

The role of Matriptase-2 during the early postnatal development in humans

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Supplementary Methods

DNA Sequence Analysis

Anticoagulated (EDTA-treated) blood samples were obtained and stored at -20°C. Genomic DNA was isolated by the QIAamp DNA Blood Mini Kit (Promega Corporation, Madison, WI), according to the manufacturer's instructions.

To analyze *TMPRSS6* gene all coding exons and splice junctions were amplified by PCR and amplified fragments were directly sequenced. The *TMPRSS6* genomic sequence from GenBank accession numbers NC_000022.9 was used as reference sequence. Detailed protocols and primer sequences are available on request.

The amplified products were isolated by electrophoresis on 1% agarose gel and purified using the QIAamp purification kit (Qiagen, Valencia, CA). Direct sequencing was performed using a fluorescence-tagged dideoxy chain terminator method in an ABI 3100 automated sequencer (Applied Biosystem, Foster City, CA), according to the manufacturer's instructions.

Hepcidin assay

Serum hepcidin was measured by means of a recently validated mass spectrometry-based approach, i.e. SELDI-TOF-MS using a PBSCIIc mass spectrometer, copper loaded immobilized metal-affinity capture ProteinChip arrays (IMAC30-Cu²⁺), and a synthetic hepcidin analogue (hepcidin-24, Peptides International, Louisville, KY) as an internal standard, as described in detail elsewhere.^{1,2}

Bioinformatic Prediction Methods

Prediction of possible impact of amino acid substitution on *TMPRSS6* protein was done using the commonly used and previously published software SIFT version 4.0.3 (<http://sift.jcvi.org>)³ and PolyPhen-2 version 2.2.2 (<http://genetics.bwh.harvard.edu/pph2/>)⁴ using default parameters. Multiple sequence alignment of *TMPRSS6* protein (MT-2) in several species was done using ClustalOmega software using default parameters (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

Results

Sequencing analysis of *TMPRSS6* gene in 7 IRIDA patients from 4 unrelated families revealed 4 mutations, two were novel: one missense (p.L689P); one frameshift (p.I158Sfs*7) (Table 1S and Figure 1). None of the novel variants has been previously reported in the examined databases (ENSEMBL: <http://www.ensembl.org/>, NCBI dbSNP: <http://www.ncbi.nlm.nih.gov/SNP/>, 1000Genomes: <http://browser.1000genomes.org/>). In two Turkish families with four patients (Table

1S) we identified the same homozygous duplication leading to a frameshift and a pre-mature stop codon (c.1904_1905dupGC, p.K636AfsX17). This mutation has been previously reported in other four unrelated families of Turkish origin at homozygous state yet.^{5,6} Hb levels in patients carrying the missense variant L689P are higher compared to patients with frameshift mutations in the *TMPRSS6* gene, probably confirming a more clinical severe phenotype for the patients with two frameshift mutations compared to patients with two missense mutations.⁷

Bioinformatic Prediction

The novel missense substitution is bioinformatically predicted to be damaging or deleterious according to two commonly used programs (SIFT and PolyPhen-2). In addition, a multiple sequence alignment of MT-2 proteins among 24 species shows that this mutation is highly conserved through evolution (identical amino acid in 100% of the sequences; Supplementary Figure S1).

References

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Supplementary Table 1: Clinical, genetics and laboratory data and response to iron treatment of IRIDA patients.

