

Metabolomic Data Analysis with MetaboAnalyst 3.0

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1 Background

MESA or Metabolite Set Enrichment Analysis is a way to identify biologically meaningful patterns that are significantly enriched in quantitative metabolomic data. In conventional approaches, metabolites are evaluated individually for their significance under conditions of study. Those compounds that have passed certain significance level are then combined to see if any meaningful patterns can be discerned. In contrast, MSEA directly investigates if a set of functionally related metabolites without the need to preselect compounds based on some arbitrary cut-off threshold. It has the potential to identify subtle but consistent changes among a group of related compounds, which may go undetected with the conventional approaches.

Essentially, MSEA is a metabolomic version of the popular GSEA (Gene Set Enrichment Analysis) software with its own collection of metabolite set libraries as well as an implementation of user-friendly web-interfaces. GSEA is widely used in genomics data analysis and has proven to be a powerful alternative to conventional approaches. For more information, please refer to the original paper by Subramanian A, and a nice review paper by Nam D, Kim SY.

2 MSEA Overview

Metabolite set enrichment analysis consists of four steps - data input, data processing, data analysis, and results download. Different analysis procedures are performed based on different input types. In addition, users can also browse and search the metabolite set libraries as well as upload their self-defined metabolite sets for enrichment analysis. Users can also perform metabolite name mapping between a variety of compound names, synonyms, and major database identifiers.

3 Data Input

There are three enrichment analysis algorithms offered by MSEA. Accordingly, three different types of data inputs are required by these three approaches:

- A list of important compound names - entered as a one column data (*Over Representation Analysis (ORA)*);
- A single measured biofluid (urine, blood, CSF) sample- entered as tab separated two-column data with the first column for compound name, and the second for concentration values (*Single Sample Profiling (SSP)*);
- A compound concentration table - entered as a comma separated (.csv) file with the each sample per row and each metabolite concentration per column. The first column is sample names and the second column for sample phenotype labels (*Quantitative Enrichment Analysis (QEA)*)

You selected Quantitative Enrichment Analysis (QEA) which requires a concentration table. This is the most common data format generated from quantitative metabolomics studies. The phenotype label can be discrete (binary or multi-class) or continuous.

4 Data Process

The first step is to standardize the compound labels. It is an essential step since the compound labels will be subsequently compared with compounds contained in the metabolite set library. MSEA has a built-in tool to convert between compound common names, synonyms, identifiers used in HMDB ID, PubChem, ChEBI, BiGG, METLIN, KEGG, or Reactome. **Table 1** shows the conversion results. Note: 1 indicates exact match, 2 indicates approximate match, and 0 indicates no match. A text file contain the result can be found the downloaded file *name_map.csv*

Table 1: Result from Compound Name Mapping

Query	Match
1 1,2-dilinoeoyl-GPC (18:2/18:2)	NA
2 1,2-dioleoyl-GPC (18:1/18:1)*	NA
3 1,2-dioleoyl-GPE (18:1/18:1)	NA
4 1,2-dipalmitoyl-GPC (16:0/16:0)	NA
5 1,2-distearoyl-GPC (18:0/18:0)	NA
6 1,5-anhydroglucitol (1,5-AG)	NA
7 1-(1-enyl-oleoyl)-GPE (P-18:1)*	NA
8 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPC (P-16:0/20:4)*	NA
9 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	NA
10 1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	NA
11 1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)*	NA
12 1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	NA
13 1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	NA
14 1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)*	NA
15 1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*	NA
16 1-(1-enyl-palmitoyl)-GPC (P-16:0)*	NA
17 1-(1-enyl-palmitoyl)-GPE (P-16:0)*	NA
18 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	NA
19 1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	NA
20 1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	NA
21 1-(1-enyl-stearoyl)-GPE (P-18:0)*	NA
22 1-(3-aminopropyl)-2-pyrrolidone	NA
23 1-arachidonoyl-GPC (20:4)*	NA
24 1-arachidonoyl-GPE (20:4)*	NA
25 1-arachidonoyl-GPI (20:4)*	NA
26 1-lignoceroyl-GPC (24:0)	NA
27 1-linolenoyl-GPC (18:3)*	NA
28 1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4)*	NA
29 1-linoleoyl-GPC (18:2)	NA
30 1-linoleoyl-GPE (18:2)*	NA
31 1-linoleoyl-GPI (18:2)*	NA
32 1-linoleoylglycerol (18:2)	NA
33 1-methylguanidine	Methylguanidine
34 1-methylhistidine	1-Methylhistidine
35 1-methylimidazoleacetate	NA
36 1-methylnicotinamide	1-Methylnicotinamide
37 1-oleoyl-2-linoleoyl-glycerol (18:1/18:2)	NA
38 1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	NA
39 1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	NA
40 1-oleoyl-3-linoleoyl-glycerol (18:1/18:2)	NA
41 1-oleoyl-GPC (18:1)	NA
42 1-oleoyl-GPE (18:1)	NA
43 1-oleoyl-GPI (18:1)*	NA
44 1-oleoyl-GPS (18:1)	NA
45 1-oleoylglycerol (18:1)	NA
46 1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	NA
47 1-palmitoleoyl-2-oleoyl-glycerol (16:1/18:1)*	NA
48 1-palmitoleoyl-3-oleoyl-glycerol (16:1/18:1)*	NA
49 1-palmitoleoyl-GPC (16:1)*	NA
50 1-palmitoleoylglycerol (16:1)*	NA
51 1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	NA
52 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	NA
53 1-palmitoyl-2-linoleoyl-glycerol (16:0/18:2)*	NA
54 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	NA
55 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	NA
56 1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	NA
57 1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	NA
58 1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	NA
59 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	NA
60 1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	NA
61 1-palmitoyl-3-linoleoyl-glycerol (16:0/18:2)*	NA
62 1-palmitoyl-GPC (16:0)	NA

