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Supplemental Data

Prodynorphin Mutations Cause the Neurodegenerative Disorder Spinocerebellar Ataxia Type 23

**Georgy Bakalkin, Hiroyuki Watanabe, Justyna Jezierska, Cloë Depoorter,
Corien Verschuuren-Bemelmans, Igor Bazov, Konstantin A. Artemenko,
Tatjana Yakovleva, Dennis Dooijes, Bart P.C. Van de Warrenburg, Roman A. Zubarev,
Berry Kremer, Pamela E. Knapp, Kurt F. Hauser, Cisca Wijmenga, Fred Nyberg,
Richard J. Sinke, and Dineke S. Verbeek**

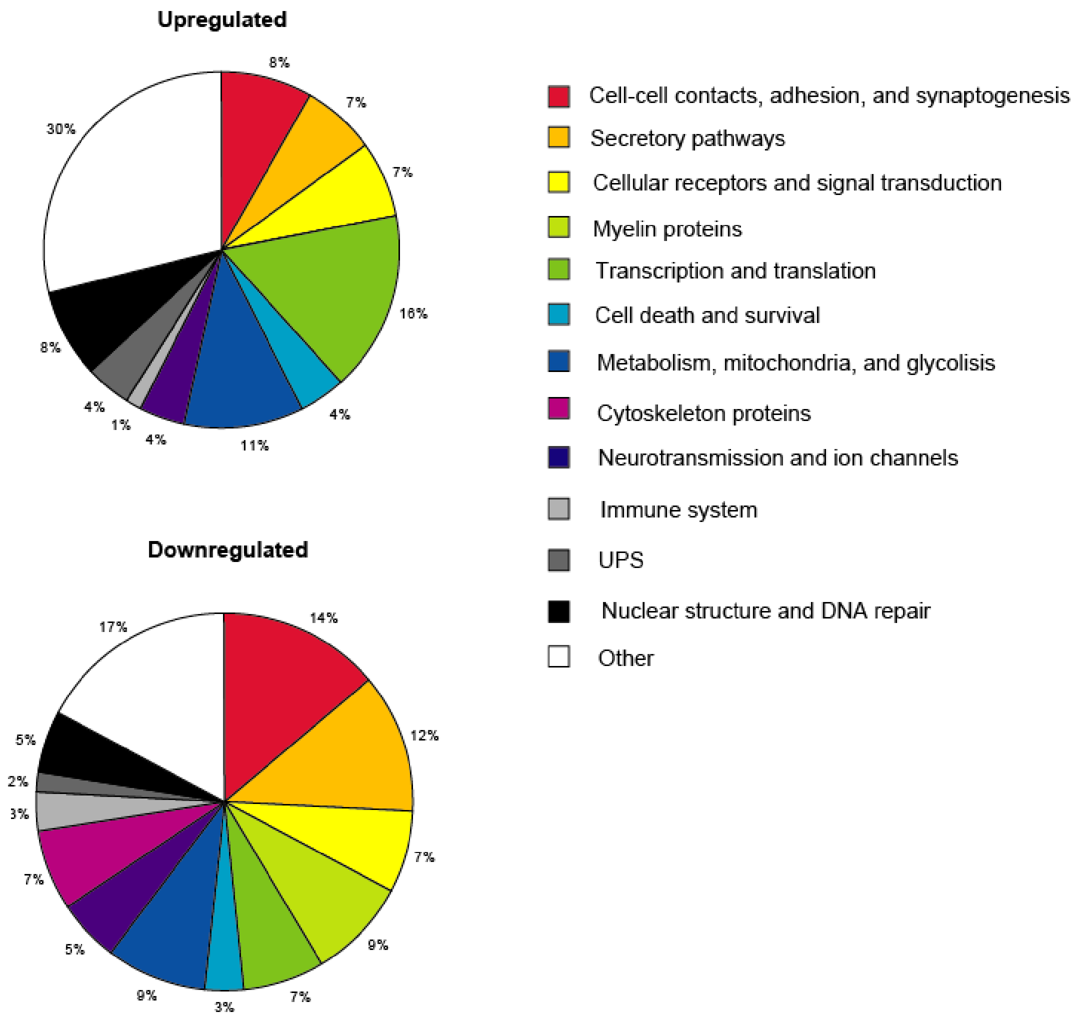

Figure S1. Functional Categorization of Proteins that Are Up- or Downregulated in SCA23 Compared to Control Cerebellum (Shown in Table S4)

