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Application News

LC-MS LCMS-8060NX
GC-MS GCMS-TQ™8040 NX

Metabolomic differential analysis of gene-mutated Drosophila using LC/MS and GC/MS

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User Benefits

- Obtained LC/MS and GC/MS data can be easily visualized by Multi-omics Analysis Package as the software has an intuitive and user-friendly interface.
- The greater number of metabolites can be comprehensively displayed by integrating the results of LC/MS and GC/MS measurements and outputting them on a metabolic map.

■ Introduction

A genetic mutation is a change in the sequence of a DNA base, which can be a substitution, an insertion, or a deletion. They are divided into innate ones inherited from parents and acquired ones due to environmental factors (e.g., radiation, chemicals, eating habits, microorganisms, etc.) or errors in DNA replication during cell division. The innate will be present in almost every cell of the body throughout life. This mutation is also called a germline mutation because it is present in the parent's germ cells[(1)].

Acquired ones, on the other hand, are called somatic mutations, mutations that occur only in specific cells of the body, not in every cell. Newborn mutations that occur in the offspring can be inherited or somatic[(1)].

Genetic mutations in the DNA sequence may prevent proteins from being synthesized correctly or from functioning. Diseases caused by one or more gene mutations are called hereditary diseases and include Lysosome disease, muscular dystrophy, and phenylketonuria.

In this application, long-lived gene mutations were generated in Drosophila yellow, and metabolites in wild and mutant Drosophila were measured by liquid chromatograph mass spectrometer (LCMS-8060NX) and gas chromatograph mass spectrometer (GCMS-TQ 8040 NX). For more details on GC-MS measurement results, please refer to Application News 01-00410.

In order to find differences between wild and mutant strains from the measurement results, we analyzed them using Multi-omics Analysis Package. Multi-omics Analysis Package is an analysis software with data visualization features such as multivariate analysis (such as principal component analysis and class cluster analysis), volcano plots, and metabolic maps. This application news presents examples of analyses using these statistical methods to compare wild and genetically mutated Drosophila and analyze differences in metabolites.

Fig. 1 LC-40 and LCMS™-8060NX

■ Experimental

In this application, 100 Drosophila were used for analysis. Of the 100 flies, 50 were wild and the remaining 50 were genetically mutated. Each sample was formed with 5 flies and a total of 20 samples were prepared : 10 wild type samples and 10 genetically mutant samples. Fly samples were ground and then pretreated according to the Metabolomics Pretreatment Handbook and measured using the primary metabolite method package and LCMS™ -8060NX[(2)].

■ Results from LC/MS measurement

Principal component analysis was performed using Multi-omics Analysis Package to visualize differences between wild type and genetically mutant metabolites. In principal component analysis using the 89 detected components, PC1 and PC2 accounted for 29.3 % and 18.0 %, respectively, giving a cumulative contribution of 47.3 % for both. The 10 wild type and 10 genetically mutant samples visually clustered into two groups (Fig. 2).

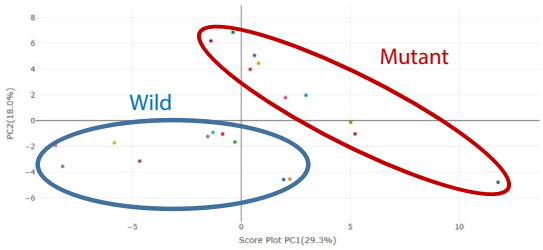

Fig. 2 Score Plot by Principal Component Analysis

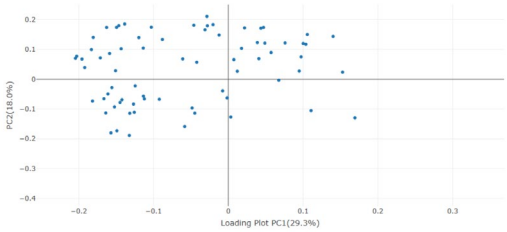

Fig. 3 Loading Plot by Principal Component Analysis

A loading plot of principal component analysis revealed that Kynurenic acid, Vitamin B12, and Methionine sulfoxide were specifically present in the wild type, while Citrulline, Proline, and Carnitine were characteristically present in the genetically mutated (Fig. 3). In addition, regarding the samples displayed in the lower right of the plot among the genetically mutated, it was shown that the content of Malic acid and Aspartic acid had an effect.

Fig. 4 shows the results of LC/MS measurements with a volcano plot. In the upper left of the volcano plot, the most detected compounds in the wild type are Kynurenic acid, Kynurenine, and Vitamin B12. Conversely, the top right of the volcano plot is a compound that is common in genetically mutated, and Citrulline is the only one shown. From these findings, it was found that compounds with significant differences in metabolites between wild type and genetic mutant were rare, and most compounds were detected equally.

