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Application News

Metabolite Analysis by Muscle Measuring System and GC/MS

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User Benefits

- Volcano plot results can identify significant differences in metabolic pathway positions.
- A multi-omics analysis package can detect digital biomarkers with a large number of features, using both primary metabolite and muscle strength measurement data.

■ Introduction

Frailty (muscle weakness) is a condition that falls between the healthy state and the care-requiring state. It is associated with physical and cognitive decline and is more likely to affect older people (Fig. 1). This condition affects 8.7 % of the elderly in Japan and 5 % in the United States, significantly impacting medical insurance in each country [1), 2)]. However, muscle strength measurement alone cannot infer and study the causes of muscle weakness and cannot provide enough information to prevent or improve frailty.

Metabolite measurements are important better to understand the mechanism and progress of muscle weakness. For example, muscle weakness can cause an imbalance in metabolites. Specific approaches to prevent or improve frailty can be explored using metabolite measurements.

In addition, metabolite measurements are essential to evaluate the therapeutic effects of frailty. By tracking changes in metabolites and muscle strength, doctors can more accurately assess the effects of treatment and prognosis. For example, if an increase in a particular metabolite indicates treatment efficacy, the treatment plan can be adjusted based on the measurement of that metabolite.

Frailty definition (>2 items applicable : frail)

- Weight loss
  (unintentional weight loss of ≥ 4.5 kg/year)
- Fatigue
  (feeling lazy to do anything for more than 3 days a week)
- Walking speed decreased
  (<1 second/second)
- Decreased grip strength
  (< 28 kg for men and < 18 kg for women)
- Decreased physical activity
  (for men <383 kcal/wk and women <270 kcal/wk)

Fig. 1 Overview of Frailty [3)]

Fig. 2 Muscle Measurement Device (Left) GCMS-TQ™8040 NX (Right)

On the other hand, measuring metabolites also presents some challenges. Metabolite measurements require specialized knowledge and skills, and caution in measurement and interpretation is required. Also, the measurement can be time-consuming and expensive.

In this study, we measured the serum of two groups of healthy subjects with normal muscle strength measurements (robust subjects) and those with muscle weakness (frail subjects) using gas chromatograph mass spectrometers GCMSTM-TQ™8040 NX and Smart Metabolites Database™ Ver. 2 (Fig. 2).

Using the database eliminates the specialized knowledge and techniques required for method development. Even though it is a simultaneous measurement of more than 500 components, it was completed in 23 minutes. For statistical analysis, the multi-omics analysis package (Garuda) was used to visualize a new visualization method that combines a volcano plot and a metabolic pathway and to visualize and analyze the correlation between muscle strength measurement data and each metabolite on the metabolic pathway map.

■ Experimental

Sera from 114 healthy subjects (Female, Japanese, robust n=75, frail n=39) aged 65 to 80 years were used for the analysis. Preprocessing was performed per the Metabolomics Preprocessing Handbook [4)], and GC-MS analysis was performed using the Smart Metabolites Database Ver. 2. Using Multiple Reaction Monitoring (MRM) as the data collection mode, we were able to sensitively measure more than 500 compounds with an analysis time of 23 minutes. For details on the sample preparation method and analysis conditions, please refer to application news No. 01-00410 "Metabolomic Differential Analysis of Gene Mutant Drosophila Using GC/MS."

As for muscle strength measurement, grip strength (Grip), walking speed (G.A.I.T.), limb muscle mass (SMI), and body mass index (BMI) were included. Previous studies have also suggested a correlation between loss of grip strength and the risk of developing dementia, and a correlation between body mass index (BMI) and the risk of death from cancer, heart disease, and cerebrovascular disease [5), 6), 7)]. Statistical analysis to make these markers (digital biomarkers) is attracting attention because they can be measured without visiting a hospital, are non-invasive, and do not cost much.

