

Sex-dependent responses to maternal exposure to PM_{2.5} in the offspring

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Materials and Method

LC/MS/MS.

Protein and metabolic pathways were examined using proteomic techniques. Samples (5 µL) were loaded onto a nanoEase Symmetry C18 trapping column (180 m x 20 mm) (3 mins/ 15 L/min) via an Acquity M-class nanoLC system (Waters, USA), and then washed onto a PicoFrit column (75 mID x 350 mm; New Objective, Woburn, MA) packed with SP-120-1.7-ODS-BIO resin. Eluted peptides were loaded into the source of a Q Exactive Plus mass-spectrometer (Thermo Scientific) using 5-30% MS buffer B (98% Acetonitrile + 0.2% Formic Acid) - 90 mins, 30-80% MS buffer B - 3 mins, 80% MS buffer B (2 mins), 80-5% for 3 min. Eluted peptides were ionised at 2400V. A data-dependent MS/MS (dd-MS2) experiment was carried out using a survey scan of 350-1500 Da at 70,000 resolution for peptides of charge state 2+ or greater with an AGC target of 3e6 and maximum Injection Time of 50ms. Peptides (top 12) were fragmented in the HCD cell using an isolation window of 1.4 m/z, an AGC target of 1e5 and a maximum injection time of 100ms. These were analysed in the Orbitrap analyser at 17,500 resolution; production fragment masses were measured (mass-range of 120 - 2000 Da). The mass of the precursor peptide was then excluded for 30 seconds.

MS/MS datafiles analysed using Peaks Studio X Pro (mouse proteome from UniProt) with a database of common contaminants with the following parameter settings. Fixed modifications: none. Variable modifications: propionamide, oxidised methionine, deamidated asparagine. Enzyme: semi-trypsin. The number of allowed missed cleavages: 3. Peptide mass tolerance: 10 ppm. MS/MS mass tolerance: 0.05 Da. Searchers were refined to include peptides with a -log₁₀P score characterised by a False Discovery Rate (FDR) of <1%. The score is that where decoy database search matches were <1% of the total matches. Label-Free Quantification (LFQ) was performed using the PEAKS Q module.

The protein's gene name and fold change (Supplementary Table 1) were submitted to StringDB (<https://string-db.org/>) for Functional Enrichment using the "Proteins with Ranks/Values" Search. The functional categories enriched are listed in Supplementary Table 2.

Supplementary Table S1. Peptide abundance area for top-3 peptides used for quantification by Peaks Studio XPro. Fold changes are compared to the F-Control.