LS-MS/MS analysis shows that proteins of several groups including those involved in cell-cell contacts, adhesion and synaptogenesis, secretory pathways (proteins of the endoplasmatic reticulum, Golgi, exocytotic machinery, secretory and plasma membrane proteins), and cellular receptors and signal transduction proteins are dysregulated (up- and downregulated) in the SCA23 cerebellum compared to control cerebella. Myelin and immune proteins and those involved in neurotransmission, including ion channels and transporters, are downregulated, while the UPS proteins are predominantly upregulated in SCA subject. Protein categorization is based on web-resources www.uniprot.org and www.biobase-international.com.

Table S1. Clinical Characteristics of the SCA23 Individuals with *PDYN* Mutations

Mutation	Age of Onset (Years)	Gait Ataxia	Limb Ataxia	Dysarthria	Oculomoter Impairment	Other
p.L211S	73	+	+	+/-	+	Parkinson-like features: polyneuropathy and pyramidal syndrome in the legs
p.R212W	54	+	+	+	-	Tremor of the head and upper and lower extremities
p.R215C Patient 1	54 Clumsy as a child	?	+	+	+	Progressive postural tremor, slowly progressive frontoparietal white matter abnormalities on MRI.
p.R215C Patient 2	42 Clumsy as a child	+	+	+	?	Tremor of the head and arms. Discrete peri - ventricular white matter lesions.

These mutations were found in four patients from a large panel of ataxia subjects (n=1100) who did not have mutations in the known SCA genes that currently are screened in diagnostic setting (SCA1-3, 6, 7, 12, 14, and 17). absent; +/- subtle; + present or mild

Table S2. Predicted Protein Conformational Changes Due to PDYN Missense Mutations Using the PolyPhen, Sift, SNP and Align GVGD Programs

		PolyPhen		SIFT		SNAP		Align GVGD	
Status	**Protein Change**	**Prediction**	**Score[a]**	**Prediction**	**Score[b]**	**Prediction**	**Expected Accuracy[c]**	**Prediction**	**C-Score[d]**
Affected	p.R138S	benign	1.362	Tolerated	0.07	Neutral	89%	Less likely	C15
Affected	p.L211S	possibly damaging	1.671	Affect protein function	0.00	Non-neutral	63%	Most likely	C65
Affected	p.R212W	probably damaging	3.143	Affect protein function	0.00	Non-neutral	82%	Most likely	C65
Affected	p.R215C	probably damaging	3.143	Affect protein function	0.00	Non-neutral	78%	Most likely	C65

[a] The lower the score, the more benign the substitution, [b] The higher the SIFT score, the more tolerated the substitution, [c] The higher the percentage of accuracy, the greater the reliability for prediction, [d] The lower the C-score, the less likely the substitution is deleterious.

Table S3. Phosphorylation Prediction Scores Corresponding to Identified Sequence Variants

Status	Protein Change	Putative Effects	Putative Phosophorylation Site	Context	Wild Type Score	Mutant Score
Affected	p.R138S	possible increased phosphorylation	S134 S138 S143	LRGL**S**DGFS SDGF***S***EGAE EGAE**S**ELMR	0.955 0 0.906	0.989 **0.546** 0.788
Affected	p.L211S	probable increased phosphorylation	S211	YGGF**S**RRIR	0	**0.729**

Phosphorylation scores range from 0.0 (minimal probability) to 1.0 (high probability). Substitutions scores for high probability phosphorylation sites (PDYN p.R138S and p.L211S) are shown in bold.