	A II1	A II3	B II3	C II2	C II3	C III1	D II1				
Age at diagnosis, years/sex	6/M	1.5/M	0.75/F	26/F	17/M	3.25/M	1/F				
Genotype	p.L689P/ p.L689P	p.L689P/ p.L689P	p.K636Afs*17/ p.K636Afs*17	p.K636Afs*17/ p.K636Afs*17	p.K636Afs*17/ p.K636Afs*17	p.K636Afs*17/ p.K636Afs*17	p.I158Sfs*7/ p.I158Sfs*7	Normal values adults (range)		Normal values children^b	
Consanguinity, Y(es)/N(o)	Y	Y	Y	N	N	N	Y	M	F	Mean	- 2SD
Hb, g/dL	9	9.4	6.2	11.5	9.18	7.9	7.2	12.0-17.5	12.0-16.0	12.5	11.5
MCV, fL	52	52	54.9	80.9	57.8	52	53	80-97		81	75
MCH, pg	16.6	17.8	15.4	27.5	17.1	15	15	25-34		33	31
MCHC, g/dL	32	34.3	28.1	33.9	29.6	29.5	28	32-37		34	31
Plt, *10³/μl	69.1	43.9	42	26.4	46.6	53.9	32.9	130-400		150	303
WBC, *10³/μl	13.6	10	6.8	5.5	5.4	7.4	6	4.8-10.8		11.4-9.1 ^c	5.5-17.5 ^c
Reticulocytes count, *10³/μl	43.2	36.4	37	n.a.	53.7	70	46.4	20- 120		n.a.	n.a.
RBC, *10⁶/μl	5.4	5.2	4.07	4.17	5.37	5	5.8	4.2- 5.6	4.0-5.4	4.6	3.9
Ferritin, μg/L	44.2	59.8		135	9.44	11.7	76	18-370	9-120	6	24
Serum Fe, μg/dL	11	13	17	14	9	10	12	16-124		22	136
Transferrin saturation, %	3.9	5.1	6.1	5	2	2	4	15- 35		7	44
Serum Hcpidin^a, nM	7.03	14.53	8.02	4.5	7.3	11	9.1	3-7		2 ± 2.6	
Iron Treatment/ Responce	Oral (No)	Oral (No)	Oral (No) and parenteral (No)	Oral iron during pregnancy	Oral (No)	Oral (No)	Oral (No)/parenteral (Partial)				

^a Reference range- adults: n=57 normal individuals (median 4.7)
 Reference range- children: mean +/- SD (range) = 2 ± 2.6 (0.55-11.3) nM
 * Values in iron deficiency anemia are 0,04-0,12 nM.

^b Reference values reported from Nathan and Oski's, Hematology of infancy and childhood, Nathan DG, Orkin SH, Ginsburg D, Look AT, VI edition.

^c WBC ranges for children aged 1-5 years

Supplementary Table 2: Time course of iron indices for A III1 proband.

Age (months)	Serum iron (µg/dL)	Transferrin saturation (%)	Serum ferritin (ng/mL)
2.5	27	8	238
3	16	4	140
8.5	10	3	154
12.5	16	6	97
14.3	13	5	59

Reference values from Nathan and Oski's, Hematology of infancy and childhood, Nathan DG, Orkin SH, Ginsburg D, Look AT, VI edition: Serum Ferritin (ng/mL): 1-6 months: male: 6-410; female: 6-340, 7-12 months: male: 6-80; female: 6-45, 1-6 years, male/female: 6-24; Serum Iron (µg/dL) :1-6 years, male/female: 22-136; Transferrin saturation (%), male/female: 7-44.

Supplementary Table 3: Reference values of healthy, term neonates.

