**Comparative assessment of Graves' disease and main extrathyroidal manifestation, Graves' ophthalmopathy, by non-targeted metabolite profiling of blood and orbital tissue**

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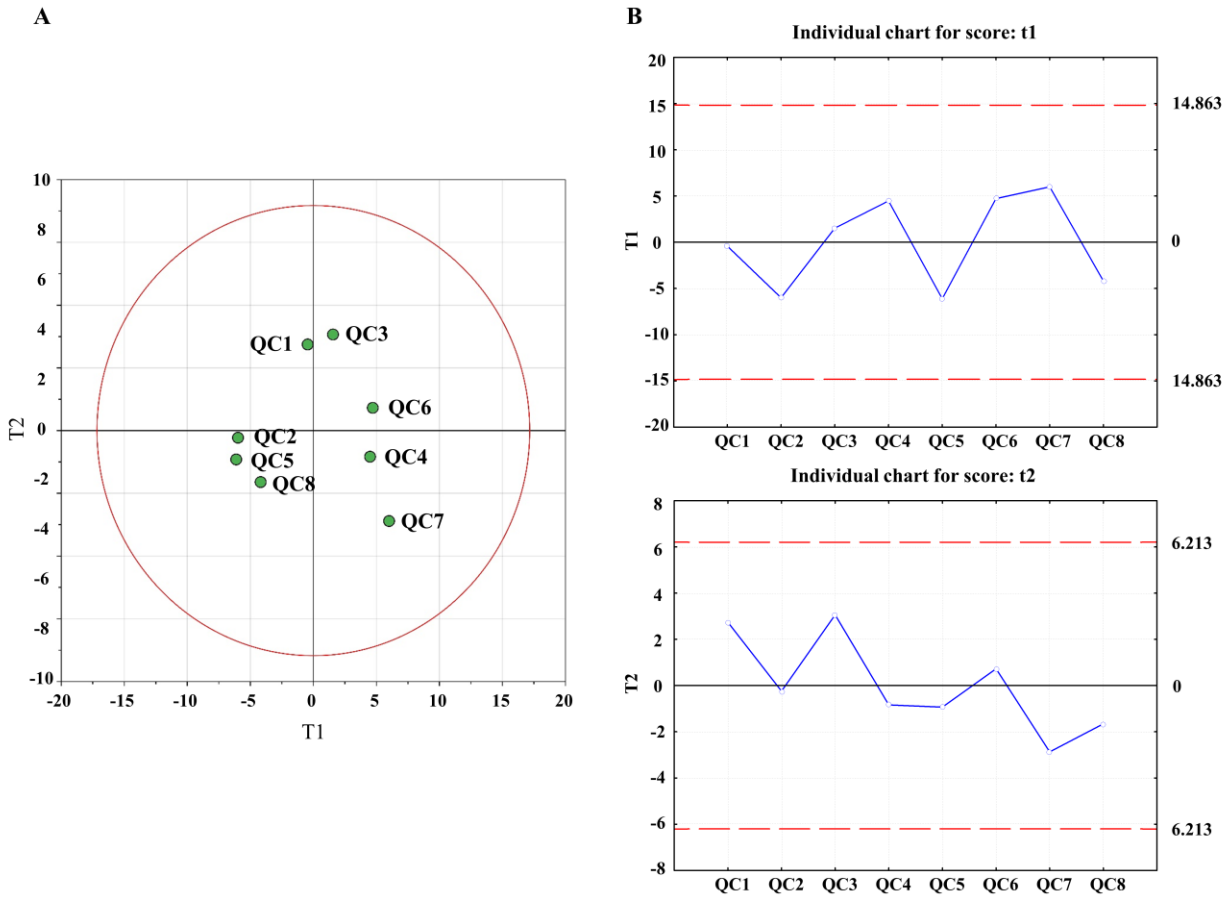
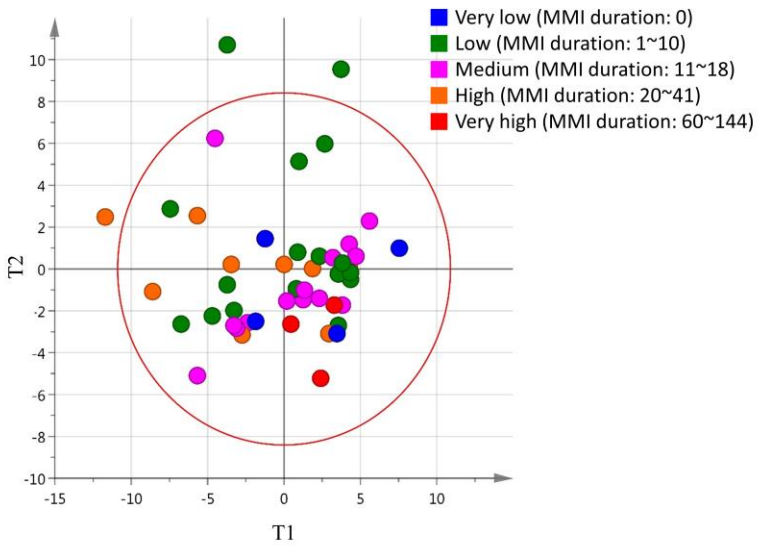
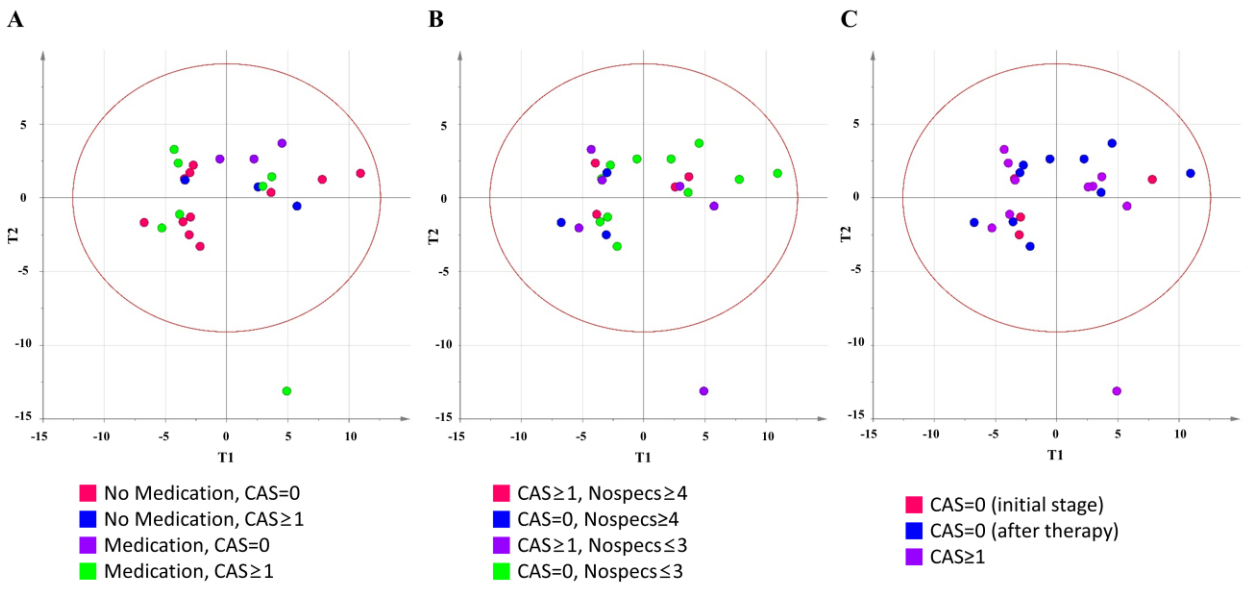