Table S4. List of Proteins that Are Up- or Downregulated in the SCA23 Cerebellum

Protein ID		Normalisation to the sum of quantified peptide intensities [1]			External normalization to total protein amount [2]		
		Median Controls	Median SCA23	$\log_{10}$ (median samples/median controls)	Median Controls	Median SCA23	$\log_{10}$ (median samples/median controls)
	PROTEINS UP-REGULATED IN SCA23 SUBJECT						
IPI00816801	Isoform 2 of DNA2-like helicase	2.26E+05	4.71E+06	1.32	2.16E+05	4.38E+06	**1.31**
IPI00656087	Isoform 1 of Tyrosine-protein phosphatase non-receptor type substrate 1 precursor	4.50E+05	7.90E+06	1.24	4.36E+05	6.35E+06	**1.16**
IPI00292496	Beta-tubulin 4Q	6.30E+06	9.76E+07	1.19	5.28E+06	1.07E+08	**1.31**
IPI00643452	Protein	8.29E+04	1.28E+06	1.19	8.23E+04	1.13E+06	**1.14**
IPI00005198	Interleukin enhancer-binding factor 2	5.54E+04	7.10E+05	1.11	4.35E+04	8.02E+05	**1.27**
IPI00018233	SAM pointed domain-containing Ets transcription factor	9.91E+05	1.22E+07	1.09	9.68E+05	1.54E+07	**1.20**
IPI00456149	Isoform 1 of Polypeptide N-acetylgalactosaminyltransferase 12	8.14E+07	9.71E+08	1.08	7.86E+07	1.00E+09	**1.11**
IPI00152625	Isoform 1 of OTU domain-containing protein 7A	4.61E+07	4.75E+08	1.01	4.55E+07	4.39E+08	**0.98**
IPI00030058	Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-4 subunit precursor	1.24E+06	1.25E+07	1.00	1.15E+06	1.34E+07	**1.06**
IPI00658128	Similar to stromal interaction molecule 2	5.74E+07	5.66E+08	0.99	4.59E+07	6.00E+08	**1.12**
IPI00011822	CDNA FLJ43077 fis. clone BRTHA3015910	5.74E+07	5.66E+08	0.99	4.59E+07	6.01E+08	**1.12**
IPI00788073	similar to Huntingtin-interacting protein 1-related protein	1.81E+04	1.69E+05	0.97	1.12E+04	2.33E+05	**1.32**
IPI00411412	similar to autism susceptibility candidate 2	5.92E+05	5.35E+06	0.96	4.84E+05	6.76E+06	**1.15**
IPI00003843	Isoform A1 of Tight junction protein ZO-2	8.24E+05	6.96E+06	0.93	7.73E+05	6.82E+06	**0.95**
IPI00786919	similar to Trichohyalin	1.80E+06	1.50E+07	0.92	1.56E+06	1.81E+07	**1.06**
IPI00183794	zinc finger protein 265 isoform 2	2.88E+04	2.26E+05	0.89	2.70E+04	2.18E+05	**0.91**
IPI00418240	LIM-nebulette	1.81E+06	1.26E+07	0.84	1.49E+06	1.45E+07	**0.99**
IPI00022799	Isoform 2 of Aquaporin-4	1.26E+07	8.35E+07	0.82	1.13E+07	9.30E+07	**0.92**
IPI00787414	hypothetical protein LOC284069	6.11E+05	3.95E+06	0.81	5.32E+05	4.27E+06	**0.90**
IPI00297859	Isoform 1 of Myeloid/lymphoid or mixed-lineage leukemia protein 2	1.68E+07	1.05E+08	0.80	1.53E+07	1.28E+08	**0.92**
IPI00014253	Ribosome biogenesis regulatory protein homolog	6.34E+05	3.95E+06	0.79	5.21E+05	3.44E+06	**0.82**
IPI00218848	ATP synthase. H+ transporting. mitochondrial F0 complex. subunit e	1.86E+06	1.16E+07	0.79	1.80E+06	1.09E+07	**0.78**