63	1-palmitoyl-GPE (16:0)	NA
64	1-palmitoyl-GPG (16:0)*	NA
65	1-palmitoyl-GPI (16:0)*	NA
66	1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	NA
67	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	NA
68	1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	NA
69	1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	NA
70	1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	NA
71	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	NA
72	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	NA
73	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	NA
74	1-stearoyl-2-oleoyl-GPS (18:0/18:1)	NA
75	1-stearoyl-GPC (18:0)	NA
76	1-stearoyl-GPE (18:0)	NA
77	1-stearoyl-GPI (18:0)	NA
78	1-stearoyl-GPS (18:0)*	NA
79	10-heptadecenoate (17:1n7)	NA
80	10-nonadecenoate (19:1n9)	NA
81	12,13-DiHOME	12,13-DHOME
82	12-HETE	12-HETE
83	15-HETE	15(S)-HETE
84	15-methylpalmitate	NA
85	16-hydroxypalmitate	NA
86	17-methylstearate	NA
87	2'-deoxycytidine	Deoxycytidine
88	2'-deoxyguanosine	Deoxyguanosine
89	2'-deoxyinosine	Deoxyinosine
90	2'-deoxyuridine	Deoxyuridine
91	2-aminoadipate	Aminoadipic acid
92	2-aminoheptanoate	NA
93	2-aminooctanoate	DL-2-Amino-octanoic acid
94	2-hydroxy-3-methylvalerate	2-Hydroxy-3-methylpentanoic acid
95	2-hydroxyadipate	2-Hydroxyadipic acid
96	2-hydroxybutyrate/2-hydroxyisobutyrate	NA
97	2-hydroxyglutarate	2-Hydroxyglutarate
98	2-hydroxystearate	NA
99	2-linoleoylglycerol (18:2)	NA
100	2-methylbutyrylcarnitine (C5)	NA
101	2-methylbutyrylglycine	2-Methylbutyrylglycine
102	2-methylcitrate/homocitrate	NA
103	2-methylmalonyl carnitine	NA
104	2-oleoylglycerol (18:1)	NA
105	2-palmitoleoyl-GPC (16:1)*	NA
106	2-palmitoyl-GPC (16:0)*	NA
107	2-stearoyl-GPE (18:0)*	NA
108	3-(4-hydroxyphenyl)lactate	3-(4-Hydroxyphenyl)lactate
109	3-aminoisobutyrate	3-Aminoisobutanoic acid
110	3-hydroxy-3-methylglutarate	3-Hydroxymethylglutaric acid
111	3-hydroxybutyrate (BHBA)	NA
112	3-hydroxybutyrylcarnitine (1)	NA
113	3-hydroxybutyrylcarnitine (2)	NA
114	3-hydroxyisobutyrate	(S)-3-Hydroxyisobutyric acid
115	3-indoxyl sulfate	Indoxyl sulfate
116	3-methylcytidine	NA
117	3-methylhistidine	3-Methylhistidine
118	3-phosphoglycerate	3-Phosphoglyceric acid
119	3-ureidopropionate	Ureidopropionic acid
120	4-cholesten-3-one	Cholestenone
121	4-ethylphenylsulfate	NA
122	4-guanidinobutanoate	4-Guanidinobutanoic acid
123	4-hydroxy-nonenal-glutathione	NA
124	4-hydroxybutyrate (GHB)	NA
125	4-imidazoleacetate	Imidazoleacetic acid
126	4-vinylphenol sulfate	NA
127	5-aminovalerate	5-Aminopentanoic acid
128	5-dodecenoate (12:1n7)	NA
129	5-hydroxylysine	5-Hydroxylysine
130	5-methylthioadenosine (MTA)	NA
131	5-oxoproline	Pyroglutamic acid
132	6-oxopiperidine-2-carboxylic acid	NA
133	6-phosphogluconate	6-Phosphogluconic acid
134	7-hydroxycholesterol (alpha or beta)	NA
135	7-methylguanine	7-Methylguanine
136	9,10-DiHOME	9,10-DHOME
137	acetylcarnitine	L-Acetylcarnitine
138	acetylphosphate	Acetylphosphate
139	aconitate [cis or trans]	NA
140	adenine	Adenine
141	adenosine	Adenosine
142	adenosine 2'-monophosphate (2'-AMP)	NA
143	adenosine 3',5'-diphosphate	Adenosine 3',5'-diphosphate
144	adenosine 3'-monophosphate (3'-AMP)	NA
145	adenosine 5'-diphosphoribose (ADP-ribose)	NA
146	adenosine 5'-monophosphate (AMP)	NA
147	adrenate (22:4n6)	NA
148	alanine	Alanine
149	allantoin	Allantoin

