Nuclear Magnetic Resonance Based Metabolomics in Patients with Rheumatoid Arthritis

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What is Rheumatoid Arthritis (RA)?

- Rheumatoid arthritis (RA); is an inflammatory autoimmune disease that is common in the population and is characterized by painful and swollen joints that seriously affect physical function and quality of life.

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Characteristics of RA

- Symptoms such as pain, swelling and limitation of movement in the musculoskeletal system are frequently encountered in patients with RA. Besides these; Patients with RA are much more prone to various diseases than the normal population. (1)

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Diagnosing RA

- The clinic of the patients has a very important place in the diagnosis of RA. Along with the clinic, X-ray and ultrasound (USG) images and laboratory results of the patients help to diagnose, further investigations are needed since RA is thought to be a genetically based disease.

What is Nuclear Magnetic Resonance (NMR)

•Nuclear Magnetic Resonance (NMR) is a very useful method in terms of examining and studying normal and pathological biochemical processes in tissues (2). With this; The ability to examine molecules at the nuclear level with NMR allows a better understanding of the basis of many diseases.

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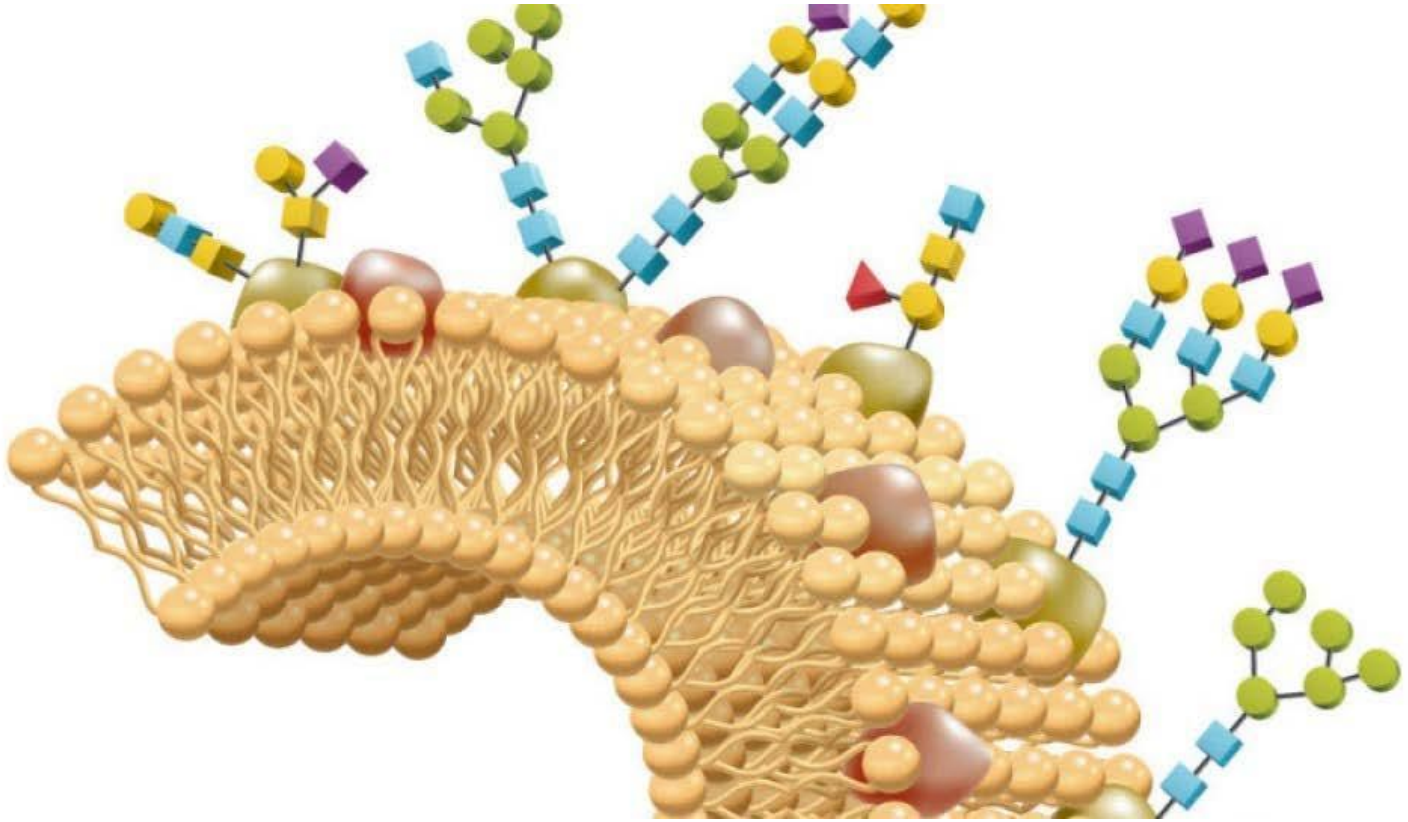
Metabolomics



The detailed study of substrates, products, intermediates or small molecules (MW<1000-1500) such as monosacarits, Amino acids and Organic acids in metabolic reactions in an organism is called ”metabolomics”.

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Hypothesis

- There could be some different types of metabolites between urine sample of a healthy person and urine sample of a RA patient.

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Aim

- The aim of this study was, to determine different types of metabolites between urine sample of a healthy person and urine sample of a RA patient.
- To work on the blood serums, proteins must be precipitated which may cause loss of metabolites. Therefore, the literature recommends working on urine samples.

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Materials and Method

- In order to do that, 120 urine samples were collected from healthy people and 120 urine samples were collected from RA patients as well.
- Then, urine samples were prepared for NMR analysis, phosphate buffer (for pH), D2O and internal standard (final concentration 250 µM DSS) were added to each sample and put into NMR tubes.

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Materials and Method

- 1D NOESY proton NMR was studied on the 500 MHz NMR device in our Bezmialem Vakif University ILMER center.
- The quantity and identity characterization of the molecules obtained with 1D NOESY was revealed using the Chenomx NMR suite profesinals program.

