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Supplemental Data

Recessive Inactivating Mutations in *TBCK*, Encoding a Rab GTPase-Activating Protein, Cause Severe Infantile Syndromic Encephalopathy

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Table S1. Candidate genes in each family.

	Candidate gene	Genomic coordinates (hg19)	Zyg	ID	Predicted amino acid change	CADD phred score	GeneDistiller score
Fam A-II-1	*TBCK*	chr4:107183260 G>A	Hom	-	p.Arg126*	37	n/a
	PRDM2	chr1:14109023 C>T	Het	rs148083107	p.1578Leu	25.2	n/a
	PRDM2	chr1:14149672 A>G	Het	rs202013308	p.Asn225Ser	16.64	n/a
	CGN	chr1:151491318 T>G	Het	rs142903144	p.Val108Gly	13.55	n/a
	CGN	chr1:151502430 C>T	Het	rs147054552	p.Arg718Trp	21	n/a
	TTN	chr2:179397433 G>A	Het	rs200716930	p.Arg34637Trp	24.8	n/a
	TTN	chr2:179457531 G>A	Het	rs72646840	p.Pro19772Leu	21.1	n/a
	DUSP19	chr2:183943771 A>G	Het	-	p.Asp37Gly	15.85	n/a
	DUSP19	chr2:183943783 A>G	Het	rs75674163	p.His41Arg	10.16	n/a
	DDX4	chr5:55075860 G>A	Het	rs147190735	p.Gly155Ser	23	n/a
	DDX4	chr5:55076956 C>A	Het	rs148485105	p.Pro187Thr	4.742	n/a
	MACC1	chr7:20199461 G>A	Het	rs200826339	p.Arg175Trp	28.5	n/a
	MACC1	chr7:20201458 G>A	Het	rs117142233	p.Arg108Trp	14.58	n/a
	DNAH11	chr7:21640660 G>A	Het	-	p.Met1096Ile	7.93	n/a
	DNAH11	chr7:21641142 C>G	Het	-	p.Ala1185Gly	21.6	n/a
	SLC7A13	chr8:87229732 T>G	Het	rs140915018	p.Arg382Ser	23.8	n/a
	SLC7A13	chr8:87241843 C>T	Het	rs148729888	p.Ala222Thr	8.719	n/a
	MRGPRG	chr11:3239259 G>A	Het	-	p.Pro262Leu	18.03	n/a
	MRGPRG	chr11:3239398 A>T	Het	-	p.Phe216Ile	0.002	n/a
	OR52W1	chr11:6221376 G>A	Hom	rs149476132	p.Arg308Gln	3.529	n/a
	SLCO1B3	chr12:21015348 T>G	Het	rs140353351	p.Cys162Gly	21.8	n/a
	SLCO1B3	chr12:21015737 C>G	Het	rs115227445	p.Leu226Val	12.22	n/a
	OTOGL	chr12:80651708 G>T	Het	rs192234924	p.Gln596His	25.6	n/a
	OTOGL	chr12:80712366 G>C	Het	rs139375212	p.Gln1216His	12.7	n/a
	ZC3H13	chr13:46538115 G>A	Het	-	p.Arg1513*	39	n/a
	ZC3H13	chr13:46541962 C>T	Het	rs75503329	p.Arg1333His	23.9	n/a
	ZNF469	chr16:88500657 A>G	Het	rs75136873	p.Lys2232Arg	0.002	n/a
