ON-LINE SUPPLEMENTAL DATA

Appendix, Part 1

Molecular Studies. Genomic DNA was extracted using either ammonium acetate extraction, or by the QiaAmp DNA Blood Mini Kit (Cat. no: 51106; Qiagen, Hilden, Germany). Thirteen overlapping primer sets were used to amplify 9 exons of the *SACS* gene. Polymerase chain reactions were carried out in the GeneAmp PCR System 9700 (Applied Biosystems) using GoTaq DNA Polymerase (Promega, cat. no: M8305; Applied Biosystems) for shorter amplicons (< 1000 bp), or the Expand Long Template PCR System (Roche, cat. no: 11 681 842 001; Germany) for longer amplicons (> 1000 bp). Dimethyl-sulfoxide 2% was added to reactions for primer sets for exons 5 and 6. Thermal cycling parameters for short amplicons were 5 minutes at 95°C, followed by 39 cycles of denaturation at 95°C for 1 minute, annealing at 56–65°C for 1 minute and extension at 72°C for 1 minute. Final extension was at 72°C for 9 minutes. Thermal cycling parameters for long amplicons were 4 minutes at 94°C, followed by 39 cycles of denaturation at 94°C for 30 seconds, annealing at 57–60°C for 50 seconds and extension at 68°C for 4 minutes. Final extension was at 68°C for 7 minutes. Polymerase chain reaction products were checked on 2% and 1% agarose gels containing ethidium bromide for shorter and longer amplicons, respectively.

Polymerase chain reaction products were purified with ExoSAP-IT (Affymetrix, cat. No: 78200; Santa Clara, California) before being sequenced on an ABI 3130 Genetic Analyzer. Cycle-sequencing reactions were carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, cat. no: 4337455) with forward and reverse primers, and longer exons 7 and 9 were divided into several overlapping sequences by using several sequencing primers to obtain the full result. Cycle-sequencing products were purified using Receiver Columns (20 μm, Macherey-Nagel, cat. no: 740522, Düren, Germany) containing 10% Sephadex G-50 (Sigma, cat no: S5897) before capillary electrophoresis. Data were analyzed with SeqScape Software v2.7 (Applied Biosystems).

Appendix, Part 2

Electrophysiologic Studies. Conventional nerve conduction studies were performed at the upper and lower extremities. In the motor-evoked potential studies, cortical motor-evoked potentials were recorded at the tibialis anterior muscle bilaterally by applying transcranial magnetic stimulation. Also, the spinal-root motor-evoked potentials were recorded bilaterally at the tibialis anterior muscle by applying the magnetic stimulation to the motor roots over the fourth lumber spinous processes. Central motor conduction time was calculated as the difference between the latency of cortical and spinal-root motor-evoked potential. Somatosensory-evoked potentials were studied bilaterally by stimulating the posterior tibial nerve and recording over the T12 spinal process and vertex.

Appendix, Part 3

Molecular Studies Results. An allele containing a double missense mutation (*F4011S+V3369A*) was found in patient 1, whereas the *V3369A* variation was found to exist in 5 of 9 patients. *S894AMB* (Stop) was found in patient 2, resulting in a truncated protein of sacsin. In a family trio, the parents of patient 3 were shown to carry a heterozygous mutation [*S894AMB* (Stop)], causing a homozygous stop codon in exon 9 in the patient. Patient 4 was shown to carry only the single-nucleotide variation *V3369A*, as mentioned before. Patient 5 was found to carry the same variation *V3369A* and, in addition, a currently unpublished mutation of *S4007F*. Patient 6 was found to carry a homozygous missense mutation *I1464T*, which resides in a particular functional domain (SRR1) of the sacsin protein. Patient 7 was shown to carry a homozygous frameshift insertion that resides in a functional domain SRR2, as his mother carried a heterozygous insertion at the same spot. Patient 8 was shown to carry a nonsense mutation in exon 9 and the last patient studied, Patient 9, was shown to have a missense mutation at the residue of 4495, which is on the functional J domain.