Protein (abundance unit)	Female Area			Male Area			Fold Change
	F-Control	F-PM	F-Pre	M-Control	M-PM	M-Pre	
Gstp1 (x10 ⁸)	1.36±0.057	1.52±0.195	1.04±0.076	17.9±1.37	17.0±2.31	19.6±1.68	1:1.13:0.81: 12.61:11.84:15.21
Selenbp2 (x10 ⁶)	4.80±1.00	3.22±1.33	1.46±0.144	55.9±4.46	51.8±3.91	72.4±11.8	1:0.67:0.30: 11.65:10.80:15.07
Mat1a (x10 ⁸)	10.5±0.343	9.96±0.350	10.1±0.330	4.61±0.160	3.88±0.143	4.44±0.235	1:0.95:0.96: 0.45:0.38:0.44
Keg1 (x10 ⁷)	5.26±0.348	4.44±0.489	4.62±0.315	13.6±0.281	15.6±0.464	12.0±1.56	1:0.84:0.83: 2.84:3.22:2.43
Cyp2d9 (x10 ⁷)	1.60±0.078	1.83±0.272	1.36±0.878	5.80±0.310	5.02±0.152	3.61±0.431	1:1.14:0.85: 3.61:3.13:2.25
Aox3 (x10 ⁷)	2.04±0.542	1.54±0.173	1.52±0.126	11.4±1.11	12.2±0.823	14.1±2.01	1:0.76:0.75: 5.32:5.74:6.66
Papss2 (x10 ⁷)	6.05±0.328	5.53±0.357	6.67±0.325	2.42±0.080	2.08±0.100	1.87±0.254	1:0.94:1.18: 0.40:0.36:0.31
Sult1d1 (x10 ⁷)	7.87±0.290	8.70±0.377	8.48±0.304	3.08±0.191	3.36±0.141	3.50±0.340	1:1.12:1.11: 0.48:0.41:0.43
Idh2 (x10 ⁸)	2.10±0.110	2.18±0.125	2.45±0.116	1.05±0.052	1.16±0.065	1.14±0.059	1:1.10:1.22: 0.54:0.57:0.54
Glyat (x10 ⁸)	1.58±0.025	1.68±0.068	1.71±0.083	2.48±0.071	2.82±0.126	2.25±0.182	1:1.12:1.05: 1.80:2.12:1.56
Sult2a8 (x10 ⁷)	3.58±0.407	3.73±0.352	4.24±0.349	13.4±0.840	14.7±1.06	14.5±1.62	1:1.05:1.20: 3.61:4.01:3.75
Ttpa (x10 ⁷)	4.64±0.229	4.02±0.244	4.70±0.179	2.29±0.146	2.20±0.179	2.08±0.128	1:0.87:1.01: 0.49:0.47:0.44
Ugdh (x10 ⁷)	8.88±0.351	8.59±0.435	8.63±0.360	15.7±0.681	18.0±1.12	13.1±1.84	1:0.98:0.99: 1.79:2.05:1.47
Upb1 (x10 ⁶)	7.04±0.385	7.21±0.088	8.08±0.709	16.7±0.918	14.7±0.611	15.2±1.75	1:1.02:1.15: 2.38:2.09:2.16
Kyat3 (x10 ⁷)	10.8±0.605	9.53±1.43	11.3±1.07	5.71±0.231	4.96±0.327	3.28±0.813	1:0.92:1.15: 0.51:0.43:0.29
Mug1 (x10 ⁷)	2.95±0.193	2.84±0.397	2.71±0.291	6.87±0.403	7.01±0.560	5.27±0.477	1:0.96:0.92: 2.32:2.38:1.79
Gstk1 (x10 ⁸)	3.03±0.081	3.18±0.048	3.31±0.169	2.02±0.076	2.14±0.060	1.80±0.188	1:1.03:1.11: 0.66:0.69:0.52
Ugt1a1 (x10 ⁸)	1.21±0.047	1.12±0.039	1.12±0.035	0.788±0.105	0.655±0.004	0.517±0.064	1:0.96:0.96: 0.63:0.53:0.43
Dpys (x10 ⁸)	2.54±0.094	2.52±0.181	2.84±0.124	1.76±0.060	1.80±0.060	1.46±0.085	1:1.04:1.23: 0.70:0.73:0.54
Serpina1c (x10 ⁸)	1.58±0.223	1.43±0.235	1.14±0.097	3.17±0.228	2.72±0.309	4.41±0.891	1:0.88:0.72: 2.10:1.74:2.83
Ces3b (x10 ⁷)	6.79±0.523	6.34±0.974	5.60±0.528	12.5±0.358	11.2±0.856	7.90±1.29	1:0.93:0.83: 1.84:1.65:1.17
Arhgdib (x10 ⁶)	3.03±0.118	3.13±0.239	3.16±0.196	2.03±0.176	1.55±0.132	2.06±0.087	1:1.03:1.04: 0.67:0.51:0.68
Serpina1b (x10 ⁷)	2.54±0.152	2.95±0.203	2.59±0.249	4.41±0.348	4.20±0.227	5.21±0.642	1:1.16:1.02: 1.74:1.66:2.05
Cyp2c29 (x10 ⁸)	1.97±0.188	1.64±0.159	1.44±0.114	3.90±0.415	3.02±0.177	2.25±0.427	1:0.81:0.71: 1.94:1.50:1.09
Hmox2 (x10 ⁵)	10.4±0.757	8.43±0.124	6.59±0.303	10.9±0.604	9.84±0.869	14.9±2.10	1:0.81:0.63: 1.04:0.94:1.43
Hacl1 (x10 ⁷)	2.93±0.204	2.85±0.280	3.50±0.264	1.44±0.184	1.63±0.219	1.56±0.241	1:0.98:1.19: 0.49:0.56:0.54

Gbp9 (x10 ⁶)	3.38±0.39	3.35±0.244	3.55±0.172	2.31±0.165	2.15±0.137	1.67±0.262	1:0.99:1.05: 0.68:0.64:0.49
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Results are expressed as mean ± SEM, n = 6. PM: offspring from dams continuously exposed to PM_{2.5}; Pre: offspring from dams exposed to PM_{2.5} during pre-gestational period only.

