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GC-MS GCMS-TQ™ 8040 NX

Application News

Differential Analysis of Aging by Sex Using Correlation Analysis of Primary Metabolites

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User Benefits

- Smart Metabolites Database™ Ver. 2 realizes an effortless implementation of a primary metabolites analysis.
- Multi-omics Analysis Package reveals characteristic compounds of each sample group by visualizing the metabolite amounts in a metabolic pathway and performing age-specific and correlation analyses on the pathway map.

■ Introduction

Since the elderly are at high risk of developing diseases and tend to develop them for a long time, Japan has a medical system for the elderly that differs from other age groups. Age-related changes in physical function are a factor contributing to the worsening of morbidity, and it has been reported in the literature that postprandial blood glucose tends to spike due to abnormal glucose metabolism, and fatty acid capacity stored in the liver decreases due to abnormal lipid metabolism[1)].

There is also a difference in the age-related health status change among the same elderly people according to the gender. Men tend to suffer from lifestyle-related diseases such as heart disease and stroke and need assistance, while women tend to have decreased mobility and independence due to bone and muscle weakness[2)]. These differences are also thought to be influenced by changes in the amount of primary metabolites. Therefore, it is of utmost importance to clarify the differences in the amount of metabolites between the genders in the elderly.

In this application, primary metabolites in the serum of healthy subjects aged 70 to 85 were measured by gas chromatograph mass spectrometer (GC-MS). In order to find differences between the genders, Multi-omics Analysis Package statistical software was used (Fig. 1). Multi-omics Analysis package is a software that provides data visualizations such as multivariate analysis (such as principal component analysis and rank cluster analysis), volcano plots, and metabolic maps. Here is an example of an analysis that visualizes the primary metabolite measurement data in a metabolic pathway diagram and analyzes the differences by sex by age and correlation analysis.

■ Experimental

Sera from ten healthy subjects aged 70 to 85 years (men: n=5, women: n=5) were used for the analysis. Preprocessing was performed in accordance with the Metabolomics Preprocessing Handbook[3)], and the GC-MS analysis was performed using the Smart Metabolites Database Ver.2. Using Multiple Reaction Monitoring (MRM) as the data collection mode, 604 compounds were measured with an analysis time of 37 minutes. For details of the sample preparation method and analysis conditions, please refer to Application News 01-00410 "Metabolomic Differential Analysis of Genetic Mutant Drosophila Using GC/MS" in the "Related Applications" section on the next page.

