

Identification of Protein Changes in the Urine of Hypothyroid Patients Treated with Thyroxine Using Proteomics Approach

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SUPPORTING INFORMATION

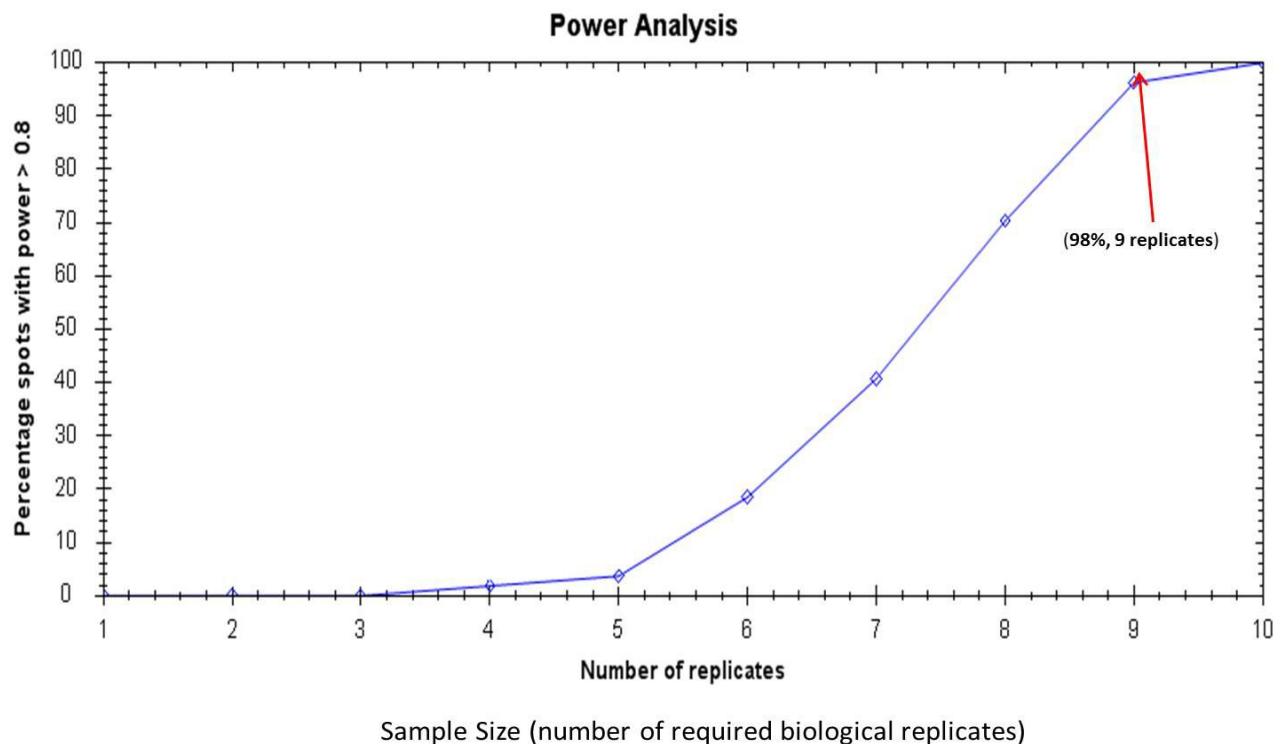


Figure S1: Power calculation for determination of the minimum number of required biological variants for 2-DIGE analysis. The power curve was used to calculate the sample size required to find significant difference with a fold-change of ≥ 1.5 between two paired groups at 98% power and $p\text{-value} \leq 0.05$

Top Networks

ID	Associated Network Functions	Score
1	Amino Acid Metabolism, Molecular Transport, Small Molecule Biochemistry Gastrointestinal Disease, Organismal Injury and Abnormalities, Connective Tissue Disorders	26
2	Cardiovascular System Development and Function, Organ Development, Cell Death and Survival	26
3		20

Top Canonical Pathways

Name	p-value	Overlap
LXR/RXR Activation	6.49E-07	4.1 % 5/121
FXR/RXR Activation	7.93E-07	4.0 % 5/126
Acute Phase Response Signaling	4.47E-06	2.8 % 5/179
Clathrin-mediated Endocytosis Signaling	2.38E-03	1.6 % 3/193
Coenzyme A Biosynthesis	4.15E-03	33.3 % 1/3

Figure S2: The figure shows the different canonical pathways obtained from IPA functional analysis.

