

# Metabo-Typing of Internet Addiction and Discovery of Potential Molecular Indicator Coupled to Clinical Parameter



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## Abstract

The internet addiction is a new disorder caused by dramatic increase of internet utilization in worldwide. Internet addiction is characterized by non-steady state in daily life, which is acquired by adverse impact through excessive use of online video games and mobile devices. Moreover, it is a disease, and often co-exist with other psychological disorder. The diagnosis of the abnormal status is often made by expert clinicians using survey tools and symptoms. However, it is difficult and limited methods for definite diagnosis. In our study, we explored new molecular indicators that metabolically define internet addiction. We examined significantly changed metabolites from plasma samples of 51 patients based GC mass spectrometry. The patient group included only internet addition and combination of attention deficit hyperactivity disorder (ADHD). Furthermore, we performed integrative analysis with clinical parameter. Consequently, we acquired internet addiction-specific model, and discovered the biomarker candidates using multiple complex statistics. Expectantly, it will provide better understanding toward internet addiction, and potential biomarker for clinical application.

## Experiment designs

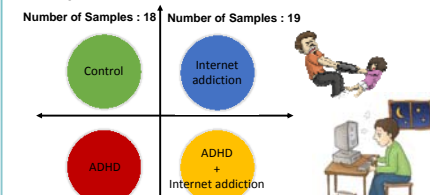
### Internet Addiction Disorder (IAD)

People with this disorder are aware that the act of using a computer to harm the health and social life. But it can not control the desire. Due to excessive internet, it appears dependence and withdrawals under the condition. it can interrupt the social, emotional, physical and financial with daily life.

### Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a disorder that appears often in childhood, refers to the lack of state attention to distractions and exhibit hyperactivity, impulsivity. Because of lowering of the frontal lobe function execution instruction for our actions.

### Group information



### Clinical parameter

Depression, Anxiety, Impulsivity, Alcohol, Attention

- Striatal dopamine is released during video gaming  
 - Compensate for the frustration they feel in real-life situations

## Methods

### Sample extraction and derivatization

Sample (1ml) were injected into 1mL of -20°C methanol. The pellet was lyophilized in freeze dryer. Cells were disrupted and added extraction solvent, methanol : isopropanol : water (3:3:2; v/v/v). The supernatant were collected and concentrated to dryness in a vacuum concentrator. The dried pellets, 5 µL of a solution of 40 mg/mL of 98% pure methoxyamine hydrochloride in pyridine (analytical grade; Pierce) were added and shaken at 30°C for 90 min, to protect aldehyde and ketone groups. Then, 45 µL of MSTFA and a mixture of internal retention index (RI) markers, fatty acid methyl esters (FAME), were added and shaken at 37°C for 30 min, for trimethylsilylation.

### Mass-spectrometry analysis

The derivatized metabolite samples were analyzed by GC/TOF MS using an Agilent 7890B GC (Agilent Technologies, Wilmington, DE) coupled with a Pegasus HT TOF MS (LECO, St. Joseph, MI).



### Data Processing

GC/TOF MS data were pre-processed using the LECO Chroma TOF software (ver. 3.34; St. Joseph, MI) to detect peaks and to deconvolute the mass spectra. The preprocessed data were processed using BinBase, an in-house programmed database built for metabolite identification.

### Statistical Analysis

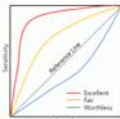
Simca (ver. 13.0.3, Umetrics, Umea Sweden) was used for Orthogonal Partial Least Squares (OPLS-DA), Classification list and univariate analysis. Hierarchical clustering analysis (HCA) was performed using MultiExperiment Viewer (MeV, ver.4.8.1) to visualize and organize metabolite profiles. Pathway enrichment analysis and pathway topological analysis was performed by software R (ver. 2.8 on the linux).

## Introduction

### Receiver operating characteristic (ROC)

Commonly used in clinical radiology research to express the diagnostic accuracy of imaging examination

**Sensitivity** (true positive rate) : measures the proportion of positives that are correctly identified as such  
**Specificity** (true negative rate) : measures the proportion of negatives that are correctly identified as such

