Molecular Diagnosis of Medium-Chain Acyl-CoA Dehydrogenase Deficiency by Oligonucleotide Ligation Assay

In the current issue of *Clinical Chemistry*, Romppanen et al. [1] report a molecular screening study in a Finnish population for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, the most common inherited disorder of fatty acid β-oxidation [2]. This metabolic disorder, which is transmitted as an autosomal recessive trait, can be regarded as a paradigm for inborn errors of metabolism. As seen in many other genetic disorders, a phenotypic heterogeneity frequently is expressed within the same family, ranging from sudden infant death to asymptomatic individuals [3]. The disorder is one of exacerbations and remissions and was initially recognized in children with hypoketogenic hypoglycemia, Reye syndrome, and (or) liver failure, frequently resulting in severe brain damage [2]. The pathophysiology of MCAD deficiency involves the inability of cells to oxidize fatty acids 4–12 carbons long. A secondary effect of the metabolic block is systemic carnitine deficiency, further compromising fatty acid metabolism. Thus, clinical attacks are precipitated during fasting or intercurrent illnesses when fat mobilization and fatty acid oxidation are induced. Biochemical markers that are diagnostic of MCAD deficiency include urine hexanoylglycine and phenylpropionylglycine [4] and plasma and urine octanoylcarnitine [5].

If the disease is recognized before severe sequelae ensue, conservative treatment results in an excellent prognosis. Therapy involves anticipation and prevention of metabolic decompensation by administration of glucose-containing fluids and L-carnitine. This treatment during an impending catabolic crisis prevents the combination of severe hypoglycemia, dehydration, and metabolic acidosis that might otherwise cause death or brain damage.

In comparison with other inborn errors of metabolism, MCAD deficiency has a relatively higher frequency in many European countries, where the prevalence can be as high as 1:10 000 [6]. The great majority of affected alleles carry an A985G transition (causing Lys329Glu substitution), which seems to have been propagated by a founder effect [6]. The G985 allele is common in middle and northern Europe but less frequent in southern Europe [6]. On the basis of the above observations, the disorder has been proposed as a target for newborn mass screening [7].

Romppanen et al. [1] applied a DNA-based screening method, the oligonucleotide ligation assay (OLA), to determine the frequency of the A985G mutant allele in a Finnish population. The OLA technology, which was described first by Landegren et al. in 1988 [8], is a combination of PCR and ELISA methods and has been developed for screening a large number of samples for one or a few mutations. Results can be interpreted by visualization of color change in microtiter plates with either the naked eye or a spectrophotometer. The test obviates the need for gel electrophoresis, radioactivity detection, or endonuclease digestion applied in other DNA-based methods. Romppanen et al., besides reporting the frequency of the prevalent MCAD allele in the screened population, conclude that OLA is applicable to population screening; thus, any specific mutation in any gene could be tested by this technique.

This paper raises important issues on the applicability of DNA-based methods for mass screening. The first point to consider is the scientific data needed for a specific method before it can be determined as suitable for mass screening. The second question is what screening method is most suitable for MCAD deficiency or other inborn errors of metabolism. The advantage of the OLA technique is its use of microtiter plates for reading many samples simultaneously. The high signal-to-noise ratio of this test is also very beneficial. Without automation, however, the sample preparation procedure reported in this paper is tedious, repetitive, and time consuming and therefore is more likely to be subject to human errors. As applied herein, the method involved genomic DNA extraction from whole blood, which may not be economically feasible for mass screening. Furthermore, amplified DNA, prepared separately by PCR, adds an additional time-consuming step, further increasing the cost of the test. A robotic workstation has been applied previously for the OLA procedure to screen for common genetic diseases [9], and such automation is essential for the use of OLA in mass screening.

In the case of newborn screening, only a very small amount of blood spotted on Guthrie cards is available, and previous studies have used these samples for successful PCR-based screening of the G985 MCAD allele [10, 11]. Before OLA can be applied for mass screening, use of the Guthrie card blood sample or some other easily obtainable source of DNA (e.g., buccal scraping) should be optimized and incorporated into the method. Carryover or external contamination, unsuccessful DNA amplification, and allele drop-out in PCR are all problems, especially when a very small amount of DNA is available. These shortcomings will need to be overcome by further method development and automation.

The method described in this issue has not been tested for false-negative results in a satisfactory fashion. The frequency of the A985G mutation in Finland by this method is very low, much lower than in neighboring countries and much lower than observed in a previous Finnish study, albeit one that dealt with a smaller population [12]. Although Romppanen et al. [1] may be reporting the true frequency (if Finland avoided the founder effect of this mutation in Europe), all samples that tested as normal should have been confirmed by an alternative
DNA assay to rule out the occurrence of false-negative results. It would also be useful to have a cost analysis of the OLA method for comparison with other currently used screening methods.

Newborn mass screening for MCAD deficiency by using tandem mass spectrometry (MS-MS) to measure acyl-carnitines has already been shown to be feasible and reliable [7, 13]. That sensitive method, which is applicable to a small amount of blood spotted on paper, does not have the shortcomings of missing patients with alleles other than A985G. Moreover, several additional inherited metabolic disorders can be screened simultaneously by the same acyl-carnitine assay with the application of computerized automation [13, 14]. On the other hand, such marker-based screening does not detect heterozygotes, who are readily identified by molecular methods. Nevertheless, MS-MS screening is currently more practical overall for MCAD deficiency than are molecular methods. Despite the many pilot studies for mass screening with PCR-based tests, current newborn screening for inherited metabolic disorders still relies on Guthrie’s bacterial inhibition assay [15] or other simple methods for detection of disease markers. The molecular tests yet need to be further developed and automated to become feasible for mass screening. Automation of the OLA and other molecular methods are likely to provide analytical tools that may be suitable for mass screening in the future; we need to ensure that they are investigated thoroughly before implementation.

Lastly, one important point must be considered. Before screening for MCAD deficiency or any other disorder is implemented, a prospective, population-based screening research study needs to be performed. Such a study should use rigorous methods for ascertaining cases diagnosed by screening or clinically to learn as much as possible about the natural history of the disease to be screened. We need to know how and the impact of screening on it. We currently have only a biased view of MCAD deficiency from clinically diagnosed cases [16] and need to know the full clinical spectrum of patients that escape diagnosis. This information is required to devise the best strategy for screening, follow up, and treatment to estimate the cost/benefit ratio of mass screening.

References

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