Table S1: Experimental design: 18 samples run on 9 2D-PAGE gels, samples were labeled randomly with Cy3 and Cy5, and a pooled sample was used as an internal standard and was stained with Cy2

Gel	Cy3	Cy5	Cy2
1	1 <i>(Euthyroid)</i>	11 <i>(hypothyroid)</i>	Pooled sample
2	12 <i>(hypothyroid)</i>	2 <i>(Euthyroid)</i>	Pooled sample
3	5 <i>(Euthyroid)</i>	13 <i>(hypothyroid)</i>	Pooled sample
4	14 <i>(hypothyroid)</i>	3 <i>(Euthyroid)</i>	Pooled sample
6	16 <i>(hypothyroid)</i>	6 <i>(Euthyroid)</i>	Pooled sample
7	7 <i>(Euthyroid)</i>	17 <i>(hypothyroid)</i>	Pooled sample
8	18 <i>(hypothyroid)</i>	8 <i>(Euthyroid)</i>	Pooled sample
9	9 <i>(Euthyroid)</i>	19 <i>(hypothyroid)</i>	Pooled sample

Table S2: Mass spectrometry list of significant differentially abundant proteins between Hypo and Euth identified in urine samples, using 2D-DIGE with. Protein name, accession number, Mascot score, MS % coverage, protein MW and pI values according to Uniprot database are listed.

Sl no:	Spot No ^a	Accession No ^b	Protein Name	MASCOT ID	Pi ^c	MW ^d	Cov%	Score ^e
1.	578	P02768	Serum albumin	ALBU_HUMAN	5.92	71317	37	81
2.	376	P02768	Serum albumin	ALBU_HUMAN	5.92	71317	41	132
3.	918	Q8IZU9	Kin of IRRE-like protein 3	KIRR3_HUMAN	6.46	86227	17	57
4.	356	P02768	Serum albumin	ALBU_HUMAN	5.92	71317	40	121
5.	644	Q96CD2	Phosphopantethenoylcysteine decarboxylase	COAC_HUMAN	5.72	22741	42	64
6.	110	Q8NFX7	Syntaxin-binding protein 6	STXB6_HUMAN	9.19	23824	39	57
7.	481	095395	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 3	GCNT3_HUMAN	8.53	51572	19	57

8.	450	095395	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 3	GCNT3_HUMAN	8.53	51572	27	58
9.	808	Q9HBZ2	Aryl hydrocarbon receptor nuclear translocator 2	ARNT2_HUMAN	6.25	79268	23	58
10.	503	P01112	GTPase HRas	RASH_HUMAN	5.16	21627	38	58
11.	700	O75409	Huntingtin-interacting protein M	HYPM_HUMAN	4.93	13547	65	60
12.	117	P02787	Serotransferrin	TRFE_HUMAN	6.81	79280	43	158
13.	170	P02768	Serum albumin	ALBU_HUMAN	5.92	71317	35	81
14.	742	P21281	V-type proton ATPase subunit B, brain isoform	VATB2_HUMAN	5.57	56807	20	78
15.	743	095395	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 3	GCNT3_HUMAN	8.53	51572	25	71
16.	735	Q5T197	E3 ubiquitin-protein ligase DCST1	DCST1_HUMAN	9.28	82142	23	58
17.	750	P07339	Cathepsin D	CATD_HUMAN	6.10	45037	27	63
18.	819	Q8N806	Putative E3 ubiquitin-protein ligase UBR7	UBR7_HUMAN	4.70	49336	21	58
19.	509	P13645	Keratin, type I cytoskeletal 10	K1C10_HUMAN	5.13	59020	30	75

20	498	P01042	Kininogen-1	KNG1_HUMAN	6.34	72556	21	57
21	655	Q8NF91	Nesprin-1	SYNE1_HUMAN	5.38	1017069	6	61
22	648	Q9UQ35	Serine/arginine repetitive matrix protein 2	SRRM2_HUMAN	12.05	300179	10	63
23	435	Q8NF91	Nesprin-1	SYNE1_HUMAN	5.38	1017069	4	64
24	493	Q8NF91	Nesprin-1	SYNE1_HUMAN	5.38	1017069	4	66
25	547	Q6FIFO	AN1-type zinc finger protein 6	ZFAN6_HUMAN	6.87	23168	27	56
26	752	Q96PF1	Protein-glutamine gamma-glutamyltransferase Z	TGM7_HUMAN	6.54	80575	17	58
27	105	A6NJ69	IgA-inducing protein homolog	IGIP_HUMAN	9.13	6161	98	58
28	104	Q9UP79	A disintegrin and metalloproteinase with thrombospondin motifs 8	ATS8_HUMAN	5.78	98890	17	59
29	730	O15212	Prefoldin subunit 6	PFD6_HUMAN	8.83	14574	35	57
30	608	Q8N7C3	Probable E3 ubiquitin-protein ligase TRIML2	TRIMM_HUMAN	5.52	44525	18	57
31	728	Q6FIFO	AN1-type zinc finger protein 6	ZFAN6_HUMAN	6.87	23168	24	57

