PD 52-09: Development of a Urinary Metabolomic Signature for Prostate Cancer Using Capillary Electrophoresis Mass Spectrometry

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Background

Prostate cancer (PCa) pathogenesis is influenced by alterations in cellular metabolism

Metabolomics measures these biochemical changes to create global tissue metabolite profiles

Tissue metabolites that are present in urine can potentially be utilized as biomarkers for detection and evaluation of PCa

Objective

We used Capillary Electrophoresis Mass Spectrometry (CE-MS) to analyze urine from men undergoing prostate biopsy for suspicion of PCa to investigate their metabolomic profiles

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Urine was prospectively collected from 150 men undergoing prostate biopsy

We retrospectively selected 40 urine samples for metabolomic analysis:

- 20 from prostate biopsies demonstrating PCa

- 20 from prostate biopsies demonstrating benign tissue

CE-MS analysis of charged metabolites and peptides was performed as previously described (J Proteome Res. 2:488; 2003)

Urinary metabolites were extracted from 100 µL urine by mixing with methanol containing 20 µM of internal standards

CE-MS experiments were performed with the Agilent CE system

Screening of metabolites was performed with the following statistical protocols:

- Metabolites with concentrations below the threshold in all samples were excluded

- Relative abundances of metabolites were normalized to levels of creatinine

- The biologic significance of metabolites was investigated using the Human Metabolome Database (HMDB)

- Metabolic pathway analyses were performed as previously described using MetaboAnalyst software (Metabolites. 9:57; 2019)

Cohort Clinical Characteristics

Median (IQR)	Benign (n = 20)	PCa (n = 20)	P value
Age, years	62 (57-69)	63 (56-68)	0.9
Prostate volume, cc	66 (39-77)	36 (29-54)	0.006
PSA, ng	6.5 (4.5-8.2)	6.0 (4.5-7.9)	0.8
PSA density, ng/mL	0.12 (0.08-0.14)	0.15 (0.12-0.23)	0.03
Prior biopsy results	11 (55%) Biopsy-naïve 9 (45%) Prior negative	15 (75%) Biopsy-naïve 5 (25%) Prior positive	–
Biopsy Gleason grade group (GG)	–	9 (45%) GG 1 6 (30%) GG 2 3 (15%) GG 3 2 (10%) GG 4	–