Supplementary Table S2. Functional Enrichment pathways

Physiological Functions	Observed Gene Count	Background Gene Count	Strength	Matching Proteins In The Network
Oxoacid metabolic process	21	800	1.14	Cyp2f2,Cyp2c29,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Ugt1a1,Aldh3a2,Oat,Cyp2d9,Idh2,Gstp1
Small molecule metabolic process	25	1450	0.96	Cyp2f2,Cyp2c29,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Ttpa,Idh2,Gstp1
Carboxylic acid metabolic process	20	754	1.14	Cyp2f2,Cyp2c29,Hacl1,Dpys,Csad,Got1,Ddah1,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Ugt1a1,Aldh3a2,Oat,Cyp2d9,Idh2,Gstp1
Sulfur compound metabolic process	11	280	1.31	Csad,Papss2,Ugdh,Gstk1,Ces1d,Glyat,Mat1a,Nudt7,Sult1a1,Sult1d1,Gstp1
Catabolic process	20	1680	0.8	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Got1,Ddah1,Ces1d,Cyp1a2,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Oat,Cyp2d9,Ces3b,Ces3a
Drug metabolic process	7	52	1.85	Cyp2f2,Cyp2c29,Cyp1a2,Aox3,Cbr1,Cyp2d9,Sult1a1
Cellular catabolic process	18	1445	0.82	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Got1,Ddah1,Ces1d,Cyp1a2,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Oat,Cyp2d9
Organic substance catabolic process	17	1425	0.8	Hmox2,Hacl1,Dpys,Csad,Got1,Ddah1,Ces1d,Cyp1a2,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Oat,Ces3b,Ces3a
Cellular amino acid metabolic process	9	236	1.3	Dpys,Csad,Got1,Ddah1,Ccbl2,Glyat,Mat1a,Upb1,Oat
Monocarboxylic acid metabolic process	11	461	1.1	Cyp2f2,Cyp2c29,Hacl1,Ces1d,Cyp1a2,Glyat,Ugt1a1,Aldh3a2,Cyp2d9,Idh2,Gstp1
Oxidation-reduction process	14	917	0.9	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Ugdh,Gstk1,Cyp1a2,Aox3,Cbr1,Stbd1,Aldh3a2,Cyp2d9,Idh2,Gstp1
Metabolic process	33	7331	0.37	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Gstk1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Stbd1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Ces3b,Ces3a,Serpina1b,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1
Organic substance metabolic process	32	6824	0.39	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Gstk1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Ces3b,Ces3a,Serpina1b,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1
Cellular metabolic process	31	6445	0.4	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Gstk1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Stbd1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Serpina1b,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1