IPI00013788	HIV Tat-specific factor 1	1.14E+05	6.70E+05	0.77	9.61E+04	7.58E+05	**0.90**
IPI00008223	UV excision repair protein RAD23 homolog B	1.04E+06	6.04E+06	0.76	9.36E+05	7.10E+06	**0.88**
IPI00218831	Glutathione S-transferase Mu 1	7.12E+06	4.05E+07	0.75	5.51E+06	4.64E+07	**0.93**
IPI00784338	Similar to APOA4 protein	4.55E+05	2.58E+06	0.75	4.16E+05	2.44E+06	**0.77**
IPI00008575	Isoform 1 of KH domain-containing. RNA-binding. signal transduction-associated protein	4.73E+05	2.59E+06	0.74	4.74E+05	3.54E+06	**0.87**
IPI00435935	PP11647	1.53E+06	7.98E+06	0.72	1.27E+06	1.06E+07	**0.92**
IPI00006854	Ras GTPase-activating protein nGAP	9.02E+04	4.59E+05	0.71	7.19E+04	5.78E+05	**0.90**
IPI00295469	Copine-6	4.28E+05	2.16E+06	0.70	4.27E+05	2.48E+06	**0.76**
IPI00219622	Proteasome subunit alpha type 2	1.48E+05	7.42E+05	0.70	1.47E+05	9.07E+05	**0.79**
IPI00292657	NADP-dependent leukotriene B4 12-hydroxydehydrogenase	4.79E+05	2.35E+06	0.69	4.53E+05	2.29E+06	**0.70**
IPI00746388	Ezrin	1.35E+08	6.50E+08	0.68	1.30E+08	7.64E+08	**0.77**
IPI00217914	Uncharacterized protein C6orf182	4.16E+06	1.99E+07	0.68	4.03E+06	2.44E+07	**0.78**
IPI00157414	Ectonucleotide pyrophosphatase/phosphodiesterase 6 precursor	4.36E+04	2.07E+05	0.68	2.71E+04	2.79E+05	**1.01**
IPI00011307	Bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase. mitochondrial prec	3.10E+05	1.46E+06	0.67	2.43E+05	1.40E+06	**0.76**
IPI00430411	Calcium/calmodulin-dependent protein kinase type IV	2.26E+06	9.90E+06	0.64	2.24E+06	7.98E+06	**0.55**
IPI00329488	Isoform IB of Tyrosine-protein kinase ABL2	1.96E+06	8.42E+06	0.63	1.69E+06	9.54E+06	**0.75**
IPI00004502	CDNA FLJ20330 fis. clone HEP10296	8.02E+06	3.39E+07	0.63	7.16E+06	2.64E+07	**0.57**
IPI00737307	similar to paraneoplastic antigen MA3	9.49E+06	3.92E+07	0.62	9.40E+06	4.12E+07	**0.64**
IPI00413641	Aldose reductase	1.41E+07	5.80E+07	0.61	1.40E+07	6.08E+07	**0.64**
IPI00219067	Glutathione S-transferase Mu 2	2.22E+07	9.09E+07	0.61	1.88E+07	1.04E+08	**0.74**
IPI00025512	Heat-shock protein beta-1	2.43E+07	9.90E+07	0.61	2.16E+07	1.07E+08	**0.69**
IPI00003799	Isoform 2 of Heme-binding protein 2	3.59E+05	1.46E+06	0.61	3.06E+05	1.79E+06	**0.77**
IPI00009030	Isoform LAMP-2A of Lysosome-associated membrane glycoprotein 2 precursor	6.91E+06	2.81E+07	0.61	6.01E+06	2.56E+07	**0.63**
IPI00031070	SCRT1 protein	1.72E+06	6.95E+06	0.61	1.67E+06	7.08E+06	**0.63**
IPI00646304	peptidylprolyl isomerase B precursor	1.43E+08	5.70E+08	0.60	1.38E+08	6.38E+08	**0.66**
IPI00012303	Selenium-binding protein 1	8.18E+05	3.23E+06	0.60	7.93E+05	3.60E+06	**0.66**
IPI00816217	Isoform 2 of Structural maintenance of chromosomes protein 6	2.57E+05	9.87E+05	0.58	1.98E+05	1.09E+06	**0.74**
IPI00024993	Enoyl-CoA hydratase. mitochondrial precursor	7.14E+06	2.73E+07	0.58	6.30E+06	3.17E+07	**0.70**