Selection of Metabolites with Greatest Differential Expression

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CE-MS analysis produced >11,000 features in combined anionic and cationic modes:
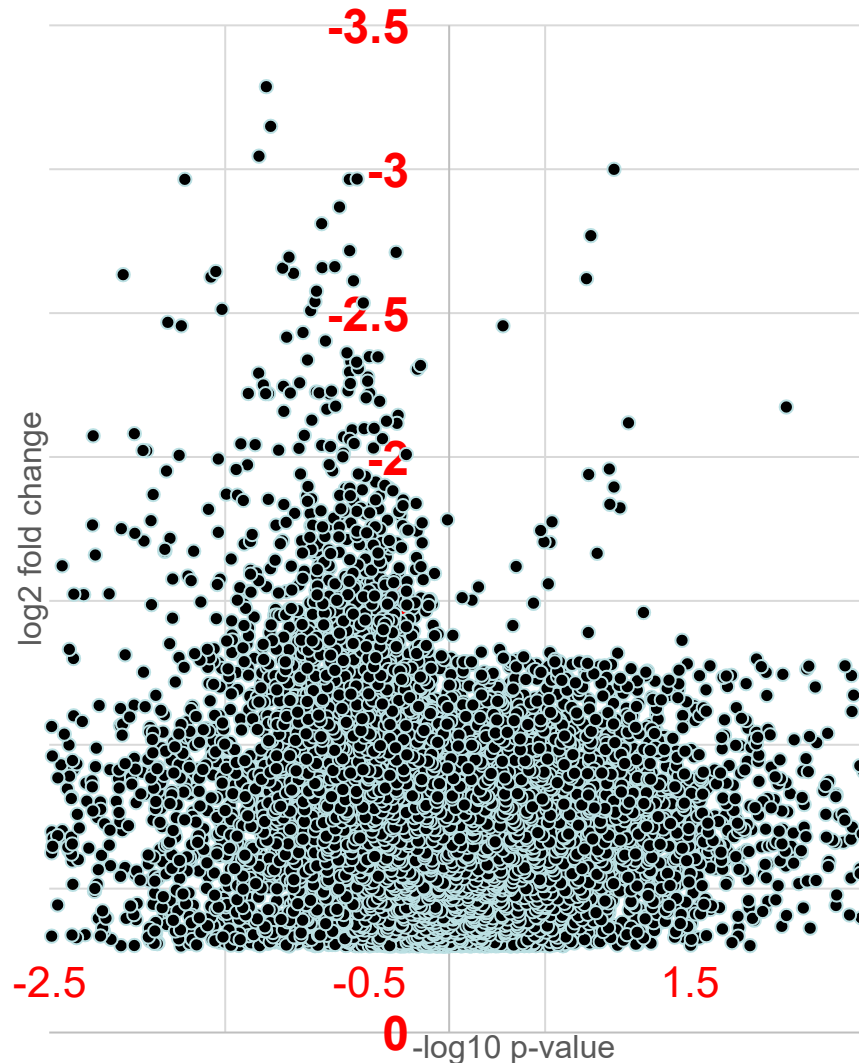