Xenobiotic metabolic process	6	74	1.63	Cyp2f2,Cyp2c29,Ugt1a1,Cyp2d9,Sult1a1,Gstp1
Long-chain fatty acid metabolic process	6	95	1.52	Cyp2f2,Cyp2c29,Hacl1,Cyp1a2,Cyp2d9,Gstp1
Alpha-amino acid metabolic process	7	173	1.33	Csad,Got1,Ddah1,Ccbl2,Glyat,Mat1a,Oat
Primary metabolic process	29	6369	0.38	Cyp2f2,Cyp2c29,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Ces3b,Ces3a,Serpina1b,Sult1a1,Idh2,Sult1d1,Gstp1
Fatty acid metabolic process	8	310	1.13	Cyp2f2,Cyp2c29,Hacl1,Ces1d,Cyp1a2,Aldh3a2,Cyp2d9,Gstp1
Nucleobase-containing small molecule metabolic process	9	440	1.03	Dpys,Papss2,Ugdh,Ces1d,Glyat,Upb1,Aox3,Nudt7,Idh2
Carboxylic acid catabolic process	7	206	1.25	Hacl1,Csad,Got1,Ddah1,Ces1d,Mat1a,Oat
Small molecule catabolic process	8	318	1.12	Hacl1,Csad,Got1,Ddah1,Ces1d,Mat1a,Upb1,Oat
Lipid metabolic process	12	1032	0.79	Cyp2f2,Cyp2c29,Hacl1,Ces1d,Cyp1a2,Aldh3a2,Cyp2d9,Ces3b,Ces3a,Sult1a1,Sult1d1,Gstp1
Organic cyclic compound metabolic process	18	2614	0.56	Cyp2f2,Hmox2,Dpys,Papss2,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Upb1,Aox3,Ugt1a1,Nudt7,Oat,Ttpa,Sult1a1,Idh2,Sult1d1
Exogenous drug catabolic process	4	33	1.8	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Alpha-amino acid catabolic process	5	86	1.48	Csad,Got1,Ddah1,Mat1a,Oat
Olefinic compound metabolic process	5	91	1.46	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9,Gstp1
Sulfur compound biosynthetic process	5	92	1.46	Csad,Papss2,Ugdh,Mat1a,Gstp1
Organic hydroxy compound metabolic process	8	420	1	Got1,Ces1d,Cyp1a2,Aldh3a2,Ttpa,Sult1a1,Idh2,Sult1d1
Icosanoid metabolic process	5	99	1.42	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9,Gstp1
Unsaturated fatty acid metabolic process	5	100	1.42	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9,Gstp1
Response to toxic substance	6	197	1.2	Cyp2f2,Gstk1,Ces1d,Glyat,Ttpa,Gstp1
Small molecule biosynthetic process	8	468	0.95	Csad,Got1,Ugdh,Ces1d,Upb1,Nudt7,Oat,Gstp1
Organic cyclic compound catabolic process	7	327	1.05	Hmox2,Dpys,Cyp1a2,Upb1,Aox3,Ugt1a1,Nudt7
Cellular aromatic compound metabolic process	16	2412	0.54	Cyp2f2,Hmox2,Dpys,Papss2,Ugdh,Ces1d,Cyp1a2,Ccbl2,Glyat,Upb1,Aox3,Ugt1a1,Nudt7,Sult1a1,Idh2,Sult1d1
Arachidonic acid metabolic process	4	53	1.6	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9

Organonitrogen compound metabolic process	22	4475	0.41	Hmox2,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Gstk1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Nudt7,Oat,Serpina1b,Sult1a1,Idh2,Sult1d1,Gstp1
2-oxoglutarate metabolic process	3	16	1.99	Got1,Ccbl2,Idh2
Biosynthetic process	15	2176	0.56	Csad,Papss2,Got1,Ddah1,Ugdh,Ces1d,Cyp1a2,Ccbl2,Mat1a,Upb1,Nudt7,Oat,Serpina1b,Idh2,Gstp1
Organic acid biosynthetic process	6	240	1.12	Csad,Got1,Ugdh,Upb1,Oat,Gstp1
Organonitrogen compound catabolic process	10	909	0.76	Hmox2,Dpys,Csad,Got1,Ddah1,Mat1a,Upb1,Aox3,Nudt7,Oat
Glutamine family amino acid catabolic process	3	22	1.85	Got1,Ddah1,Oat
Sulfur compound catabolic process	3	24	1.82	Csad,Mat1a,Nudt7
Dicarboxylic acid metabolic process	4	89	1.37	Got1,Ccbl2,Oat,Idh2
Ribonucleoside biphosphate metabolic process	4	102	1.31	Papss2,Ces1d,Glyat,Nudt7
Purine nucleoside biphosphate metabolic process	4	102	1.31	Papss2,Ces1d,Glyat,Nudt7
Sulfate assimilation	2	4	2.42	Papss2,Sult1d1
Cellular biosynthetic process	13	2025	0.53	Csad,Papss2,Got1,Ddah1,Ugdh,Cyp1a2,Mat1a,Upb1,Nudt7,Oat,Serpina1b,Idh2,Gstp1
Beta-alanine metabolic process	2	5	2.32	Dpys,Upb1
Carboxylic acid biosynthetic process	5	238	1.04	Got1,Ugdh,Upb1,Oat,Gstp1
Lipid catabolic process	5	260	1	Hacl1,Ces1d,Cyp1a2,Ces3b,Ces3a
Sulfur amino acid catabolic process	2	9	2.07	Csad,Mat1a
Arginine catabolic process	2	9	2.07	Ddah1,Oat
Cellular nitrogen compound catabolic process	5	279	0.97	Hmox2,Dpys,Upb1,Aox3,Nudt7
Nucleobase catabolic process	2	9	2.07	Dpys,Aox3
Heterocycle catabolic process	5	282	0.97	Hmox2,Dpys,Upb1,Aox3,Nudt7
Organonitrogen compound biosynthetic process	9	1096	0.63	Csad,Papss2,Got1,Ugdh,Upb1,Oat,Serpina1b,Idh2,Gstp1
Cellular amino acid biosynthetic process	3	58	1.43	Got1,Upb1,Oat
Alcohol metabolic process	5	292	0.95	Got1,Ces1d,Cyp1a2,Aldh3a2,Idh2