IPI00642432	Glutathione S-transferase M5	1.49E+06	5.69E+06	0.58	1.21E+06	6.56E+06	**0.73**
IPI00796704	11 kDa protein	1.84E+07	7.00E+07	0.58	1.85E+07	7.08E+07	**0.58**
IPI00441187	MGAT5B protein	1.47E+06	5.50E+06	0.57	1.30E+06	5.99E+06	**0.66**
IPI00097257	Uncharacterized protein C20orf152	3.84E+05	1.41E+06	0.57	3.20E+05	1.64E+06	**0.71**
IPI00013174	Isoform 1 of RNA-binding protein 14	4.15E+05	1.51E+06	0.56	3.69E+05	1.66E+06	**0.65**
IPI00012382	U1 small nuclear ribonucleoprotein A	3.38E+06	1.22E+07	0.56	2.61E+06	1.44E+07	**0.74**
IPI00062599	Isoform 1 of SWI/SNF-related matrix-associated actin-dependent regulator of chromatin	1.83E+06	6.62E+06	0.56	1.54E+06	7.66E+06	**0.70**
IPI00003377	Isoform 1 of Splicing factor. arginine/serine-rich 7	1.15E+07	4.06E+07	0.55	1.15E+07	3.71E+07	**0.51**
IPI00396243	Isoform 1 of WD repeat protein 19	1.31E+06	4.54E+06	0.54	1.20E+06	4.50E+06	**0.57**
IPI00031411	Cadherin-related tumor suppressor homolog precursor	1.65E+05	5.62E+05	0.53	1.27E+05	4.34E+05	**0.53**
IPI00783082	Isoform 1 of tRNA isopentenyltransferase. mitochondrial precursor	5.85E+06	1.98E+07	0.53	5.15E+06	1.79E+07	**0.54**
IPI00386444	Isoform 1 of Nesprin-1	1.75E+07	5.89E+07	0.53	1.66E+07	6.54E+07	**0.60**
IPI00102118	MLX interacting protein	2.06E+05	6.91E+05	0.53	1.85E+05	7.59E+05	**0.61**
IPI00215918	ADP-ribosylation factor 4	5.19E+07	1.72E+08	0.52	5.00E+07	1.83E+08	**0.56**
IPI00411743	CCDC15 protein (Fragment)	6.73E+06	2.23E+07	0.52	6.48E+06	1.98E+07	**0.48**
IPI00290553	10-formyltetrahydrofolate dehydrogenase	4.13E+07	1.36E+08	0.52	3.68E+07	1.60E+08	**0.64**
IPI00783455	G2	1.33E+07	4.30E+07	0.51	1.17E+07	4.29E+07	**0.56**
IPI00782981	Isoform 1 of ATP-binding cassette sub-family A member 12	3.51E+06	1.13E+07	0.51	3.04E+06	1.09E+07	**0.56**
IPI00015351	AD039	3.82E+06	1.21E+07	0.50	3.10E+06	1.61E+07	**0.72**
IPI00293655	ATP-dependent RNA helicase DDX1	2.48E+06	7.83E+06	0.50	2.06E+06	6.49E+06	**0.50**
IPI00787639	similar to centaurin. gamma-like family. member 1 isoform 1	1.31E+07	4.09E+07	0.50	1.02E+07	4.76E+07	**0.67**
IPI00009104	RuvB-like 2	3.70E+06	1.15E+07	0.49	3.19E+06	1.05E+07	**0.52**
IPI00220301	Peroxiredoxin-6	2.74E+08	8.34E+08	0.48	2.28E+08	9.03E+08	**0.60**
IPI00022429	Alpha-1-acid glycoprotein 1 precursor	2.33E+06	7.04E+06	0.48	2.07E+06	8.47E+06	**0.61**
	PROTEINS DOWN REGULATED IN SCA23 SUBJECT						
IPI00023780	**Isoform 2 of DnaJ homolog subfamily C member 5**	7.29E+06	2.43E+06	-0.48	6.71E+06	1.72E+06	**-0.59**
IPI00760924	**Isoform 2 of Rho GTPase-activating protein 18**	5.53E+06	1.81E+06	-0.49	5.39E+06	1.58E+06	**-0.53**
IPI00016837	**GRID2 protein**	1.73E+07	5.30E+06	-0.51	1.61E+07	3.57E+06	**-0.65**
IPI00743456	**G protein-coupled receptor 124**	6.47E+07	1.97E+07	-0.52	6.10E+07	1.72E+07	**-0.55**
IPI00383598	**Cytochrome C oxidase subunit VIa homolog**	5.46E+06	1.57E+06	-0.54	5.46E+06	1.65E+06	**-0.52**