150	alpha-hydroxyisocaproate	Leucinic acid
151	alpha-hydroxyisovalerate	2-Hydroxy-3-methylbutyric acid
152	alpha-ketoglutarate	NA
153	alpha-tocopherol	Alpha-Tocopherol
154	anserine	Anserine
155	arabitol/xylitol	NA
156	arabonate/xylonate	NA
157	arachidate (20:0)	NA
158	arachidonate (20:4n6)	NA
159	arachidonoyl ethanolamide	NA
160	arginine	L-Arginine
161	argininosuccinate	Argininosuccinic acid
162	ascorbate (Vitamin C)	NA
163	asparagine	L-Asparagine
164	aspartate	L-Aspartic acid
165	azelate (nonanedioate)	NA
166	behenoyl sphingomyelin (d18:1/22:0)*	NA
167	beta-alanine	Beta-Alanine
168	beta-guanidinopropanoate	NA
169	beta-hydroxyisovaleroylcarnitine	NA
170	beta-muricholate	NA
171	betaine	Betaine
172	betaine aldehyde	Betaine aldehyde
173	butyrylcarnitine	Butyrylcarnitine
174	C-glycosyltryptophan	NA
175	campesterol	Campesterol
176	carboxyethyl-GABA	N-Carboxyethyl-g-aminobutyric acid
177	carnitine	Carnitine
178	carnosine	Carnosine
179	catechol sulfate	NA
180	cholesterol	Cholesterol
181	choline	Choline
182	choline phosphate	Phosphorylcholine
183	citrate	Citric acid
184	citrulline	Citrulline
185	corticosterone	Corticosterone
186	creatine	Creatine
187	creatine phosphate	Phosphocreatine
188	creatinine	Creatinine
189	cystathionine	L-Cystathionine
190	cysteine	Cysteine
191	cysteine s-sulfate	NA
192	cysteine sulfinic acid	3-Sulfinioalanine
193	cystine	L-Cystine
194	cytidine	Cytidine
195	cytidine 3'-monophosphate (3'-CMP)	NA
196	cytidine 5'-diphosphocholine	Citicoline
197	cytidine 5'-monophosphate (5'-CMP)	NA
198	cytidine 5'-monophospho-N-acetylneuraminic acid	NA
199	cytidine-5'-diphosphoethanolamine	NA
200	cytosine	Cytosine
201	decanoylcarnitine	Decanoylcarnitine
202	dehydroascorbate	Dehydroascorbate
203	deoxycarnitine	4-Trimethylammoniobutanoic acid
204	dihomo-linoleate (20:2n6)	NA
205	dihomo-linolenate (20:3n3 or n6)	NA
206	dihydroxyphenylalanine (L-DOPA)	NA
207	dihydroxyacetone phosphate (DHAP)	NA
208	dimethyl sulfone	Dimethyl sulfone
209	dimethylarginine (SDMA + ADMA)	NA
210	dimethylglycine	Dimethylglycine
211	docosadienoate (22:2n6)	NA
212	docosahexaenoate (DHA; 22:6n3)	NA
213	docosapentaenoate (n3 DPA; 22:5n3)	NA
214	docosapentaenoate (n6 DPA; 22:5n6)	NA
215	dopamine sulfate (2)	NA
216	eicosapentaenoate (EPA; 20:5n3)	NA
217	eicosenoate (20:1)	NA
218	equol sulfate	NA
219	ergothioneine	Ergothioneine
220	erucate (22:1n9)	NA
221	erythronate*	NA
222	ethylmalonate	Ethylmalonic acid
223	flavin adenine dinucleotide (FAD)	NA
224	fructose	D-Fructose
225	fumarate	Fumaric acid
226	galactitol (dulcitol)	NA
227	galactonate	Galactonic acid
228	gamma-aminobutyrate (GABA)	NA
229	gamma-carboxyglutamate	NA
230	gamma-glutamyl-epsilon-lysine	NA
231	gamma-glutamylalanine	5-L-Glutamyl-L-alanine
232	gamma-glutamylglutamate	Gamma Glutamylglutamic acid
233	gamma-glutamylglutamine	Gamma-Glutamyl Glutamine
234	gamma-glutamylglycine	NA
235	gamma-glutamylhistidine	NA
236	gamma-glutamylisoleucine*	NA