■ Volcano plot on pathways

Volcano plot is a technique to detect statistically significant variables between two groups, where the horizontal axis is the ratio of the values of each compound or muscle strength measurement item (robust/frail), and the vertical axis is an index of statistical significance (p-value). Compounds and plyometrics with a horizontal axis close to 0 (middle) indicate similar areas between the two groups, whereas compounds and plyometrics with a high vertical axis indicate statistically significant differences (low p-values).

Octanoic acid, glyceric acid, and 4-hydroxyphenylacetic acid were detected at low concentrations in frail subjects. On the other hand, the compound detected at higher concentrations was 2-deoxyglucose, which is enclosed in a red box. G.A.I.T and BMI were also found to be higher in people with frailty. Octanoic acid, a medium-chain fatty acid, is known to suppress fat accumulation and has been reported to reduce waist circumference, body fat, and visceral fat in human clinical studies [8]. Low levels of octanoic acid and high body mass index (BMI) in frail patients suggest an association between obesity and frailty.

Glyceric acid is also known as a compound that exists in fat cells as a neutral fat by combining with fatty acids such as octanoic acid. The low levels of fatty acids and glycerin may be due to the high levels of triglycerides, which are their precursors, suggesting a decrease in the function of lipase, which hydrolyzes triglycerides.

2-Deoxyglucose, which was detected at high levels in frail subjects, is a glucose derivative in which the 2-hydroxyl group of glucose is replaced by a hydrogen atom. Like glucose, 2-deoxyglucose is taken up into cells using the glucose transporter GLUT1. Since it cannot be metabolized by glycolytic enzymes, it accumulates in cells and is known to inhibit the glycolytic pathway. The finding that such inhibitory metabolites were detected at significantly higher concentrations in individuals with frailty suggests that glucose metabolism may play an important role in the health of the elderly.

The volcano plot results (i.e., color-coded dots: red, green, and black) were projected onto a metabolic pathway map to explore the impact of frailty symptoms on metabolic pathways (Fig. 3). Metabolites in the glycerolipid pathway, shikimate pathway, and tryptophan production pathway were found to be affected.

