Editorial

Importance of phenotype estimation for Krabbe disease with a newly developed diagnostic procedure in the era of advanced medical treatment

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Comment on: Liao HC, Spacil Z, Ghomashchi F, *et al*. Lymphocyte Galactocerebrosidase Activity by LC-MS/MS for Post-Newborn Screening Evaluation of Krabbe Disease. Clin Chem 2017;63:1363-9.

Received: 10 October 2017; Accepted: 30 October 2017; Published: 10 November 2017.

doi: 10.21037/jlpm.2017.10.04

View this article at: http://dx.doi.org/10.21037/jlpm.2017.10.04

Krabbe disease is one of the metabolic leukodystrophies caused by dysfunction of the hydrophobic lysosomal enzyme, galactocerebrosidase (EC 3.2.1.46) which is encoded by the *GALC* gene. Krabbe disease was first reported as a clinical entity 100 years ago, in 1916, by Knud Krabbe (1). The disease frequency is about 1/100,000–1/200,000 (2). It is recognized as one of the predominant genetic diseases, showing leukodystrophy from infancy to adulthood.

The clinical phenotype of Krabbe disease has been classified as infantile and late-onset types based on age at the onset of disease manifestation (2). Loonen and colleagues (3) sub-classified the late-onset phenotype into late-infantile (onset from 7 months to 3 years), juvenile (onset from 3 to 8 years), and adult (onset after 9 years) types. It has been reported that most patients have the infantile phenotype, with the late-onset phenotype being recognized in about 10% of patients. However, there are several reports indicating a higher frequency of patients belonging to the late-onset phenotype (4,5).

Diagnosis has been basically performed with an enzyme assay for galactocerebrosidase using lymphocytes or skin fibroblasts. For the enzyme assay, galactocerebroside was originally used as the natural substrate with tritium labeling (6); however, the artificial fluorescent substrate 6-hexadecanoylamino-4-methylumbelliferyl-β-D-galactopyranoside has recently been used for disease diagnosis (7). The assay using galactocerebrosidase is reliable as it uses a natural substrate. The assay with fluorescent substrate is also reliable and does not need to be performed in an environment requiring radioactive containment.

However, there are several problems with enzyme assays for Krabbe disease. First, the specific enzyme activity of *GALC* for healthy people is rather low compared to other lysosomal enzymes, which makes it difficult to differentiate patients from normal controls. This means that it is more difficult to differentiate residual enzyme activity for patients in order to estimate their clinical phenotypes on the assumption that clinical severity is dependent on residual activity (8). Second, the specific activity might differ between natural substrates and the artificial fluorescent substrate, although it is believed to reliable for biochemical diagnosis. Third, as a more complicated situation, the specific activity might differ between different natural substrates. Endogenous galactocerebrosidase can digest several endogenous substrates in tissues, such as galactocerebroside, lactosyl cerebroside, and psychosine. Psychosine, in particular, is recognized as a pathogenic material from its toxic effect towards neuronal cells in patients. This concept has been called the "psychosine hypothesis" (9). Harzer and colleagues (10) analyzed substrate specificity for several mutations and recognized the G270D mutation, which contributes to the mild phenotype, has nearly normal activity for psychosine, although it leads to a loss of enzymatic activity for galactocerebroside. This result is still controversial (11), but it may be important to consider the real enzyme activity

towards the actual pathogenic substrate.

Krivit and colleagues (12) reported the first five patients with Krabbe disease treated by hematopoietic stem cell transplantation (HSCT) in 1998. So far, this is the only clinical treatment for Krabbe disease proven to be effective, but only when it is applied at a very early stage of the disease. For patients with infantile type disease, it is necessary to perform HSCT at less than 2 months of age (13).

It is an essential concept for newborn screening that it is used for diseases where it is possible to diagnose and treat sufficiently early. With newborn screening for Krabbe disease, it is not only essentially important to diagnose the disease precisely but also distinguish its clinical phenotype, i.e., infantile or later-onset phenotypes. Enzymatic diagnosis of dried blood spots using tandem mass spectrometry (MS/MS) and a synthetic substrate has been reported as a newborn screening method in New York State (14). The established criteria to diagnose the infantile phenotype, which mostly depends on genetic testing, uses the knowledge that a homozygous state for the most frequent infantile mutation (the 30-kb deletion) reveals the infantile phenotype (15), although this is not sufficient for all affected babies (16). Another method to evaluate the clinical phenotype before clinical onset is to measure the level of serum psychosine in dried blood spots (17,18). This showed that the serum level of psychosine for the infantile phenotype of the disease is higher than that of later onset phenotypes, suggesting the possibility of evaluating clinical severity. However, there are always exceptional cases and evaluation is not perfect.

Dr. Liao and colleagues (19) have recently reported a new enzyme assay for galactocerebrosidase in T-lymphocytes using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This assay includes several characteristics that need to be pointed out. First, the assay uses T-lymphocytes separated from venous blood using magnetic beads containing antibody to the cell surface CD3 protein. Second, a close structural analog (galactosylceramide with a heptanoyl fatty acyl chain) of the natural substrate is used in the reaction and the internal standard is the same material with deuterium substitution. Third, the reaction buffer is optimized to use citrate-phosphate buffer pH 4.2, which provides better activity for the *GALC* enzyme.

The authors found that analytical range of this assay was 20-fold greater than the conventional radiometric *GALC* assay. This means that this new assay can distinguish low activities down to 0.3% of normal samples, which is generally within the error range of the conventional method. The authors observed that there is a correlation between residual enzyme activity and disease severity in a few patient samples they tested. This suggests the important possibility of phenotype estimation but many patient samples to be tested for confirmation. Another point for the assay is that it was checked its specificity under different conditions and it was influenced by other beta-galactosidases at very small extent.

It is very important to have a very sensitive and specific enzyme assay for accurate diagnosis for positive samples during newborn screening for Krabbe disease. In this sense, this study has succeeded in setting up new method for enzyme assay with high sensitivity and specificity. However, it is not perfect for evaluation of the disease severity to depend on this very sensitive assay alone. To succeed in setting-up newborn screening for Krabbe disease, it might be necessary to develop a new algorithm to differentiate the disease phenotype according to residual enzyme activity, serum level of psychosine, and detailed genotype information.

The New York model of newborn screening for Krabbe raises the problem of efficacy and safety for HSCT in very early infancy (20). We are still straggling in the development of better treatment options such as enzyme replacement into the central nervous system or gene therapy. However, we can now hope to have better chance of developing a sophisticated protocol of newborn screening for Krabbe disease by combining the new assay method with more established treatment options in near future.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Executive Editor Dr. Zhi-De Hu (Department of Laboratory Medicine, General Hospital of Ji'nan Military Region, Ji'nan, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2017.10.04). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/jlpm.2017.10.04
Cite this article as: Sakai N. Importance of phenotype estimation for Krabbe disease with a newly developed diagnostic procedure in the era of advanced medical treatment. J Lab Precis Med 2017;2:87.