60 metabolites significantly different between urine of men with/without PCa: *P* value vs fold change
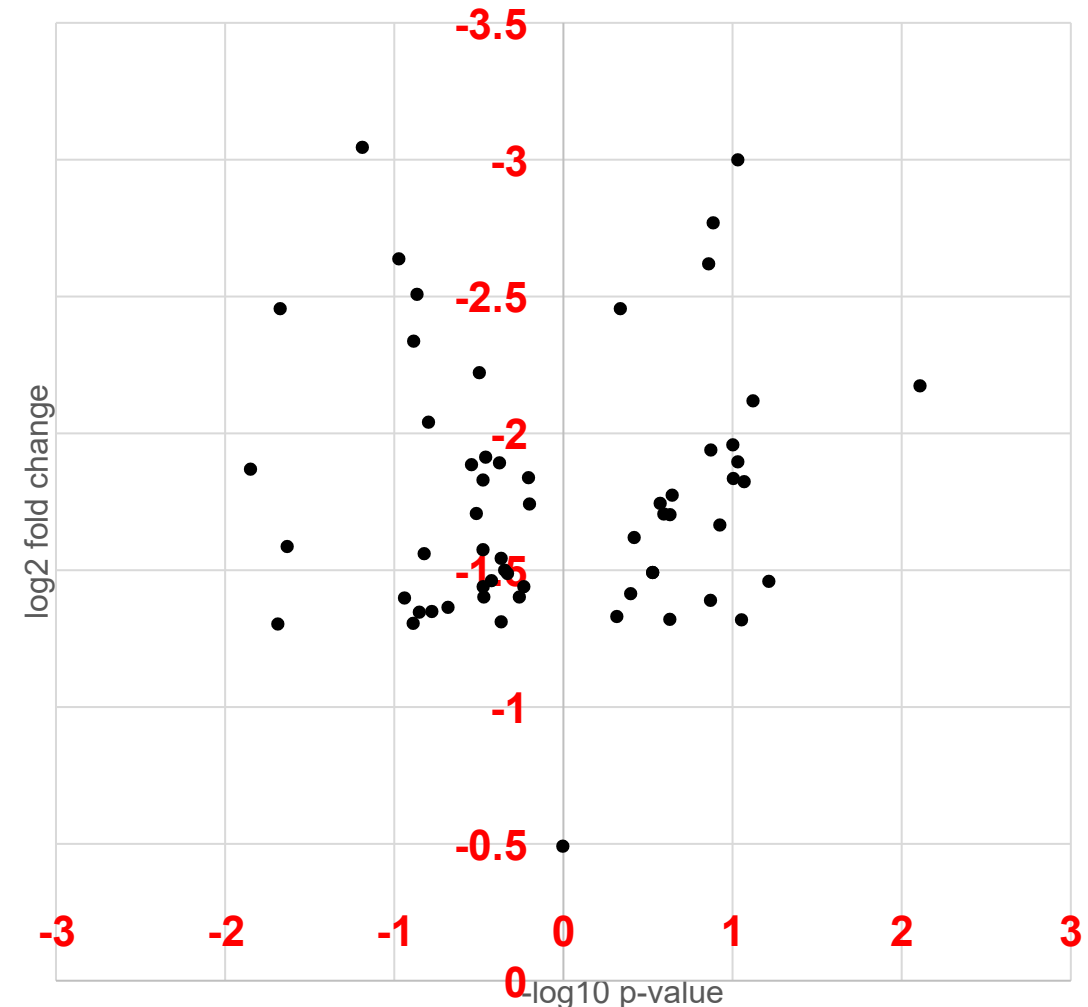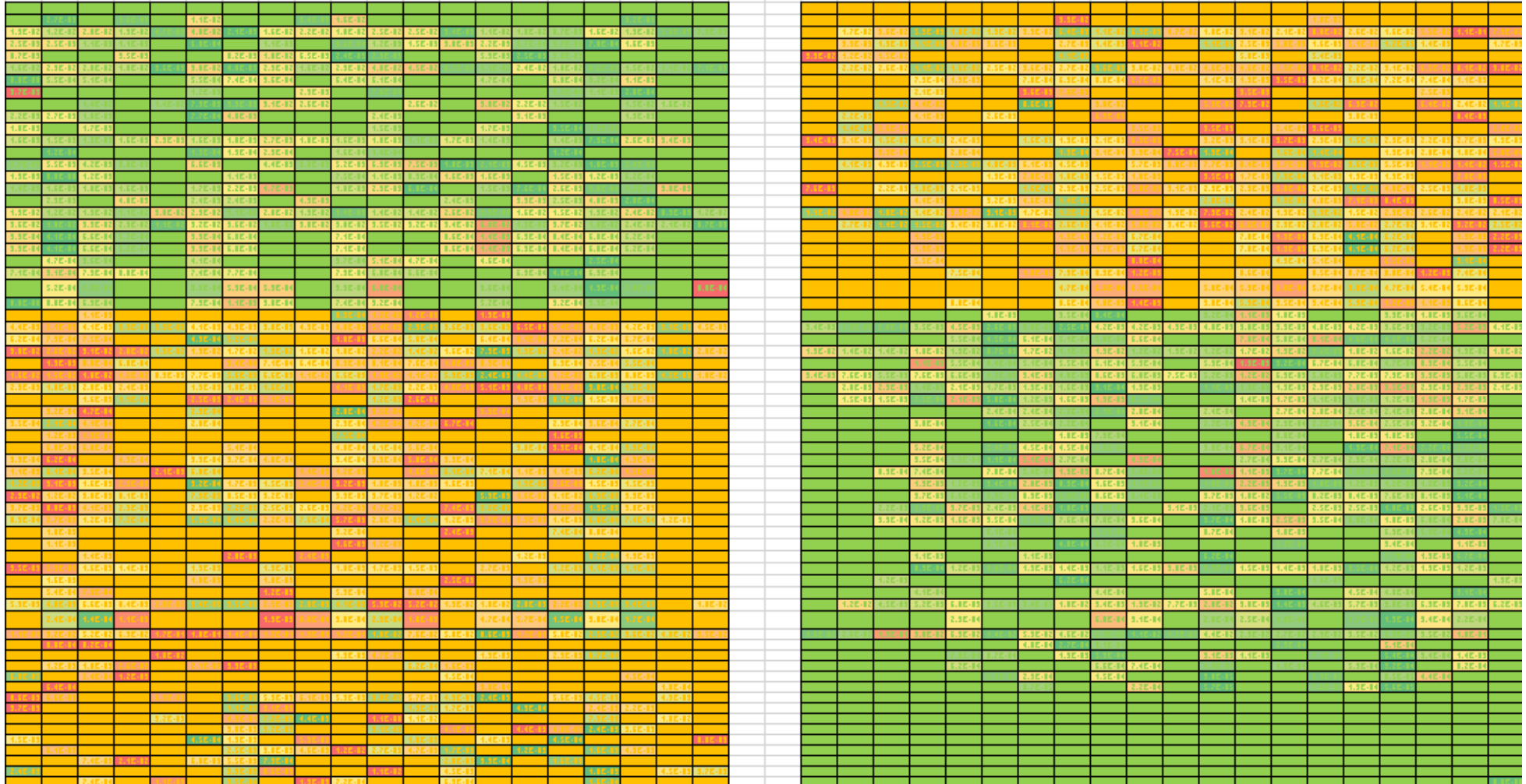
Heat Map of 60 Metabolites of Interest