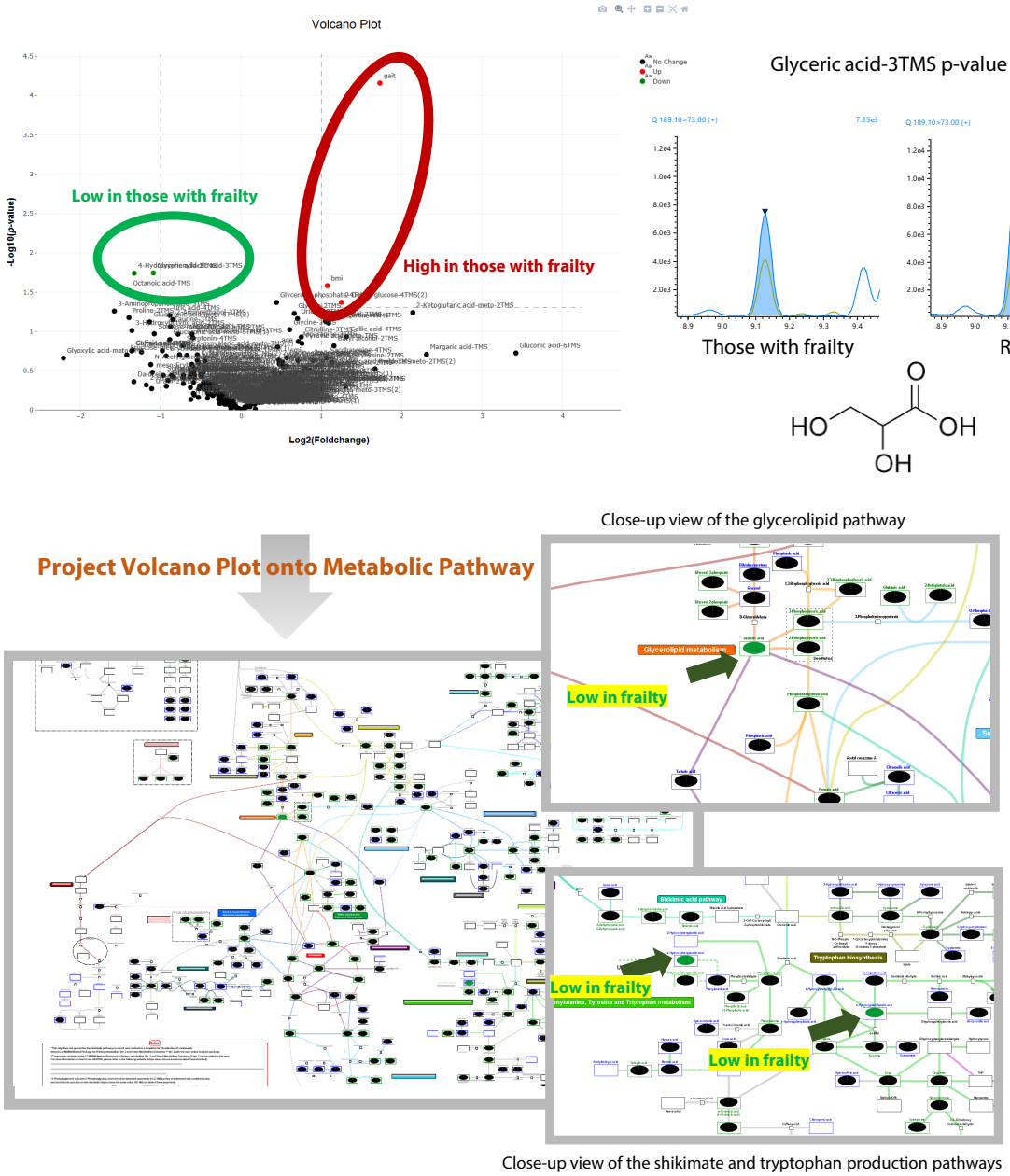

Fig. 3 Volcano Plot Projected onto a Metabolic Pathway Map

■ Correlation Analysis of Muscle Strength Measurement and Metabolite Measurement Data on Metabolic Pathway Map

Using the multi-omics analysis package, we searched for metabolic pathways and metabolites that were highly correlated with each of the following measures: grip strength (Grip), gait speed (G.A.I.T.), limb muscle mass (SMI), and body mass index (BMI) (Fig. 4). In Fig. 4, using Grip as a reference (yellow background box), compounds and muscle strength measurement items that have a high positive correlation with Grip have a red background strength, and compounds and muscle strength measurement items that have a high negative correlation have a blue background color.

It was found that there was a positive correlation with SMI and quinic acid as test items with high correlation with Grip. Quinic acid is an antioxidant and anti-inflammatory metabolite. On the other hand, a negative correlation was found for G.A.I.T and malonic acid. A scatter plot was automatically created using SMI, which was positively correlated, and 2-aminoethanol, which was not correlated. Blue indicates people with frailty and red indicates robustness. The positive correlation between Grip and SMI was strong from the scatter plot.

■ Simple Discriminant Analysis of Unknown Samples by Volcano Plot and Class Cluster Analysis

By performing a class cluster analysis on the metabolites and muscle strength measures that are identified as having significant differences on the volcano plot, the number of items is reduced and the heatmap is easier to review (next page, Fig. 5). Review of the dendrogram suggested that there were multiple sample groups as well as robust and frail individuals. Statistical analysis is not just one method, but a combination of methods to visualize and analyze can provide new insights.

■ Summary

In this study, sera from 114 healthy subjects (Women and Japanese) aged 65 to 80 years were analyzed using GCMS-TQ8040 NX and Smart Metabolites Database Ver. 2. By integrating plyometric data with metabolite concentrations and using a multi-omics analysis package for statistical analysis, we were able to detect multiple compounds and test items that could serve as biomarkers or digital biomarkers.