32.	828	Q6FIF0	AN1-type zinc finger protein 6	ZFAN6_HUMAN	6.87	23168	27	61
33.	698	13647	Keratin, type II cytoskeletal 5	K2C5_HUMAN	7.59	62568	20	70
34.	734	P02760	Protein AMBP	AMBP_HUMAN	3.95	39886	23	70
35.	702	Q12907	Vesicular integral-membrane protein VIP36	LMAN2_HUMAN	6.46	40545	30	75
36.	756	Q96KA5	Cleft lip and palate transmembrane protein 1-like protein	CLP1L_HUMAN	8.71	62531	25	57
37.	726	P02760	Protein AMBP	AMBP_HUMAN	3.95	39886	28	65
38.	718	A8K0R7	Zinc finger protein 839	ZN839_HUMAN	6.11	88570	21	75
39.	741	P02760	Protein AMBP	AMBP_HUMAN	3.95	39886	23	60
40.	802	Q93099	Homogentisate 1,2-dioxygenase	HGD_HUMAN	6.54	50625	25	58
41.	700	Q9UQ35	Serine/arginine repetitive matrix protein 2	SRRM2_HUMAN	12.05	300179	11	72
42.	753	P02753	Retinol-binding protein 4	RET4_HUMAN	5.76	23337	61	93

^a Spot number.

^b Protein accession number for SWISSPROT Database.

^c Theoretical isoelectric point.

^d Theoretical relative mass.

^e MASCOT score

Table S3: Biochemical parameters of the study subjects at baseline and after L-thyroxine therapy

	Hypothyroid	Euthyroid	<i>p</i> -value
N	9 (6 f)	9 (6 f)	
Age (years)	39.3 ± 12.9	39.3 ± 12.9	
Fasting glucose (mmol/L)	5.3 ± 0.4	5.0 ± 0.5	0.078
Urea (mmol/L)	4.7 ± 0.7	4.6 ± 0.9	0.392
Creatinine (mmol/L)	72.5 ± 13.1	76.1 ± 23.3	0.404
Aspartate transaminase (IU/L)	33.4 ± 6.6	37.5 ± 9.0	0.407
Alanine transaminase (IU/L)	18.0 ± 5.8	17.4 ± 3.9	0.169
Alkaline phosphatase (IU/L)	94.9 ± 25.9	96.8 ± 31.2	0.133
FT4 (pmol/L)	8.3 ± 5.5	18.8 ± 3.7	0.000
TSH (mIU/l)	33.9 ± 22.1	1.6 ± 0.9	0.000
Total Cholesterol (mmol/L)	4.6 ± 0.6	4.8 ± 0.7	0.193
Triglycerides (mmol/L)	1.2 ± 0.3	1.4 ± 0.3	0.184
LDL cholesterol (mmol/L)	2.9 ± 0.8	3.0 ± 0.6	0.373
HDL cholesterol (mmol/L)	1.2 ± 0.4	1.0 ± 0.3	0.087

Table S4: Identified proteins, with changes in abundance of significantly differentially abundant proteins between hypothyroid and euthyroid states in urine samples. Table 2 shows values for the average ratio between the two states, with their corresponding levels of fold changes and one-way ANOVA (p-value < 0.05) using 2D-DIGE. [Analysis type: MALDI-TOF; database: SwissProt; taxonomy: Homo sapiens].

