**Metabolome analysis**

Metabolome analysis was conducted by *C-SCOPE* package of HMT (Human Metabolome Technologies, Tsuruoka, Japna) using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) for cation analysis and CE-tandem mass spectrometry (CE-MS/MS) for anion analysis based on the methods described previously (1, 2)

Briefly, CE-TOFMS analysis was carried out for cation mode metabolome using an Agilent CE capillary electrophoresis system equipped with an Agilent 6210 time-of-flight mass spectrometer (Agilent Technologies, Waldbronn, Germany) as shown in Table 1. CE-MS/MS analysis for anionic metabolome were measured in the positive or negative mode of metabolome using an Agilent CE capillary electrophoresis system equipped with Agilent 6460 TripleQuad LC/MS (Agilent Technolgies, Santa Clara, CA, USA) as shown in Table 2.

The systems were controlled by Agilent G2201AA ChemStation software version B.03.01 for CE (Agilent Technologies) and connected by a fused silica capillary (50μm *i.d.*×80cm total length) with commercial electrophoresis buffer (H3301-1001 and I3302-1023 for cation and anion analyses, respectively, HMT) as the electrolyte. The spectrometer was scanned from m/z 50 to 1,000 (1).

**Table 1: Device and Analytical Condition in Cation Measurement**

Device CE-MS Agilent CE-TOFMS system (Agilent Technologies)

Capillary Fused silica capillary, i.d. 50 μm × 80 cm

Analytical Condition

Run buffer Cation buffer solution (p/n: H3301-1001)

Rinse buffer Cation buffer solution (p/n: H3301-1001)

Sample injection Pressure injection at 50 mbar, 10 s

CE voltage Positive, 27 kV

MS ionization ESI Positive

MS capillary voltage 4,000 V

MS scan range *m/z* 50–1,000

Sheath liquid HMT sheath liquid (p/n: H3301-1020)

**Table 2: Device and Analytical Condition in Anion Measurement**

Device CE Agilent CE system

MS Agilent 6460 TripleQuad LC/MS

Capillary Fused silica capillary, i.d. 50 μm × 80 cm

Analytical Condition

Run buffer Anion buffer solution (p/n: I3302-1023)

Rinse buffer Anion buffer solution (p/n: I3302-1023)

Sample injection Pressure injection at 50 mbar for 25 s

CE voltage 30 kV

MS ionization Positive and negative

MS capillary voltage 4000 V for positive and 3500V for negative mode

Sheath liquid 50% Methanol/water (v/v)

Peaks were extracted using MasterHands, automatic integration software (Keio University, Tsuruoka, Yamagata, Japan) (3) and MassHunter Quantitative Analysis B.04.00 (Agilent Technologies) in order to obtain peak information including *m/z*, peak area, and migration time (MT). Signal peaks were annotated according to the HMT metabolite database based on their *m*/*z* values with the MTs. The peak area of each metabolite was normalized with respect to the area of the internal standard and metabolite concentration was evaluated by standard curves with three-point calibrations using each standard compound.

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed by HMT’s proprietary software, PeakStat and SampleStat, respectively. Detected metabolites were plotted on metabolic pathway maps using VANTED software (4).

**References**

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