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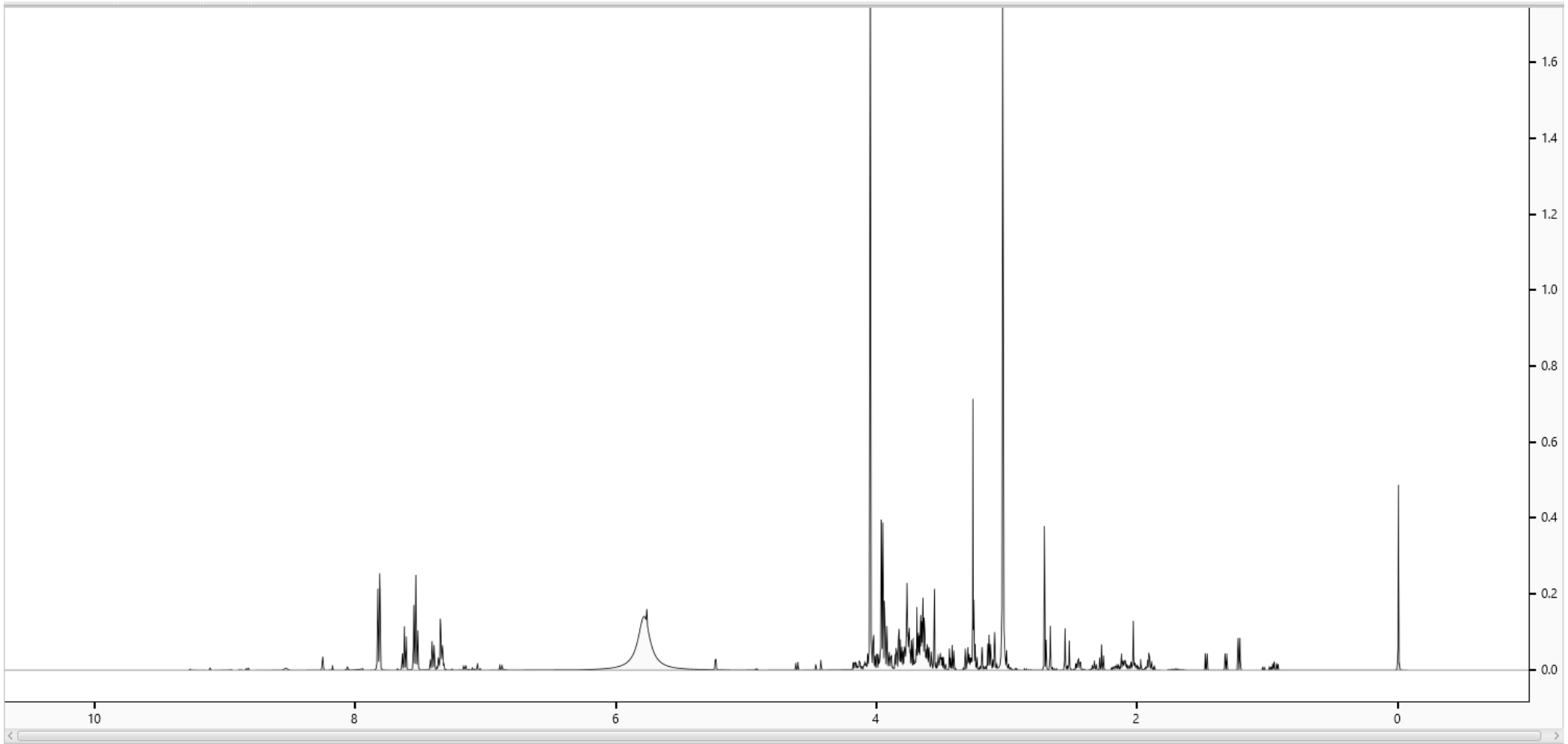


Find in Table (Ctrl+L)

Compound Name	Concentration (mM)	Maximum (mM)	Match Factor	Weight (Da)	Author	Area (sa)
Alanine	0.1384	0.1212	99.22	89.0932	Chenomx Inc.	0.1455
Betaine	0.0989	0.3428	55.41	117.1463	Chenomx Inc.	0.2910
Citrate	0.6392	0.6443	96.73	192.1235	Chenomx Inc.	0.6423
Creatine	0.2373	0.2441	81.23	131.1332	Chenomx Inc.	0.3305
Creatine phosphate	0.5343	1.2342	98.76	211.1131	Chenomx Inc.	0.6678
Creatinine	8.3709	7.4867	99.76	113.1179	Chenomx Inc.	10.5931
Dimethylamine	0.3403	0.3400	99.74	45.0837	Chenomx Inc.	0.3776

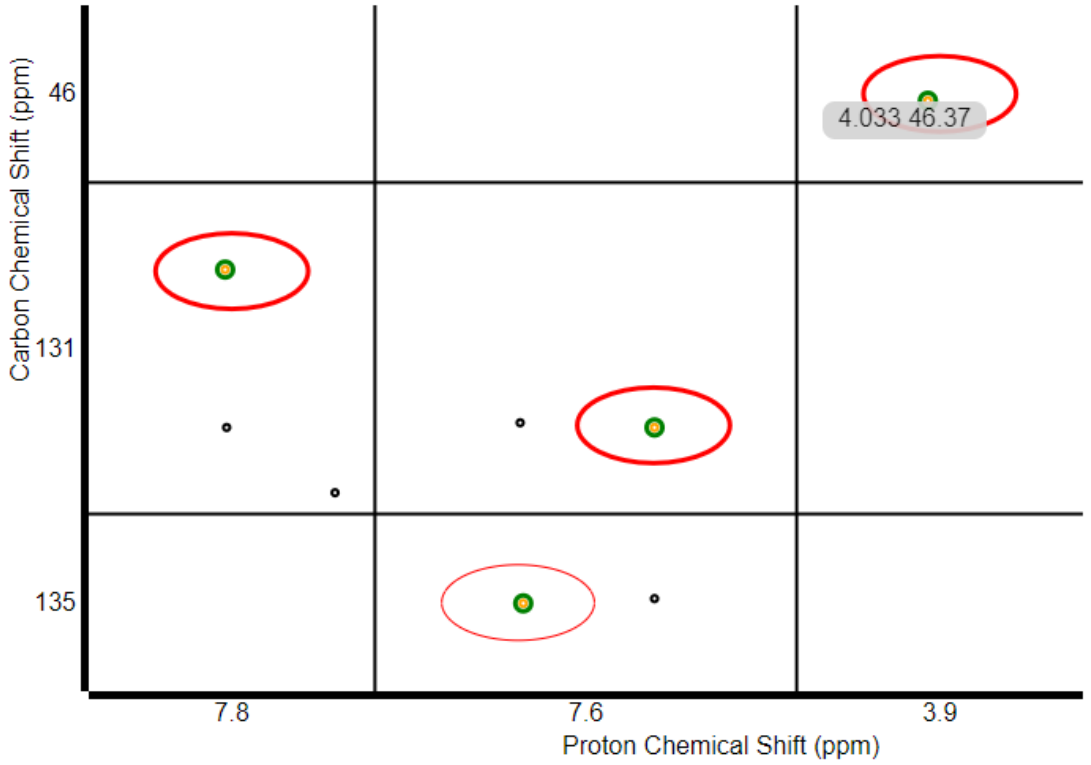
Show detail of one matched spin-system: Show previous compound | Hippuric_acid | Show next compound | Good | Fair | Poor | Not sure