Aromatic compound catabolic process	5	297	0.95	Hmox2,Upb1,Aox3,Ugt1a1,Nudt7
Catalytic activity	33	5224	0.52	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Keg1,Papss2,Got1,Ddah1,Ugdh,Gstk1,Arhgdib,Iigp1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Ugt1a1,Nudt7,Aldh3a2,Oat,Cyp2d9,Ces3b,Ces3a,Sult1a1,Idh2,Sult1d1,Gstp1
Oxidoreductase activity	12	710	0.95	Cyp2f2,Cyp2c29,Hmox2,Ugdh,Gstk1,Cyp1a2,Aox3,Cbr1,Aldh3a2,Cyp2d9,Idh2,Gstp1
Small molecule binding	18	2406	0.59	Cyp2f2,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Ugdh,Iigp1,Ccbl2,Mat1a,Aox3,Ugt1a1,Oat,Ttpa,Sult1a1,Idh2,Gstp1
Vitamin binding	6	134	1.37	Hacl1,Csad,Got1,Ccbl2,Oat,Ttpa
Methyl indole-3-acetate esterase activity	3	8	2.29	Ces1d,Ces3b,Ces3a
Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	4	41	1.71	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Sterol esterase activity	3	12	2.12	Ces1d,Ces3b,Ces3a
Steroid hydroxylase activity	4	47	1.65	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Oxidoreductase activity, acting on ch or ch2 groups	3	13	2.08	Cyp2c29,Cyp1a2,Aox3
Heme binding	5	131	1.3	Cyp2f2,Cyp2c29,Hmox2,Cyp1a2,Cyp2d9
Pyridoxal phosphate binding	4	52	1.61	Csad,Got1,Ccbl2,Oat
Carboxylic acid binding	6	218	1.16	Dpys,Got1,Ddah1,Mat1a,Ugt1a1,Gstp1
Transaminase activity	3	22	1.85	Got1,Ccbl2,Oat
Iron ion binding	5	162	1.21	Cyp2f2,Cyp2c29,Cyp1a2,Aox3,Cyp2d9
Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	5	169	1.19	Cyp2f2,Cyp2c29,Hmox2,Cyp1a2,Cyp2d9
Triglyceride lipase activity	3	27	1.77	Ces1d,Ces3b,Ces3a

Ion binding	23	5439	0.35	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Iigp1,Cyp1a2,Ccbl2,Mat1a,Upb1,Aox3,Ugt1a1,Nudt7,Oat,Cyp2d9,Ttpa,Sult1a1,Idh2,Gstp1
Aromatase activity	3	32	1.69	Cyp2c29,Cyp1a2,Cyp2d9
Identical protein binding	13	1962	0.54	Hacl1,Dpys,Iigp1,Ccbl2,Mat1a,Upb1,Aox3,Ugt1a1,Serpina1c,Aldh3a2,Oat,Serpina1b,Sult1a1
Aryl sulfotransferase activity	2	4	2.42	Sult1a1,Sult1d1
Caffeine oxidase activity	2	4	2.42	Cyp2c29,Cyp1a2
Glycine n-acyltransferase activity	2	4	2.42	Keg1,Glyat
Organic cyclic compound binding	21	4858	0.36	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ugdh,Iigp1,Cyp1a2,Ccbl2,Mat1a,Aox3,Ugt1a1,Nudt7,Oat,Cyp2d9,Ttpa,Sult1a1,Idh2
Anion binding	15	2678	0.47	Hacl1,Dpys,Csad,Papss2,Got1,Ddah1,Iigp1,Ccbl2,Mat1a,Aox3,Ugt1a1,Oat,Ttpa,Sult1a1,Gstp1
Transition metal ion binding	9	962	0.69	Cyp2f2,Cyp2c29,Dpys,Ddah1,Cyp1a2,Upb1,Aox3,Nudt7,Cyp2d9
Oxidoreductase activity, acting on ch-oh group of donors	4	131	1.2	Ugdh,Cbr1,Aldh3a2,Idh2
Carbon-carbon lyase activity	3	48	1.52	Hacl1,Csad,Got1
Heterocyclic compound binding	20	4771	0.34	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Papss2,Got1,Ugdh,Iigp1,Cyp1a2,Ccbl2,Mat1a,Aox3,Nudt7,Oat,Cyp2d9,Ttpa,Sult1a1,Idh2
Transferase activity, transferring alkyl or aryl (other than methyl) groups	3	59	1.43	Gstk1,Mat1a,Gstp1
Estrogen 16-alpha-hydroxylase activity	2	10	2.02	Cyp2c29,Cyp1a2
NAD binding	3	62	1.4	Ugdh,Aox3,Idh2
Amino acid binding	3	69	1.36	Dpys,Ddah1,Mat1a
Transferase activity	12	2088	0.48	Keg1,Papss2,Got1,Gstk1,Ccbl2,Glyat,Mat1a,Ugt1a1,Oat,Sult1a1,Sult1d1,Gstp1
Lyase activity	4	179	1.07	Hacl1,Csad,Got1,Ccbl2
Cytoplasm	36	10283	0.26	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Keg1,Got1,Ddah1,Ugdh,Gstk1,Arhgdib,Iigp1,Ces1d,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Stbd1,Ugt1a1,Nudt7,Serpina1c,Aldh3a2,Oat,Cyp2d9,Selenbp2,Ces3b,Ces3a,Serpina1b,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1
Intracellular	37	12596	0.19	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Keg1,Got1,Ddah1,Ugdh,Gstk1,Arhgdib,Iigp1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Stbd1,Ugt1a1,Nudt7,Serpina1c,Aldh3a2,Oat,Cyp2d9,Selenbp2,Ces3b,Ces3a,Serpina1b,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1