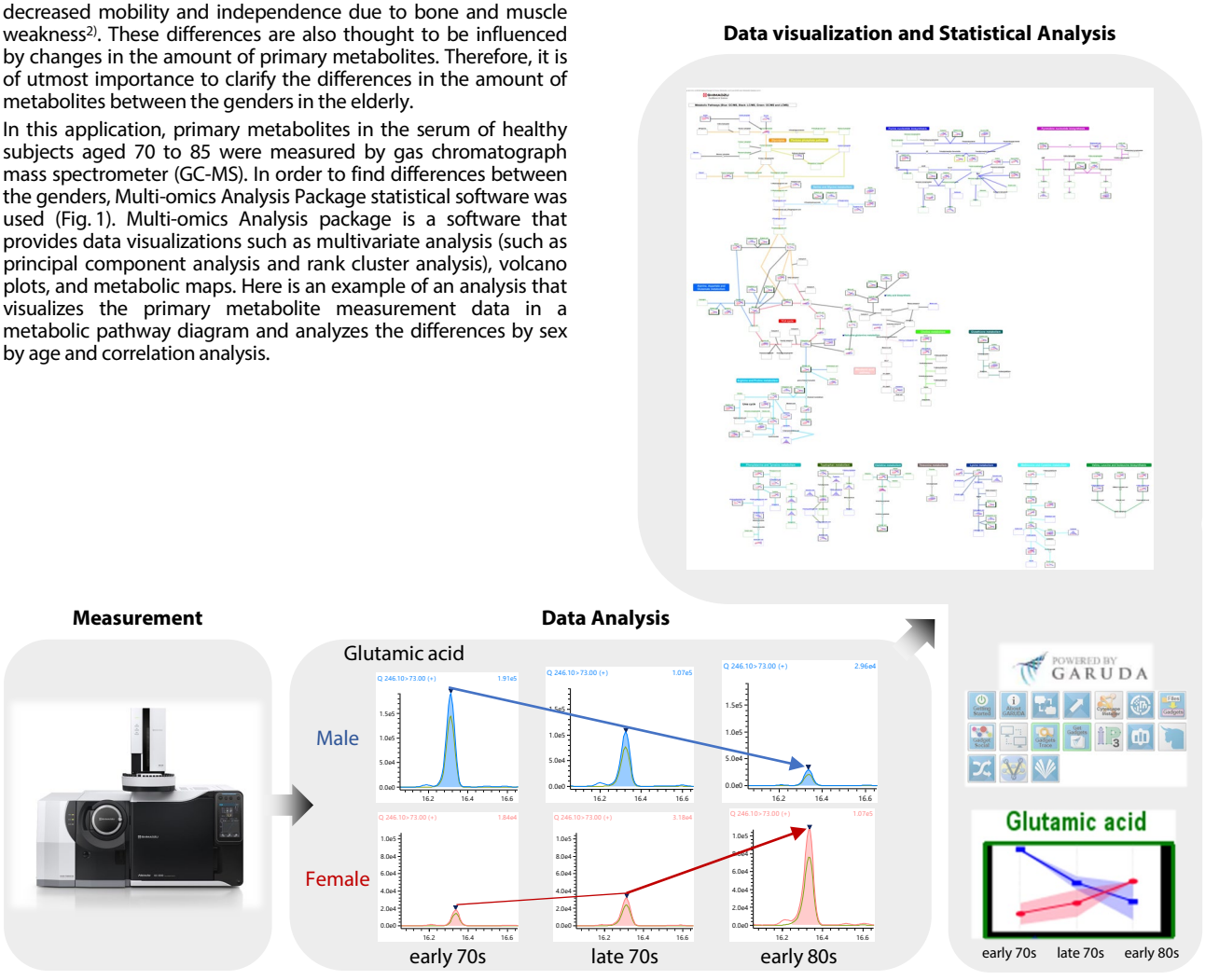

Fig. 1 Workflow from measurement with GCMS-TQ™8040 NX to statistical analysis with the Multi-omics Analysis Package

■ Chronological and correlation analyses on a metabolic pathway

When 604 primary metabolites were measured by GC-MS, 243 were detected in all serum samples. These components were projected onto a metabolic pathway map by age group and sex, which was divided into three categories: early 70s, late 70s, and early 80s, using Multi-omics Analysis Package. For each metabolite, age-related changes in metabolite levels are visualized with line graphs in blue for the male and red for the female (Fig. 2).

Glutamate was one of the components that showed differences in the amount between sexes. Serum glutamate levels increase with age in women but tend to decrease in men. Glutamate, an amino acid abundant in the brain, is produced from glutamine in neurons and plays an important role as an excitatory neurotransmitter. Glutathione, an antioxidant present in the body, is produced from glutamate and has a defense mechanism against various oxidative stresses in the body[4]. Glutamate is found to be higher in serum in men, but it is reported to be higher in women in the body tissues from which it flows into the blood[5].