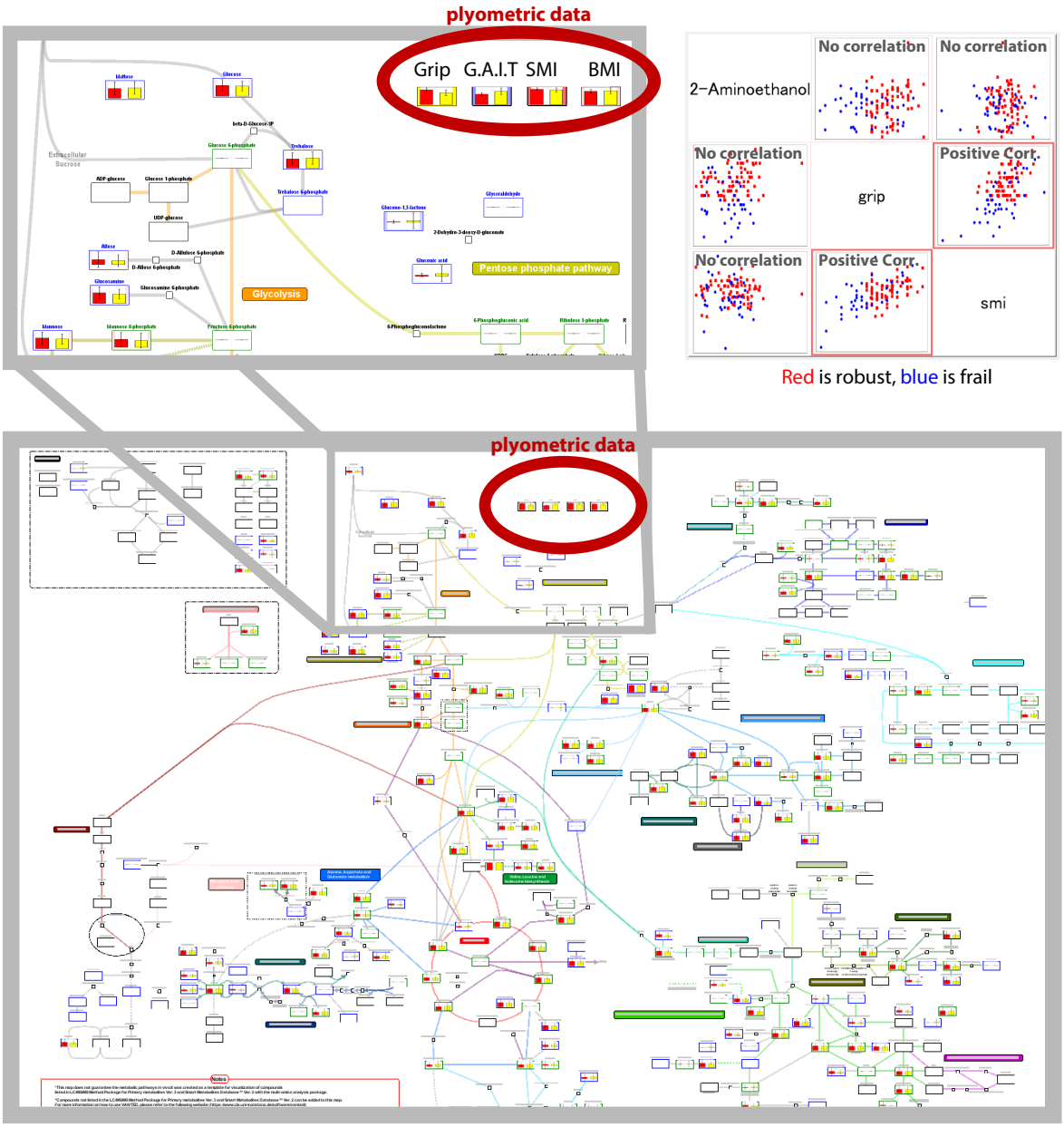

Fig. 4 Correlation analysis between muscle strength measurement results and metabolites in the metabolic pathway diagram
(Images of multi-omics analysis packages: robust red, frailty yellow)