For compound Hippuric_acid, matching ratio is 1.00 ,carbon rmsd is 0.04 proton rmsd is 0.005 User_selected is not processed yet

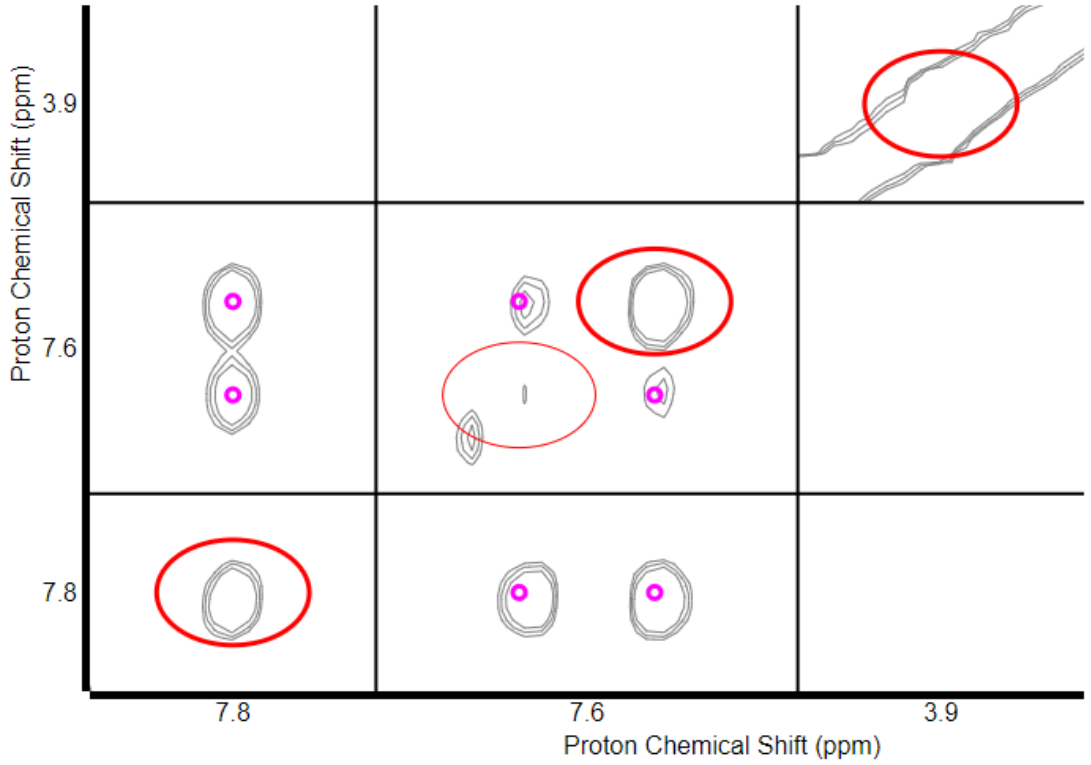
Index	database 1H	database 13C	exp 1H	exp 13C	Sharing	intensity	
0	3.94	46.50	3.95	46.57		4.031e+10	4.031e+10
1	7.81	129.87	7.81	129.85		7.033e+10	7.033e+10
2	7.53	131.50	7.53	131.52		7.603e+10	7.603e+10
3	7.62	134.90	7.62	134.91	29 29	4.138e+10	4.138e+10