237	gamma-glutamylleucine	L-gamma-glutamyl-L-leucine
238	gamma-glutamylphenylalanine	Glutamylphenylalanine
239	gamma-glutamylvaline	L-gamma-glutamyl-L-valine
240	gamma-tocopherol/beta-tocopherol	NA
241	gluconate	Gluconic acid
242	glucose	D-Glucose
243	glucuronate	D-Glucuronic acid
244	glutamate	D-Glutamic acid
245	glutamate, gamma-methyl ester	NA
246	glutamine	L-Glutamine
247	glutarate (pentanedioate)	NA
248	glutarylcarntine (C5)	NA
249	glutathione, oxidized (GSSG)	NA
250	glutathione, reduced (GSH)	NA
251	glycerate	Glyceric acid
252	glycerol	Glycerol
253	glycerol 3-phosphate	Glycerol 3-phosphate
254	glycerophosphoethanolamine	Glycerylphosphorylethanolamine
255	glycerophosphoglycerol	NA
256	glycerophosphoinositol*	NA
257	glycerophosphorylcholine (GPC)	NA
258	glycine	Glycine
259	glycosyl-N-palmitoyl-sphingosine	NA
260	glycosyl-N-stearoyl-sphingosine	NA
261	glycylleucine	Glycyl-L-leucine
262	glycylvaline	NA
263	guanidinoacetate	Guanidoacetic acid
264	guanidinossuccinate	Guanidinossuccinic acid
265	guanine	Guanine
266	guanosine	Guanosine
267	guanosine 5'- monophosphate (5'-GMP)	NA
268	gulonic acid*	NA
269	heme	Heme
270	hexadecanedioate	Hexadecanedioic acid
271	hexanoylcarnitine	Hexanoylcarnitine
272	hexanoylglycine	Hexanoylglycine
273	hippurate	Hippuric acid
274	histamine	Histamine
275	histidine	L-Histidine
276	homoarginine	Homo-L-arginine
277	homocitrulline	Homocitrulline
278	homostachydrine*	NA
279	hypotaurine	Hypotaurine
280	hypoxanthine	Hypoxanthine
281	imidazole lactate	Imidazole lactate
282	imidazole propionate	NA
283	indolelactate	Indolelactic acid
284	inosine	Inosine
285	inosine 5'-monophosphate (IMP)	NA
286	Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate, myo-inositol 1,4 or 1,3-diphosphate	NA
287	isobutyrylcarnitine	NA
288	isocitrate	Isocitric acid
289	isoleucine	(+/-)-erythro-Isoleucine
290	isoleucylglycine	NA
291	isovalerylcarnitine	Isovalerylcarnitine
292	isovalerylglycine	Isovalerylglycine
293	kynurenate	Kynurenic acid
294	kynurenine	L-Kynurenine
295	lactate	L-Lactic acid
296	laurylcarnitine	NA
297	leucine	L-Leucine
298	leucylglycine	NA
299	linoleate (18:2n6)	NA
300	linolenate [alpha or gamma; (18:3n3 or 6)]	NA
301	linoleoylcarnitine*	NA
302	lysine	L-Lysine
303	malate	NA
304	malonylcarnitine	Malonylcarnitine
305	maltose	D-Maltose
306	maltotriose	Maltotriose
307	mannitol/sorbitol	NA
308	mannose	D-Mannose
309	margarate (17:0)	NA
310	mead acid (20:3n9)	NA
311	methionine	NA
312	methionine sulfoxide	Methionine sulfoxide
313	methyl glucopyranoside (alpha + beta)	NA
314	methylmalonate (MMA)	NA
315	methylphosphate	NA
316	methylsuccinate	Methylsuccinic acid
317	myo-inositol	Myoinositol
318	myristate (14:0)	NA
319	myristoleate (14:1n5)	NA
320	myristoylcarnitine	Tetradecanoylcarnitine
321	N-acetyl-aspartyl-glutamate (NAAG)	NA
322	N-acetyl-beta-alanine	N-Acetyl-beta-alanine
323	N-acetyl-glucosamine 1-phosphate	N-Acetyl-glucosamine 1-phosphate