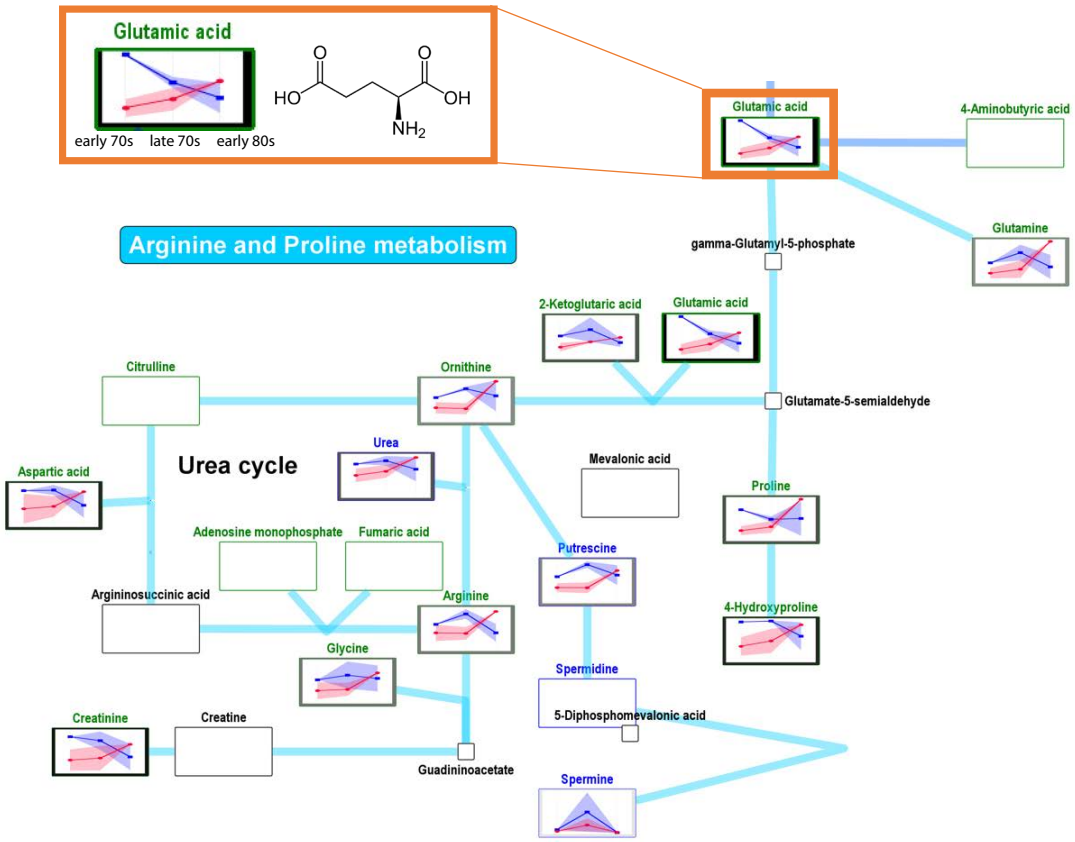

Fig. 2 Correlation Results on glutamate metabolic pathway (Image from Multi-omics Analysis Package)