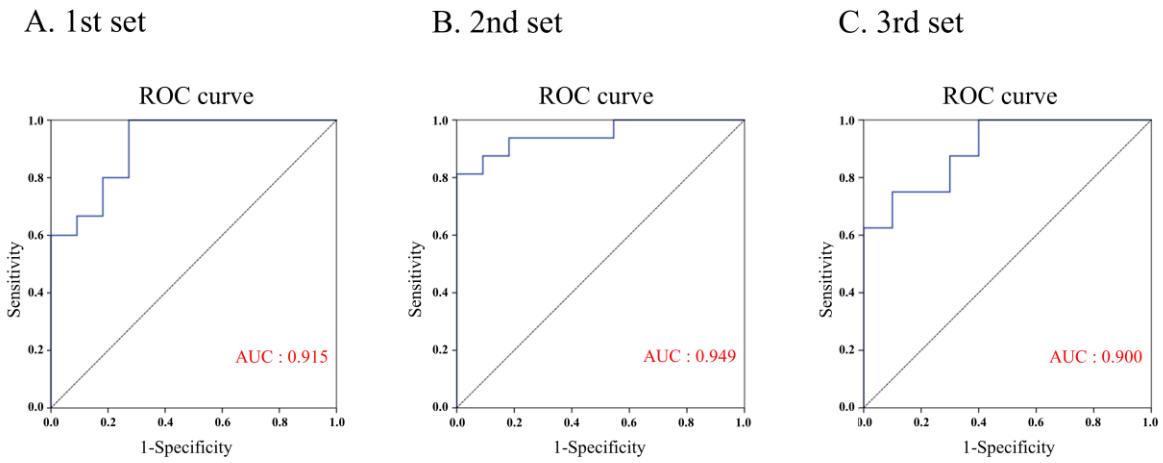

**Supplementary Figure S1. Principal component analysis of QC mixture compounds.** (A) Score scatter plot of metabolite profiles of 8 QC samples with T1 and T2. X (T1)- and Y(T2)-axis indicate the most and the second most discriminant vectors, respectively. 0.05 as significance level. (B) Line plots (score control chart) with T1 (upper panel) and T2 (lower panel).




**Supplementary Figure S2. Principal component analysis for the potential effects of Methimazole on blood plasma metabolite profiles.** Score scatter plot of 47 samples with T1 and T2. X (T1)- and Y(T2)-axis indicate the most and the second most discriminant vectors, respectively. Red circle is the two-dimensional boundary of 47 standard deviation. Color indicates different MMI duration sorted by five periods.

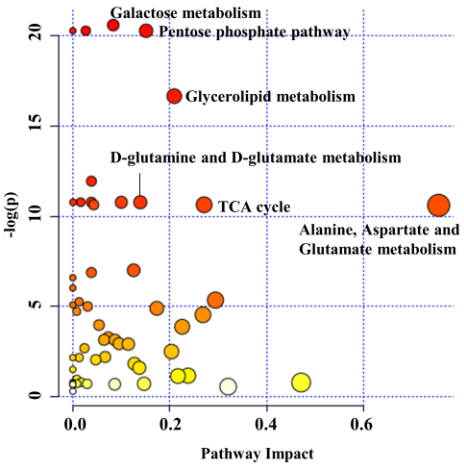



**Supplementary Figure S3. Score scatter plots by PCA for four groups categorized by the combination of CAS and steroid medication (A), NOSPECS and CAS (B), and for three groups, CAS=0 (initial stage), CAS=0 (after therapy), and the others (CAS≥1) (C) in the GO group.**




**Supplementary Figure S4. Receiver operating characteristic (ROC) analysis of 3 independent test set that thirty percent (26 subjects) of all subjects (79 subjects) are randomly selected and used.**