324	N-acetylalanine	N-Acetyl-L-alanine
325	N-acetylarginine	NA
326	N-acetylaspargine	N-Acetylaspargine
327	N-acetylaspargate (NAA)	NA
328	N-acetylglucosamine 6-phosphate	N-Acetylglucosamine 6-phosphate
329	N-acetylglutamate	N-Acetylglutamic acid
330	N-acetylglutamine	N-Acetylglutamine
331	N-acetylglycine	Acetyl glycine
332	N-acetylhistidine	N-Acetylhistidine
333	N-acetylleucine	N-Acetylleucine
334	N-acetylmethionine	N-Acetyl-L-methionine
335	N-acetylneuraminate	NA
336	N-acetylphenylalanine	N-Acetyl-L-phenylalanine
337	N-acetylputrescine	N-Acetylputrescine
338	N-acetyserine	N-Acetyserine
339	N-acetyltaurine	NA
340	N-acetylthreonine	NA
341	N-alpha-acetylornithine	NA
342	N-carbamoylaspartate	Ureidosuccinic acid
343	N-delta-acetylornithine	NA
344	N-formylmethionine	NA
345	N-formylphenylalanine	NA
346	N-glycolylneuraminate	N-Glycolylneuraminic acid
347	N-methyl-4-aminobutyric acid	NA
348	N-monomethylarginine	NA
349	N-palmitoyl-sphinganine (d18:0/16:0)	NA
350	N-palmitoyl-sphingosine (d18:1/16:0)	NA
351	N-palmitoyltaurine	NA
352	N-stearoyltaurine	NA
353	N1-Methyl-2-pyridone-5-carboxamide	N1-Methyl-2-pyridone-5-carboxamide
354	N1-methyladenosine	1-Methyladenosine
355	N2-acetyllysine/N6-acetyllysine	NA
356	N6,N6,N6-trimethyllysine	NA
357	N6-carboxymethyllysine	NA
358	N6-succinyladenosine	NA
359	nicotinamide	Niacinamide
360	nicotinamide adenine dinucleotide (NAD+)	NA
361	nicotinamide riboside	Nicotinamide riboside
362	nonadecanoate (19:0)	NA
363	O-sulfo-L-tyrosine	NA
364	octanoylcarnitine	L-Octanoylcarnitine
365	oleamide	Oleamide
366	oleate/vaccenate (18:1)	NA
367	oleoyl ethanolamide	NA
368	oleoylcarnitine	Oleoylcarnitine
369	ornithine	Ornithine
370	orotate	Orotic acid
371	orotidine	Orotidine
372	oxalate (ethanedioate)	NA
373	p-cresol sulfate	p-Cresol sulfate
374	p-cresol-glucuronide*	NA
375	palmitate (16:0)	NA
376	palmitoleate (16:1n7)	NA
377	palmitoyl dihydrosphingomyelin (d18:0/16:0)*	NA
378	palmitoyl ethanolamide	Palmitoylethanolamide
379	palmitoyl sphingomyelin (d18:1/16:0)	NA
380	palmitoylcarnitine	NA
381	pantothenate	Pantothenic acid
382	phenol sulfate	NA
383	phenylacetyl glycine	Phenylacetyl glycine
384	phenylalanine	L-Phenylalanine
385	phenylalanyl glycine	NA
386	phenyllactate (PLA)	NA
387	phosphate	Phosphate
388	phosphoenolpyruvate (PEP)	NA
389	phosphoethanolamine	O-Phosphoethanolamine
390	phosphopantetheine	Pantetheine 4'-phosphate
391	pipecolate	Pipecolic acid
392	pro-hydroxy-pro	NA
393	proline	L-Proline
394	prolylglycine	L-prolyl-L-glycine
395	propionylcarnitine	Propionylcarnitine
396	pseudouridine	Pseudouridine
397	putrescine	Putrescine
398	pyridoxal	Pyridoxal
399	pyridoxamine	Pyridoxamine
400	pyridoxamine phosphate	NA
401	pyridoxate	NA
402	pyroglutamine*	NA
403	quinolinate	Quinolinic acid
404	retinol (Vitamin A)	NA
405	ribitol	Ribitol
406	riboflavin (Vitamin B2)	NA
407	ribonate	Ribonic acid
408	ribose	D-Ribose
409	ribose 5-phosphate	D-Ribulose 5-phosphate
410	S-adenosylhomocysteine (SAH)	NA

411	S-adenosylmethionine (SAM)	NA
412	saccharopine	Saccharopine
413	salicylate	Salicylic acid
414	sarcosine (N-Methylglycine)	NA
415	sebacate (decanedioate)	NA
416	sedoheptulose-7-phosphate	D-Sedoheptulose 7-phosphate
417	serine	L-Serine
418	spermidine	Spermidine
419	sphinganine	Sphinganine
420	sphingomyelin (d18:1/14:0, d16:1/16:0)*	NA
421	sphingomyelin (d18:1/15:0, d16:1/17:0)*	NA
422	sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	NA
423	sphingomyelin (d18:1/18:1, d18:2/18:0)	NA
424	sphingomyelin (d18:1/20:0, d16:1/22:0)*	NA
425	sphingomyelin (d18:1/20:1, d18:2/20:0)*	NA
426	sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	NA
427	sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	NA
428	sphingomyelin (d18:1/24:1, d18:2/24:0)*	NA
429	sphingomyelin (d18:2/14:0, d18:1/14:1)*	NA
430	sphingomyelin (d18:2/16:0, d18:1/16:1)*	NA
431	sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	NA
432	sphingomyelin (d18:2/24:1, d18:1/24:2)*	NA
433	sphingosine	Sphingosine
434	stachydrine	Proline betaine
435	stearate (18:0)	NA
436	stearidonate (18:4n3)	NA
437	stearoyl ethanolamide	Stearoylethanolamide
438	stearoyl sphingomyelin (d18:1/18:0)	NA
439	stearoylcarnitine	Stearoylcarnitine
440	succinate	Succinic acid
441	succinylcarnitine	NA
442	sucrose	Sucrose
443	sulfate*	NA
444	tartronate (hydroxymalonate)	NA
445	taurine	Taurine
446	tauro-beta-muricholate	Tauro-b-muricholic acid
447	taurochenodeoxycholate	Taurochenodesoxycholic acid
448	taurocholate	Taurocholic acid
449	taurocyamine	Taurocyamine
450	taurodeoxycholate	NA
451	tauroursodeoxycholate	Tauroursodeoxycholic acid
452	tetradecanedioate	Tetradecanedioic acid
453	thiamin (Vitamin B1)	NA
454	thiamin monophosphate	Thiamine monophosphate
455	threonate	Threonic acid
456	threonine	L-Threonine
457	thymidine	Thymidine
458	tiglylcarnitine	Tiglylcarnitine
459	trans-4-hydroxyproline	4-Hydroxyproline
460	trans-urocanate	NA
461	tricosanoyl sphingomyelin (d18:1/23:0)*	NA
462	trigonelline (N'-methylnicotinate)	NA
463	trimethylamine N-oxide	Trimethylamine N-oxide
464	tryptophan	D-Tryptophan
465	tyrosine	L-Tyrosine
466	tyrosylglycine	NA
467	UDP-galactose	Uridine diphosphategalactose
468	UDP-glucose	Uridine diphosphate glucose
469	UDP-glucuronate	Uridine diphosphate glucuronic acid
470	UDP-N-acetylgalactosamine	Uridine diphosphate-N-acetylgalactosamine
471	UDP-N-acetylglucosamine	Uridine diphosphate-N-acetylglucosamine
472	uracil	Uracil
473	urate	Uric acid
474	urea	Urea
475	uridine	Uridine
476	uridine 5'-diphosphate (UDP)	NA
477	uridine 5'-monophosphate (UMP)	NA
478	valine	L-Valine
479	valylglycine	NA
480	xanthine	Xanthine
481	xanthosine	Xanthosine