IPI00402145	**similar to CG10671-like**	1.03E+07	2.97E+06	-0.54	9.21E+06	2.30E+06	**-0.60**
IPI00018768	**Translin**	4.29E+06	1.22E+06	-0.55	4.03E+06	1.35E+06	**-0.48**
IPI00063903	**Up-regulated during skeletal muscle growth protein 5**	2.72E+07	7.70E+06	-0.55	2.47E+07	7.38E+06	**-0.52**
IPI00718942	**dipeptidyl-peptidase 6 isoform 2**	8.18E+06	2.31E+06	-0.55	7.93E+06	2.14E+06	**-0.57**
IPI00000041	**Rho-related GTP-binding protein RhoB precursor**	1.96E+07	5.23E+06	-0.57	1.89E+07	4.42E+06	**-0.63**
IPI00220993	**Isoform CNPI of 2'.3'-cyclic-nucleotide 3'-phosphodiesterase**	7.85E+08	2.08E+08	-0.58	6.93E+08	1.77E+08	**-0.59**
IPI00386258	**Isoform 1 of Mitochondrial carrier homolog 1**	2.94E+06	7.59E+05	-0.59	2.40E+06	7.35E+05	**-0.51**
IPI00473011	**Hemoglobin subunit delta**	7.82E+07	2.00E+07	-0.59	7.80E+07	1.83E+07	**-0.63**
IPI00478296	**OTTHUMP00000031447**	2.09E+06	5.27E+05	-0.60	1.79E+06	5.35E+05	**-0.52**
IPI00009688	**Phosphatidylinositol-4-phosphate 5-kinase type-2 alpha**	1.67E+06	4.06E+05	-0.61	1.29E+06	4.21E+05	**-0.48**
IPI00028509	**Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-7 subunit precursor**	5.10E+07	1.22E+07	-0.62	4.39E+07	1.02E+07	**-0.63**
IPI00169259	**Small VCP/p97-interacting protein**	1.07E+07	2.51E+06	-0.63	9.44E+06	2.80E+06	**-0.53**
IPI00647009	**OTTHUMP00000017655**	9.44E+07	2.21E+07	-0.63	9.39E+07	1.81E+07	**-0.72**
IPI00478968	**Sec23 homolog B**	2.80E+07	6.48E+06	-0.64	2.46E+07	6.58E+06	**-0.57**
IPI00031697	**Transmembrane protein 109 precursor**	7.01E+06	1.60E+06	-0.64	5.84E+06	1.66E+06	**-0.55**
IPI00026846	**Sodium- and chloride-dependent GABA transporter 1**	3.49E+06	7.49E+05	-0.67	2.85E+06	7.88E+05	**-0.56**
IPI00239406	**Isoform 2 of Nesprin-2**	1.82E+07	3.90E+06	-0.67	1.61E+07	3.07E+06	**-0.72**
IPI00025142	**immunoglobulin superfamily. member 6**	2.53E+07	5.25E+06	-0.68	2.34E+07	6.70E+06	**-0.54**
IPI00000581	**Hypothetical protein DKFZp564E242**	9.30E+06	1.87E+06	-0.70	8.16E+06	1.85E+06	**-0.64**
IPI00215997	**CD9 antigen**	6.11E+07	1.23E+07	-0.70	6.11E+07	1.00E+07	**-0.79**
IPI00017510	**Cytochrome c oxidase subunit 2**	1.82E+07	3.54E+06	-0.71	1.28E+07	3.00E+06	**-0.63**
IPI00394876	**basigin isoform 3**	3.69E+08	6.90E+07	-0.73	3.56E+08	7.21E+07	**-0.69**
IPI00787193	**similar to Golgin subfamily A member 2**	4.33E+05	7.74E+04	-0.75	4.31E+05	8.65E+04	**-0.70**
IPI00291607	**Inositol 1.4.5-trisphosphate receptor type 3**	2.89E+06	5.16E+05	-0.75	2.46E+06	3.96E+05	**-0.79**
IPI00173461	**Junctophilin-1**	1.00E+06	1.73E+05	-0.76	9.63E+05	1.12E+05	**-0.93**
IPI00305865	**LOC348174 protein (Fragment)**	7.09E+06	1.21E+06	-0.77	5.49E+06	1.35E+06	**-0.61**
IPI00444262	**CDNA FLJ45706 fis. clone FEBRA2028457. highly similar to Nucleolin**	9.32E+08	1.59E+08	-0.77	8.35E+08	1.68E+08	**-0.70**
IPI00187000	**CDNA FLJ38262 fis. clone FCBBF3001594. moderately similar to Chlamydomonas**	7.99E+06	1.35E+06	-0.77	6.97E+06	1.50E+06	**-0.67**