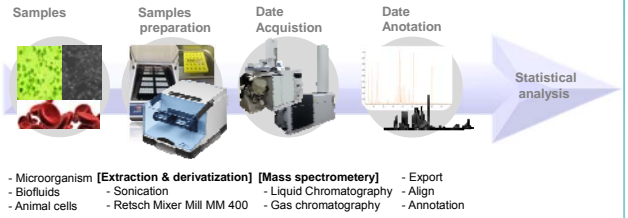


$$1\text{-specificity} = \frac{FP}{FP+TN}$$

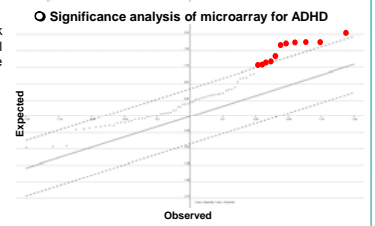
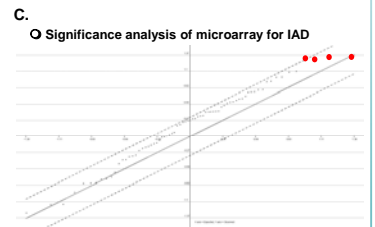
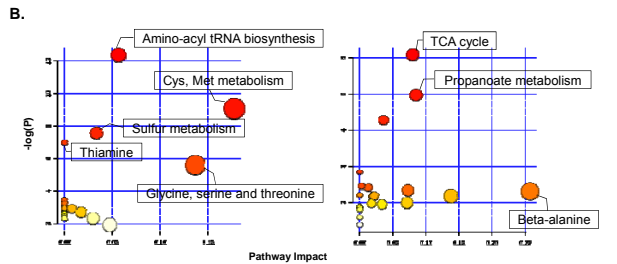
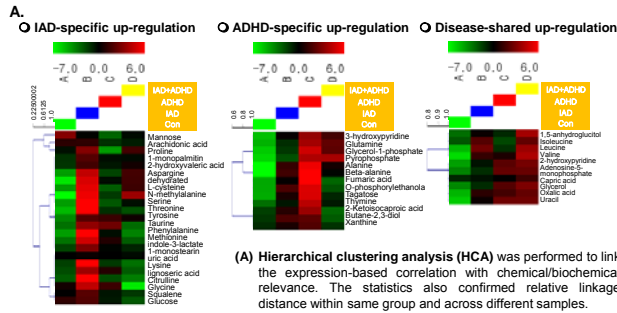
AUC (Area Under the Curve)

- 0.90 - 1.00 → Excellent
- 0.80 - 0.90 → Good
- 0.70 - 0.80 → Fair
- 0.60 - 0.70 → Poor
- 0.50 - 0.60 → Fail

### Work flow



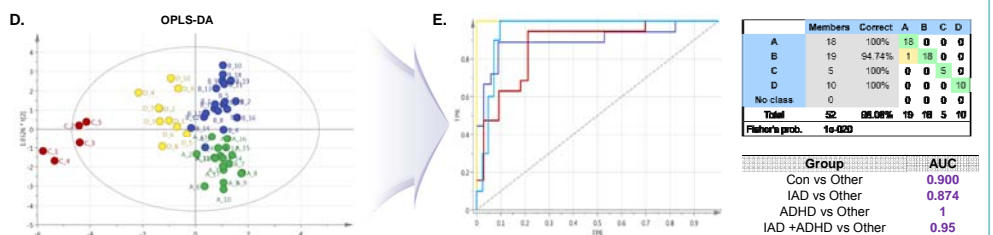
## Results



IAD	ADHD
L-cysteine	Citric acid
Methionine	Tagatose
Serine	Alanine
Tyrosine	Pyrophosphate
	Isothreonic acid
	Glycerol-1-phosphate
	Glyceric acid
	Fumaric acid
	Beta-alanine
	Lactic acid
	O-phosphorylethanolamine

(C) Significance analysis of microarray (SAM) were performed for screening genotype-specific metabolite alteration. False discovery rate was maintained as zero. Metabolites located up-right (red circle) indicates significantly up-regulated ones with existence of IAD and ADHD

(B) Pathway over-representation and topological analysis were conducted using Hypergeometry method and Relative-betweenness centrality. The analysis pinpoints each of internet addition disorder (IAD) in sulfur metabolism, cysteine / methionine metabolism, which can imply the direct functional points by IAD group. The analysis pinpoints each of Attention Deficit Hyperactivity Disorder (ADHD) in TCA cycle, beta-alanine metabolism, and carbohydrate metabolism, which can imply the direct functional points by ADHD group



## Conclusions

- Molecular characterization of Internet addition disorder
- Model construction of IAD and associated disorders (e.g. ADHD)
- Disorder-specific biomarker discovery
- Mechanical understanding of the biochemical consequences

## References

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