

Micronutrient Deficiencies and the Human Plasma Nutriproteome

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Background

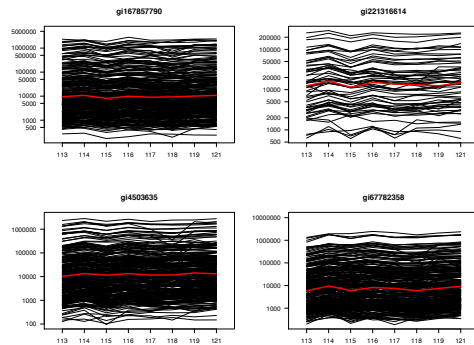
- Micronutrient deficiencies remain a complex and “hidden” aspect of malnutrition worldwide.
- Timely and thorough estimation of the prevalence of multiple micronutrient deficiencies is hampered by dependence on complex, time and resource-intensive laboratory indicators of nutritional status.
- We are seeking to establish the validity of a “plasma nutriproteome” that could be used as a basis for developing tests to assess multiple micronutrient deficiencies in populations on a single methodological platform.

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Project overview

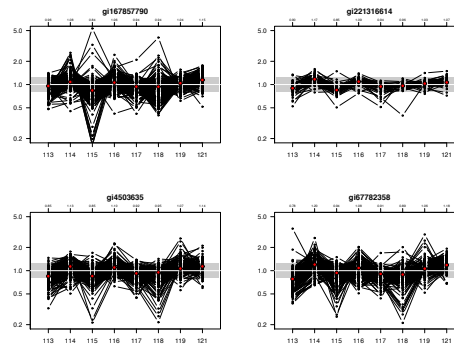
- Using 8plex iTRAQ based proteomics, we are identifying plasma proteins that covary with conventional plasma indicators of micronutrient status.
- Plasma samples collected and archived from 1000 Nepalese children, 6-9 years of age, were selected for comprehensive nutritional analyses.
- Over 2,500 unique plasma proteins have been identified (with a false discovery rate set at 95%) in pooled plasma and individual specimen (n=198 to date, n=500 anticipated by spring 2012).
- Biostatistical methods are developed to improve the quantitation of the relative protein abundances in the samples (see the following slides), and to detect a protein signature predictive of indicators of micronutrient status.

Reporter ion intensities



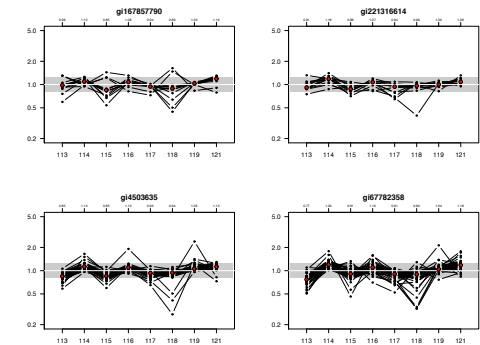
→ Run with 8 technical replicates of a master pool

Log reporter ion intensities (median polish)



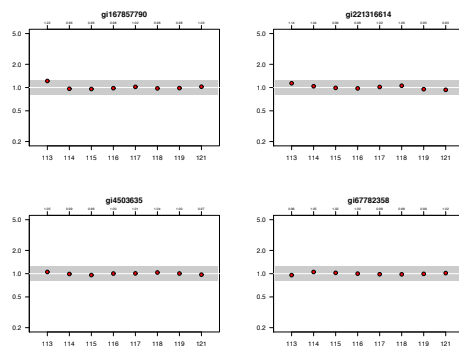
→ Run with 8 technical replicates of a master pool

Log reporter ion intensities (median polish, by peptide)



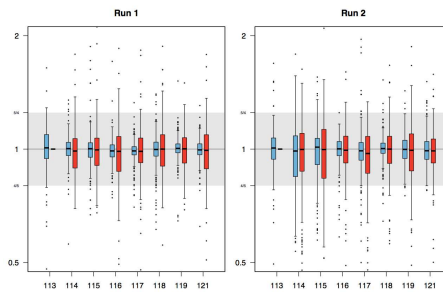
→ Run with 8 technical replicates of a master pool