	ZNF469	chr16:88502882 A>C	Hom	rs141776185	p.Met2974Leu	0.025	n/a
	C19orf21	chr19:757509 G>C	Het	rs78455638	p.Arg188Thr	0.001	n/a
	C19orf21	chr19:757641 A>G	Het	rs3746175	p.Lys232Arg	0.001	n/a
	PPP6R1	chr19:55742803 G>A	Het	rs200736594	p.Arg812Cys	12.56	n/a
	PPP6R1	chr19:55751610 C>T	Het	rs201089630	p.Arg452His	15.89	n/a
	DNAJC28	chr21:34861335 ATCTTCT>A	Hom	-	p.Glu120_Glu121del	16.62	n/a
	DNAJC28	chr21:34861444 G>A	Hom	rs144266803	p.Thr86Ile	15.77	n/a
	GART	chr21:34889888 A>G	Hom	rs151206034	p.Met577Thr	19.71	n/a
	GART	chr21:34897281 G>C	Hom	rs139633075	p.Leu365Val	14.62	n/a
	GART	chr21:34903861 C>A	Hom	rs142038738	p.Glu177Asp	8.326	n/a
	SON	chr21:34925936 A>G	Hom	rs142751481	p.Ile1467val	0.001	n/a
	DONSON	chr21:34951753 T>G	Hom	rs146664036	p.Lys489Thr	10.7	n/a
	DMD	chrX:31854852 C>T	Hem	rs72466590	p.Ala3686Thr	15.64	n/a
	CXorf30	chrX:36337351 T>C	Hem	rs145065748	p.Met237Thr	0.002	n/a
	KDM5C	chrX:53222017 G>C	Hem	-	p.Pro1350Arg	2.121	n/a
	FAM104B	chrX:55170246 T>C	Hem	rs182883175	p.Asn106Ser	0.627	n/a
	ACRC	chrX:70832746 C>T	Hem	rs138927483	p.Arg664Cys	17.56	n/a
	SLC16A2	chrX:73744567 C>T	Hem	rs144755294	p.Arg317Cys	33	n/a
	MAGEE1	chrX:75649404 G>A	Hem	rs145927168	p.Gly361Ser	10.64	n/a
	ARMCX2	chrX:100911031 C>T	Hem	rs192734301	p.Arg515Cys	25.5	n/a
	MAGEC3	chrX:140985243 G>T	Hem	rs143730187	p.Val567Phe	11.39	n/a
Fam B-IV-4	*TBCK*	chr4:107156512 T>A	Hom	rs376699648	p.Lys455*	42.0	n/a
Fam B-IV-6	*VPS50*	chr7:92953034 G>A	Hom	-	p.Arg626Gln	22.1	n/a
	LRCH4	chr7:100173865 C>T	Hom	rs370008127	p.Arg545His	22.7	n/a
Fam C-II-1[1]	*TBCK*	chr4:107154202, C>T	Hom	-	p.Arg511His	35.0	17.0
	TRPM4	chr19:49685865, G>A	Hom	rs201907325	p.Ala432Thr	33.0	10.6
		chr19:49691898, G>A	Hom	rs172149856	p.Gly582Ser	20.3	
	HAUS3	chr4:2238026, G>C	Hom	-	p.Arg503Gly	23.9	8.9
	ARHGEF28	chr5:73128166, G>A	Hom	rs187897891	p.Arg343His	21.4	6.9
	CMBL	chr5:10290679, TC>T	Hom	-	frameshift	35.0	6.0
	TMEM131	chr2:98612283, G>A	Hom	-	start gain	15.2	4.2
	EYS	chr6:64791875, A>G	Hom	-	p.Ser2149Pro	24.7	1.0
	KCTD14	chr11:77728003, C>T	Hom	-	p.Arg135Gln	34.0	-0.3