On-line Table 1: Demographic and clinical features of patients with ARSACS

Clinical Features	Pt No. 1	Pt No. 2	Pt No. 3	Pt No. 4	Pt No. 5	Pt No. 6	Pt No. 7	Pt No. 8	Pt No. 9
Age (y)/sex	14/M	19/M	5/F	42/F	42/M	18/F	20/M	37/M	16/M
Base change	5019a>G	6945a>G	12841t>A 6945a>G	6945a>G	6945a>G 5031 g>A	12660a>G	8346–8347insT	5677 g>A	5566delC
Age at onset/age at diagnosis (y)	1/11	3/16	2/3	7/41	4/37	4/16	2/18	3/35	3/16
Presenting symptoms	Ataxic gait	Delayed motor development, dystonia	Delayed motor development	Ataxic gait	Delayed motor development	Delayed motor development	Delayed motor development, frequent falls	Delayed motor development, ataxic gait	Delayed motor development
Prenatal and natal history	Uneventful/term	Uneventful/term	C/S, mild hypoxia at birth	Uneventful/term	Uneventful/term	Uneventful/term	Uneventful/term	Uneventful/term	Uneventful/term
Consanguinity/family history	+/−	+/−	+/−	−/−	−/−	−/−	−[a]	−/−	+/−
Initial diagnosis	Cerebral palsy	Static cerebellar ataxia	Cerebral palsy	Hereditary spastic paraparesis	Hereditary spastic paraparesis	Cerebral palsy	Hereditary spastic paraparesis	Hereditary spastic paraparesis	Cerebral palsy
Cerebellar ataxia/dysarthria/nystagmus	+/+/−	+/+/+	−/−/−	+/−/−	−/−/−	−/−/−	+/+/−	+/+/−	−/−/−
Spasticity	+	+	−	+	+	−	+	+	+
Extremity deformity	−	−	Mild pes cavus	−	−	−	Mild pes cavus	−	−
Retinal myelinated fibers	+	Thick peripapillary retinal fibers	−	−	−	−	+	−	−
Peripheral neuropathy	−[b]	−[b]	+	+	+	+	+	+	+

Note:—C/S indicates cesarean section; F, female; M, male.

[a] There was no known consanguinity between the parents, but they were from the same village.

[b] Peripheral neuropathy was not evident clinically, though it was revealed by electrophysiologic studies.

On-line Table 2: Summary of results of molecular studies collected from all patients

Pt No.	Base Change	ROI	Type	Effect	Aa change	Description	Domain
1	5019a>G	Exon 9	Sub	Missense	*F4011S*	Not defined	—
	6945a>G	Exon 9	Sub	Missense	*V3369A*	rs17078605	—
2	14370g>T	Exon 9	Sub	Nonsense	*S894AMB (Stop)*	Not defined	—
3	12841t>A	Exon 9	Sub	Nonsense	*K1404OCH (Stop)*	Not defined	—
	6945a>G	Exon 9	Sub	Missense	*V3369A*	rs17078605	—
4	6945a>G	Exon 9	Sub	Missense	*V3369A*	rs17078605	—
5	6945a>G	Exon 9	Sub	Missense	*V3369A*	rs17078605	—
	5031g>A	Exon 9	Sub	Missense	*S4007F*	Not defined	—
6	12660a>G	Exon 9	Sub	Missense	*I1464T*	Not defined	SRR1
7	8346−8347insT	Exon 9	Ins	Frameshift insertion	*G2902V*	Not defined	SRR2
8	5677g>A	Exon 9	Sub	Nonsense	*R3801OPA (Stop)*	Not defined	—
9	5566delC	Exon 9	Del	Missense	*K4495N*	Not defined	DnaJ

Note:—Base and amino acid change positions are based on the NCBI sequence ref. No: NG_012342.
Del indicates deletion; Ins, insertion; Sub, substitution.

On-line Table 3: Summary of electrophysiologic studies of the patients with ARSACS

Pt No.	Nerve	DML (ms)	Motor CV (m/s)	CMAP (mV)	F Wave Latency (ms)	Sensory CV (m/s)	SNAP (μV)
1	R median	4,2 (3,8)	42 (50)	3,9	28,1 (30)	NR (50)	NR
	R ulnar	3,4 (3,3)	44 (50)	3,7	27,6 (30)	NR (50)	NR
	R tibial	7,8 (5,8)	43 (40)	4,7	47,5 (50)		
	R peroneal	5,5 (5,9)	38 (40)	2,9	48,7 (50)		
	R/L sural						
2	Not performed						
3	R median	3,1	44	4,70	20,5	45	4,4
	R ulnar	2,9	41	3,50	20,5	41	3,3
	R tibial	3,8	34	6,20	32,2		
	R peroneal	3,8	32	0,80	32,3		
	R/L sural					NR	NR
4	R median	4,9	44	11,1	32,7	NR	NR
	R ulnar	3,2	45	7,6	28,8	32,1	2,3
	R tibial	5,8	26	3,2	64,1		
	R peroneal	7,3	28	1,8	NR		
	R/L sural					NR	NR
5	R median	4,8	44	1,80	36,1	NR	NR
	R ulnar	3,8	46	5,10	34,3	NR	NR
	R tibial	5,5	29	1,10	67,6		
	R peroneal	5,7	26	0,20	NR		
	R/L sural					NR	NR
6	R median	4	38	6	34,3	NR	NR
	R ulnar	3,6	41	7,1	35,3	NR	NR
	R tibial	7,9	26	1,1	NR		
	R/L sural					NR	NR
7	R median	5,5	37	5,5	37,2	NR	NR
	R ulnar	4,4	39	5,8	36,5	NR	NR
	R tibial	6,8	36	0,2	NR		
	R/L sural					NR	NR
8	R median	7,6	37	3,3	NR	NR	NR
	R ulnar	4	38	3,1	NR	NR	NR
	R tibial	8,5	34	0,7	69		
	R/L sural					NR	NR
9	R median	5	45	6,60	32	NR	NR
	R ulnar	3,2	40	5,90	32,3	NR	NR
	R tibial	4,9	33	2,40	51,2		
	R peroneal	5,1	31	1,30	NR		

