Emerging Role for Tandem Mass Spectrometry in Detecting Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by a deficiency of one of five enzymes required for the synthesis of cortisol and aldosterone. The most common type of CAH is 21-hydroxylase deficiency (21OHD), which is attributable to mutations in the gene CYP21 and a pseudogene, CYP21P, located on the short arm of chromosome 6. As with other genetic disorders, genotype does not always predict phenotype.

The phenotypic spectrum associated with 21OHD ranges from asymptomatic to early onset (as early as the fourth day of life) of severe salt-wasting crisis characterized by hypotension and electrolyte abnormalities that, if uncorrected, lead to death. Increased androgenic steroids, attributable to accumulated precursors proximal to the enzyme defect, cause ambiguous genitalia in female infants, leading to earlier clinical evaluation in girls. Virilized infant boys, however, are not always recognized, and many will present with acute catastrophic symptoms. Approximately 70% of infants with classic 21OHD have the salt-wasting form.

All newborns in the US are offered medical screening that uses blood collected shortly after birth on filter-paper cards, but the disorders screened for vary from state to state. All US states screen for phenylketonuria (PKU) and congenital hypothyroidism, and 36 states screen for CAH (1). A filter-paper immunoassay to screen for CAH has been available since 1977 (2). This approach relies on measurement of 17-hydroxyprogesterone (17OHP), which is normally converted to 11-deoxycortisol by CYP21 and is therefore increased when the enzyme is deficient. Several commercial immunoassays are now available.

The analytical and clinical characteristics of available tests affect whether a condition is screened for. Important characteristics include whether a test is available; its cost, precision, and accuracy; and whether it can be done on a large-scale screening basis. Some states have been reluctant to screen for CAH because the most commonly used assays have poor positive predictive values, especially in preterm and acutely ill neonates, in whom the adrenal steroids are physiologically increased. Immunoassays also have cross-reactivities with similar compounds. For these reasons, and because of the dynamic changes in the newborn adrenal axis after birth, establishing reference intervals and cutoffs for 17OHP is challenging, despite adjustments for gestational age and/or birth weight (3). False-positive results increase cost because of the need for repeat specimens or referral for clinical evaluation and quantitative steroid and electrolyte measurement to confirm or exclude the diagnosis. False-positive results add considerably to stress and anxiety in the families of infants with other inborn errors of metabolism (4). Although this has not been studied for this population, it may also be a factor.

The gold standard for diagnosis of CAH is serum steroid profiling. An advantage of profiling is that ratios of compounds can be calculated, and the same criteria for diagnosis can be used regardless of age at sampling, except in the case of preterm newborns (5). Mitchell and Hermos (6) previously used cortisol concentrations, as measured by an immunoassay, to distinguish newborns with transiently increased 17OHP from those with 17OHP increases produced by 21OHD. Although the method was reliable and simple, the overlapping cortisol values in the affected and unaffected newborns were not amenable to establishing a suitable cortisol cutoff.

In this issue of Clinical Chemistry, Lacey et al. (7) present a method for steroid profiling of newborn dried blood specimens by tandem mass spectrometry (MS/MS). After measurement of 17OHP, androstenedione, and cortisol, interpretation relies on the whole profile rather than on the 17OHP concentration alone (8, 9) and thus represents a modification of the authors’ earlier method (10). MS/MS enables more accurate quantification of the steroids, without the cross-reactivity problems of the immunoassays. The requirement for both 17OHP to be >12.5 mg/L and the ratio to be >3.75 markedly improved specificity, and the positive predictive value was improved dramatically to 50%. The sensitivity and negative predictive value were, by design, 100% because the cutoffs were chosen so that no cases would be missed in the cohort being analyzed.

These techniques of profile and ratio interpretation are well established in MS/MS screening for other inborn errors of metabolism (11). MS/MS has been recently adapted and introduced to newborn screening in this country and others around the world; currently, one-half of US states utilize this methodology in some capacity for routine newborn screening. MS/MS is particularly elegant in that it can measure multiple analytes in a single dried blood spot after a simple extraction and sample preparation technique. It also has high sensitivity and specificity and is suitable for the high-throughput analyses necessary for mass screening. Many screening programs use a method that allows quantitative profiling of amino acids and acylcarnitine species to detect >30 inborn errors of metabolism, including amino acidopathies, organic acidemias, and fatty acid oxidation defects.

The method described by Lacey et al. (7) is not the same as is used at present for amino acid and acylcarnitine profiling and notably uses a water elution, followed by a diethyl ether extraction, drying, and reconstitution in a methanol–water solution. Additionally, it requires use of a narrow-bore liquid chromatography column for separation of the steroids, with a 12-min total run time, which is long by newborn-screening standards but necessary to elute an unidentified, unrelated compound that was, nevertheless, important because it caused signal contamination by “ghosting”. As the authors indicate, this technique modification and the longer analysis time makes
this method more suitable for “second-tier” testing of samples that have abnormal results in the initial immunoassay screen. A potential disadvantage of the method for use as a second-tier screen is the possible delay in notification of a positive result until after the additional testing is completed, particularly because this method requires greater technical sophistication, which might not be readily available in many of the screening programs that have recently introduced or are currently introducing MS/MS for a limited number of disorders, thus requiring that samples with abnormal results be sent to regional laboratories for confirmation. Alternatively, this method may be well suited for diagnostic testing, rather than for mass screening, if it is in fact suitable for use in preterm and acutely ill newborns.

MS/MS is a technology that is increasingly being adapted to newborn screening, allowing greater numbers of disorders to be detected presymptomatically. Early detection coupled with early intervention reduces morbidity and/or mortality. This report by Lacey et al. (7) presents an important advance in the screening and early diagnosis of CAH. MS/MS steroid profiling also offers the opportunity to better characterize the time course changes in adrenal synthetic function in the newborn period, especially in the context of newborns who are premature or have acute illnesses. If the positive predictive value can be improved with an improved cost:benefit ratio, other screening programs, both in the US and in other countries, may be more willing to add CAH to their newborn-screening panels. Once more is known about these issues and the method can be replicated in other laboratories and other populations, it may be possible to consider using the method as part of routine newborn screening.

References

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