There are more than 40 lysosomal disorders, and although individually rare, they are collectively a significant group of disorders with life-altering manifestations not only for patients but with equally devastating effects for their families (1). The cost of healthcare for an individual affected with one of these disorders is exceedingly high over an often shortened life span. The diseases are a result of the failure of one or more lysosomal enzymes to degrade the generally polymeric biomaterials that are brought into the organelle for digestion. In the case of the mucopolysaccharidoses, a single enzyme defect leads to a block in the sequential degradation of glycosaminoglycans, and the undigested fragments accumulate in the lysosomes, which become distended and gradually compromise cellular function. The symptoms organomegaly, skeletal deformity, and mental retardation result from this accumulation, and the latter two are generally irreversible in nature. Early intervention with therapies has therefore become the goal of laboratories involved in the diagnosis and treatment of these patients. For this to be possible, because so many of the symptoms that develop are irreversible, presymptomatic diagnosis is essential. Since the advent of methods for the treatment of many of the lysosomal disorders, screening tests are being called for more often. Although bone marrow transplantation treatment for lysosomal storage disorders (LSDs) has been in use for over 15 years, enzyme replacement therapy trials are now ongoing for several lysosomal disorders. Many of them are into the phase 3 stage before adoption as fully approved treatments.

Diagnosis of the LSDs by enzyme assay is performed in only a small number of specialist laboratories worldwide. The assays are performed using an array of substrates that may variously be colorimetric, fluorogenic, or radiolabeled. To narrow the focus down to a few of the 40 possible disorders, the testing laboratory is dependent on the use of a screening test. Although urine screening tests have been in use for some time to measure concentrations of stored glycosaminoglycans (2) or neutral oligosaccharides (3), they tend to be less effective for the less severe cases. These tests are not presymptomatic, but they are useful because, although they are specific for a small subset of the LSDs, they help to narrow down the possibilities to just a few enzymes that need to be tested. Many attempts have been made to develop quantitative versions of these tests, mainly HPLC based, but these have not been widely adopted (4). Another approach is to develop screening tests for the lysosomal disorders based on the ELISA detection of lysosomal membrane proteins, such as LAMP 1 and/or saposins, as markers of lysosomal proliferation (5). This approach has the advantage of detecting almost all of the 40-plus lysosomal disorders and requires follow-up with second-tier screening by enzyme assay for confirmation.

Mass spectrometry has become the tool of choice for the detection of many, perhaps all, metabolic disorders. Mass spectrometry can be said to now lead the field of clinical chemistry methodologies. The possibilities seem limited only by the ability of chemists to design appropriate standards and by the collective will to advance the field.

The aim of Li et al. (6) is to use the mass spectrometer as a common platform for the screening of more, perhaps all, of the lysosomal disorders. Their approach is to develop a set of substrates with which to assay the activities of the lysosomal enzymes in blood spots punched from Guthrie cards. Assaying the enzyme activity directly as a screening test gets around the question of whether there has been time to accumulate substrate in those patients with a small amount of residual enzyme activity, who have less severe symptoms. The enzyme deficiency will always exist, no matter how old or young the patient. The test requires specific substrates made more hydrophobic synthetically to allow the required sample clean up via silica adsorption to prepare the sample for mass spectrometry. This is primarily for the removal of salts and other small components that interfere with the spray ionization of the samples. Each of the products of digestion of these synthetic substrates is paired with an internal standard of identical chemical structure but that differs in mass by containing either a longer aliphatic chain or a per-deuterated phenyl group. Li et al. (6) have shown that because these substrates have unique molecular weights, they are then capable of being “multiplexed” for the mass spectrometric detection step by being mixed and analyzed together. A further display of the ingenuity in this approach is the use of the collision-induced degradation cell within the tandem mass spectrometer to allow the detection of a fragment ion common to several of the substrates. Thus, multiple-reaction monitoring pair detection is run in conjunction with Q1 scanning to develop the full power of this approach.

The whole approach benefits from the long-term plans of the Gelb/Scott collaboration to develop a single mass spectrometry platform for as many of the lysosomal disorders as can be arranged within one multiplexed assay pass. They have previously signaled their intent with the development of a single substrate with which to assay all four of the Sanfilippo subtypes within the mucopolysaccharidoses group of disorders (7). We await with interest the further development of this series of substrates with which to assay the remainder of the lysosomal enzymes in blood spots by this novel approach.

References

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