Data base peak Matched experimental peak Expected cross peaks

HSQC Lowest contour level: =105559260462.64

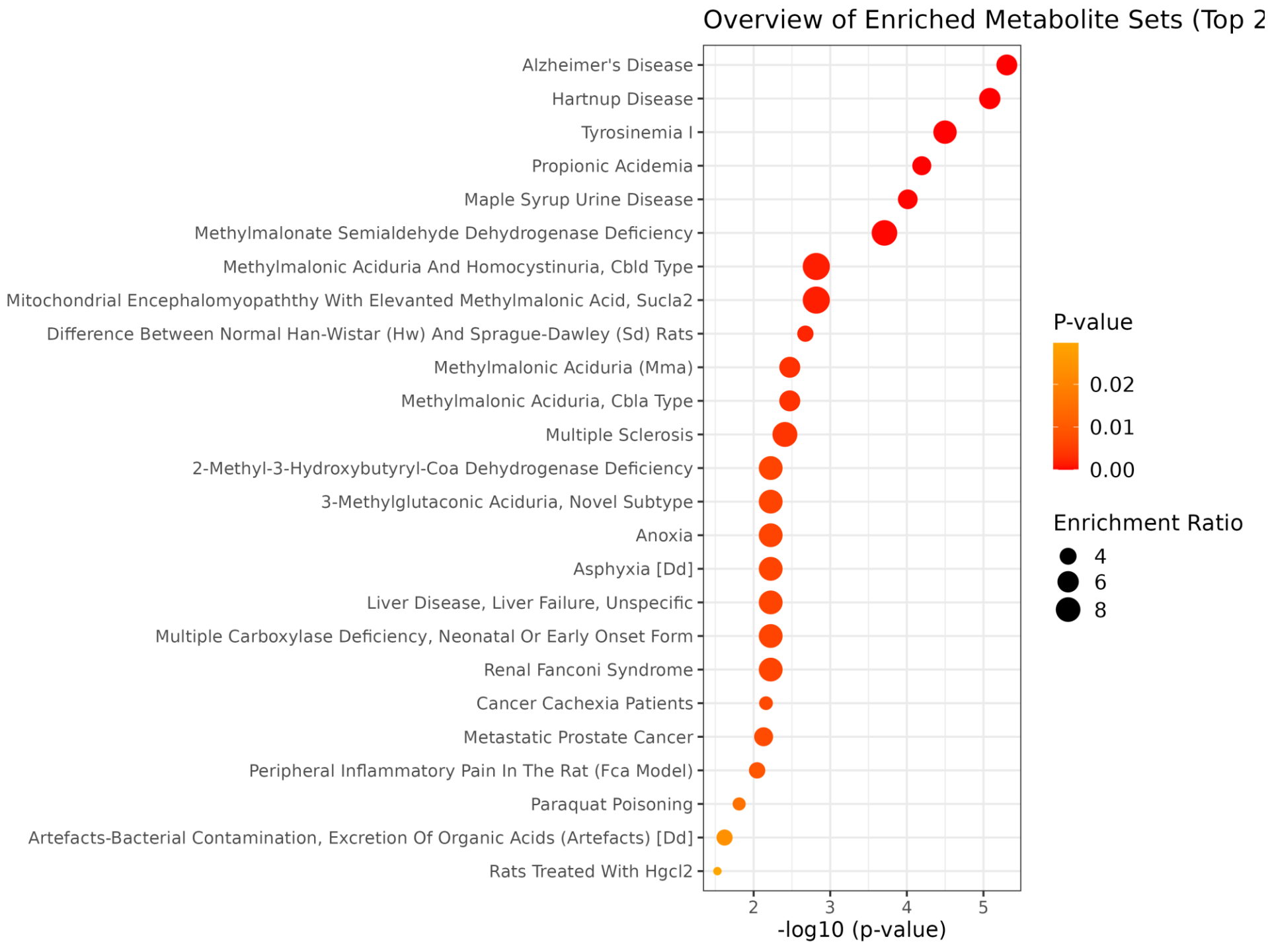


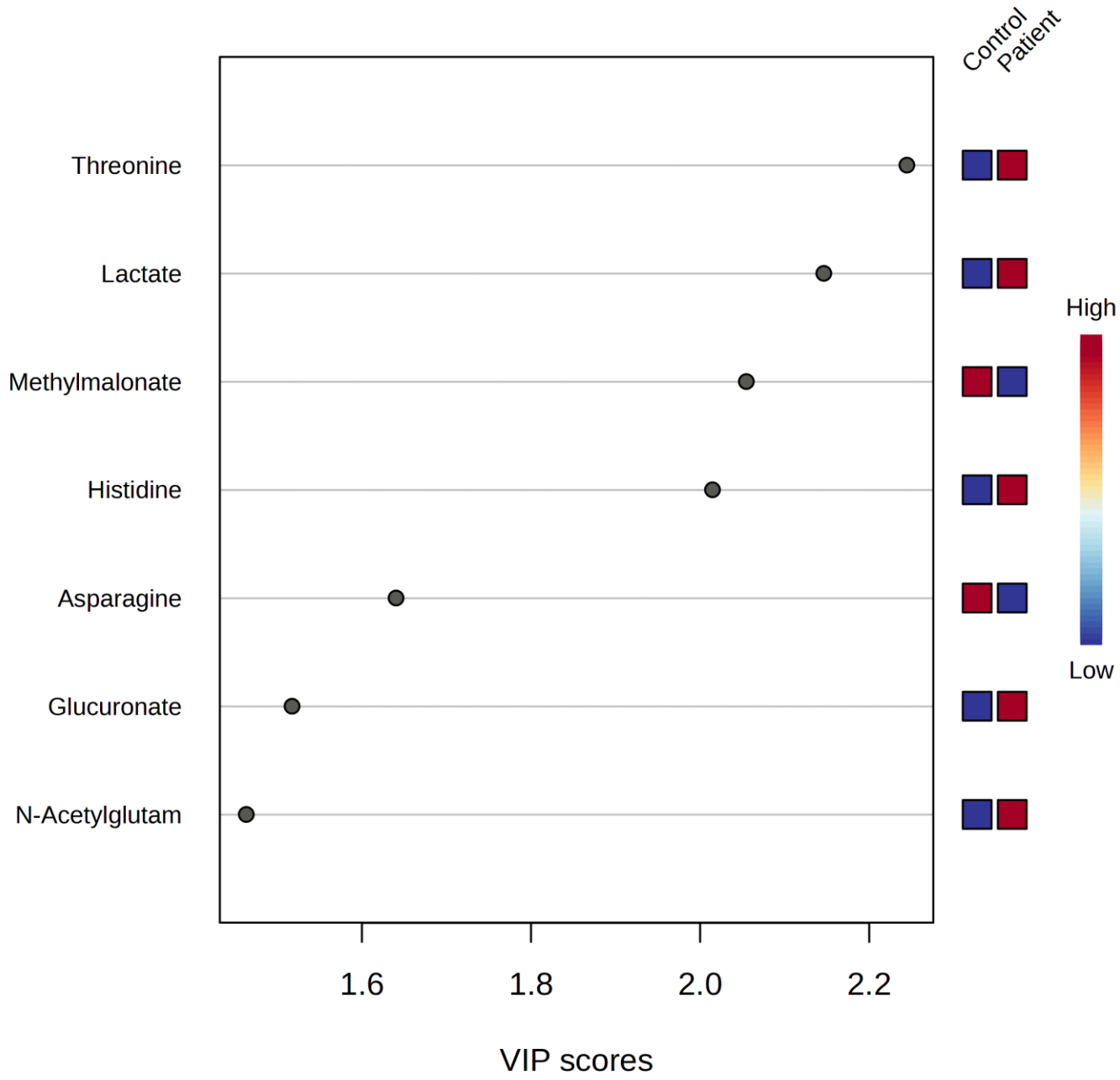
TOCSY Lowest contour level: =68437243519.78