	reinhardtii						
IPI00026237	**Myelin-associated glycoprotein precursor**	5.34E+06	8.60E+05	-0.79	5.31E+06	9.63E+05	**-0.74**
IPI00018934	**Isoform 1 of Sodium channel protein type 1 subunit alpha**	6.87E+04	1.06E+04	-0.81	6.04E+04	5.65E+03	**-1.03**
IPI00000949	**Mu-crystallin homolog**	8.71E+08	1.29E+08	-0.83	8.06E+08	1.49E+08	**-0.73**
IPI00014068	**Isoform 1 of Serine/threonine-protein kinase PAK 4**	3.41E+06	4.89E+05	-0.84	3.42E+06	6.59E+05	**-0.71**
IPI00010766	**Excitatory amino acid transporter 4 (EAAT4)**	7.14E+07	8.64E+06	-0.92	6.06E+07	1.16E+07	**-0.72**
IPI00021841	**Apolipoprotein A-I precursor**	5.42E+07	6.16E+06	-0.94	4.84E+07	5.58E+06	**-0.94**
IPI00550206	**Zinc finger protein 576**	8.03E+06	8.69E+05	-0.97	6.07E+06	9.18E+05	**-0.82**
IPI00304809	**hypothetical protein LOC23281 isoform a**	5.53E+05	5.94E+04	-0.97	5.33E+05	5.86E+04	**-0.96**
IPI00007812	**Vacuolar ATP synthase subunit B. brain isoform**	7.28E+08	7.80E+07	-0.97	6.17E+08	7.90E+07	**-0.89**
IPI00179109	**Isoform 1 of NAD-dependent deacetylase sirtuin-2**	2.55E+07	2.66E+06	-0.98	2.15E+07	3.02E+06	**-0.85**
IPI00032485	**Plasmolipin**	1.03E+06	1.02E+05	-1.00	1.03E+06	7.15E+04	**-1.16**
IPI00657720	**Protein**	3.68E+06	3.39E+05	-1.04	2.87E+06	4.33E+05	**-0.82**
IPI00000779	**Isoform 1 of ADAM 22 precursor**	5.14E+06	4.27E+05	-1.08	4.53E+06	2.67E+05	**-1.23**
IPI00790000	**54 kDa protein**	6.64E+05	4.39E+04	-1.18	5.26E+05	5.11E+04	**-1.01**
IPI00017530	**Ficolin-2 precursor**	1.87E+06	1.20E+05	-1.19	1.47E+06	1.71E+05	**-0.93**
IPI00410163	**Isoform 1 of WD repeat protein 78**	6.01E+06	3.55E+05	-1.23	5.18E+06	3.13E+05	**-1.22**
IPI00402501	**CDNA FLJ32731 fis. clone TESTI2001134**	4.12E+06	2.22E+05	-1.27	4.11E+06	2.50E+05	**-1.22**
IPI00008896	**Palmitoyltransferase ZDHHC2**	6.71E+06	3.17E+05	-1.33	5.87E+06	3.63E+05	**-1.21**
IPI00021907	**Isoform 1 of Myelin basic protein**	1.02E+09	4.26E+07	-1.38	8.96E+08	5.34E+07	**-1.23**
IPI00556079	**Isoform 1 of Myelin-oligodendrocyte glycoprotein precursor**	1.15E+08	4.35E+06	-1.42	1.03E+08	3.26E+06	**-1.50**
IPI00386849	**KIAA1812 protein (Fragment)**	6.36E+05	2.18E+04	-1.46	5.83E+05	2.01E+04	**-1.46**
IPI00746412	**splicing factor. arginine/serine-rich 2. interacting protein**	5.15E+06	1.58E+05	-1.51	4.38E+06	1.78E+05	**-1.39**
IPI00099834	**Ubinuclein**	2.50E+06	5.67E+04	-1.64	2.39E+06	5.60E+04	**-1.63**
IPI00183002	**Isoform 1 of Protein phosphatase 1 regulatory subunit 12A**	1.21E+05	2.01E+03	-1.78	1.21E+05	2.54E+03	**-1.68**
IPI00219661	**proteolipid protein 1 isoform 1**	3.43E+09	4.88E+07	-1.85	2.85E+09	4.82E+07	**-1.77**
IPI00026337	**Isoform 1 of Ran-binding protein 3**	1.14E+09	6.39E+06	-2.25	9.62E+08	7.67E+06	**-2.10**