Note:—Upper limits of normal values for F wave and DML, and lower limits for nerve CVs are given within the parenthesis at the first case.
CMAP indicates compound muscle action potential; CV, conduction velocity; DML, distal motor latency; L, left; NR, no response; R, right; SNAP, sensory nerve action potential.

On-line Table 4: Diffusion measures obtained from major WM tracts with significant alterations in patients with ARSACS and corresponding regions in control participants

	Patients with ARSACS				Control Participants			
Affected Structure	FA	RD ($\times10^{-3}$ mm^2/s)	AD ($\times10^{-3}$ mm^2/s)	MD ($\times10^{-3}$ mm^2/s)	FA	RD ($\times10^{-3}$ mm^2/s)	AD ($\times10^{-3}$ mm^2/s)	MD ($\times10^{-3}$ mm^2/s)
TPF	0.73	0.32	1.31	0.70	0.59	0.42	1.15	0.66
Pyramids, R/L	0.32/0.35	0.63/0.56	1.24/1.18	0.75/0.70	0.59/0.59	0.44/0.37	1.23/1.13	0.72/0.65
CST, pons, R/L	0.42/0.44	0.63/0.60	1.27/1.39	0.78/0.78	0.64/0.63	0.46/0.36	1.25/1.26	0.70/0.67
CST, midbrain, R/L	0.53/0.48	0.64/0.59	1.16/1.37	0.76/0.77	0.67/0.68	0.39/0.39	1.17/1.36	0.65/0.71
Anterior LIC, R/L	0.61/0.55	0.52/0.67	1.20/1.18	0.79/0.77	0.65/0.58	0.57/0.54	1.13/1.11	0.75/0.73
Posterior LIC, R/L	0.72/0.66	0.45/0.46	1.38/1.41	0.68/0.71	0.74/0.63	0.38/0.40	1.3/1.18	0.60/0.66
Corona radiata, R/L	0.47/0.47	0.55/0.55	1.11/1.11	0.72/0.73	0.56/0.58	0.42/0.44	1.08/1.19	0.64/0.70
Precentral gyrus, R/L	0.44/0.49	0.53/0.54	1.11/1.16	0.73/0.77	0.59/0.55	0.43/0.47	1.23/1.15	0.70/0.67
Cingulum, R/L	0.37/0.34	0.79/0.85	1.21/0.98	0.86/0.79	0.42/0.47	0.71/0.75	1.11/1.0	0.84/0.67
Fornix, R/L	0.23/0.26	1.4/1.5	1.78/2.1	1.4/1.3	0.38/0.32	1.4/1.1	1.97/1.78	0.70/0.71
Superior longitudinal fasciculus, R/L	0.54/0.45	0.48/0.46	1.23/1.34	1.5/1.7	0.64/0.62	0.37/0.41	1.15/1.18	0.91/0.93
CC, genu	0.69	0.50	1.55	0.79	0.75	0.35	1.3	0.71
CC, body	0.62	0.84	1.64	0.90	0.84	0.78	2.01	0.85
CC, splenium	0.75	0.91	1.81	1.1	0.83	0.72	1.93	0.82
Thalamus, R/L	0.34/0.31	0.66/0.78	1.05/0.98	0.79/0.74	0.36/0.35	0.58/0.52	0.96/1.02	0.70/0.71
IFOF, R/L	0.45/0.47	0.66/0.72	1.40/1.46	0.86/0.86	0.51/0.50	0.46/0.50	1.05/1.01	0.67/0.67

Note:— CC indicates corpus callosum; FA, mean fractional anisotropy; IFOF, inferior fronto-occipital fasciculus; L, Left; LIC, limb of internal capsule; MD, mean diffusivity; R, Right.