Supplementary Online Content

Ozaki K, Doi H, Mitsui J, et al. A novel mutation in *ELOVL4* leading to spinocerebellar ataxia (SCA) with the hot cross bun sign but lacking erythrokeratodermia: a broadened spectrum of SCA34. *JAMA Neurol*. Published online May 26, 2015. doi:10.1001/jamaneurol.2015.0610.

eAppendix: Parameters used for the analysis with SNP HiTLink and MERLIN

Parameters used for parametric analysis with SNP HiTLink and MERLIN were as follows: SNP exclusion: Hardy-Weinberg equilibrium ($p < 0.001$), call rate (0.95), minor allele frequency (MAF) zero test: +, and an interval of 80,000 to 120,000 bp; disease frequency: 0.0001; penetrance: w/w (homozygous for wild type allele): 0, w/m (heterozygous for mutant allele): 0.99, m/m (homozygous for mutant allele): 0.99; Linkage disequilibrium: D' (0.6), r2 (0.6).

eTable 1: Detailed neurological and radiological findings in members of the two Japanese families with SCA

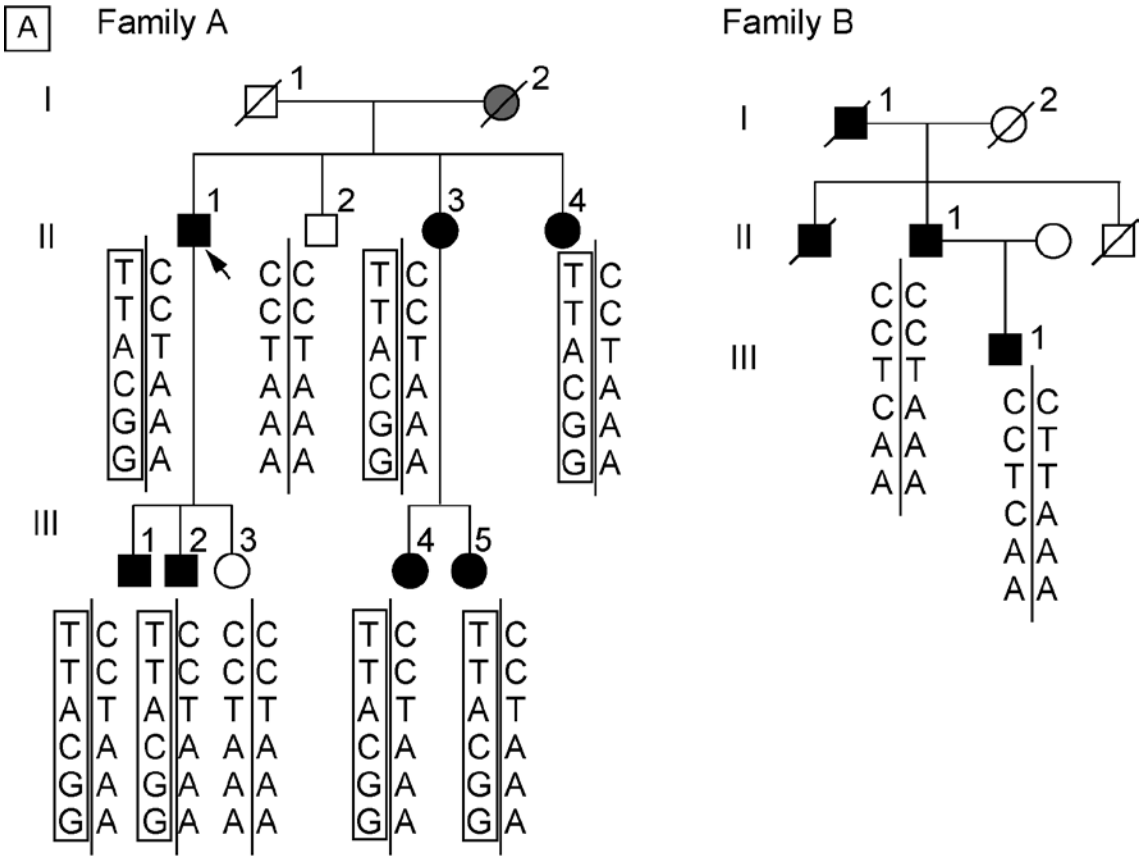
Member/family	Age/sex	Age of onset	Limb and truncal ataxia/dysarthria	Nystagmus	Autonomic disturbance	Severity of symptoms	Other findings and complications	MRI findings
II-1/A	82/M	46	+/+	+	BD (FU), Co, negative ST	Severe (WC. A cane was used from the age of 60.)	OP, DC, iDTR, BS, trivial akinesia, and rare myoclonus	CeA, PoA, CoA, HCBS. Incidental old CIs.
II-3/A	70/F	56	+/+	+	BD (FU)	Moderate (walker)	OP, DC, dDTR, BS. SAH (post clipping), DM, HTN.	ND (CeA, PoA on CT)
II-4/A	68/F	25	+/+	+	BD, Co	Moderate	OP, DC, iDTR in PaT, dDTR in UL and AcT, BS. DM.	CeA, PoA, PMLH. High intensities at MCP on FLAIR.
III-1/A	47/M	37	+/+	−	(−)	Mild	SPEM, iDTR in LL.	CeA, PoA, HCBS
III-2/A	44/M	33	+/+	−	(−)	Mild	SPEM.	ND
III-4/A	47/F	35	+/+	+	(−)	Mild	SPEM, iDTR in LL.	CeA, PoA, PMLH
III-5/A	43/F	30	+/+	+	(−)	Mild	SPEM, iDTR in jaw and limbs.	CeA, PoA, HCBS
II-2/A	78/M		−/−	−	(−)	None	None	ND
III-3/A	41/F		−/−	−	(−)	None	None	ND (Normal CT)
II-1/B	80/M	30	+/+	+	BD (FU)	Moderate to severe (walker)	SPEM, iDTR in limbs. BPH.	ND (BsA, CeA on CT)
III-1/B	56/M	13	+/+	+	None	Moderate	iDTR in LL	CeA, PoA, HCBS