Peroxisome	5	142	1.27	Hacl1,Gstk1,Nudt7,Aldh3a2,Idh2
Steroid hormone biosynthesis, and Glutathione metabolism	10	194	1.43	Cyp2f2,Cyp2c29,Ugt2b1,Gstk1,Ces1d,Cyp1a2,Ugt1a1,Cyp2d9,Sult1d1,Gstp1
Steroid hormone biosynthesis, and Fatty acids	8	131	1.51	Cyp2f2,Cyp2c29,Ugt2b1,Ces1d,Cyp1a2,Ugt1a1,Cyp2d9,Sult1d1
Mixed, incl. complement and coagulation cascades, and histidine metabolism	5	46	1.76	Mug1,Serpina1c,Ces3b,Ces3a,Serpina1b
Steroid hormone biosynthesis, and Fatty acids	6	123	1.41	Cyp2f2,Cyp2c29,Ugt2b1,Cyp1a2,Ugt1a1,Cyp2d9
Methyl indole-3-acetate esterase activity, and tonb box, conserved site	3	5	2.5	Mug1,Ces3b,Ces3a
Alpha-amino acid metabolic process, and beta-alanine metabolism	5	109	1.38	Dpys,Mat1a,Upb1,Aldh3a2,Oat
Steroid hormone biosynthesis, and Fatty acids	5	111	1.37	Cyp2c29,Ugt2b1,Cyp1a2,Ugt1a1,Cyp2d9
Retinol metabolism, and Cytochrome P450, E-class, group I, CYP2J-like	4	70	1.48	Cyp2c29,Ugt2b1,Cyp1a2,Ugt1a1
Citrate cycle (TCA cycle), and Phenylalanine, tyrosine and tryptophan biosynthesis	3	34	1.67	Got1,Ccbl2,Idh2
Testosterone 6-beta-hydroxylase activity, and oxidative deethylation	2	5	2.32	Ugt2b1,Cyp1a2
Retinol metabolism	3	45	1.54	Ugt2b1,Cyp1a2,Ugt1a1
Mixed, incl. folic acid-containing compound metabolic process, and s-adenosylmethionine metabolic process	3	52	1.48	Dpys,Mat1a,Upb1

