Galactosemia Diagnosis Gets an Upgrade
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Classic galactosemia, a defect of the metabolism of galactose, was one of the earliest defects of intermediary metabolism to be recognized in the mid 20th century (1, 2). The disease is caused by a defect of galactose-1-phosphate uridylyltransferase (GALT), an enzyme central to the Leloir pathway in which galactose is converted into glucose. Galactose is primarily derived from the lactose content of milk that a newborn receives. The well-described and frequently taught clinical phenotype of an untreated newborn with classic galactosemia begins shortly after the onset of regular milk feeding and includes feeding intolerance with vomiting and diarrhea caused by galactose fermentation in the gut; hepatic failure leading to jaundice; bleeding manifestations; hypoalbuminemia and hypoglycemia; a renal Fanconi syndrome with loss of phosphate, glucose, and amino acids; congenital bilateral cataracts; and susceptibility to fatal gram-negative sepsis (primarily Escherichia coli). The hepatic and renal manifestations are caused by intracellular accumulation of galactose 1-phosphate, the substrate for GALT. Cataracts are due to an alternative metabolic pathway of galactose in the lens that leads to galactitol accumulation. Treatment is relatively simple. Once the diagnosis is suspected or confirmed, lactose and lactose-containing products are removed from the diet to reduce the metabolic burden, and most of the neonatal toxicities are alleviated.

Subsequent to the recognition of GALT deficiency, 2 additional enzymatic defects of galactose metabolism that also caused galactose accumulation were identified. Galactokinase (GALK) converts galactose to galactose 1-phosphate. GALK deficiency leads to failure to generate the cytoytic galactose 1-phosphate and therefore does not have the potentially fatal hepatic and renal manifestations. GALK-deficient patients are still at high risk for developing cataracts, which may be congenital or may develop later in life (3). The third enzyme is UDP-galactose 4-epimerase (GALE), which converts UDP-galactose to UDP-glucose, which subsequently is converted to glucose or glycogen as a source of energy. Patients with GALE deficiency do accumulate intracellular galactose 1-phosphate, and they may have hepatic and renal disease but typically not to the extent of that seen in GALT deficiency (4).

The diagnosis of GALT deficiency became even more complex when the mild Duarte GALT variant was identified. Individuals who are homozygous for the Duarte variant are without symptoms and do not require treatment (5). Individuals who are compound heterozygotes for classic galactosemia and the Duarte variant have also been identified. Whether treatment helps patients with these variants despite quite low GALT activities and galactosemia has not conclusively been demonstrated, and there is some debate as to whether the restrictive treatment required for classic GALT deficiency is necessary.

Most newborn-screening programs for galactosemia in the US monitor blood spot galactose concentrations with a fluorescence assay as a first-line screen and follow up with a fluorometric blood spot enzyme assay for GALT, known as the Beutler test (6). The Beutler test is essentially a screening procedure with the potential for false-positive results, particularly when it is used to attempt to differentiate the variant forms of galactosemia. Because of the urgent need to initiate dietary treatment in patients with classic galactosemia and a lesser degree of urgency or perhaps no need for treatment in the case of the compound heterozygotes, it is very important to be able to quickly and accurately distinguish between the 2 groups. A recent review of cutoff values for total galactose concentrations revealed that the fraction of false-positive cases may be high as 89% in some screening programs (7). The importance of any delay in getting a confirmatory result cannot be overemphasized, in terms of parental stress induced by waiting for an absolute diagnosis. It is also important to consider that a positive galactose screening result with a typical GALT screen does not exclude the possibility of GALK or GALE deficiencies or other genetic or infectious causes of hepatic dysfunction.

The metabolic clinical laboratory has long been involved in confirmatory testing for all 3 enzyme defects to determine the clinical importance of a positive

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2 Nonstandard abbreviations: GALT, galactose-1-phosphate uridylyltransferase; GALK, galactokinase; GALE, UDP-galactose 4-epimerase; UPLC-MS/MS, ultra-performance liquid chromatography–tandem mass spectrometry.
screening result. Following diagnostic confirmation, the laboratory is then required to longitudinally monitor the effectiveness of therapy in patients by measuring the concentrations of metabolic intermediates. Typically, the concentrations of red blood cell galactose 1-phosphate and urine galactitol are monitored for this purpose. These metabolites are initially monitored frequently while dietary regimens are being introduced and are monitored less frequently as patients stabilize on treatment.

