Editorial

A specific substrate assay for lysosomal acid lipase paves the way to neonatal screening and better identification of patients with potentially treatable genetic disease

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There are numerous problems with identifying people who have rare diseases (1): these include:

- Ethical: it would not right to seek to find people who have a problem that cannot be treated because they would derive no benefit from being identified but could suffer negative psychological and economic consequences of the disease, even if it was not affecting them physically;
- Educational: in the words of Donald Rumsfeld, "*There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know.*" When a doctor does not know that certain diseases exist, they are unlikely to know that a specific test is needed, and therefore they are unlikely to initiate appropriate investigations;
- Organisational: even when the index of suspicion has been raised sufficiently highly to suggest that a specific disease should be considered, it may be difficult to find a centre where the test can be carried out or it may be logistically difficult to arrange for the relevant test to be carried out;
- Financial: screening programs are generally only instituted when the cost of identifying a problem has a good chance of providing a cost benefit or can be achieved at a low cost.

Some of these issues are now being dealt with for lysosomal acid lipase (LAL) deficiency, which causes Wolman disease (the severe infantile form) and Cholesteryl Ester Storage Disorder (CESD) (the milder adult form of the deficiency). Continuous accumulation of cholesteryl esters and triglycerides occurs in the liver, blood vessel walls and other tissues as a result of reduced/absent activity of the LAL enzyme. This can result in progressive organ damage in the affected tissues (2,3). Treatment is now possible using a recombinant LAL enzyme—sebelipase alfa (Kanuma®) (Alexion Pharmaceuticals Ltd., Boston, USA), which is a recombinant human LAL synthesized in chickens genetically engineered to secrete the protein into the egg white. Sebelipase alfa is a glycoprotein (molecular weight 55 kDa) having a human-identical amino acid sequence. Carbohydrate-containing elements of sebelipase alfa include *N*-acetylglucosamine, mannose-6-phosphate (M6P), and mannose-terminated *N*-linked glycan structures allow targeted delivery of LAL to the lysosomal compartment due to specific binding to the macrophage mannose receptor on reticuloendothelial cells and the M6P receptor (4,5).

Since treatment is now possible, it becomes ethical to find people who may have LAL deficiencies so they can be treated and a number of studies are underway to try to identify the best strategy to use to find affected individuals. One such strategy is to use information already available in laboratory databases to try to target people who have signs that may indicate they are at a higher risk of having the disease in question. Thus, for adult onset CESD, it is known that liver damage occurs, which could lead to increased serum alanine or aspartate transaminase activity and that lipid biology is also affected leading to low serum

concentrations of high-density lipoprotein-cholesterol (6).

This is where the organisational issues become important. Traditional tests for lysosomal disorders relied upon assaying activity of the LAL enzyme in peripheral lymphocytes or cultured fibroblast cells. 'Wet' samples have the major problem that they must arrive at the testing laboratory very quickly because lymphocytes lyse after death, and the lysosomal enzyme of interest is degraded by other serum enzymes. In addition to speed of sample delivery to the testing laboratory, it is essential that samples arrive when there is sufficient capacity for analysis to commence immediately (e.g., not at weekends) because of the sample instability which limits the days when screening can be carried out to early in the week.

To make testing more practical, a system for stabilisation of the sample is essential. This has been developed using dried blood spot (DBS) samples in which the LAL present in lymphocytes is preserved by drying onto filter paper. Unfortunately, this has one fundamental problem. Whereas assaying activity in lymphocytes assays only the lysosomal enzyme in the lymphocytes, analysis of blood spot samples also assays the activity of enzymes in serum that can act upon the same substrate as the lysosomal enzyme. This was initially solved by Hamilton *et al.*, who developed a specific inhibitor (Lalistat-2) which meant that the activity of lysosomal enzyme in a DBS punch could be estimated by subtracting the Lalistat-inhibited activity from the total DBS punch activity (7).

Obviously, carrying out 2 assays to derive a result by subtraction has its disadvantages such as increasing imprecision and a single assay that allows the specific enzyme of interest to be measured in a single assay is preferable. Thus, the identification of a substrate that is specifically acted upon by the LAL enzyme is an important development. Masi *et al.* (8), tested known LAL substrates and a large number of novel fatty acid and cholesteryl esters to identify 3 compounds that appeared to be specific for LAL, and finally developed a functioning tandem mass spectrometric assay using P-PMHC (palmitoyl-4-propyl-8-methyl-7-hydroxycoumarin) which is hydrolysed to palmitoleate and PMHC by LAL. A 13C substituted PMHC internal standard was used to calculate results. Having thus identified a specific substrate, it was then possible to create a fluorometric method because PMHC fluoresces, that means that assays can be carried out using a standard plate reader assay technique.

The large number of substrates tested and the complex chemistry needed to create many of the novel molecules is extremely impressive and clearly it took a massive research effort to identify the optimum molecule for assaying LAL. This is however very worthwhile because by making this into a single stage assay, many potential errors are removed so the precision of the assay of LAL must be greatly enhanced which as stated in their discussion could reduce the cut off threshold for neonatal screening and thus the false positive rate. However, it has to be stated that the Hamilton assay (7) was very effective because the activity causing disease was so low that false positives were very unlikely.

In addition to the replacement enzyme that has been developed for LAL deficiency, similar drugs are also available for other equally rare lysosomal storage diseases:

- Pompé disease [alglucosidase alfa, (Lumizyme®), Sanofi-Genzyme, Cambridge, Massachusetts, USA];
- Gaucher disease [imiglucerase, (Cerezyme®), Sanofi-Genzyme; velaglucerase alfa, (VPRIV®), Shire Pharmaceutical Holdings, Ireland; taliglucerase alfa, (Elelyso®), Pfizer, New York, USA];
- Fabry disease [agalsidase beta (Fabrazyme®), Sanofi-Genzyme].

It therefore becomes appropriate for healthcare providers to consider offering screening programs to identify people who could benefit from these products. Therefore, perhaps the most exciting prospect of this research into a specific substrate for LAL is the possibility that similar techniques to identify substrates could be used to develop assays for other lysosomal enzymes to create a multiplex assay to be used in a neonatal screening program for a number of lysosomal storage defects.

In conclusion, rare diseases occur rarely and therefore are often not identified until it is too late. With the development of new drugs for effective treatment of these diseases, it is becoming essential that we are able to identify those affected so they can receive effective treatment early before irreversible damage has built up. The detailed work described by Masi *et al.* shows that we have the technology to be able to identify specific substrates that could allow the development of an effective neonatal screening test for lysosomal storage disorders. More research of this type is needed but this is an extremely good start!

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