁎,1, Stefan Riedl b,1, Gernot Kriegshäuser a, c, Sabina Baumgartner-Parzer d, Paola Concolino e, Vassos Neocleous f, Leonidas A. Phylactou f, Maryla Borucka-Mankiewicz g, Hüseyin Onay h, Aijan Tukun i, Christian Oberkanins a

a ViennaLab Diagnostics GmbH, Gaudenzdorfer Gürtel 43-45, 1120 Vienna, Austria
b Department of Pediatrics, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria
c Ludwig Boltzmann Gesellschaft, Cluster Translational Oncology, Währinger Gürtel 18-20, 1090 Vienna, Austria
d Department of Molecular Genetics, Function & Therapy, The Cyprus Institute of Neurology & Genetics, PO Box 23463, 1683 Nicosia, Cyprus
e Laboratory of Molecular Biology, Institute of Biochemistry and Clinical Biochemistry, Catholic University, Largo F. Vito 1, 00168 Rome, Italy
f Department of Medical Genetics, Ankara University Faculty of Medicine, Sihhiye, 06100 Ankara, Turkey
g Department of Medical Genetics, Ege University Faculty of Medicine, 35100 Bornova/Izmir, Turkey
h Department of Medical Genetics, The Children’s Memorial Health Institute, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland
i Department of Medical Genetics, Ankara University Faculty of Medicine, Sihhiye, 06100 Ankara, Turkey
j Division of Medical Genetics, Duzen Laboratory Groups, Tunus Caddesi No: 95, 06680 Ankara, Turkey

⁎ Corresponding author at: ViennaLab Diagnostics GmbH, Gaudenzdorfer Guertel 43-45, A-1120 Vienna, Austria. Tel.: +43 1 8120156 43; fax: +43 1 8120156 19.
E-mail addresses: nemeth@viennalab.co.at (S. Németh), stefan.riedl@meduniwien.ac.at (S. Riedl), kriegshauser@viennalab.co.at (G. Kriegshäuser), sabina.baumgartner-parzer@meduniwien.ac.at (S. Baumgartner-Parzer), paolaconcolino78@libero.it (P. Concolino), vassos@cing.ac.cy (V. Neocleous), m.borucka@czd.pl (M. Borucka-Mankiewicz), onayhuseyin@gmail.com (H. Onay), aijan.tukun@medicine.ankara.edu.tr (A. Tukun).
1 These authors contributed equally.

A B S T R A C T

Background: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder most commonly caused by defects in the CYP21A2 gene. Neonatal CAH-screening based on 17-hydroxyprogesterone (17-OHP) measurements prevents life-threatening salt wasting conditions in newborns, but results in a considerable false-positive rate. Therefore, efficient second tier tests are required.

Methods: We developed a reverse-hybridization test strip-based assay (CAH StripAssay) covering the most prevalent CYP21A2 point mutations/small insertions/deletions occurring in Middle European populations. Assay specificity was validated using plasmid clones, and wild-type and mutant reference DNAs. Its practicability was evaluated in 271 samples from patients with CAH, suspected CAH, and dried blood spots from screening-positive newborns.

Results: All eleven point mutations and 51% of large deletions/conversions could be unambiguously identified when compared to reference methods (DNA sequencing, MLPA). After exclusion of rare mutations (6.4%) not covered by the StripAssay, the overall detection rate was 85%. Undetected heterozygous deletions/conversions caused a lack of information, but did not result in an incorrect prediction of phenotypes.

Conclusions: Our novel CAH StripAssay proved to be a fast (7 h) and reliable method for detection of common CYP21A2 mutations. Implemented as a second-tier test in CAH newborn screening, it has the potential to significantly reduce recall rates.

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1. Introduction

Congenital adrenal hyperplasia (CAH; OMIM ID: 201910) is an autosomal recessive disorder of the adrenal cortex (incidence 1:10,000–15,000) caused in about 95% of cases by genetic defects in the steroid 21-hydroxylase gene CYP21A2. The resulting disorder of adrenal steroid metabolism is characterized by a lack of cortisol and aldosterone biosynthesis. As a consequence, steroid precursors upstream of the enzymatic block are shunted into the androgen pathway, leading to androgen excess starting from early intrauterine life. Depending on severity of the different CYP21A2 mutations, CAH results in a wide range of clinical manifestations including classic salt-wasting (SW) CAH, classic simple-virilizing (SV) CAH as well as mild non-classic (NC) forms of the disease [1]. Various degrees of genital virilization...