The second step is to check concentration values. For SSP analysis, the concentration must be measured in *umol* for blood and CSF samples. The urinary concentrations must be first converted to *umol/mmol_creatinine* in order to compare with reported concentrations in literature. No missing or negative values are allowed in SSP analysis. The concentration data for QEA analysis is more flexible. Users can upload either the original concentration data or normalized data. Missing or negative values are allowed (coded as *NA*) for QEA. Please note, MSEA does not perform data normalization. If normalization is important, you should first normalize your data before upload. You can use our companion website **MetaboAnalyst** www.metaboanalyst.ca for a variety of data processing and normalization methods.

5 Selection of Metabolite Set Library

Before proceeding to enrichment analysis, a metabolite set library has to be chosen. There are seven built-in libraries offered by MSEA:

- Metabolic pathway associated metabolite sets (*currently contains 88 entries*);
- Disease associated metabolite sets (reported in blood) (*currently contains 416 entries*);
- Disease associated metabolite sets (reported in urine) (*currently contains 346 entries*);
- Disease associated metabolite sets (reported in CSF) (*currently contains 124 entries*);
- Metabolite sets associated with SNPs (*currently contains 4500 entries*);
- Predicted metabolite sets based on computational enzyme knockout model (*currently contains 912 entries*);
- Metabolite sets based on locations (*currently contains 57 entries*);

In addition, MSEA also allows user-defined metabolite sets to be uploaded to perform enrichment analysis on arbitrary groups of compounds which researchers want to test. The metabolite set library is simply a two-column comma separated text file with the first column for metabolite set names and the second column for its compound names (**must use HMDB compound name**) separated by "; ". Please note, the built-in libraries are mainly from human studies. The functional grouping of metabolites may not be valid. Therefore, for data from subjects other than human being, users are suggested to upload their self-defined metabolite set libraries for enrichment analysis.

6 Enrichment Analysis

Quantitative enrichment analysis (QEA) will be performed when the user uploads a concentration table. The enrichment analysis is performed using package **globaltest**¹. It uses a generalized linear model to estimate a *Q-statistic* for each metabolite set, which describes the correlation between compound concentration profiles, X, and clinical outcomes, Y. The *Q statistic* for a metabolite set is the average of the *Q* statistics for each metabolite in the set. **Figure 2** below summarizes the result.

¹Jelle J. Goeman, Sara A. van de Geer, Floor de Kort and Hans C. van Houwelingen. *A global test for groups of genes: testing association with a clinical outcome*, Bioinformatics Vol. 20 no. 1 2004, pages 93-99

Enrichment Overview (top 50)

