

Searching for changes in metabolome of women with polycystic ovary syndrome with the use untargeted metabolomics

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex, endocrine disorder with unexplained pathogenesis, also found as the main reason for infertility of women of reproductive age. The main components of PCOS are:

1. Oligoovulation or lack of ovulation
2. Clinical and/or biochemical symptoms of hyperandrogenism
3. Presence of polycystic ovaries in ultrasound examination

AIMS OF STUDY

Determination and comparison of metabolomic profiles of serum samples obtained from patients with PCOS and healthy volunteers

Determination of biochemical pathways involved in PCOS development

SUBJECTS

	PCOS(n=30)	Control (n=30)
AGE	22-38	17-39
BMI	24,99 ± 5,09	23,61 ± 5,51

SAMPLE PREPARATION

GC-QqQ/MS

Precipitation
Cold MeOH

IS addition
Pentadecanoic acid (1 mg/ml)

Derivatization
Methoxyamine in pyridine (16h) BSTFA : TMCS (99:1, v)

Dilution
Heptane

HPLC-TOF/MS

Precipitation
Cold MeOH : EtOH, 1:1, v/v

IS addition
1-(4-Fluorobenzyl)-5-oxoproline (1 µg/ml)

Filtration
Nylon filters, d=0,22 µm

STATISTICAL ANALYSIS

DATA PROCESSING

- Filtration
- Normalization
- Identification

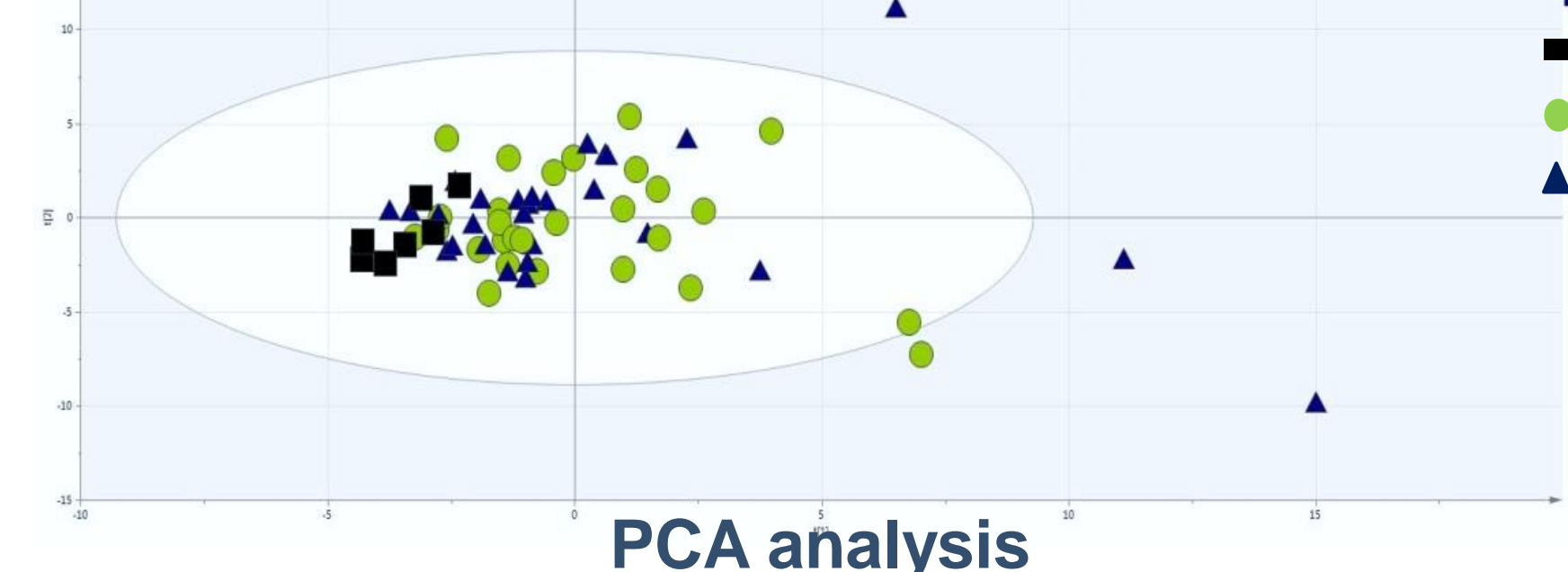
MULTIVARIATE STATISTICAL ANALYSIS

PCA analysis
VIP and SR methods

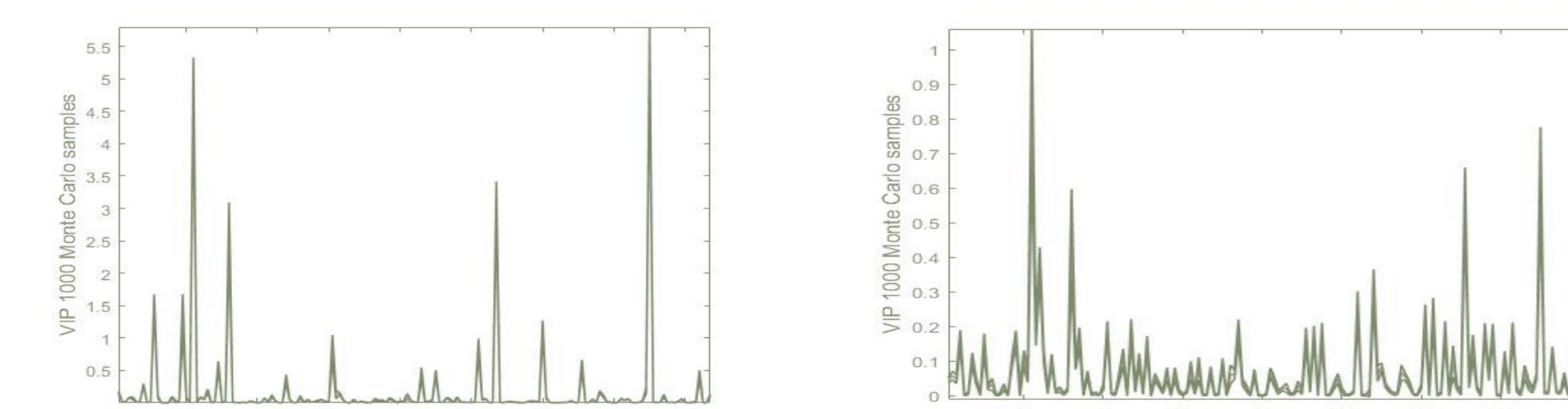
ONE-DIMENSIONAL STATISTICAL ANALYSIS

The t-Student or Mann-Whitney U test
Bonferroni and Benjamini-Hochberg correction