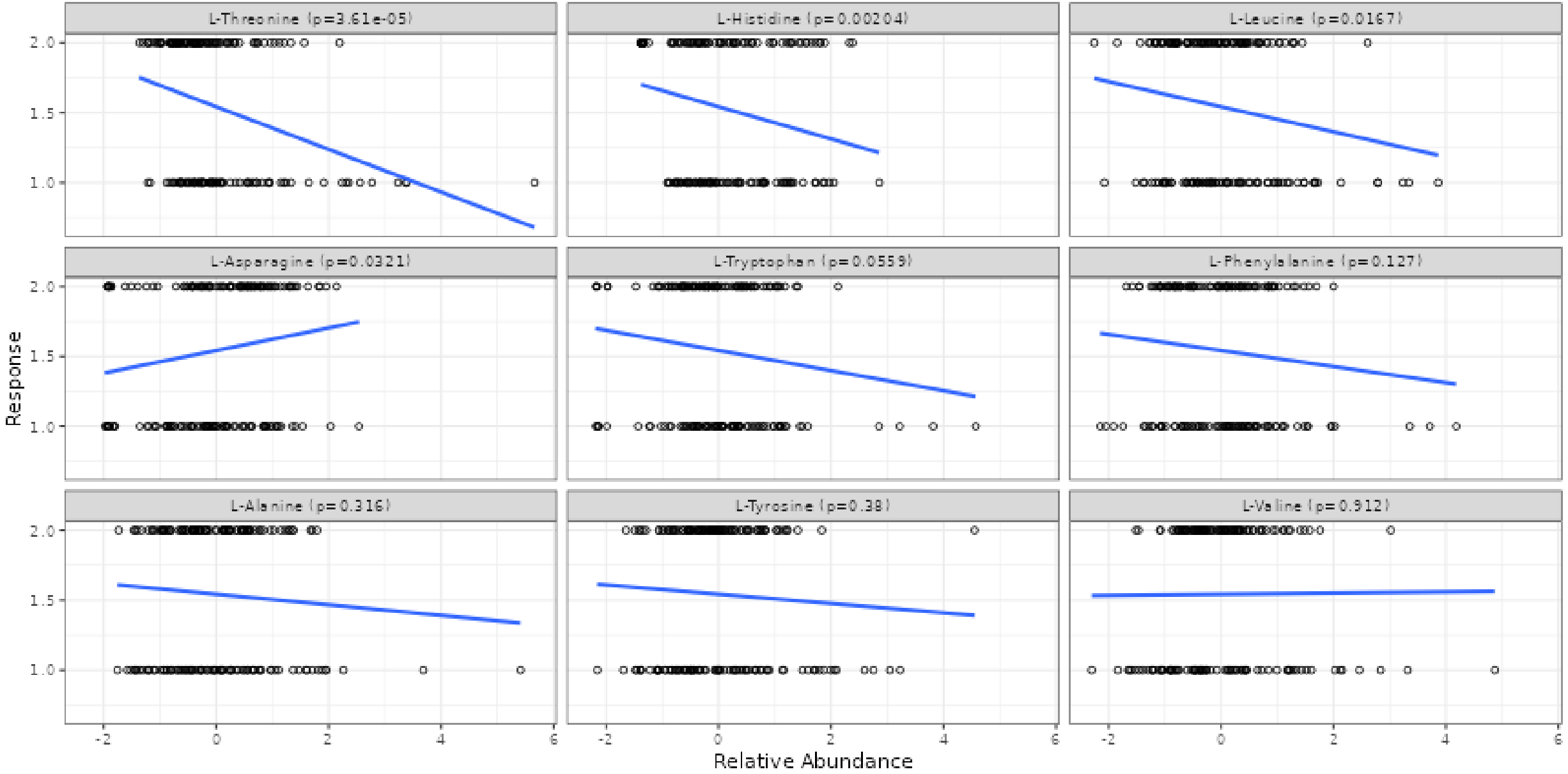


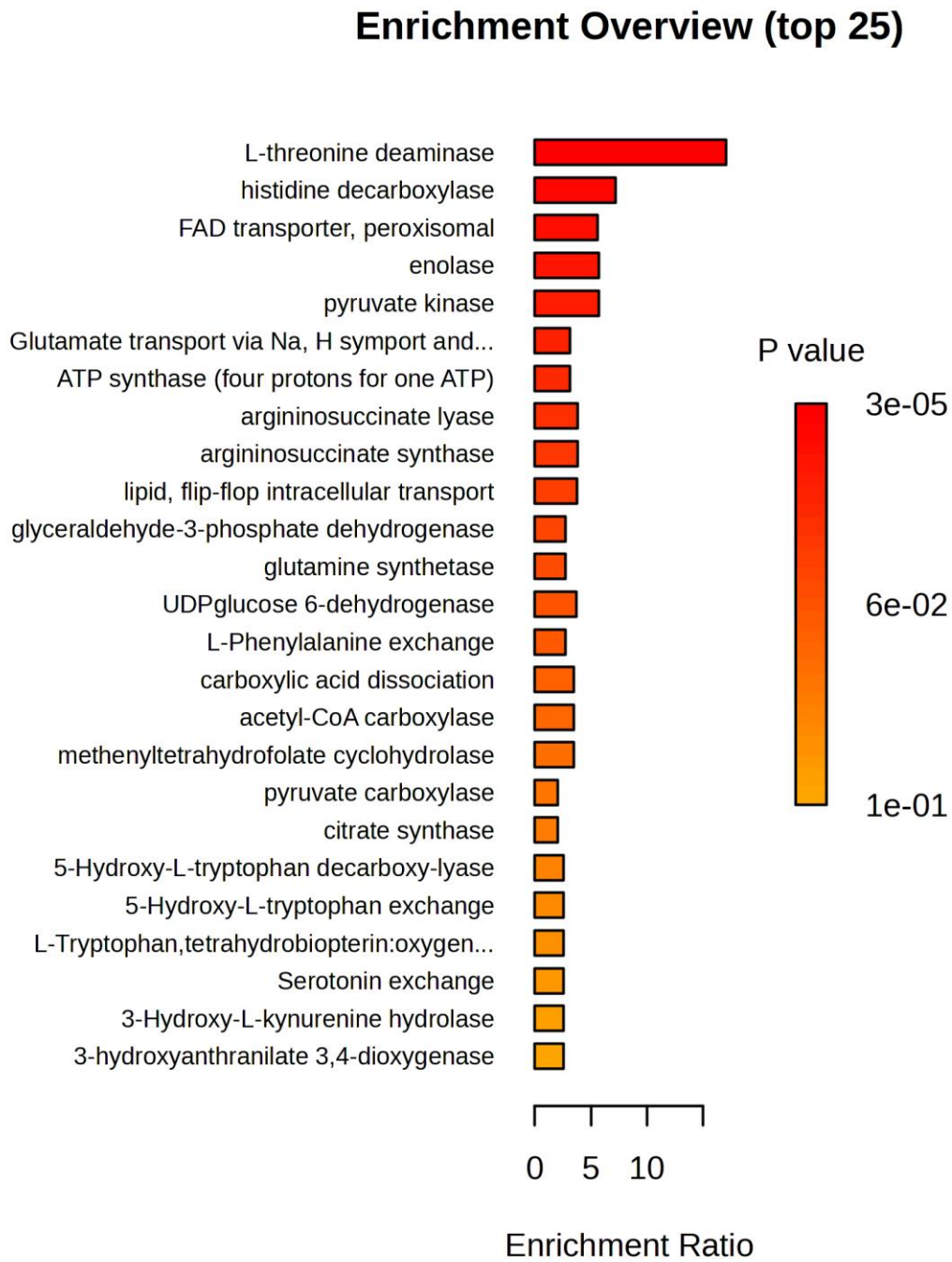
RESULTS

	RAH-U1	RAH-U2	RAH-U3	RAH-U6	RAH-U7	RAH-U8	RAH-U10	RAH-U11	RAH-U12	RAH-U13	RAH-U15	RAH-U16	RAH-U17	RAH-U18	RAH-U19	RAH-U20	RAH-U21	RAH-U22
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4-Aminobutyrate	39.47007	65.3349	44.51118	26.57032	45.46993	25.4712	17.69231	15.44193	40.44829	26.90899	25.35547	46.63437	54.60504	46.95868	32.11371	24.57013	43.44401	16.00601
Acetate	6.725681	13.95417	7.705054	17.0863	10.38023	11.6906	10.72874	14.25409	7.270826	4.391818	8.006991	8.778489	10.78357	13.23325	6.819102	11.19163	6.534682	7.387387
Alanine	16.53347	37.80846	37.05468	24.83911	19.49114	26.40645	21.1336	36.20215	21.81248	45.44683	25.01299	22.70486	37.68964	41.77372	29.51393	28.38643	25.62215	19.12913
Arabinitol	100.2401	153.2462	92.46065	114.4104	168.4249	83.25619	113.4818	142.6219	90.81133	68.98309	85.23076	110.674	154.8112	158.4739	76.58704	77.91261	242.0157	78.22823
Asparagine	8.935718	15.24677	18.10273	44.10824		8.398149	9.02834	51.02316	10.42777	45.51962	58.95413	53.76554	22.21909	72.68225	70.83342	46.95339	33.69364	23.75375
Betaine	11.81474	7.168038	13.75311	4.516202	11.14183	4.867109	15.91093	18.81648	8.740776	2.790382	8.609287	9.41791	22.39529	9.286488	10.35651	17.58072	4.959126	8.108108
Citrate	76.35977	345.2409	112.821	389.6353	161.0628	156.5491	161.5385	272.1505	189.7419	104.9911	200.8479	546.4664	352.9681	379.5697	523.2595	342.3524	189.8157	10.81081
Creatine	28.34821	29.61222	26.55344	23.37134	40.90037	17.86515	36.11336	29.72302	19.34612	17.0092	9.896547	25.38176	62.587	53.16514	28.15011	37.51983	17.89935	98.04805
Creatine phosphate	63.82826	6.653937	76.90555	35.71563	35.11791	52.37391	113.3198	62.7126	52.81954	71.36098	101.847	69.02494	59.64442	59.34066	59.28357	28.85811	39.55678	76.36637
Dimethyl sulfone	3.727198	7.109283	4.86744	7.865718	2.284779	2.633965	4.574899	5.021327	6.80715	0.776454		0.249266	3.999789	1.238198	2.152279	4.37374	0.503661	4.204204
Dimethylamine	40.65274	50.30846	52.85833	48.54917	44.87758	44.61516	48.54251	50.13228	42.92451	35.76541	46.0343	54.96852	47.60982	51.40071	48.18122	58.57382	47.30541	48.10811
Erythritol	101.9962	210.1058	139.9337	155.6961	355.7486	119.5305	116.3563	132.2823	170.6227	92.0826	111.3302	151.4994	176.7307	173.2704	104.9502	167.3599	279.829	141.021
Ethanolamine	65.78743	92.15629	107.3115	64.20534	91.19373	58.07129	64.2915	62.57761	40.44829	62.26191	88.11233	70.07619	95.46632	112.3665	81.25386	65.09155	139.3721	62.34234
Glucuronate	97.57613	78.89248	86.72328	77.00124	178.1846	118.4902	94.97976	70.81151	79.67326	66.70225	51.41953	54.37245	100.6467	125.5378	58.00499	71.05184	252.98	21.83183
Glutamine	51.27286	95.69624	70.48467	53.93098	149.5825	59.64594	67.53036	72.35031	59.18275	67.30886	42.37328	36.43615	87.06148	90.88376	65.12242	76.8835	48.50645	30.63063
Glycine	49.2898	109.3713	132.0423	108.2383	39.65926	121.3628	79.79757	89.97894	90.70281	334.773	96.95782	103.1527	194.9853	196.4866	160.9308	169.9756	120.0134	30.9009
Glycolate	18.17009	19.34489	13.93952	9.408754	14.38565	14.42	79.06883	12.82328	17.96496	4.731517	14.58501		30.46535	33.35397	19.02956	33.74641	49.15217	15.28529
Hippurate	254.9547	245.4025	271.4374	500.5457	509.1391	132.2899	782.5506	380.271	306.3514	6.769709	190.6089	32.06858	677.4972	771.9858	201.5258	146.5632	522.1417	208.8288
Histamine	10.28563	13.92479	29.05965	25.96816	17.51664	6.451305	19.67611	9.961665	9.194586	18.39225	18.36412	15.48699	15.0829	21.15772	26.91414	39.96398	21.4508	12.19219