Healthy Controls	WBC,* 10 ³ /μl	Hb, g/dl	RBC, *10 ⁶ /μl	MCV, fl	MCHC, g/dl	MCH, pg	RDW, %	PLT, 10 ³ /μl	AGE (days)	Sex
1	16230	16.9	5.2	104.8	31	32.4	16.9	450000	2	M
2	10960	18.3	5.51	108	30.7	33.2	18	302000	2	M
3	10960	18.2	5.76	104.1	30.3	31.6	16.7	110000	2	M
4	11400	18.9	5.65	106.9	31.2	33.4	17.2	286000	3	M
5	10250	19.4	5.43	116.8	30.6	35.8	16.2	292000	3	M
6	10060	16.7	4.57	116	31.5	36.6	15.9	277000	3	M
7	16950	13.9	4.08	104.9	32.4	34	14.3	327000	3	M
8	10220	18.5	5.32	107.8	32.3	34.8	15.8	329000	3	M
9	10640	14.9	4.43	104.5	32.1	33.5	15.2	317000	3	M
10	10380	20	6.16	108.1	30	32.5	17.8	246000	3	M
11	8690	17.2	4.78	112.9	31.8	35.9	15	232000	3	M
12	10270	13	4.03	99.1	32.5	32.2	16.4	295000	3	M
13	7970	16.5	4.74	114.9	30.3	34.9	16.8	67000	3	M
14	8030	14.3	3.68	124.4	31.3	30.3	12.8	289000	4	M
15	10410	19.5	5.86	111.5	29.8	33.2	16.3	202000	4	M
16	9290	17.1	5	108.5	31.5	34.1	16	209000	4	M
17	15160	17.3	5.07	109	31.3	34.2	15.6	309000	4	M
18	11380	17.7	5.18	111	30.8	34.2	15.2	129000	4	M
19	9060	18.7	5.48	113.9	30.1	34.2	16.4	255000	4	M
20	8290	16.2	4.92	110.4	29.8	32.9	15.6	365000	4	M
21	10400	18.7	5.76	101.9	31.9	32.5	14.4	239000	4	M
22	9740	17.3	4.88	109.6	32.4	35.5	16	256000	4	M
23	8520	8.7	2.95	88	33.4	29.4	14.4	318000	4	M
24	9560	18.7	5.02	114.7	32.5	37.3	15.6	224000	4	M
25	9540	18.8	5.73	109.3	30	32.8	16.7	86000	5	M
26	11870	18.3	5.47	104.9	31.8	33.4	15.3	305000	5	M
27	8060	18.4	6.34	98.2	29.5	28.9	20	275000	5	M
28	10690	14.2	4.12	107	32.1	34.3	14.2	276000	5	M
29	12160	17.2	5.16	107.4	31	33.3	15.4	372000	5	M
30	8920	18.6	4.86	110.7	34.6	38.3	15.5	272000	5	M
31	11270	18.2	4.9	110.8	33.4	37.1	15.6	418000	5	M
32	9540	19.7	5.69	110.2	31.5	34.7	16.7	313000	6	M
33	8560	14.5	4.33	105.8	31.7	33.5	16.7	92000	6	M
34	10800	11	3.04	110.9	32.5	36.1	15.9	477000	6	M
35	14250	13.7	4.06	107.4	31.5	33.8	14.5	319000	6	M
36	11850	20.9	6.37	110.7	29.6	32.8	16.4	170000	6	M
37	12700	13.5	4.11	102	32.2	32.9	14.9	543000	6	M
38	8110	16.6	4.81	105.6	32.6	34.4	15.1	305000	6	M

39	10920	17.7	5.03	112.2	31.4	35.3	16.7	240000	7	M
40	11450	17.7	5.66	95.4	32.8	31.2	14.2	428000	7	M
41	17480	15.7	4.57	106.9	32.2	34.4	14.8	473000	7	M
42	13470	19.1	5.42	110.5	31.9	35.3	16.7	189000	7	M
43	10450	14.7	4.47	101.6	32.4	32.9	15.2	385000	7	M
44	10840	16	4.94	104.1	31.2	32.4	17.2	264000	7	M
45	11770	12.8	4.13	101.3	30.5	31	16.5	572000	7	M
46	9910	19.5	5.52	113.5	31.1	35.3	16	196000	8	M
47	12910	16.6	4.9	106.9	31.7	33.9	16	352000	8	M
48	12860	16.7	5.07	105.8	31.2	33	16.7	252000	8	M
49	9390	15.8	4.38	112.1	32.3	36.2	15.2	466000	9	M
50	11180	14.7	4.18	112.5	31.3	35.3	14.5	197000	9	M
51	12090	14.9	4.26	106.7	32.8	35	15.4	412000	9	M
52	18910	17.5	5.09	110.8	31	34.4	15.8	447000	9	M
53	10390	14.5	4.24	106.2	32.1	34.1	14.5	488000	9	M
54	9590	18.5	5.26	108.8	32.3	35.2	16.3	352000	9	M
55	10640	16.2	5.08	103.5	30.8	31.8	15.4	348000	10	M
56	10600	14.5	4.24	108.2	31.6	34.2	13.9	277000	10	M
57	12210	17.1	4.99	110.7	30.9	34.2	14.6	700000	10	M
58	9110	16.2	5.04	102	31.6	32.2	15.3	454000	10	M
59	8640	15.2	4.53	104.9	32	33.6	14.2	251000	11	M
60	9560	15.9	4.54	107.4	32.5	35	15.5	353000	11	M
61	17080	15	4.56	103.6	31.7	32.9	16.1	331000	11	M
62	9510	19.3	5.67	108	31.5	34	16	387000	11	M
63	10750	16.8	5.02	105.1	32	33.6	16.4	226000	11	M
64	9690	14.7	4.48	107	30.7	32.9	15.5	357000	12	M
65	14160	16.9	5.18	103.3	31.5	32.5	16.5	294000	12	M
66	9740	17	5.21	106.7	30.6	32.7	16.9	296000	12	M
67	14880	16.4	4.69	110	31.7	34.9	15.7	369000	13	M
68	3750	17.2	4.92	109.2	32	35	15.4	360000	13	M
69	8510	14.9	4.58	101.8	31.9	32.4	16	481000	14	M
70	12600	15.5	4.68	105.5	31.4	33.1	15.5	332000	15	M
71	9900	14.1	4.13	104.2	32.8	34.2	14.9	541000	15	M
72	10760	12.3	3.57	101.2	33.9	34.3	14.9	496000	15	M
73	10710	19.6	5.97	106.3	30.8	32.8	15	419000	15	M
109	11770	16.9	5.02	109.6	30.6	33.6	15.9	310000	2	F
110	19830	20	6.1	108.5	30.2	32.7	16.7	260000	2	F
111	10280	18.5	5.59	110.8	29.9	33.1	16.7	228000	2	F
112	15250	15.1	4.56	104.1	31.8	33.1	15.9	297000	2	F
113	11090	16.8	4.81	111	31.5	34.9	15.8	368000	2	F
114	9890	17.3	4.94	113.2	30.9	35	15.7	514000	3	F
115	15760	17.9	5.38	108.3	30.7	33.2	15.7	285000	3	F
116	15860	15.1	4.15	120.7	30	36.2	15.9	337000	3	F