[1]Based on the hypothesis of homozygosity by descent, 16 candidate genes with private or rare (MAF <0.001), homozygous variants were identified. Genes were ranked taking into account their predicted biological relevance on the developmental processes altered in the disorder (GeneDistiller; using the following keywords: brain atrophy, optic atrophy, corpus callosum, cerebellum, hypotonia and dysphagia). Only genes with variants predicted to have damaging impact (CADD_phred > 15) were retained.

Table S2. Diagnostic evaluations of patients with *TBCK*-related encephalopathy.

	Family A-II-1	**Family B-IV-4**	**Family B-IV-6**	**Family C-II-1**	**Family D-II-1**
EMG/nerve conduction	normal at 2 yr	axonal neuropathy	axonal neuropathy	Normal at 2 yr	At 8mo: Nerve conduction studies normal, EMG with possible myopathic changes
EEG	slow, disorganized, multifocal sharps, maximal R frontal	slowing, paroxysmal fast activity, bifrontal spikes, no normal sleep architecture	diffuse fast activity, generalized paroxymsal fast activity, spikes, no normal sleep architecture	slow and high-voltage monomorphic and diffuse high-voltage slow (delta) activity, paroxysmal high-voltage burst of spikes in left temporal/occipital region	mixed focal and generalized seizures
Lactate	elevated only when severe illness; elevated in urine OA	Normal	Normal	Normal	Normal
Creatine kinase	Unknown	Normal	Unknown	Unknown	Normal
Plasma amino acids	incr glutamine	Normal	Unk, CSF aa normal	Normal	Normal
Urine organic acids	incr lactate	Unknown	Unknown	Normal	Normal
Biopsies	Muscle: rare scattered small fibers Liver: panlobular hepatocellular ballooning	Unknown	Nerve: chronic axonal degeneration	Not done	Muscle: angiocentric perimysial lymphocytic infiltrates, no overt myopathic changes, dispersed moderately atrophic fibers, type II predominance Nerve: patchy loss of large caliber axons and moderately swollen axonal profiles
Respiratory chain assays	Muscle: Complex IV (COX) activity 37%, mildly low complex II and I Fibroblasts: Nl PC, CS, PDH	Pending	low respiratory chain enzyme levels, non-diagnostic	Not done	Muscle: low complex I&III activity (30-40% of control)

Genetic testing	Nl MitoMet, oligonucleotide array, Nuclear complex IV gene sequencing (*SCO1*, *SCO2*, *SURF1* and *COX10*)	Normal CGH array	CGH array (1.2 Mb) normal	CGH array: pat del11p15.4 (81 Kb)	NA

CGH: comparative genomic hybridization, CS: citrate synthase, incr: increased, Kb; Kilobases, mo: months, NA: Not Available, OA: Organic Acids, pat: paternal, PC: pyruvate carboxylase, PDH: pyruvate dehydrogenase, yr: years

Table S3. Brain imaging features of individuals with *TBCK* mutations.

Feature	Family A-II-1	Family B-IV-4	Family B-IV-6	Family C-II-1	Family D-II-1
Age at MRI	22 d, 18 mo, 2, 3, and 8 yr	18 mo	4 and 6 yr	21 mo	14 yo
Extra-axial space	Incr	Incr	Incr	Incr	Incr
Ventriculomegaly	Yes	Yes	Yes	Yes	Mild
Cortical gray and WM volume	Decr	Decr	Decr	Decr	Decr
Periventricular WM T2/FLAIR signal	Incr	Incr	Incr	Incr	Incr
CC thinning	Yes	Yes	Yes	Yes	Yes
CC partial agenesis	Yes	No	No	Yes	No
Basal ganglia	Small	Small	Small	Small	Small
Cerebellar hypoplasia (vermis smaller than hemispheres)	Yes	Yes	Yes	Yes	Yes
Relatively spared brainstem	Yes	Yes	Yes	No	Yes
Magnetic resonance spectroscopy	Normal except for decr NAA c/w atrophy, no lactate	NA	Normal except for decr NAA c/w atrophy, no lactate	Normal except for decr NAA c/w atrophy, no lactate	NA
Other findings	History of restricted diffusion and edema of right parietal-occipital cortex, subdural hematoma, dural thickening	turricephaly, right posterior plagiocephaly	synechiae in right frontal horn	brainstem atrophy and dysplasia	thick frontal skull

CC: Corpus Callosum, d: days, Decr: Decreased, Incr: Increased, mo: months, NA: Not Available, NAA: N-acetylaspartate, T2/FLAIR; FLuid-Attenuated Inversion Recovery, Unk: Unknown, WM: White Matter, yr: years