Benign Patient Group

PCa Patient Group

Reduced Expression

Relative Metabolite Expression

Elevated Expression

Metabolites Elevated in Urine of Men with PCa

| Annotation	General Pathway(s)	P value	Cancer/Benign Fold Change
AC(10:0)	SCFA	0.0067	4.3
19-Hydroxyandrostenedione	Steroid	0.0110	2.2
Phe Tyr	Aromatic metabolism	0.0076	2.2
L-Tyrosine methyl ester 4-sulfate	Microorganism end product	0.0150	2.1
Sulfanilamide	Microorganism end product	0.0480	2.0
Pantetheine	Energy Metabolism	0.0010	2.0
Phe	Aromatic metabolism	0.0146	2.0
3-Methylhistidine	Histidine metabolism	0.0216	1.9
5-Hydroxyindoleacetic acid (5-HIAA)	Prostate tumor marker	0.0017	1.8
Coenzyme Q10	Energy Metabolism	0.0115	1.8
Lactosylceramide(d18:1/24:1)	Ceramide	0.0407	1.8
TrpTrp Pro Ala	Aromatic metabolism	0.0024	1.8
Tyr Tyr Ser Glu	Aromatic metabolism	0.0168	1.6
Gluconic acid	Chelator SCFA	0.0478	1.5
PE(P-16:0/20:4)	Phospholipid	0.0198	1.5
Homocysteinesulfinic acid	Glutamate Receptor Agonist	0.0197	1.5
Tyr Tyr Asp Thr	Aromatic metabolism	0.0180	1.5
Glycerol 3-phosphate	Prostate tumor marker	0.0322	1.4
Melatonin	Prostate tumor risk marker (increases)	0.0385	1.3
His His Pro Ala	Histidine metabolism	0.0035	1.3
Cer(t20:0/22:0(2OH))	Ceramide	0.0240	1.3
Glucosylceramide(d18:1/24:1)	Ceramide	0.0467	1.2

Metabolites Reduced in Urine of Men with PCa

Annotation	General Pathway(s)	P value	Cancer/Benign Fold Change
3-Amino-2-piperidone	Ornithine metabolism Urea Cycle	0.0145	0.9
Succinic acid	Energy Metabolism	0.0181	0.9
Androstanolone	Steroid	0.0286	0.8
Ser	Ser Thr metabolism	0.0396	0.8
Thr	Ser Thr metabolism	0.0316	0.8
2-Hydroxyvaleric acid	SCFA	0.0325	0.8
N-Acetylhyalobiuronic acid	Prostate tumor marker (Hyaluronic acid)	0.0363	0.8
4-Oxovaleric acid	SCFA	0.0396	0.7
2-Aminobutyric acid	SCFA	0.0060	0.7
Guanine	RNA turnover	0.0148	0.7
2-guanidinoethanol	Ornithine metabolism Urea Cycle	0.0266	0.7
5-Hydroxylysine	Histone modification	0.0363	0.7
Asp-Asp	Chelator Calcium binder	0.0122	0.7
Val	BCAA	0.0196	0.7
Testosterone	Steroid	0.0275	0.6
Adipic acid	SCFA	0.0091	0.6
Pro	Prostate tumor marker	0.0450	0.6
Digalacturonic acid	SCFA	0.0046	0.5
4-(β-Acetylaminoethyl)imidazole	Histidine metabolism	0.0399	0.5
N-Acetylglutamine	GlcNac Metabolism	0.0031	0.5
2,3-Dioctanoylglyceramide	ceramide	0.0023	0.5
Homocitrulline (hCIT)	Ornithine metabolism Urea Cycle	0.0009	0.4
β-Estradiol 17-glucuronide	Steroid	0.0135	0.3
o-Hydroxyhippuric acid	Microorganism end product	0.0035	0.3
Creatine	Energy Metabolism	0.0259	0.3
2,6-Diamino-7-hydroxy-azelaic acid	SCFA	0.0001	0.1
Chondroitin	Prostate tumor marker (Hyaluronic acid)	0.0001	0.1
Met His Met Cys	Cys metabolism	0.0001	0.1
His Thr Ala	His Metabolism	0.0001	0.1
Met Cys	Cys metabolism	0.0001	0.1