117	13040	18.1	5.23	110.8	31.2	34.6	19.4	298000	3	F
118	17510	16.9	5.04	109.3	30.6	33.5	15.3	416000	3	F
119	7750	14.1	4.1	108.7	31.7	34.4	14.8	352000	3	F
120	11750	18.4	5.23	110.8	31.7	35.1	15.2	256000	3	F
121	6600	16.4	4.77	110.8	31	34.4	15.2	198000	3	F
122	14200	18.1	5.6	99	32.7	32.4	16.8	244000	3	F
123	9230	17.3	4.86	113	31.4	35.5	15.9	303000	3	F
124	10000	19.9	5.67	108.4	32.4	35.1	17.2	234000	3	F
125	8850	20.2	5.7	107.8	32.8	35.4	15.6	195000	3	F
126	12410	16.4	4.72	110.2	31.5	34.7	15.3	314000	4	F
127	9400	17.6	5.07	107.8	32.1	34.6	15.6	131000	4	F
128	10340	18.4	5.72	105.6	30.4	32.1	17.1	328000	4	F
129	9900	19	5.53	110	31.3	34.4	16.2	406000	4	F
130	13480	15.7	4.28	113.9	32.1	36.6	16	292000	4	F
131	7630	13.6	3.94	106.4	32.4	34.5	14.6	314000	4	F
132	10910	16.3	4.46	107.8	33.8	36.5	14.9	384000	4	F
133	7300	16.7	4.84	107.9	34.9	34.5	15.8	316000	5	F
134	13890	18	5.15	110.1	31.8	35	16.4	372000	5	F
135	11030	16.3	5	115.7	28.1	32.5	15.5	199000	5	F
136	8280	16.4	4.77	110.9	30.9	34.3	15.7	338000	5	F
137	11760	15.9	4.78	107.2	31.1	33.4	16.2	348000	5	F
138	15190	12.1	4.37	80.7	34.3	27.7	12.1	328000	5	F
139	6330	17.4	5.64	101.6	30.4	30.9	16.4	200000	5	F
140	8930	16.3	5.35	101.9	29.9	30.5	14.9	300000	5	F
141	7510	16.8	4.63	118.7	30.6	36.3	15.1	323000	5	F
142	9140	17	5.15	105.9	31.2	33	15.8	290000	5	F
143	16570	16.4	4.83	107.9	31.4	33.9	15.2	335000	5	F
144	12020	19.2	5.8	111.9	29.6	33.1	16.4	311000	5	F
145	8650	17	5.02	110.5	30.7	33.9	15.8	260000	5	F
146	12250	17.8	5.1	111.3	31.4	35	16.3	335000	5	F
147	14930	16.7	5.06	103.9	31.7	32.9	15.5	344000	6	F
148	9320	16.6	4.83	107.9	31.9	34.5	16.1	203000	6	F
149	9160	15.9	4.41	114.2	31.6	36.1	16.4	407000	6	F
150	9550	18.1	5.38	110.3	30.5	33.6	14.5	281000	6	F
151	12150	17.8	5.48	105	30.9	32.5	15.9	270000	6	F
152	10260	14	4.32	102.4	31.8	32.6	14.2	373000	6	F
153	12260	15.1	4.43	108.3	31.4	34	16.5	260000	6	F
154	11930	18	5.14	109.2	32	35	15.5	212000	6	F
155	9380	18.4	5.77	109.3	29.1	31.8	17.1	327000	7	F
156	11730	19.7	5.63	102.6	35	34.1	16.2	489000	7	F
157	8470	16	4.6	111.4	31.3	34.8	15.4	261000	7	F
158	17220	20.3	6.19	102.8	31.9	32.7	15.3	148000	7	F
159	11950	15.5	4.32	111.6	32.2	35.9	14.1	450000	7	F