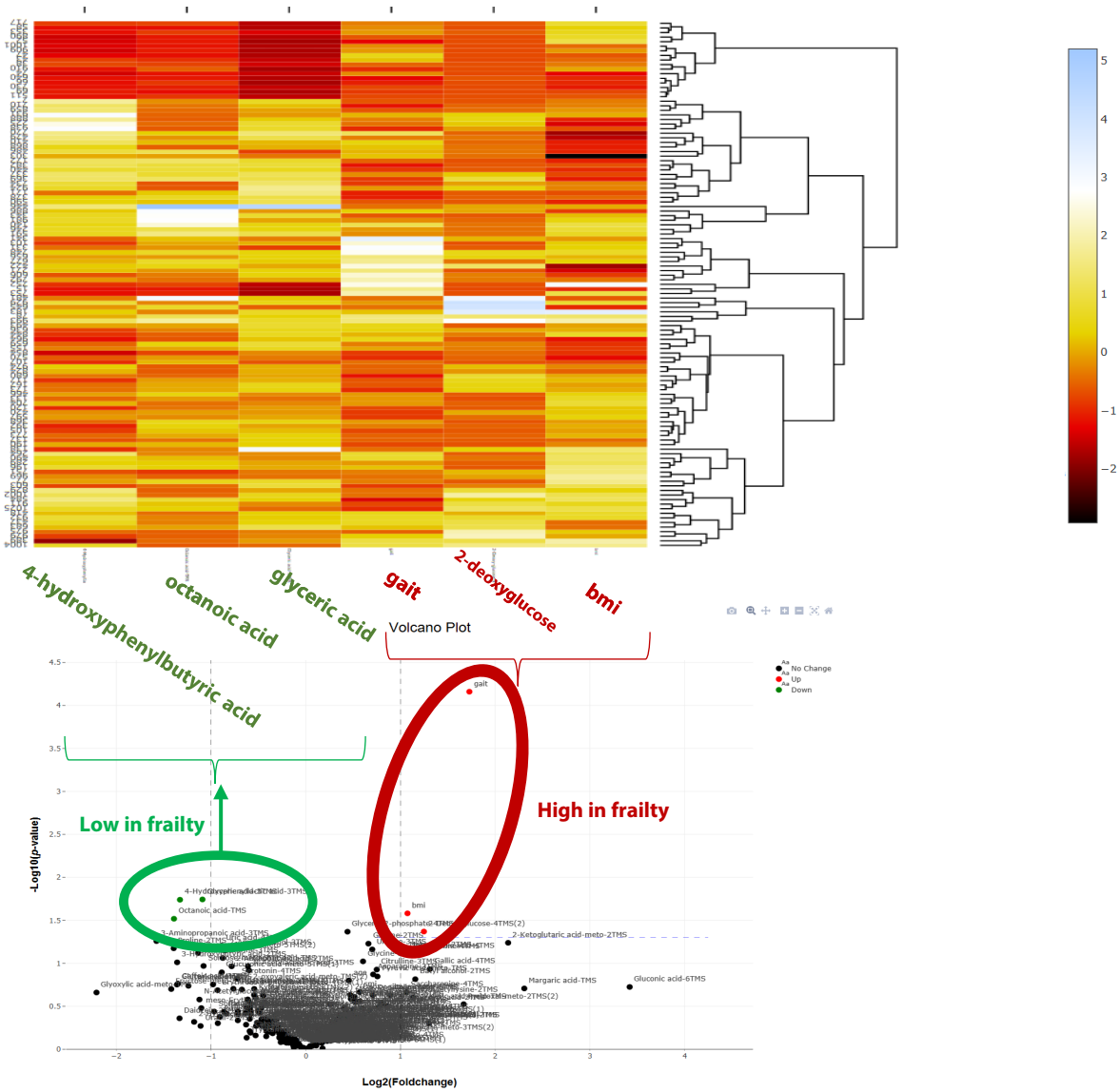

Fig. 5 Correlation analysis between muscle strength measurement results and metabolites in the metabolic pathway map
(Images of multi-omics analysis packages: robust red, frailty yellow)

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<Related Applications>

1. Application News 01-00410 “Metabolomic Differential Analysis of Genetic Mutant Drosophila Using GC/MS”

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