Two aliquots of SCA23 sample were processed and each analyzed by LC-MS/MS with three technical replicates; two aliquots of each of three control samples were processed and each analyzed by LC-MS/MS. Protein amounts were normalized to (1) the sum of quantified protein intensities, and to (2) the total protein load. Averaging of protein intensities was done by calculating median values for 6 replicates of SCA23 and controls. A protein was considered to be up- or downregulated if its intensity differed between SCA23 and controls more than 3-fold ([ABS ($\log_{10}$ (ratio of median of affected sample to median of controls)] > 0.47).

Table S5. Key Protein Signaling Molecules (Key-Nodes) Affected in SCA23 Cerebellum

Key Node	***Average Score, SCA23***	***Average Score, Controls***	***Delta Score***	***Pathway / Function***	***Ref***
Aspartate transaminase	37709.14	26108.94	11600.20	Key enzyme in glutamate cycling in brain	1
Pyruvate dehydrogenase (acetyl-transferring)	43482.71	31966.66	11516.05	Enzyme defects may cause neurodegenerative disorder with lesions in brain areas including cerebellum	2
Phosphopyruvate hydratase /enolase	37797.45	28086.55	9710.90	Has neurotrophic and neuro-protective properties; levels increased in cerebral trauma	3
YAP	25532.72	17317.41	8215.31	65 kDa Yes-associated protein 1, a transcriptional activator; acts in apoptosis, regulation of cell proliferation and migration	4
PAK3	3073.33	10026.54	-6953.21	Serine/threonine-protein kinase; defects in PAC3 cause of mental retardation	5
Aldolases EC 4.1.2.13	30554.61	39642.10	-9087.49	Strongly expressed in a subset of cerebellar Purkinje cells	6
PRK1	2970.80	12668.41	-9697.61	Serine/threonine-protein kinase N1	7
KIS	5802.18	16776.34	-10974.16	Serine/threonine-protein kinase Kist	8
ATPase alpha	21341.43	45947.15	-24605.72	Catalyzes ATP hydrolysis coupled with the exchange of sodium and potassium ions across the plasma membrane	9

A total of 419 key-node molecules were presented in ExPlain™ 2.4.1 output list (corresponding to 1-21 distance setting). The average score for each key-node molecule in the SCA23 and control sets, and the difference Delta in scores between SCA23 and controls (Delta score) were calculated. The standard deviation (σ) for Delta score values was 2258. Key-node molecules with absolute Delta score exceeded 3σ, i.e. 6774, were considered as affected and shown in the table.

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Table S6. Demographic Data of SCA23 and Control Subjects, Whose Postmortem Specimens Were Used for Molecular and Biochemical Analysis

Subject ID	*Disease*	*Cause of Death*	*Postmortem Interval in Hours*		*Age at Death (Years)*	*Brain pH*
			Warm Time	*Cold Time*		
96S237	cerebellar ataxia, SCA23	dehydration, aspiration	5.0	0	80	NA
94S79	cardiovascular disease	bronchopneumonia	4.0	0	68	NA
ND081	none	multiple injuries, ACC	4.2	34.5	53	6.75
ND083	asthma, hypertension	spontaneous SAB	10.9	39.1	58	7.00
ND099	depression	intoxication with propoxyphene	9.8	18.2	61	6.52

SAB subarachnoid hemorrhage; ACC accidental death; NA not analyzed

Table S7. *PDYN* Primer Sequences and PCR Conditions for Mutation Screening

Exons	**Sequence (5'->3')**	**Ta**	**Mg**	**Size (bp)**
3	Forward: GCTGTGGGCAGGAGTTAGAG	60	1	397
	Reverse: GCCATCTATAGGGCAGGACA			
4-1	Forward: TAGCAGTGGCGTTCATTTTG	56	1	545
	Reverse: TCTGAGCTCCTCTTGGGGTA			
4-2	Forward: GGCACACTCTATCTCGCTGA	56	1	484
	Reverse: ACCCTTCCCCATCACAGAC			

Ta annealing temperature (°C); Mg $MgCl_2$ (mM)

Table S8. Mutagenesis Primers to Introduce Mutations in *PDYN* cDNA

Mutation	**Sequence (5'->3')**
p.R138S	Forward: CTCTGACGGGTTTAGTGAGGGAGCAGAGTCT
	Reverse: AGACTCTGCTCCCTCACTAAACCCGTCAGAG
p.L211S	Forward: CTATGGGGGCTTCTCGCGGCGCATTCGTC
	Reverse: GACGAATGCGCCGCGAGAAGCCCCCATAG
p.R212W	Forward: TATGGGGGCTTCTTGTGGCGCATTCGTCCCAAG
	Reverse: TTGGGACGAATGCGCCACAAGAAGCCCCCATAG
p.R215C	Forward: CTTGCGGCGCATTTGTCCCAAGCTCAAG
	Reverse: CTTGAGCTTGGGACAAATGCGCCGCAAG