A



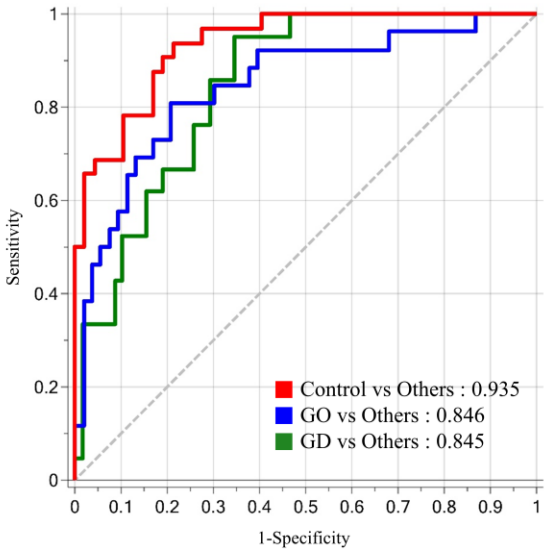

B

| Pathway name | Holm p | FDR | Impact |
|---|---|---|---|
| Galactose metabolism | 6.59E-08 | 2.26E-08 | 0.08333 |
| Starch and sucrose metabolism | 8.83E-08 | 2.26E-08 | 0.02678 |
| Pentose phosphate pathway | 8.83E-08 | 2.26E-08 | 0.15109 |
| Amino sugar and nucleotide sugar metabolism | 8.83E-08 | 2.26E-08 | 0 |
| Glycerolipid metabolism | 3.15E-06 | 6.76E-07 | 0.20907 |
| Fructose and mannose metabolism | 3.44E-04 | 6.28E-05 | 0.03835 |
| Ascorbate and aldarate metabolism | 1.08E-03 | 1.03E-04 | 0.01617 |
| Vitamin B6 metabolism | 1.08E-03 | 1.03E-04 | 0.03828 |
| Histidine metabolism | 1.08E-03 | 1.03E-04 | 0.00051 |
| D-Glutamine and D-glutamate metabolism | 1.08E-03 | 1.03E-04 | 0.13904 |
| Lysine biosynthesis | 1.08E-03 | 1.03E-04 | 0.09993 |
| Citrate cycle (TCA cycle) | 1.13E-03 | 1.03E-04 | 0.27058 |
| Glyoxylate and dicarboxylate metabolism | 1.13E-03 | 1.03E-04 | 0.04303 |
| Alanine, aspartate and glutamate metabolism | 1.13E-03 | 1.03E-04 | 0.75404 |

**Supplementary Figure S5. Pathway enrichment analysis of plasma metabolite profiles of the Graves' disease group compared to the healthy control.** The analysis is conducted on all continuous variables (metabolites) with the relative concentrations without pre-determined statistical cutoff. (A) Pathway impact values, estimated from the number of connection of a node (a metabolite), are plotted against X-axis. The values are computed by number of shortest routes passing through the node (betweenness centrality). The higher score suggests that the metabolite is located in topologically and putatively biochemically important position. P-values are plotted against Y-axis, which presents the significant levels of pathway alteration cumulatively computed by the significance of metabolite changes in a pathway. For visual clarification, the pathway importance and the statistical significance are proportional to node radius and color respectively. (B) The list of metabolic pathways with significant difference the GD group compared to healthy controls. The statistical significance is

evaluated and presented by adjusted p values (Holm-Bonferroni p values) and false discovery rate (FDR).

A




B

| Metabolite panel |
|---|
| Glucose |
| Pelargonic acid |
| 2-(4-hydroxyphenyl)ethanol |
| Gluconic acid lactone |
| Glycerol |
| Pentadecanoic acid |
| Mannose |
| Threose |
| Fumaric acid |
| Pyruvate |
| 1,5-anhydroglucitol |
| Proline |

**Supplementary Figure S6. (A) Receiver operating characteristic (ROC) analysis of multiple metabolite panels for discriminating the three different groups (GO, GD, and healthy control). (B) The list of metabolite panel is provided in the right panel.**

**Supplementary Table S1. Clinical characteristics of the patients with Graves' ophthalmopathy included in the tissue analysis**

| Case | Age/Sex | Duration of GO, m | Smoking | Previous treatment | CAS | Proptosis Rt/Lt, mm | Surgical treatment |
|---|---|---|---|---|---|---|---|
| 1 | 36/F | 36 | Yes | GC | 1/7 | 21/20 | Decompression |
| 2 | 51/M | 12 | Yes | GC | 3/7 | 21/23.5 | Decompression |
| 3 | 59/M | 192 | Yes | GC | 2/7 | 22/21 | Decompression |
| 4 | 61/M | 4 | Yes | GC | 3/7 | 21/21 | Decompression |
| 5 | 61/F | 24 | No | GC | 3/7 | 20.5/21 | Decompression |

The range of CAS was from 0 to 7. GO, Graves' ophthalmopathy; CAS, clinical activity score, GC, glucocorticoid