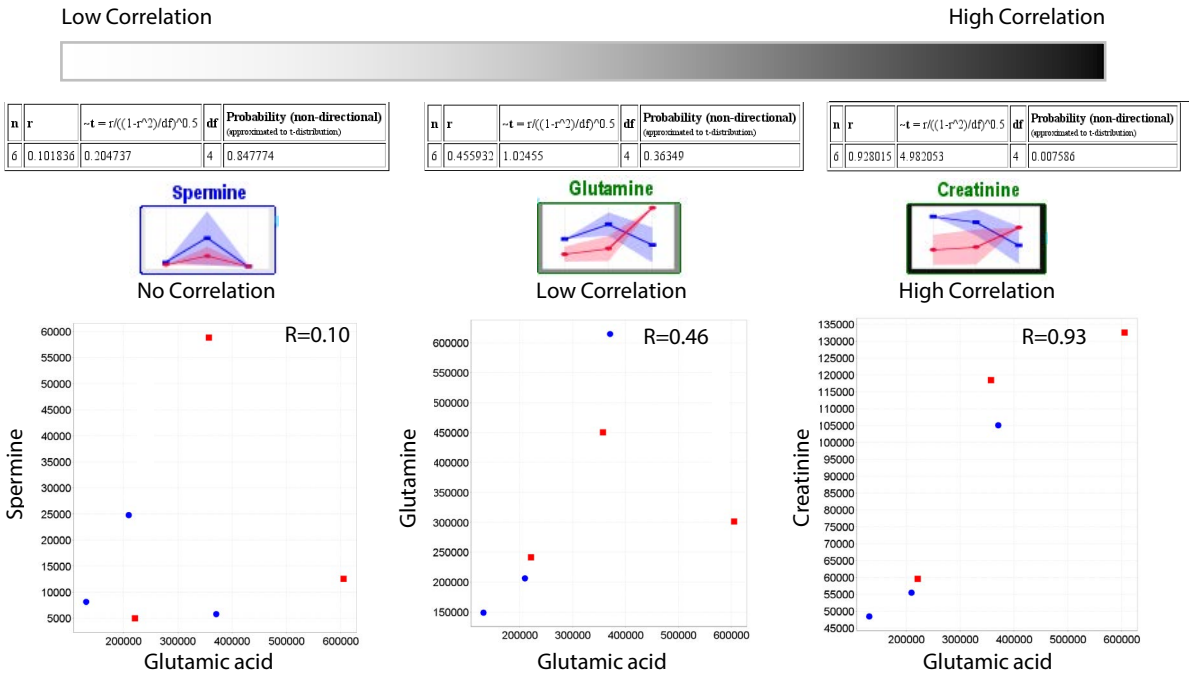

Fig. 3 Scatter plots showing the correlation between glutamate and each compound (image from Multi-omics Analysis Package)
(Compound name: blue compound is measured by GC-MS, green compounds can be measured by both GC-MS and LC-MS)

In addition, the metabolic pathway map of Multi-omics Analysis Package has a function to automatically color the metabolites that are similar (= highly correlated) to the changes of specific metabolic components by age. Using this function, we searched for age-specific changes similar to those of glutamate in the metabolic pathway map. As a result, it was found that creatinine, which is produced by the decomposition metabolic pathway of arginate and proline as well as glutamate, was highly correlated.

Creatinine is a waste product in the blood that is filtered by the liver and excreted in the urine. It is known that the blood concentration decreases at a younger age in men than in women[6].

Glutamine, a non-essential amino acid, was also confirmed to be correlated with glutamate. However, the correlation was not as high as that of creatinine because the amount of glutamine in the blood decreased due to injury and stress. Spermine was also detected as a component that had no correlation with glutamate. It has been reported that the amount of spermine in the blood is more affected by individual differences than by age[7].

Multi-omics Analysis Package provides scatter plots showing the correlation between creatine, glutamine, and spermine compounds and glutamate (Fig. 3).

The scatter plot shows the age-specific area values for glutamate on the horizontal axis and the age-specific area values for each comparison compound on the vertical axis, with blue representing the male and red representing the female. The correlation coefficients of each component with glutamate were R=0.93 for creatine, R=0.46 for glutamine, and R=0.1 for spermine.

■ Conclusion

In this study, sera (men: n=5, women: n=5) from 10 healthy subjects aged 70 to 85 years were analyzed using GCMS-TQ8040 NX and Smart Metabolites Database Ver. 2. Based on the 243 components detected, Multi-omics Analysis Package was used to project them onto a metabolic pathway map, and to perform chronological and correlation analyses. As a result, primary metabolites that differed by sex and age were detected.

<References>

1) 高齢者の代謝特性 [Metabolic characteristics of the elderly], 上垣 佐登子, 外科と代謝・栄養52巻1号, accessed on July 26th, 2023

2) 第6回 年を重ねると「健康状態」はどのように変化するのか [Part 6: How does your health change as you age?], 日本生命, accessed on July 26th, 2023

3) Pretreatment Procedure Handbook for Metabolite Analysis, Shimadzu Corporation, accessed on July 26th, 2023

4) 高齢者とグルタミン酸機能 [Elderly and Glutamate Function], 橋本謙二, accessed on July 26th, 2023

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6) Use of serum creatinine concentrations to determine renal function, T D Bjornsson , accessed on July 26th, 2023

7) アンチエイジングの本命＝高ポリアミン食 [Anti-Aging Favorite: High-Polyamine Diet], 自治医科大学大宮医療センター, accessed on July 26th, 2023

<Related Applications>

1. Application News No.01-00410 “Metabolomic Differential Analysis of Genetic Mutant Drosophila Using GC/MS”
2. Application News No.01-00498A “Culture Medium Analysis for a Metabolic Analysis of Antibody-Producing Cells Using LC-MS/MS and ICP-MS”

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