Pathways differentiating metabolomic profiles from urine of men with & without PCa
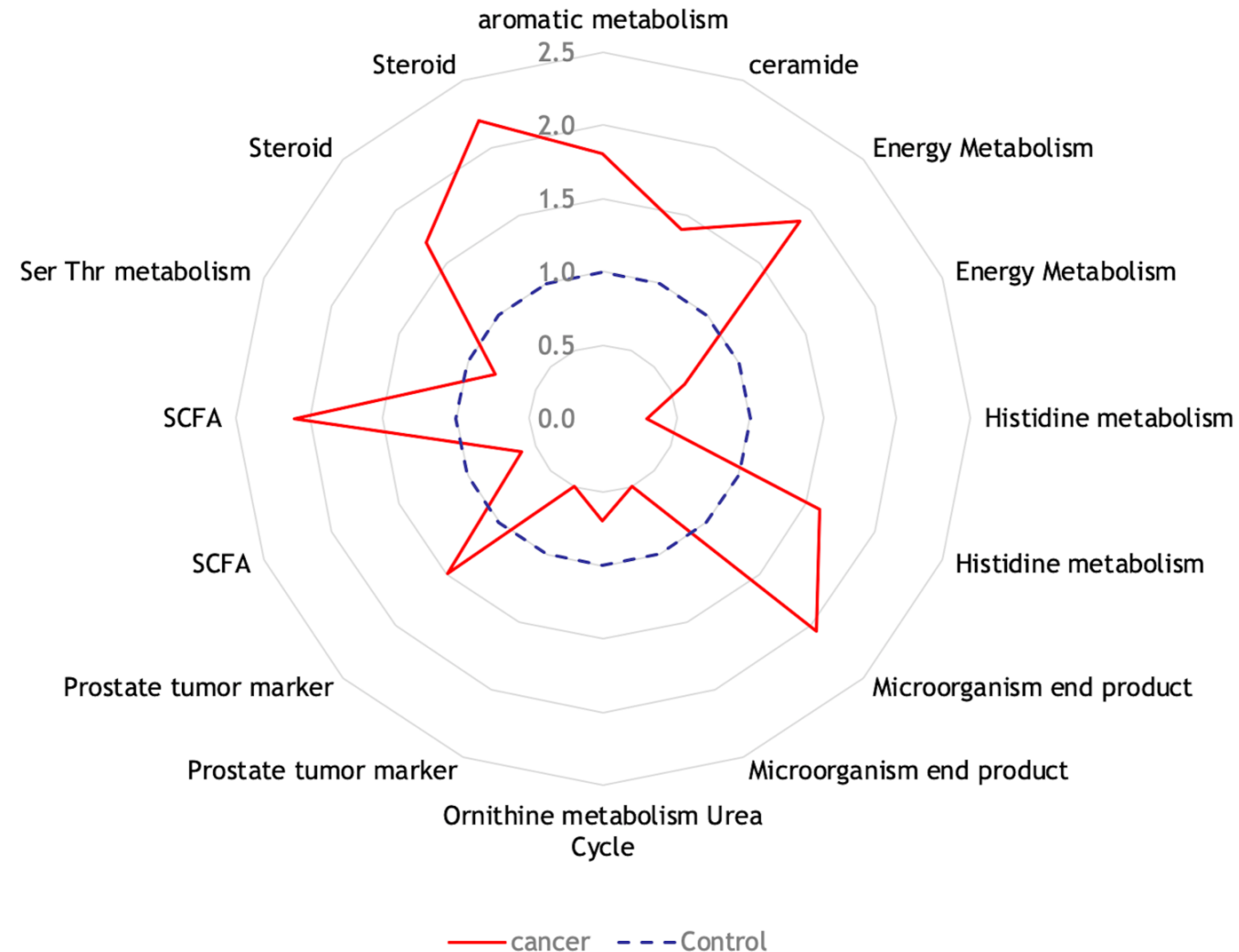
Overview of Pathway Analysis