Although methods for the measurement of these metabolites have been developed with state-of-the-art technology, primarily stable isotope–dilution selected ion–monitoring mass spectrometry (8–10), the methods that have been available for enzymatic confirmation have lagged behind. The methods are labor-intensive and time-consuming. The gold standard red cell GALT assay requires measurement of radioactive isotope exchange between UDP-galactose to UDP glucose, followed by a secondary linking enzyme reaction and chromatographic separation of the radiolabeled products before scintillation counting (6). The batch size is limiting, and over time we have begun to realize that the method lacks sufficient sensitivity at the low end to easily predict which patient will benefit and which may not benefit from treatment. To distinguish patients who have classic galactosemia (at high clinical risk) from those who are compound heterozygotes for a galactosemia mutation and the Duarte variant (at low clinical risk) requires an additional complex GALT isoelectric-focusing procedure to demonstrate the electrophoretic mobility of the variant proteins. Although molecular diagnostic testing is available for GALT deficiency, >200 different mutations have been described so far, and an assay of enzyme function remains central to defining a mutation’s importance (11). In some instances, a typical GALT activity in a galactose-positive screen needs to be followed up with either GALK or GALE enzymatic analysis, which is equally labor-intensive (6).

In this issue of Clinical Chemistry, 2 reports push the boundaries of analytical sensitivity and versatility for galactosemia diagnosis with ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) (12, 13). Both reports describe methods for measurement of GALT activity based on the use of a stable isotope–labeled substrate ([13C]galactose 1-phosphate). The enzymatic assay is performed offline, and the product, [13C]-labeled UDP-galactose, is separated by a short UPLC run, ionized by negative ion electrospray ionization, and monitored by multiple reaction monitoring with appropriate isototope-labeled internal standards. Both methods produce typical values for GALT that are consistent with those obtained with the traditional enzymatic assays. The study by Li et al. (12) focuses particularly on method sensitivity, and they demonstrate a CV of approximately 10% at enzyme activities that are 0.2% of typical, a degree of imprecision unobtainable with existing methodologies. Indeed, existing methodologies are mostly unable to distinguish below 5% residual activity. In clinical application of the method, the authors studied a large number of patients with classic and variant galactosemia and were able to clearly separate these groups. Interestingly, one patient in the study thought to have classic galactosemia was found instead to have measurable residual activity. Resequencing of the GALT gene demonstrated that the patient indeed had variant galactosemia but had previously been misclassified after a diagnosis based on the insensitive isotope-exchange enzyme assay.

The report by Ko et al. (13) describes an equally analytically sensitive GALT assay with a reference interval identical to that provided in the report by Li et al. This study did not focus on the ability of the GALT assay to distinguish variant forms of galactosemia, but rather on the ability to multiplex all 3 enzyme assays in a single test. The authors used additional isotopelabeled internal standards in performing 3 consecutive enzyme assays for GALT, GALK, and GALE. The products of the 3 reactions were then pooled for a single multiplex UPLC-MS/MS assay for all 3 enzymes that uses multiple reaction monitoring. Patients with GALK and GALE deficiencies were clearly distinguishable from control values. The shortcoming in this study of a relative paucity of material from real patients was overcome by analysis of enzyme activities in mutant cells developed by standard mutagenic procedures.

With relatively simple enzymological procedures and on-column analytical run times of <30 min per sample with unlimited batch size, these studies taken together have clearly redefined the gold standard for laboratory confirmation of galactosemia diagnosis. Variant forms of galactosemia can now be readily identified, and both GALK and GALE can be assayed simultaneously, saving considerable additional efforts. At this point, neither study has addressed issues related to improving turnaround times for confirmatory diagnosis. Intuitively, however, the assays are less time-consuming than the older assays, and we anticipate an improved performance leading to shorter times to result availability, with consequently less parental stress from the use of this advanced methodology.

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