Spot No ^a	Accession No ^b	Protein Name	MASCOT ID	P-Value (ANOVA)	Ratio	EXP ^c
				Hypo\Euth		
376	P02768	Serum albumin	ALBU_HUMAN	0.009	1.9	UP
356	P02768	Serum albumin	ALBU_HUMAN	0.015	2.8	UP
700	O75409	Huntingtin-interacting protein M	HYPM_HUMAN	0.002	3.3	UP
117	P02787	Serotransferrin	TRFE_HUMAN	0.044	4.5	UP
742	P21281	V-type proton ATPase subunit B, brain isoform	VATB2_HUMAN	0.006	2.3	UP

743	O95395	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 3	GCNT3_HUMAN	0.028	2.8	UP
735	Q5T197	E3 ubiquitin-protein ligase DCST1	DCST1_HUMAN	0.014	2.4	UP
750	P07339	Cathepsin D	CATD_HUMAN	0.026	2.5	UP
819	Q8N806	Putative E3 ubiquitin-protein ligase UBR7	UBR7_HUMAN	0.008	1.9	UP
509	P13645	Keratin, type I cytoskeletal 10	K1C10_HUMAN	0.016	1.5	UP
608	Q8N7C3	Probable E3 ubiquitin-protein ligase TRIML2	TRIMM_HUMAN	0.036	1.5	UP
728	Q6FIFO	AN1-type zinc finger protein 6	ZFAN6_HUMAN	0.038	2.1	UP
828	Q6FIFO	AN1-type zinc finger protein 6	ZFAN6_HUMAN	0.039	1.8	UP
698	P13647	Keratin, type II cytoskeletal 5	K2C5_HUMAN	0.044	1.6	UP
734	P02760	Protein AMBP	AMBP_HUMAN	0.021	2.8	UP

702	Q12907	Vesicular integral-membrane protein VIP36	LMAN2_HUMAN	0.018	2.2	UP
498	P01042	Kininogen-1	KNG1_HUMAN	0.0015	1.8	UP
578	P02768	Serum albumin	ALBU_HUMAN	0.009	1.6	UP
918	Q8IZU9	Kin of IRRE-like protein 3	KIRR3_HUMAN	0.014	-3	DOWN
644	Q96CD2	Phosphopantethenoylcysteine decarboxylase	COAC_HUMAN	0.021	-2.3	DOWN
110	Q8NFX7	Syntaxin-binding protein 6	STXB6_HUMAN	0.042	-1.7	DOWN
481	O95395	Beta-1,3-galactosyl-O-glycosyl- glycoprotein beta-1,6-N- acetylglucosaminyltransferase 3	GCNT3_HUMAN	0.039	1.8	UP

450	O95395	Beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1,6-N-acetylglucosaminyltransferase 3	GCNT3_HUMAN	0.041	2.0	UP
808	Q9HBZ2	Aryl hydrocarbon receptor nuclear translocator 2	ARNT2_HUMAN	0.04	-2.1	DOWN
503	P01112	GTPase HRas	RASH_HUMAN	0.05	-2.2	DOWN
170	P02768	Serum albumin	ALBU_HUMAN	0.049	3	UP
655	Q8NF91	Nesprin-1	SYNE1_HUMAN	0.04	-3.4	DOWN
648	Q9UQ35	Serine/arginine repetitive matrix protein 2	SRRM2_HUMAN	0.006	-2.6	DOWN
435	Q8NF91	Nesprin-1	SYNE1_HUMAN	0.010	-2.5	DOWN
493	Q8NF91	Nesprin-1	SYNE1_HUMAN	0.016	-2.1	DOWN
547	Q6FIFO	AN1-type zinc finger protein 6	ZFAN6_HUMAN	0.016	-2.5	DOWN

752	Q96PF1	Protein-glutamine gamma-glutamyltransferase Z	TGM7_HUMAN	0.022	-3.9	DOWN
105	A6NJ69	IgA-inducing protein homolog	IGIP_HUMAN	0.023	-2.1	DOWN
104	Q9UP79	A disintegrin and metalloproteinase with thrombospondin motifs 8	ATS8_HUMAN	0.042	-1.9	DOWN
730	O15212	Prefoldin subunit 6	PFD6_HUMAN	0.030	-2.1	DOWN
756	Q96KA5	Cleft lip and palate transmembrane protein 1-like protein	CLP1L_HUMAN	0.021	1.7	UP
726	P02760	Protein AMBP	AMBP_HUMAN	0.022	2.4	UP
718	A8K0R7	Zinc finger protein 839	ZN839_HUMAN	0.026	2.5	UP
741	P02760	Protein AMBP	AMBP_HUMAN	0.052	2.1	UP
802	Q93099	Homogentisate 1,2-dioxygenase	HGD_HUMAN	0.053	1.6	UP
700	Q13438	Protein OS-9	OS9_HUMAN	0.058	1.9	UP

753	P02753	Retinol-binding protein 4	RET4_HUMAN	0.058	1.9	UP
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^a Spot number.

^b Protein accession number for SWISSPROT Database.

^c Protein expression between hypothyroid and euthyroid states.