Conclusion
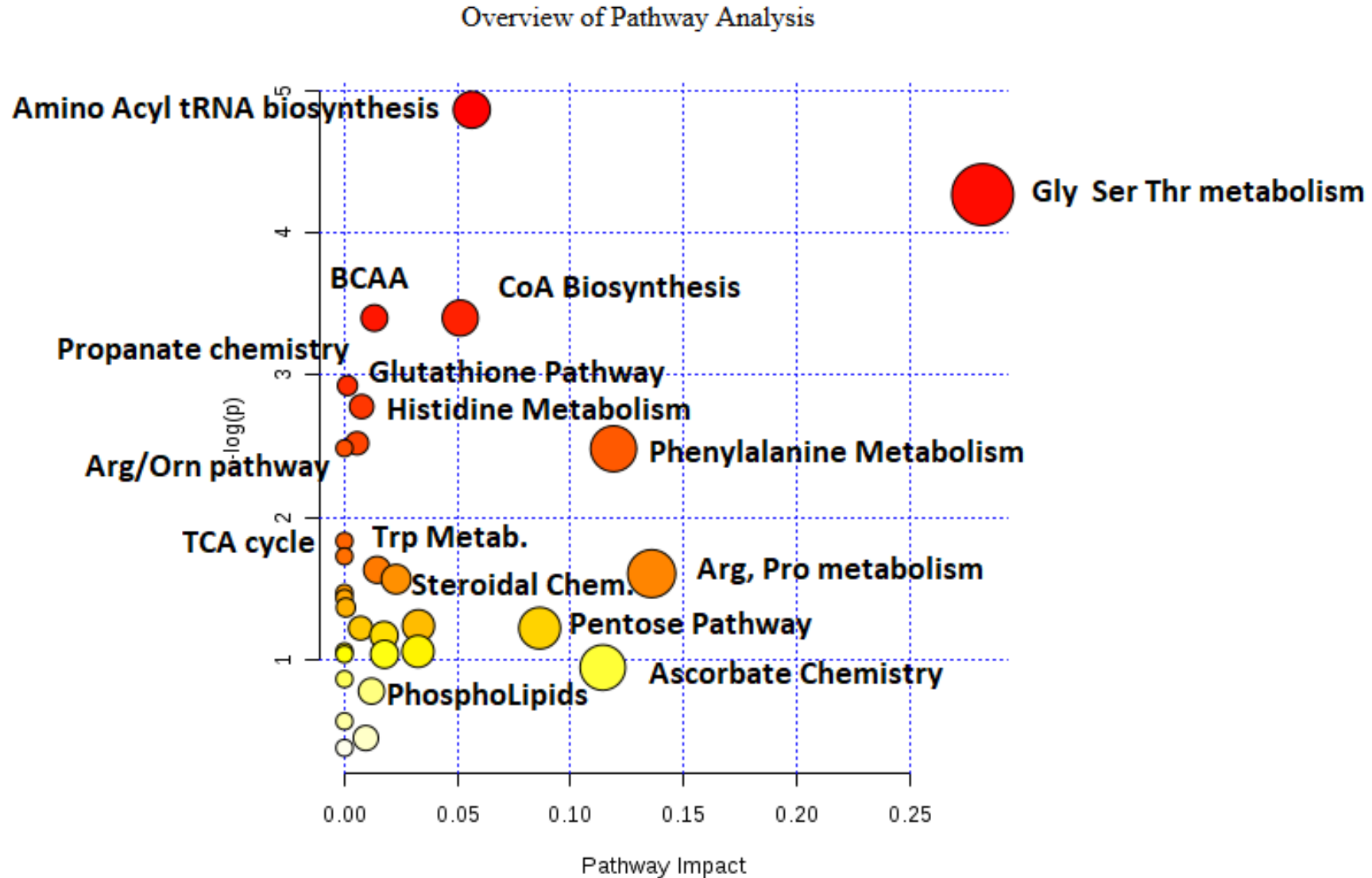
Capillary Electrophoresis Mass Spectrometry (CE-MS) analysis identified several metabolic pathways that were upregulated in urine of men with PCa

These metabolites are involved in steroid, aromatic, and SCFA processes, as well as glycine, serine, and threonine metabolism and warrant targeted studies which are underway in our lab

If validated, they have potential to serve as non-invasive biomarkers for PCa diagnosis and therapeutics

Thank You!