Histidine	4.348397	56.30141	139.3123	21.90358	18.75776	82.57861	34.93927	90.97781	103.2023	92.66494	127.9111	16.76583	107.078	124.8259	37.56899	101.0677	19.26827	53.66366
Homoserine	65.85911	97.32667	58.53355	113.5448	68.51518	46.90557	81.7004	122.2396	43.2402	65.19787	48.52615	41.59487	63.67945	46.16932	78.22789	92.44887	33.91319	27.65766
Lactate	13.40358	53.6134	25.53853	11.32814	18.64493	23.13308	15.82996	23.62183	18.24119	29.3354	40.77897	33.44496	31.04682	28.32379	21.18184	118.9057	15.38104	15.85586
Leucine	10.45288	20.07932	15.01657	10.38726	18.64493	10.21139	11.7004	11.66244	11.61162	12.66591	12.82536	15.97468	20.66851	25.58427	12.72189	12.34938	15.67807	10.06006
Methylmalonate	26.28152	19.81492	21.12676	15.76907	20.95791	19.64976	19.27126	21.13817	35.03216	16.23274	34.012	39.11305	16.98589	18.23247	11.97605	15.52249	13.93463	10.33033
N-Acetylglutamine	22.20789	26.95358	28.99751		6.713302	20.62318	8.744939	20.73322	17.77751		21.29293	27.10494	29.5491	16.54543	13.82999	12.26363	32.05352	18.10811
N-Phenylacetylglycine	27.63144	41.12808	33.82353	24.08641	68.34593	24.11605	44.0081	36.01317	46.63391	18.87754	29.47707	23.3226	50.11189	44.07986	26.99938	23.66965	64.26201	28.67868
O-Phosphocholine	3.59579	6.389542	6.151616	8.166798	7.13641	20.07921	10.24291	11.50046	7.339884	10.67624	5.609618	3.988252	7.911476	5.231388	22.99316	7.289567	7.761549	14.11411
Oxypurinol	84.20839	159.9001	125.2071	46.36634	259.5058	197.1465	261.417	116.8673	437.8971	244.3889	140.89	171.2998	90.42694	67.65207		141.0746		163.4234
Phenylalanine	74.46033	85.25264	51.38774	62.32359	128.0041	30.90137	115.7895	136.3857	69.38361	7.400577	41.01516	60.1164	67.83782	85.94645	37.05756	42.02221	105.9755	42.31231
Proline	23.11579	32.2121	40.92792	101.9909	38.44635	30.00429	47.00405	46.21781	38.92901	68.08531	25.67434	27.27834	35.24043	43.77031	39.55079	60.54629	41.17108	32.55255
Taurine	49.17034	23.36957	62.38608	25.89289	43.52364	59.15923	76.03239	28.21122	48.96216	81.21224	26.26482	75.10485	91.9599	86.72032	31.92192	86.31705	15.31647	53.66366
Threonate	112.3535	43.15511	142.6471	74.85605	117.4264	98.98363	81.65992	142.6489	87.3683	80.31446	68.40191	99.03437	78.12803	134.7469	108.2959	59.90309	145.881	61.47147
Threonine	3.858605	9.856052	8.202154	13.73678	11.48031	4.924369	10.08097	8.314886	3.640346	6.963822	8.609287	5.483846	17.42639	9.085281	6.137192	29.75859	7.038343	9.429429
Trigonelline	12.10145	5.948884	5.343828	68.11938	24.00429	15.83242	25.82996	13.33621	20.79634	3.566836	16.48637	6.871065	8.739626	12.05696	19.96718	4.716779	49.28131	8.468468
Trimethylamine N-oxic	35.24113	55.11163	34.42419	35.11347	54.94753	48.00305	59.19028	39.44171	45.32181	6.308689	9.081676	22.93245	38.20062	42.59403	30.345	45.58124	24.24031	32.7027
Tryptophan	2.520637	17.64101	29.92958	14.8282	34.21528	31.77936	52.06478	35.20328	24.96942		22.99353	28.102	55.04555	62.69927	33.26443	8.747481	59.21248	19.48949
Tyrosine	10.76348	9.371328	25.10356	10.38726	9.167325	7.11934	11.417	17.95259	15.94254	13.85485	14.75034	15.31359	19.73464	21.31249	8.609116	10.97723	6.224736	5.255255
Urea	3761.543	6109.004	2503.625	5939.257	6910.358	2805.583	4860.162	8486.934	6447.615	7049.475	4669.033	3969.665	10234.31	7060.486	7068.788	9976.116	4853.163	8838.378
Valine	3.201567	5.317274	4.660315	3.838772	4.484937	4.046381	2.874494	4.157443	4.459177	4.294761	5.337994	3.955739	5.250824	5.417118	5.58314	5.703014	2.208361	4.204204
cis-Aconitate	41.56064	33.32844	14.58161	25.21546	39.29256	30.40512	8.259109	27.23935	22.06898	22.1532	26.51283	37.78002	26.64176	35.16484	35.24623	24.22709	9.104645	18.07808