Metabolic pathways	23	1536	0.9	Cyp2c29,Hmox2,Dpys,Csad,Keg1,Papss2,Got1,Ugdh,Ugt2b1,Gstk1,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Cbr1,Ugt1a1,Aldh3a2,Oat,Selenbp2,Idh2,Gstp1
Chemical carcinogenesis	10	97	1.73	Cyp2c29,Ugt2b1,Gstk1,Cyp1a2,Ccbl2,Cbr1,Sult2a8,Ugt1a1,Sult1a1,Gstp1
Metabolism of xenobiotics by cytochrome P450	8	70	1.78	Cyp2f2,Ugt2b1,Gstk1,Cyp1a2,Cbr1,Sult2a8,Ugt1a1,Gstp1
Drug metabolism - cytochrome P450	6	68	1.67	Ugt2b1,Gstk1,Cyp1a2,Aox3,Ugt1a1,Gstp1
Drug metabolism - other enzymes	6	89	1.55	Dpys,Ugt2b1,Ces1d,Upb1,Ugt1a1,Gstp1
Ascorbate and aldarate metabolism	4	27	1.89	Ugdh,Ugt2b1,Ugt1a1,Aldh3a2
Steroid hormone biosynthesis	5	86	1.48	Cyp2c29,Ugt2b1,Cyp1a2,Ugt1a1,Cyp2d9
Retinol metabolism	5	92	1.46	Cyp2c29,Ugt2b1,Cyp1a2,Aox3,Ugt1a1
Tryptophan metabolism	4	51	1.61	Cyp1a2,Ccbl2,Aox3,Aldh3a2
Pantothenate and CoA biosynthesis	3	20	1.9	Dpys,Upb1,Aldh3a2
Phenylalanine metabolism	3	22	1.85	Keg1,Got1,Glyat
Peroxisome	4	85	1.39	Hacl1,Gstk1,Nudt7,Idh2
beta-Alanine metabolism	3	30	1.72	Dpys,Upb1,Aldh3a2
Pentose and glucuronate interconversions	3	34	1.67	Ugdh,Ugt2b1,Ugt1a1
Porphyrin and chlorophyll metabolism	3	41	1.58	Hmox2,Ugt2b1,Ugt1a1
Cysteine and methionine metabolism	3	50	1.5	Got1,Ccbl2,Mat1a
Arginine and proline metabolism	3	53	1.47	Got1,Aldh3a2,Oat
Sulfur metabolism	2	11	1.98	Papss2,Selenbp2
Glutathione metabolism	3	69	1.36	Gstk1,Idh2,Gstp1
Biosynthesis of amino acids	3	77	1.31	Got1,Mat1a,Idh2
Selenocompound metabolism	2	17	1.79	Papss2,Ccbl2
2-Oxocarboxylic acid metabolism	2	20	1.72	Got1,Idh2
Bile secretion	3	98	1.21	Ugt2b1,Sult2a8,Ugt1a1
Tyrosine metabolism	2	39	1.43	Got1,Aox3
Biological oxidations	13	213	1.51	Cyp2f2,Cyp2c29,Papss2,Ugdh,Ugt2b1,Gstk1,Ces1d,Glyat,Mat1a,Ugt1a1,Ces3a,Sult1a1,Gstp1
Metabolism	24	1779	0.85	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Papss2,Got1,Ddah1,Ugdh,Ugt2b1,Gstk1,Ces1d,Glyat,Mat1a,Upb1,