BD: bladder disturbance; FU: frequent urination; Co: constipation; ST: Schellong test; WC: wheel chair bound; OP: ophthalmoplegia (vertical > horizontal); DC: disturbed convergence; i/dDTR: increased/decreased deep tendon reflexes; BS: positive Babinski signs; SAH: subarachnoid hemorrhage; DM: diabetes mellitus; HTN: hypertension; PaT: patellar tendon; AcT: Achilles tendon; UL: upper limbs; LL: lower limbs; SPEM: mildly impaired smooth pursuit eye movements; BPH: benign prostate hypertrophy; ND: no available data of MRI; CeA; cerebellar atrophy; PoA: pontine atrophy; CoA: cortical atrophy; CIs: infarctions in left thalamus and right cingulated gyrus; HCBS: hot cross bun sign; PMLH: pontine midline linear hyperintensity; MCP: middle cerebellar peduncles; BsA: brainstem atrophy.

eTable 2: Statistical analysis of the exome and whole genome sequencing data

Sequencing method	Individual examined (family A)	Mapping rate (%)	Mean depth of coverage (times)	Coverage with 10 times or more depth (%)	Number of non-synonymous single nucleotide variations identified
Exome	II-1	98.75	152.11	93.54	10,616
Exome	III-1	98.96	124.72	92.00	10,426
Exome	III-2	98.97	85.25	89.49	11,189
Whole genome	III-1	93.78	79.19	99.90	11,488

eFigure: Haplotyping of single nucleotide polymorphisms (SNPs) in the 211 kb region harboring the *ELOVL4* gene locus



B

Chr.	Pos.	dbSNP # or mutation	Ref.	Alt.	Mean allele frequency in dbSNP	Deduced haplotype in family A	Deduced haplotype in family B
6	80567887	rs144001800	C	T	T = 0.00275	T	C
6	80575142	rs55768285	C	T	T = 0.06152	T	C
6	80607855	rs188681357	T	A	A = 0.00092	A	T
6	80626534	c.736T>G in *ELOVL4*	A	C	None	C	C
6	80776485	rs3812150	A	G	G = 0.08219	G	A
6	80779221	rs2277101	A	G	G = 0.03994	G	A

Rare SNPs identified by whole genome sequencing were used to deduce the haplotype that cosegregates with the disease in family A. We first analyzed 17 SNPs located in the vicinity of the *ELOVL4* locus regarding their cosegregation with the disease, and 5 SNPs

showed cosegregation. Margins of the haplotype block were not determined. Genotyping of these SNPs in two affected members of family B (II-1 and III-1) suggested that the two families do not share a common ancestor.