Overview of Enriched Metabolite Sets (Top 2
Alzheimer's Disease
Hartnup Disease
Tyrosinemia I
Propionic Acidemia
Maple Syrup Urine Disease
Methylmalonate Semialdehyde Dehydrogenase Deficiency
Methylmalonic Aciduria And Homocystinuria, Cbld Type
Mitochondrial Encephalomyopaththy With Elevanted Methylmalonic Acid, Sucla2
Difference Between Normal Han-Wistar (Hw) And Sprague-Dawley (Sd) Rats
Methylmalonic Aciduria (Mma)
Methylmalonic Aciduria, Cbla Type
Multiple Sclerosis
2-Methyl-3-Hydroxybutyryl-Coa Dehydrogenase Deficiency
3-Methylglutaconic Aciduria, Novel Subtype
Anoxia
Asphyxia [Dd]
Liver Disease, Liver Failure, Unspecific
Multiple Carboxylase Deficiency, Neonatal Or Early Onset Form
Renal Fanconi Syndrome
Cancer Cachexia Patients
Metastatic Prostate Cancer
Peripheral Inflammatory Pain In The Rat (Fca Model)
Paraquat Poisoning
Artefacts-Bacterial Contamination, Excretion Of Organic Acids (Artefacts) [Dd]
Rats Treated With Hgcl2
2
3
4
5
-log10 (p-value)
P-value
0.02
0.01
0.00
Enrichment Ratio
4
6
8

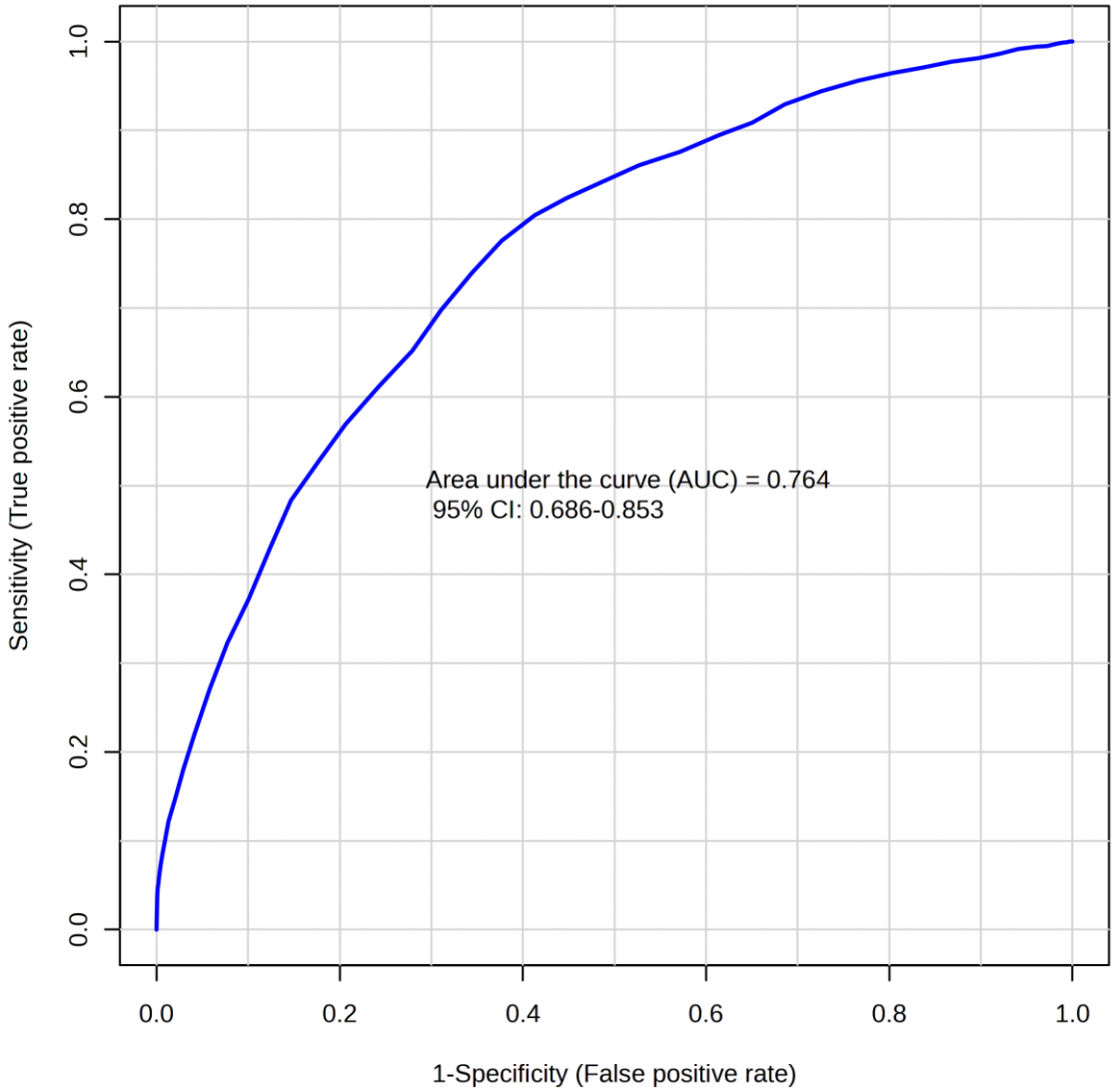

Control
Patient
Threonine
Lactate
Methylmalonate
Histidine
Asparagine
Glucuronate
N-Acetylglutam
High
Low
1.6
1.8
2.0
2.2
VIP scores