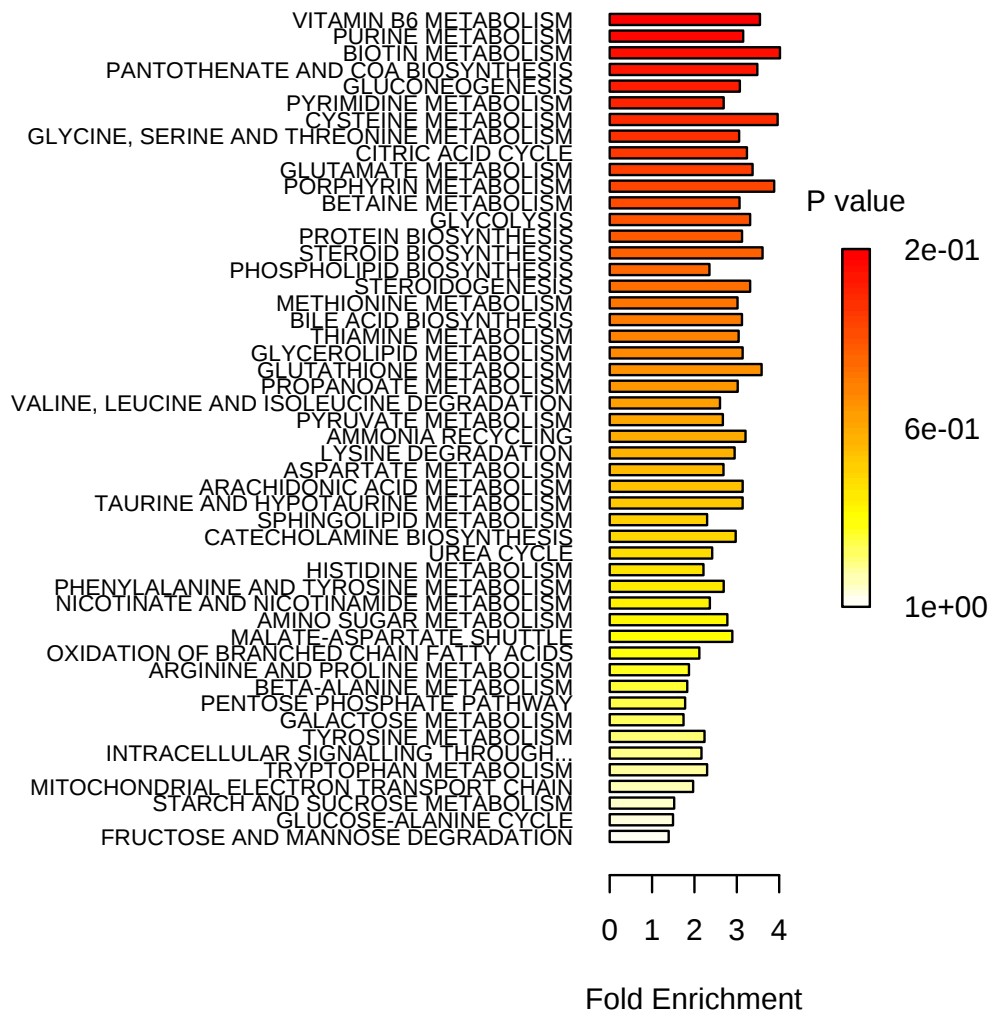


Figure 1: Summary Plot for Quantitative Enrichment Analysis (QEA)