				Cbr1,Ugt1a1,Aldh3a2,Oat,Ces3a,Ttpa,Sult1a1,Idh2,Gstp1
Phase II - Conjugation of compounds	9	95	1.7	Papss2,Ugdh,Ugt2b1,Gstk1,Glyat,Mat1a,Ugt1a1,Sult1a1,Gstp1
Glucuronidation	3	20	1.9	Ugdh,Ugt2b1,Ugt1a1
Protein localization	4	104	1.31	Hacl1,Gstk1,Nudt7,Aldh3a2
Phase I - Functionalization of compounds	4	112	1.27	Cyp2f2,Cyp2c29,Ces1d,Ces3a
Alpha-oxidation of phytanate	2	6	2.24	Hacl1,Aldh3a2
Metapathway biotransformation	7	139	1.42	Cyp2f2,Gstk1,Cyp1a2,Glyat,Ugt1a1,Sult1a1,Gstp1
Glucuronidation	3	15	2.02	Ugdh,Ugt2b1,Ugt1a1
Estrogen metabolism	3	13	2.08	Cyp1a2,Ugt1a1,Sult1a1
Tryptophan metabolism	3	44	1.55	Cyp2f2,Cyp1a2,Aldh3a2
Irinotecan pathway	2	10	2.02	Ces1d,Ugt1a1
Oxidative stress and redox pathway	3	92	1.23	Gstk1,Idh2,Gstp1
Liver	32	1546	1.04	Cyp2f2,Cyp2c29,Hacl1,Dpys,Csad,Keg1,Papss2,Got1,Ddah1,Ugdh,Gstk1,Mug1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Oat,Cyp2d9,Selenbp2,Ces3b,Ces3a,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1
Whole body	33	9158	0.28	Cyp2f2,Cyp2c29,Hmox2,Hacl1,Dpys,Csad,Keg1,Papss2,Got1,Ddah1,Ugdh,Gstk1,Mug1,Ces1d,Cyp1a2,Ccbl2,Glyat,Mat1a,Upb1,Aox3,Stbd1,Ugt1a1,Nudt7,Oat,Cyp2d9,Selenbp2,Ces3b,Ces3a,Ttpa,Sult1a1,Idh2,Sult1d1,Gstp1
Peroxisome	5	132	1.3	Hacl1,Gstk1,Nudt7,Aldh3a2,Idh2
Cytoplasm	25	6579	0.3	Hmox2,Hacl1,Dpys,Csad,Keg1,Got1,Ugdh,Gstk1,Arhgdib,Iigp1,Ces1d,Cyp1a2,Ccbl2,Mat1a,Upb1,Ugt1a1,Nudt7,Serpina1c,Aldh3a2,Oat,Ces3a,Ttpa,Sult1a1,Idh2,Gstp1
Microsome	7	139	1.42	Cyp2f2,Cyp2c29,Hmox2,Cyp1a2,Ugt1a1,Aldh3a2,Cyp2d9
Oxidoreductase	10	623	0.93	Cyp2f2,Cyp2c29,Hmox2,Ugdh,Cyp1a2,Aox3,Cbr1,Aldh3a2,Cyp2d9,Idh2
Endoplasmic reticulum	12	1099	0.76	Cyp2f2,Cyp2c29,Hmox2,Iigp1,Ces1d,Cyp1a2,Stbd1,Ugt1a1,Aldh3a2,Cyp2d9,Ces3b,Ces3a
Transferase	14	1805	0.61	Keg1,Papss2,Got1,Ugt2b1,Gstk1,Ccbl2,Glyat,Mat1a,Sult2a8,Ugt1a1,Oat,Sult1a1,Sult1d1,Gstp1
Pyridoxal phosphate	4	57	1.57	Csad,Got1,Ccbl2,Oat
Aminotransferase	3	19	1.92	Got1,Ccbl2,Oat
Heme	5	165	1.2	Cyp2f2,Cyp2c29,Hmox2,Cyp1a2,Cyp2d9
Serine esterase	3	34	1.67	Ces1d,Ces3b,Ces3a
Iron	6	362	0.94	Cyp2f2,Cyp2c29,Hmox2,Cyp1a2,Aox3,Cyp2d9
Monooxygenase	4	127	1.22	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9

Lyase	4	159	1.12	Hacl1,Csad,Cyp1a2,Ccbl2
Serine protease inhibitor	3	83	1.28	Mug1,Serpina1c,Serpina1b
Pyridoxal phosphate-dependent transferase, major domain	4	42	1.7	Csad,Got1,Ccbl2,Oat
Pyridoxal phosphate-dependent transferase	4	42	1.7	Csad,Got1,Ccbl2,Oat
Carboxylesterase type B, active site	3	22	1.85	Ces1d,Ces3b,Ces3a
Carboxylesterase, type B	3	26	1.78	Ces1d,Ces3b,Ces3a
Cytochrome P450, E-class, group I	4	82	1.41	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Cytochrome P450, conserved site	4	93	1.35	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Carboxylesterase type B, conserved site	3	26	1.78	Ces1d,Ces3b,Ces3a
Sulfotransferase domain	3	40	1.6	Sult2a8,Sult1a1,Sult1d1
Cytochrome P450	4	101	1.32	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9
Glycine N-acyltransferase	2	4	2.42	Keg1,Glyat
Glycine N-acyltransferase, C-terminal	2	4	2.42	Keg1,Glyat
Pyridoxal phosphate-dependent transferase domain 1	3	34	1.67	Got1,Ccbl2,Oat
Glycine N-acyltransferase, N-terminal	2	4	2.42	Keg1,Glyat
Cytochrome P450 superfamily	4	101	1.32	Cyp2f2,Cyp2c29,Cyp1a2,Cyp2d9