HPLC-TOF/MS



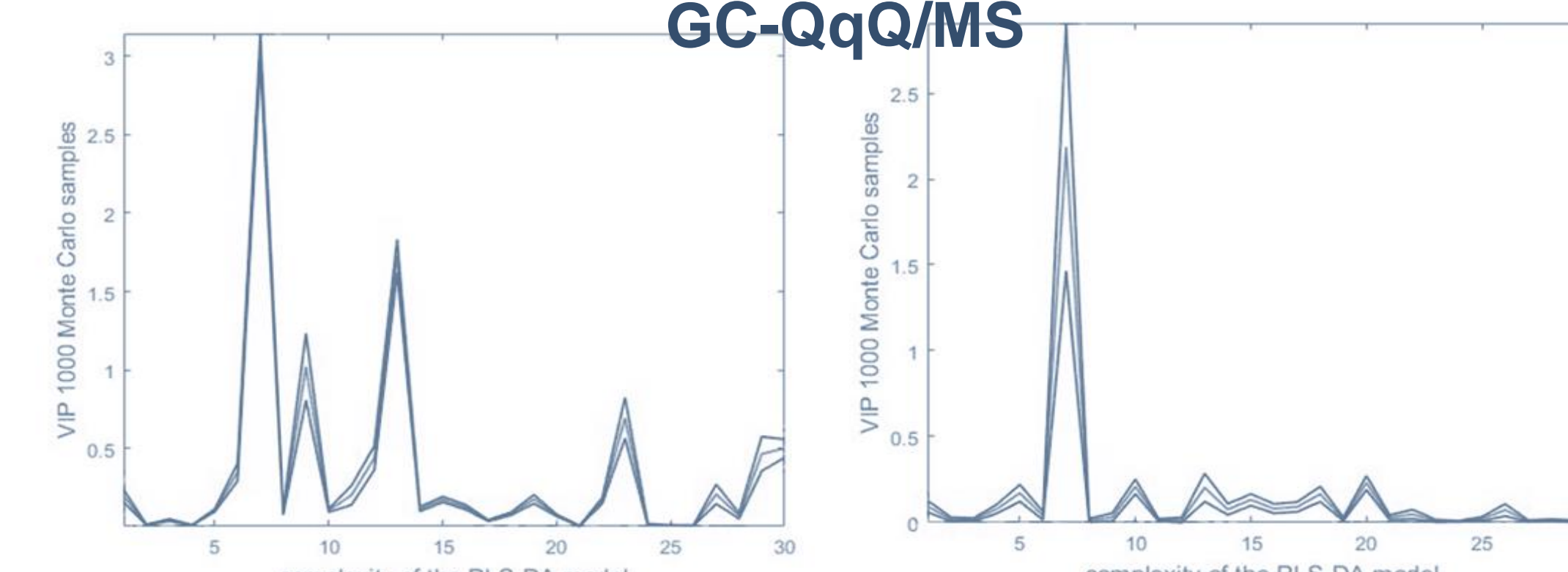
HPLC-TOF/MS



VIP analysis

SR analysis

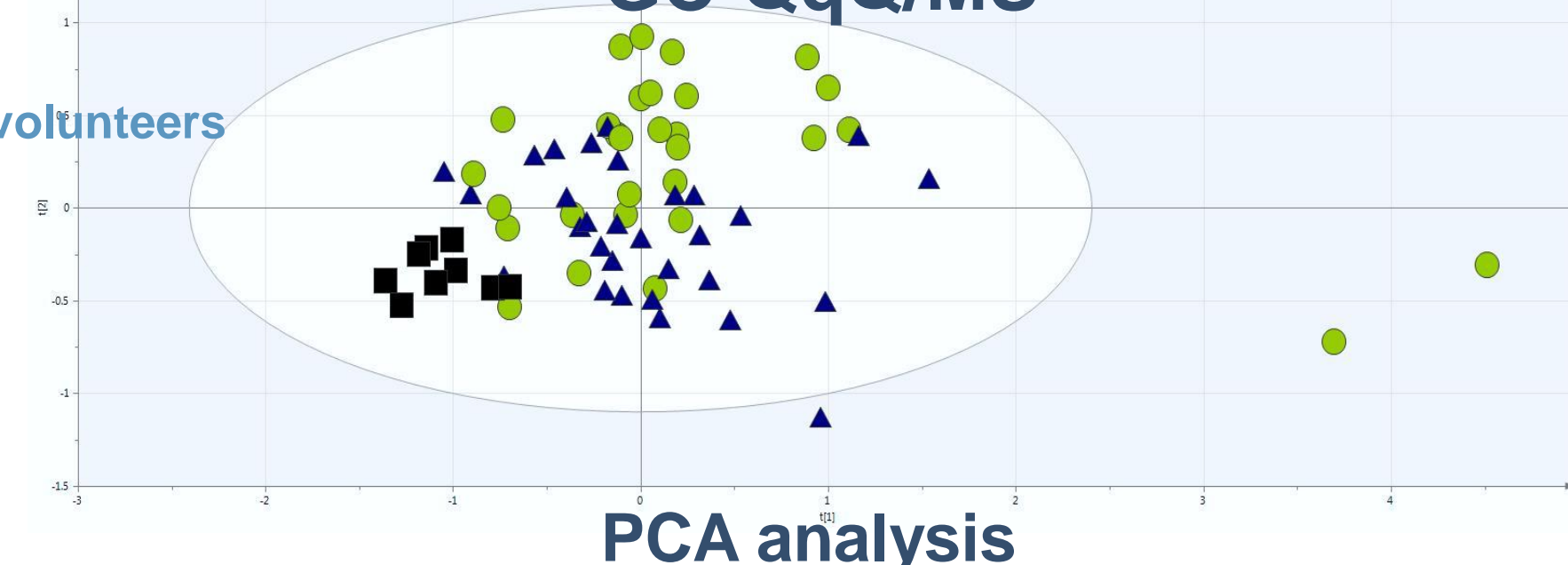
GC-QqQ/MS



SR analysis

VIP analysis

GC-QqQ/MS



METHODS

Liquid chromatography		Mass spectrometry	
Mobile phase	A: 0.1% HCOOH in water	Nebulizer pressure	30 psig
	B: 0.1% HCOOH in acetonitrile	Capillary voltage	3250 V
Acquisition mode	SCAN	Skimmer voltage	65 V
Gas flow	10 L/min	Fragmentor voltage	150 V
Drying gas temp.	325 °C	Mass range	m/z 50 -1000
Gas chromatography		Mass spectrometry	
Gas pressure	53.5 kPa	Gas flow	10 ml/min
Column oven temp.	60 °C (1 min) (8°C/min)	Interface temp.	200 °C
	320 °C (5 min)	Ion source temp.	300 °C
Injection mode	Splitless	Acquisition mode	SCAN
Injection temp.	250 °C	Mass range	m/z 50 -600

RESULTS