Estimated relative protein abundances



→ Run with 8 technical replicates of a master pool

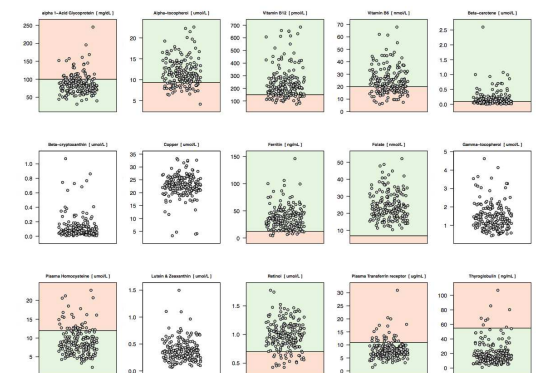
Variability



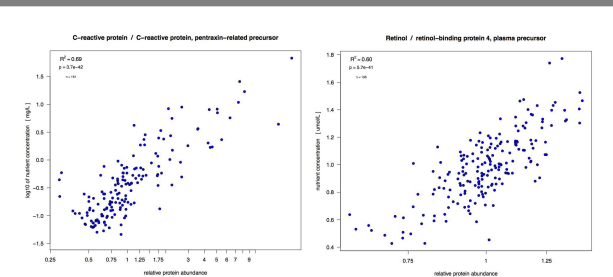
Using the new quantitation method, we obtain more precise measurements (blue boxplots) compared to Protein Discovery estimates (red boxplots).

→ Two runs with 8 technical replicates of a master pool

Micronutrient and marker measurements

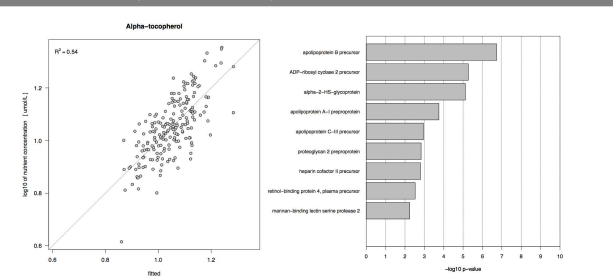


Does it work..?



The most striking correlations are observed between the C-reactive protein measured independently as an inflammation marker in the serum and by iTraQ ($r = 0.83$, $p < 10^{-40}$), and between retinol measured in the serum and the retinol binding protein quantified by iTraQ ($r = 0.78$, $p < 10^{-40}$).

Prediction (alpha-tocopherol)



While many proteins appear to be co-expressed and thus are correlated, some relative abundances contribute independent information about the nutrient concentration, and thus, can be used for modeling and prediction.

Alpha-tocopherol

	A	B	C	D	E	F
1	44557321	apolipoprotein A-I precursor	1.51E-11	2.17E-09	0.20662777	0.45785125
2	94520149	apolipoprotein A-II precursor	1.99E-08	8.26E-07	0.15213563	0.39006878
4	44557323	apolipoprotein C-III precursor	7.21E-08	8.26E-07	0.15148974	0.38921426
5	0197333753	interferon-related 5-polyomavirus regulator 2	2.55E-07	9.12E-07	0.1587072	0.38321917
6	955743122	retinol-binding protein 4, plasma precursor	1.73E-07	6.65E-06	0.12913973	0.35936017
7	g32130518	apolipoprotein C-I precursor	2.87E-07	6.88E-06	0.1272512	0.35672286
8	945020161	apolipoprotein IV precursor	4.10E-07	8.26E-06	0.12412622	0.35231151
9	g22091452	apolipoprotein M	4.58E-07	8.25E-06	0.12315996	0.35094154
10	0189181724	proteoglycan 4 isoform D	7.53E-07	1.18E-05	0.12319958	0.35099798
11	0185990323	lecucine-rich alpha-2-glycoprotein 1 precursor	8.26E-07	1.05E-05	0.11739326	0.34551937
12	046276889	proteoglycan 2 prepropeptide	2.85E-05	0.00037273	0.10525885	0.32443312
13	94502157	apolipoprotein C-II precursor	0.00053118	0.00637529	0.06014477	0.24524431
14	945056115	protein C prepropeptide	0.00082365	0.00125115	0.05617461	0.23701141
15	0167614506	plastin-2	0.0015849	0.01065496	0.03903943	0.22321748
16	94506121	vitamin K-dependent protein 2 precursor	0.0017451	0.01065496	0.05921668	0.24334478
17	04773960	cadherin 1, type 1, prepropeptide	0.0018367	0.01065496	0.0298934	0.24506126
18	04507725	transhytrin precursor	0.00135436	0.0147425	0.05167527	0.27321196
19	94507509	tissue inhibitor of metalloproteinase 1	0.00145027	0.01469419	0.06474316	0.25446477
20	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	0.00163967	0.01242974	0.04949742	0.23248986
21	0205277441	serin (or cysteine) proteinase inhibitor, beta-2-microglobulin precursor	0.00170105	0.01242974	0.04961525	0.22744811
22	04773960	cadherin 1, type 1, prepropeptide	0.0018367	0.01065496	0.0298934	0.24506126
23	g21071030	carnosinase 1 precursor	0.0036032	0.0235699	0.0428333	0.20701406
24	010518053	coagulation factor VII isoform B	0.00396277	0.0248148	0.0454377	0.21361122
25	04773960	cadherin 1, type 1, prepropeptide	0.0018367	0.01065496	0.0298934	0.24506126
26	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
27	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
28	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
29	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
30	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
31	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
32	04502133	serum amyloid P component precursor	0.0041141	0.02485371	0.04122705	0.20304437
33	955025676	insulin-like growth factor-binding protein 2	0.0074594	0.0357327	0.03805879	0.19508431