160	10450	16.3	4.65	110.2	31.8	35.1	15.6	273000	7	F
161	11970	16.4	4.53	119	30.5	36.2	15.1	197000	7	F
162	5730	18.4	5.3	103.6	33.5	34.7	15.4	272000	7	F
163	15750	17.2	5.18	103.7	32	33.2	14.3	370000	7	F
164	9270	18.2	5.17	109.4	32.3	35.3	16.5	317000	7	F
165	7670	11.8	3.33	108.7	32.7	35.5	14.6	586000	7	F
166	8980	15.9	4.59	109.7	31.6	34.7	15.2	450000	8	F
167	8950	16.3	4.98	105.1	31.2	32.8	15.7	459000	8	F
168	12900	16.5	4.57	111.3	32.4	36	16.8	443000	8	F
169	15600	19.5	5.7	106.7	32.1	34.3	15.5	425000	8	F
170	10990	14.1	4.22	110.3	30.2	33.3	16.3	480000	9	F
171	14530	16.3	4.76	109	31.5	34.3	14.6	471000	9	F
172	10720	20	6.09	105.2	31.2	32.8	14.8	439000	10	F
173	10510	17.1	5.21	105.9	31	32.9	16.1	444000	10	F
174	10160	12.8	3.87	105.2	31.5	33.2	14.5	459000	10	F
175	8940	17.9	5.23	107.9	31.8	34.3	15.9	482000	10	F
176	10130	15.8	4.79	99.4	33.2	33	16.2	427000	10	F
177	9890	11.7	3.47	103.6	32.6	33.8	14.5	553000	11	F
179	16910	14.3	4.51	100.3	31.6	31.7	14.9	701000	12	F
180	13250	13.7	3.67	112.3	33.2	37.3	16.4	350000	12	F
181	10400	12.4	3.54	109.8	31.9	35	13.8	575000	12	F
182	12150	17.1	5.23	102.8	31.7	32.6	14.4	378000	12	F
183	10080	15.6	4.43	109.8	32.2	35.3	14.5	318000	12	F
184	8470	18.8	5.42	109.6	31.6	34.6	16.6	325000	12	F
185	9300	15.7	4.27	114.7	32	36.8	19.3	549000	13	F
186	12880	15	4.49	106.2	31.5	33.5	15	266000	13	F
187	11560	16.3	5.04	104.9	30.7	32.3	16	440000	13	F
188	10650	15	4.54	103.5	32	33.1	15.5	771000	14	F
189	13880	15.1	4.73	100.9	31.7	32	16.9	594000	14	F
190	8380	15.9	4.76	107.5	31.1	33.4	15.3	226000	14	F
191	13340	15.8	4.33	113.7	32.1	36.5	15.4	480000	14	F
192	11350	16.9	5.11	103.3	32	33.1	14.8	580000	14	F
193	9920	17.8	5.36	103.1	32.3	33.2	16.2	308000	14	F
194	8970	15	4.55	102.7	32.1	32.9	15.9	504000	14	F
195	9840	15.2	4.57	109.6	30.4	33.3	15.7	373000	15	F
196	8210	15.7	5.06	101	30.8	31.1	15.6	278000	15	F
197	9290	9.9	3.38	104.6	28	29.3	15.1	379000	15	F
198	13540	18.2	5.35	106.8	31.9	34	15.3	404000	15	F
199	13170	19.2	5.81	108.7	30.4	33	15.3	424000	15	F
200	9750	16.1	4.82	107.8	31	33.4	15.1	344000	15	F
201	9570	15.6	4.47	106.8	32.7	31.5	15.2	463000	15	F
202	9910	14.2	4.07	102.9	34	34.9	14	633000	15	F
203	11380	14.5	4.39	104.7	31.5	33	14.5	608000	15	F