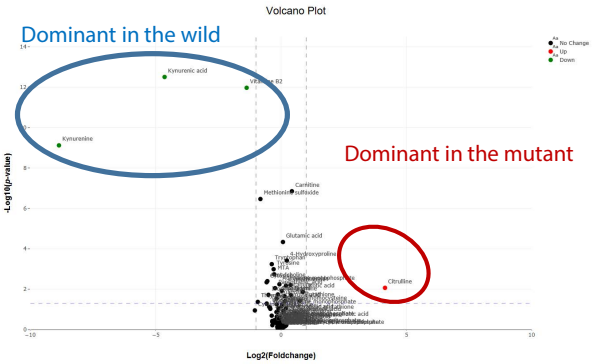

Fig. 4 Volcano plot

Multi-omics Analysis Package can display detected compounds as box plots. For example, Kynurenine, which was characteristically detected in the wild type in Fig. 4 above, is shown below in a box plot (Fig. 5). The 10 on the left are 10 wild types and the 10 on the right are 10 genetic mutants, but it can also be seen that Kynurenine is detected only in the wild type.

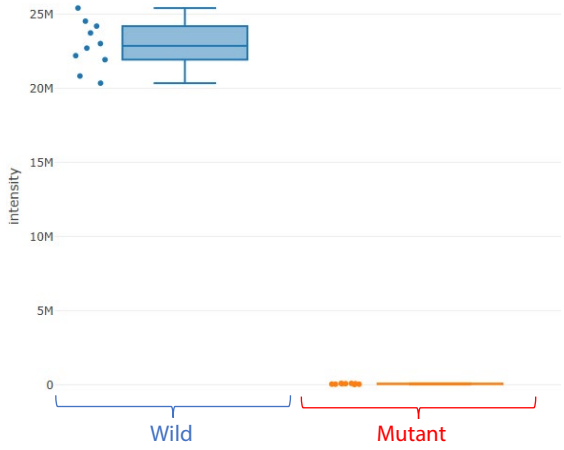

Fig. 5 Box plot

■ Results from integrated analysis of LC/MS and GC/MS

After identifying the compounds that are unique to each wild type and genetic mutant type by principal component analysis and a volcano plot, the comparison of the area values of each compound was displayed by bar graph using a metabolic map showing the metabolic pathways. GC/MS, which required more Drosophila per sample, measured only 5 samples (50 animals), so the LC/MS results (10 wild type samples and 10 genetically mutated samples) showed only 2 representative samples of wild type and 3 samples of genetic mutant (Fig. 6). The metabolic pathway of Kynurenic acid, which was characteristically detected in the wild type on the volcano plot, is enlarged and shown in Fig. 7. In addition to compounds detected by GC/MS (3-Hydroxy-kynurenine, Kynurenine, Tryptophan, 5-Hydroxy-tryptophan), Kynurenic acid measured by LC/MS was added to the metabolic map. This showed that all compounds in this metabolic pathway had high content only in the wild type.

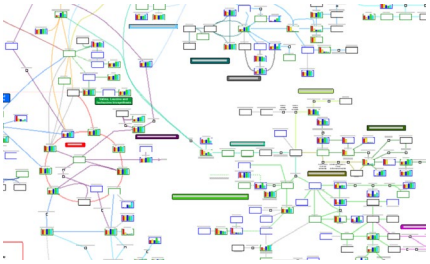

Fig. 6 Metabolic map

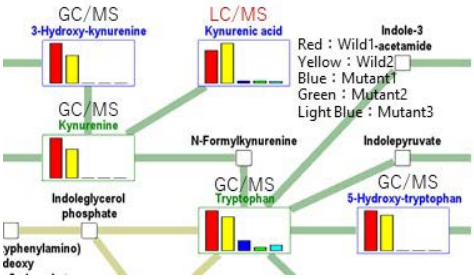

Fig. 7 Close-up of metabolic pathway map
(integration of LC/MS and GC/MS measurements)

■ Conclusion

Metabolites from wild and genetically mutated yellow Drosophila were analyzed by liquid chromatograph mass spectrometer LCMS-8060NX. The results of the comprehensive detection of metabolites were analyzed using Multi-omics Analysis Package with a variety of statistical analysis methods, including principal component analysis and a volcano plot. As a result, the metabolites that differed due to genetic mutations were visualized. Metabolic maps were projected with GC/MS and LC/MS measurements to show metabolic pathways. By using Multi-omics Analysis Package, it is possible to objectively judge the results obtained using LC-MS.

<Reference>
(1) What is genetic disease plus, genetic mutation? How does it happen?, accessed 20th June 2022 https://genetics.qlife.jp/tutorials/Variants-and-Health/What-is-a-gene-mutation-and-how-do-mutations-occur
(2) Shimadzu Corporation, Metabolomics Pretreatment Handbook, accessed 9th June 2022 https://www.an.shimadzu.co.jp/pdf/c146-2181.pdf

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