Copper

	A	B	C	D	E	F
1	44557485	catuloplasmin precursor	1.95E-16	4.13E-14	0.29502781	0.53416662
2	9116256481	TNFAIP3-interacting protein 1	6.87E-09	7.72E-07	0.1908696	0.36868265
4	94505047	lumican precursor	2.70E-05	0.00098543	0.08971105	0.29951804
5	000093825	serin (or cysteine) proteinase inhibitor, clade A, alpha-2-macroglobulin precursor	2.14E-05	0.00098543	0.08971105	0.29951804
6	94501987	afamin precursor	2.06E-05	0.00098543	0.08682348	0.29465824
7	946242068	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
8	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
9	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
10	916878799	orosomucoid 1 precursor	1.00E-04	0.00232585	0.07542569	0.27430947
11	0185990323	lecucine-rich alpha-2-glycoprotein 1 precursor	8.26E-07	1.05E-05	0.11739326	0.34551937
12	04503625	coagulation factor X prepropeptide	0.00126675	0.02299342	0.05227995	0.22864811
13	955743122	retinol-binding protein 4, plasma precursor	0.00130263	0.02299342	0.05227995	0.22864811
14	0115392404	inter-alpha (globulin) inhibitor H3 prepropeptide	0.00145445	0.0231473	0.04893032	0.21201198
15	916271030	alpha 1B-glycoprotein precursor	0.00183539	0.0231473	0.04883947	0.20999653
16	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
17	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
18	946299759	ectopic viral integration site 5	0.00195631	0.0231473	0.05871002	0.24230115
19	016418467	lecucine-rich alpha-2-glycoprotein 1 precursor	0.00196702	0.0231473	0.04893032	0.21201198
20	04626772	insulin-like growth factor, beta-2-microglobulin precursor	0.00268451	0.02927868	0.04545165	0.21330065
21	0115392809	inter-alpha (globulin) inhibitor H3 prepropeptide	0.00326237	0.0347031	0.04384049	0.2093812
22	945056239	transforming growth factor, beta, intracellular, 68kDa	0.00342897	0.0347031	0.04384049	0.2093812
23	046271843	cartilage oligomeric matrix protein precursor	0.00403269	0.03827229	0.0418333	0.2045319
24	44557321	apolipoprotein A-I precursor	0.00431328	0.04007321	0.04114994	0.19547737
25	945056239	transforming growth factor, beta, intracellular, 68kDa	0.00431328	0.04007321	0.04114994	0.19547737
26	04749007	cluserin isoform 2 prepropeptide	0.00596248	0.04918984	0.0382123	0.1957880
27	04502261	serin (or cysteine) proteinase inhibitor, clade C, alpha-1, type 1, prepropeptide	0.00663787	0.04918984	0.0382123	0.1957880
28	945056239	transforming growth factor, beta, intracellular, 68kDa	0.00663787	0.04918984	0.0382123	0.1957880
29	945056239	transforming growth factor, beta, intracellular, 68kDa	0.00663787	0.04918984	0.0382123	0.1957880
30	945056239	transforming growth factor, beta, intracellular, 68kDa	0.00663787	0.04918984	0.0382123	0.1957880
31	04502149	apolipoprotein A-II precursor	0.00713684	0.05212821	0.03671533	0.19161246
32	955179042	C-reactive protein, pentraxin-related precursor	0.00781516	0.05316115	0.04570753	0.20984265
33	0115392809	inter-alpha (globulin) inhibitor H3 prepropeptide	0.00812447	0.05451615	0.0357511	0.18866203
34	910356965	serum amyloid A-4 precursor	0.00823258	0.05451615	0.03543634	0.18824543