Table 2: Result from Quantitative Enrichment Analysis

	Total Cmpd	Hits	Statistic Q	Expected Q	Raw p	Holm p	FDR
VITAMIN B6 METABOLISM	10	2	35.09	9.90	2.71E-03	1.57E-01	3.70E-02
PURINE METABOLISM	45	12	31.17	9.89	3.02E-03	1.72E-01	3.70E-02
BIOTIN METABOLISM	4	1	39.46	9.82	4.68E-03	2.62E-01	3.70E-02
PANTOTHENATE AND COA BIOSYNTHESIS	10	2	34.43	9.88	5.98E-03	3.29E-01	3.70E-02
GLUCONEOGENESIS	27	3	30.41	9.90	6.23E-03	3.36E-01	3.70E-02
PYRIMIDINE METABOLISM	36	11	26.65	9.90	6.44E-03	3.41E-01	3.70E-02
CYSTEINE METABOLISM	8	1	39.13	9.87	7.81E-03	4.06E-01	3.70E-02
GLYCINE, SERINE AND THREONINE METABOLISM	26	9	30.26	9.91	8.59E-03	4.38E-01	3.70E-02
CITRIC ACID CYCLE	23	4	32.09	9.91	8.97E-03	4.49E-01	3.70E-02
GLUTAMATE METABOLISM	18	2	33.35	9.89	8.99E-03	4.49E-01	3.70E-02
PORPHYRIN METABOLISM	22	2	38.46	9.91	9.13E-03	4.49E-01	3.70E-02
BETAINE METABOLISM	10	4	30.35	9.90	9.56E-03	4.49E-01	3.70E-02
GLYCOLYSIS	21	2	32.81	9.90	9.81E-03	4.51E-01	3.70E-02
PROTEIN BIOSYNTHESIS	19	12	30.82	9.87	1.06E-02	4.75E-01	3.70E-02
STEROID BIOSYNTHESIS	31	1	35.37	9.80	1.09E-02	4.78E-01	3.70E-02
PHOSPHOLIPID BIOSYNTHESIS	19	4	23.23	9.87	1.09E-02	4.78E-01	3.70E-02
STEROIDOGENESIS	32	2	32.71	9.87	1.11E-02	4.78E-01	3.70E-02
METHIONINE METABOLISM	24	8	29.89	9.91	1.23E-02	5.03E-01	3.70E-02
BILE ACID BIOSYNTHESIS	49	4	30.89	9.90	1.36E-02	5.44E-01	3.70E-02
THIAMINE METABOLISM	4	1	29.28	9.62	1.43E-02	5.59E-01	3.70E-02
GLYCEROLIPID METABOLISM	13	4	31.08	9.91	1.47E-02	5.59E-01	3.70E-02
GLUTATHIONE METABOLISM	10	2	35.51	9.90	1.58E-02	5.85E-01	3.70E-02
PROPANOATE METABOLISM	18	2	29.70	9.83	1.59E-02	5.85E-01	3.70E-02
VALINE, LEUCINE AND ISOLEUCINE DEGRADATION	36	3	25.69	9.87	1.59E-02	5.85E-01	3.70E-02
PYRUVATE METABOLISM	20	2	26.35	9.87	1.61E-02	5.85E-01	3.70E-02
AMMONIA RECYCLING	18	6	31.72	9.89	1.66E-02	5.85E-01	3.70E-02
LYSINE DEGRADATION	13	3	29.02	9.84	1.73E-02	5.85E-01	3.72E-02
ASPARTATE METABOLISM	12	6	26.58	9.90	1.96E-02	6.06E-01	3.92E-02
ARACHIDONIC ACID METABOLISM	37	1	30.49	9.71	1.96E-02	6.06E-01	3.92E-02
TAURINE AND HYPOTAURINE METABOLISM	7	3	31.10	9.91	2.08E-02	6.06E-01	4.01E-02
SPHINGOLIPID METABOLISM	15	4	22.71	9.88	2.24E-02	6.28E-01	4.20E-02
CATECHOLAMINE BIOSYNTHESIS	5	1	29.05	9.77	2.60E-02	7.03E-01	4.72E-02
UREA CYCLE	20	8	23.96	9.91	2.81E-02	7.32E-01	4.95E-02
HISTIDINE METABOLISM	11	4	21.92	9.90	3.36E-02	8.39E-01	5.73E-02
PHENYLALANINE AND TYROSINE METABOLISM	13	3	26.57	9.87	3.64E-02	8.73E-01	6.03E-02
NICOTINATE AND NICOTINAMIDE METABOLISM	13	4	23.41	9.90	3.91E-02	8.98E-01	6.27E-02
AMINO SUGAR METABOLISM	15	1	27.21	9.80	4.00E-02	8.98E-01	6.27E-02
MALATE-ASPARTATE SHUTTLE	8	1	28.58	9.87	4.20E-02	8.98E-01	6.41E-02
OXIDATION OF BRANCHED CHAIN FATTY ACIDS	14	2	20.87	9.86	5.07E-02	1.00E+00	7.44E-02
ARGININE AND PROLINE METABOLISM	26	11	18.51	9.89	5.23E-02	1.00E+00	7.44E-02
BETA-ALANINE METABOLISM	13	7	18.14	9.91	5.26E-02	1.00E+00	7.44E-02
PENTOSE PHOSPHATE PATHWAY	18	4	17.52	9.84	6.25E-02	1.00E+00	8.56E-02
GALACTOSE METABOLISM	25	9	17.27	9.91	6.35E-02	1.00E+00	8.56E-02
TYROSINE METABOLISM	38	2	22.04	9.85	8.06E-02	1.00E+00	1.06E-01
INTRACELLULAR SIGNALING THROUGH ADENOSINE RECEPTOR A2A AND ADENOSINE INTRACELLULAR SIGNALING THROUGH ADENOSINE RECEPTOR A2B AND ADENOSINE	7	1	21.06	9.72	9.36E-02	1.00E+00	1.19E-01
TRYPTOPHAN METABOLISM	34	1	22.76	9.90	9.43E-02	1.00E+00	1.19E-01
MITOCHONDRIAL ELECTRON TRANSPORT CHAIN	15	3	19.43	9.87	9.93E-02	1.00E+00	1.23E-01
STARCH AND SUCROSE METABOLISM	14	6	15.07	9.90	1.10E-01	1.00E+00	1.33E-01
GLUCOSE-ALANINE CYCLE	12	1	14.61	9.78	2.38E-01	1.00E+00	2.82E-01
FRUCTOSE AND MANNOSE DEGRADATION	18	2	13.72	9.86	2.46E-01	1.00E+00	2.85E-01
NUCLEOTIDE SUGARS METABOLISM	9	3	12.18	9.88	2.82E-01	1.00E+00	3.21E-01
GLYCEROL PHOSPHATE SHUTTLE	8	1	12.04	9.78	3.27E-01	1.00E+00	3.57E-01
PHENYLACETATE METABOLISM	4	1	12.04	9.78	3.27E-01	1.00E+00	3.57E-01

INTRACELLULAR SIG- NALLING THROUGH HIS- TAMINE H2 RECEPTOR AND HISTAMINE	5	1	9.30	9.80	4.26E-01	1.00E+00	4.58E-01
INOSITOL METABOLISM	19	2	9.31	9.89	4.36E-01	1.00E+00	4.60E-01
INSULIN SIGNALLING	19	2	9.68	9.86	4.49E-01	1.00E+00	4.65E-01
SULFATE/SULFITE METABOLISM	7	1	6.29	9.85	5.68E-01	1.00E+00	5.78E-01
BETA OXIDATION OF VERY LONG CHAIN FATTY ACIDS	14	1	6.49	9.72	5.85E-01	1.00E+00	5.85E-01

The report was generated on Sun Aug 30 19:37:20 2015 with R version 3.0.3 (2014-03-06). Thank you for using MetaboAnalyst! For suggestions and feedback please contact Jeff Xia (jeff.xia@mcgill.ca).