Chr.: Chromosome; Pos.: Position in GRCh37; Ref.: Reference allele; Alt.: Alternative allele

eTable 3: Topology prediction of the ELOVL4 protein using bioinformatics tools

Prediction tool		MEMSAT-SVM	MEMSAT3	ENSEMBLE 3.0	Phobius	TMHMM2
Algorithm		SVMs	NN	NN and HMMs	HMM	HMM
	Non-cytoplasmic	1–40	1–39	1–46	1–44	1–46
	TM1	41–62	40–64	47–64	45–63	47–64
	Cytoplasmic	63–77	65–77	65–75	64–74	65–76
	TM2	78–101	78–102	76–100	75–94	77–99
	Non-cytoplasmic	102–125	103–120	101–122	95–127	100–124
	TM3	126–143	121–141	123–147	128–147	125–147
	Cytoplasmic	144–158	142–154	148–153	148–158	148–153
Regions	TM4	159–176	155–178	154–173	159–177	154–173
	Non-cytoplasmic	177–182	179–181	174–183	178–188	174–182
	TM5	183–203	182–206	184–204	189–208	183–205
	Cytoplasmic	204–219	207–217	205–213	209–219	206–216
	TM6	220–238	218–237	214–236	220–240	217–239
	Non-cytoplasmic	239-246	238-245	237-245	241-245	240-248
	TM7	247–267	246–265	246–268	246–267	249–267
	Cytoplasmic	268–314	266–314	269–314	268–314	268–314

MEMSAT-SVM: support vector machine-based MEMSAT; MEMSAT3: membrane protein structure and topology 3; TM: transmembrane helix; SVM: support vector machine; NN: neural network, HMM: hidden Markov model

Numbers in the table indicate the first and last amino acid residues in the respective regions in the ELOVL4 protein. All five tools predicted a seven transmembrane helix protein, with its n-terminus in the non-cytoplasmic compartment, and its c-terminus in the cytoplasmic compartment. Since the ELOVL4 protein is known to localize in the endoplasmic reticulum, the non-cytoplasmic compartment corresponds to the endoplasmic reticulum lumen.

eTable 4: Topology prediction of the ELOVL5 protein using bioinformatics tools

Prediction tool		MEMSAT-SVM	MEMSAT3	ENSEMBLE 3.0	Phobius	TMHMM2
Algorithm		SVMs	NN	NN and HMMs	HMM	HMM
	Non-cytoplasmic	1–27	1–28	1–31	1–31	1–25
	TM1	28–48	29–52	32–50	32–49	26–48
	Cytoplasmic	49–63	53–63	51–61	50–60	49–64
	TM2	64–88	64–88	62–86	61–80	65–87
	Non-cytoplasmic	89–110	89–105	87–108	81–113	88–109
	TM3	111–131	106–127	109–132	114–132	110–132
	Cytoplasmic	132–141	128–137	133–139	133–143	133–138
Regions	TM4	142–159	138–161	140–158	144–161	139–158
	Non-cytoplasmic	160–167	162–163	159–167	162–166	159–167
	TM5	168–186	164–188	168–190	167–186	168–187
	Cytoplasmic	187–204	189–196	191–196	187–206	188–206
	TM6	205–222	197–220	197–218	207–224	207–224
	Non-cytoplasmic	223-230	221-226	219-229	225-229	225-228
	TM7	231–251	227–250	230–252	230–251	229–251
	Cytoplasmic	252–299	251–299	253–299	252–299	252–299

The abbreviations used are as defined in the legend to eTable 3. Numbers in the table indicate the first and the last amino acid residues in the respective regions of the ELOVL5 protein. All five tools predicted a seven transmembrane helix protein, with its n-terminus in the non-cytoplasmic compartment, and its c-terminus in the cytoplasmic compartment. Since the ELOVL5 protein is known to localize in the endoplasmic reticulum, the non-cytoplasmic compartment corresponds to endoplasmic reticulum lumen.