C-reactive protein

	A	B	C	D	E	F
1	955770842	C-reactive protein, pentraxin-related precursor	3.70E-42	5.38E-40	0.6888836	0.83999012
2	9116256481	TNFAIP3-interacting protein 1	4.38E-23	3.19E-21	0.39705169	0.63012038
4	94505047	lumican precursor	1.29E-12	6.24E-11	0.39037488	0.62479597
5	000093825	serin (or cysteine) proteinase inhibitor, clade A, alpha-2-macroglobulin precursor	1.29E-12	6.24E-11	0.39037488	0.62479597
6	94501987	afamin precursor	1.29E-12	6.24E-11	0.39037488	0.62479597
7	916878799	orosomucoid 1 precursor	3.94E-15	9.56E-14	0.2731143	0.52260338
8	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.45E-16	2.32E-14	0.2324746	0.47032122
9	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	1.52E-13	2.76E-12	0.24565393	0.49561617
10	946299759	ectopic viral integration site 5	1.38E-12	2.22E-11	0.2731143	0.52260338
11	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.45E-16	2.32E-14	0.2324746	0.47032122
12	0115392809	inter-alpha (globulin) inhibitor H3 prepropeptide	3.01E-12	3.98E-11	0.26444131	0.51423857
13	946271843	cartilage oligomeric matrix protein precursor	1.85E-09	2.24E-08	0.17029677	0.41262709
14	94505047	lumican precursor	2.06E-05	0.00098543	0.08682348	0.29465824
15	0115392809	inter-alpha (globulin) inhibitor H3 prepropeptide	7.97E-09	8.28E-08	0.15801793	0.39751469
16	945056239	transforming growth factor, beta, intracellular, 68kDa	9.23E-09	9.01E-08	0.15671691	0.39587407
17	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
18	946299759	ectopic viral integration site 5	0.002197	1.99E-06	0.12907536	0.29537076
19	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	9.75E-05	0.00098543	0.08672724	0.29449516
20	916878799	orosomucoid 1 precursor	3.94E-15	9.56E-14	0.2731143	0.52260338
21	94502167	serin (or cysteine) proteinase inhibitor, clade C, alpha-1, type 1, prepropeptide	6.87E-07	5.97E-06	0.11353807	0.33695411
22	94502261	serin (or cysteine) proteinase inhibitor, clade C, alpha-1, type 1, prepropeptide	3.01E-06	2.05E-05	0.10658821	0.32647649
23	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
24	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
25	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
26	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
27	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
28	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
29	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
30	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
31	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
32	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088
33	916271030	alpha 1B-glycoprotein precursor	3.09E-06	2.05E-05	0.106347	0.3261088

Retinol

	A	B	C	D	E	F
1	955743122	retinol-binding protein 4, plasma precursor	5.09E-41	q	0.0481557	0.77768989
2	9116256481	TNFAIP3-interacting protein 1	1.69E-17	8.31E-16	0.31548309	0.56167889
4	94505047	lumican precursor	1.24E-12	1.21E-11	0.22949268	0.48966867
5	000093825	serin (or cysteine) proteinase inhibitor, clade A, alpha-2-macroglobulin precursor	1.24E-12	1.21E-11	0.22949268	0.48966867
6	94501987	afamin precursor	1.24E-12	1.21E-11	0.22949268	0.48966867
7	946242068	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.58E-08	5.36E-07	0.51133691	0.89002405
8	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.58E-08	5.36E-07	0.51133691	0.89002405
9	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.58E-08	5.36E-07	0.51133691	0.89002405
10	916878799	orosomucoid 1 precursor	5.91E-08	1.51E-06	0.14091456	0.37585757
11	0185990323	lecucine-rich alpha-2-glycoprotein 1 precursor	9.26E-08	1.05E-06	0.11739326	0.34551937
12	04618467	lecucine-rich alpha-2-glycoprotein 1 precursor	2.51E-07	5.24E-06	0.12741449	0.35695167
13	g32130518	apolipoprotein C-I precursor	3.08E-07	5.24E-06	0.12662089	0.35583833
14	94501987	afamin precursor	1.24E-12	1.21E-11	0.22949268	0.48966867
15	94503625	coagulation factor X prepropeptide	5.43E-07	7.56E-06	0.12166592	0.34881158
16	0167614506	plastin-2	9.40E-07	1.20E-05	0.11688782	0.34184743
17	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.58E-08	5.36E-07	0.51133691	0.89002405
18	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.58E-08	5.36E-07	0.51133691	0.89002405
19	04626772	insulin-like growth factor binding protein, beta-2-microglobulin precursor	7.5			