L-Threonine (p=3.61e-05)
L-Histidine (p=0.00204)
L-Leucine (p=0.0167)
L-Asparagine (p=0.0321)
L-Tryptophan (p=0.0559)
L-Phenylalanine (p=0.127)
L-Alanine (p=0.316)
L-Tyrosine (p=0.38)
L-Valine (p=0.912)
Response
Relative Abundance
2.0
1.5
1.0
-2
0
2
4
6


Enrichment Overview (top 25)
L-threonine deaminase
histidine decarboxylase
FAD transporter, peroxisomal
enolase
pyruvate kinase
Glutamate transport via Na, H symport and...
ATP synthase (four protons for one ATP)
argininosuccinate lyase
argininosuccinate synthase
lipid, flip-flop intracellular transport
glyceraldehyde-3-phosphate dehydrogenase
glutamine synthetase
UDPglucose 6-dehydrogenase
L-Phenylalanine exchange
carboxylic acid dissociation
acetyl-CoA carboxylase
methenyltetrahydrofolate cyclohydrolase
pyruvate carboxylase
citrate synthase
5-Hydroxy-L-tryptophan decarboxy-lyase
5-Hydroxy-L-tryptophan exchange
L-Tryptophan,tetrahydrobiopterin:oxygen...
Serotonin exchange
3-Hydroxy-L-kynurenine hydrolase
3-hydroxyanthranilate 3,4-dioxygenase
P value
3e-05
6e-02
1e-01
0
5
10
Enrichment Ratio

BIOMARKER?
ROC analysis on selected features

Threonine
Histidine
Acetate
Methylmalonate
N-Acetylglutamine
Asparagine
Lactate



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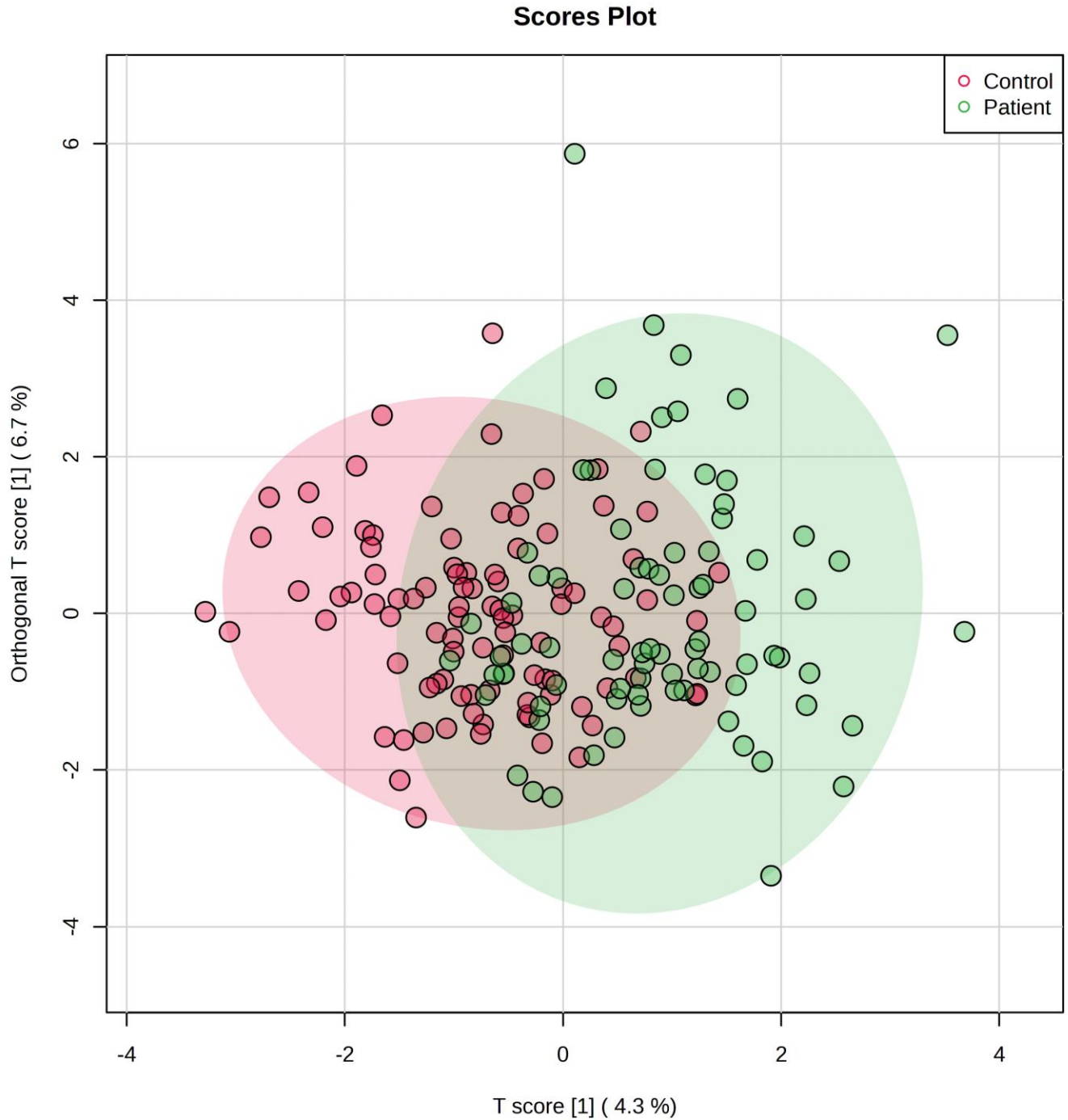
Scores Plot
Control
Patient
Orthogonal T score [1] (6.7 %)
6
4
2
0
-2
-4
-4
-2
0
2
4
T score [1] (4.3 %)

Conclusion and Future Directions

- In this study, some significant metabolites were found different clearly between urines of RA patients and healthy people.
- In further studies, those metabolites can be examined specificly, to understand if they are effective on diagnosing and treating RA patients earlier.

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