204	9080	14.3	4.68	93.2	32.8	30.6	13.7	514000	15	F
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Supplementary Figures Legend

Figure S1: Multiple amino acid sequence alignment of MT-2 protein in 24 species. New missense mutation reported in this work is marked with a vertical arrow. Uniprot accession number and entry name are reported for each sequence. Below the alignment, a star indicates that the amino acid at this position is identical for all the species, semicolons and dots indicate amino acids with similar but not identical properties. Species correspond as following (common name is reported): TMPS6_HUMAN= Human, G3SKP5_GORGO= Lowland gorilla, F7HLZ8_MACMU= Rhesus macaque, H2P4A0_PONAB= Pongopygmaeusabelii, F7IFY5_CALJA= White-tufted-ear marmoset, G1RXE8_NOMLE= Northern white-cheeked gibbon, H0WT78_OTOGA= Small-eared galago, F6ZMU8_HORSE= Horse, F1PGA1_CANFA= Dog, TMPS6_MOUSE=Mouse, D3ZF49_RAT = Rat, I3NFA4_SPETR= Thirteen-lined ground squirrel, M3W9I6_FELCA=Cat, M3YMK0_MUSPF= European domestic ferret, F6WLV0_MONDO=Gray short-tailed opossum, G3WMX0_SARHA=Tasmanian devil, F7FHE7_ORNAN= Duckbill platypus, F1NDU7_CHICK=Chicken, G1NJ34_MELGA=Common turkey, H0ZK80_TAEGU=Zebra finch, K7FBU9_PELSI=Chinese softshell turtle, F6X0X4_XENTR=Western clawed frog, H3DNB6_TETNG=Spotted green pufferfish, M4A0S3_XIPMA=Southern platyfish.

Figure S1

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647 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GPI SNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 802 Q81U80 TMSF6 HUMAN
648 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGGRALAAAG--GSHFPCPRPTGPI SNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 813 G3SEFP5 G3SKP5 GORGO
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGRKRPAPFAMYQQRHRRGSGCLGPTSNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 813 F7HL28 F7HL28 MACMU
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGRKRPAPFAMYQQRHRRGSGCLGPTSNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 704 E2F4A0 E2F4A0 FOMAB
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGRKRPAPFAMYQQRHRRGSGCLGPTSNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 605 F71FY5 F71FY5 CALJA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGRKRPAPFAMYQQRHRRGSGCLGPTSNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 812 G1RKE8 G1RKE8 NOME
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGGRKRPAPFAMYQQRHRRGSGCLGPTSNALQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 813 H0WT78 H0WT78 OTYGA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGG-----FTSNGLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 793 F6Z908 F6Z908 HOMSE
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREGG-----FTSNGLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 800 F1PGA1 F1PGA1 CANFA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 802 Q9DB10 TMSF6 MOUSE
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 772 D3E4F9 D3E4F9 RAT
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 799 I3NF44 I3NF44 SPETR
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 805 M3W916 M3W916 FELCA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 796 M3Y980 M3Y980 MURFP
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 816 F6W1V0 F6W1V0 MONDO
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 801 G3W950 G3W950 SARHA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 820 F7FHE7 F7FHE7 ORNAN
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 778 F1NDU7 F1NDU7 CHICK
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 693 G1N374 G1N374 MELGA
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 747 H0ZK80 H0ZK80 TAEGD
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 781 K7EB09 K7EB09 PELS1
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 769 F6X0X4 F6X0X4 XENTR
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 751 H31986 H31986 TETRE
649 VSFVSRLLHFPYEEESHHDYDALLQLDHPV--VRSAAVRVCLPARSHFFEPGLHCWITGWGALREG-----GVSNTLQKVVDQLI PQDLCEAYRYQVT PMLCAGYRKGKDDACQDSGGPLVCKALSGRWFAGLVSWGLGCGRPNFYGVYTRITGV 744 M4A053 M4A053 XIPMA
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