Supplemental data

S1. Methods

Procedures. *TBC1D24* variants were identified through a variety of methods, including whole exome sequencing and Sanger validation (patients #4, #6a, #7a-c, #9, #12a, #13, #16, #19, #20a, #21, #22, #23a, #26, #27), Sanger sequencing (patients #1a-h, #6b, #8a-b, #12b, #14, #20b, #23b, #24, #25, #28, #29, #30), next-generation sequencing panels (patients #2, #3, #15, #17a-b, #18, #21) and multipoint linkage analysis followed by targeted sequence enrichment and massively parallel sequencing (patients 5#a-d). The longest *TBC1D24* isoform, harbouring exon 3, was referenced (isoform 1, NM_001199107.1, GRCh37/hg19). Forty-seven patients had biallelic mutations. In only one patient (#24) was a mutation not identified in the second allele. For this patient, real-time PCR of genomic DNA for all coding exons was performed to detect a deletion or insertion of a whole exon, and the last intron was sequenced. None of these procedures identified a second mutation. The other mutation for this patient is probably in the promoter or in a deep intronic region, affecting, for example, a branch site. We did not perform further tests to explore this, since there was no clinical doubt regarding the diagnosis of DOORS syndrome.

Bioinformatics. TBC1D24 sequences from *H. sapiens*, *M. musculus*, *R. norvegicus* and *D. melanogaster* Sky, and sequences from seven other insect Sky proteins were selected as the positive set, then compared to a discriminative set of TBC-domain proteins, consisting of human and mouse TBC1D1 and TBC1D7. *E*-values and *p*-values were calculated. *E*-values estimate the likelihood of a conserved motif based on all input sequences against the amino acid composition background of the input

(Sky/TBC1D24 sequence), whereas p-values estimate the probability of conservation for each protein and motif.

***In vitro* modelling.** Cortical cells from wild-type C57BL/6J P1 mouse pups were prepared and cultured as previously described.[e1] 2 x 10^6 cells were electroporated with 1.5 μg of each DNA construct by nucleofection (Amaxa). Cells were cultured for 4 days before fixation with 4% paraformaldehyde and staining with rat anti-HA (Roche) overnight at 4ºC. Transfected cells were visualized using an anti-rat Alexa 459 secondary antibody (Life Technologies) and images were taken under fluorescence (Leica). A minimum of 60 neurites from transfected neurons representing each construct were measured using Image-J software (NIH). Human wild-type *TBC1D24* was generated by RT-PCR, adding an in-frame C-terminal HA-tag, followed by cloning into pcDNA3.1 (Invitrogen). *TBC1D24* mutants were generated by site-directed mutagenesis and were sequenced prior to cloning.

Table e-1. Details of the epilepsy phenotype and mutations identified in TBC1D24. Longest isoform, harbouring exon 3, is used (isoform 1, NM_001199107.1, hg19).

Patient number/ gender, current age*	Age at seizure onset	Seizure types and frequency	Details of Mc or clonic seizures, if present	Syndrome classification	Current AEDs	AEDs previously tried	Epilepsy outcome/ clinical evolution	Alleles, DNA (protein)
1a/ W, 54 years	4 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental,	familial infantile myoclonic	VPA	none	still sporadic Mc, free from GTC	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

			precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements					
1b/ W, 52 years	7 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements	familial infantile myoclonic	PB	PHT	still sporadic Mc, free from GTC	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

1c/ M, 50 years	5 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements	familial infantile myoclonic	PB	PHT	still sporadic Mc, free from GTC	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)
1d/ M, 47 years	5 months	GTC, occasional (12-16 years); Mc, weekly to	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters,	familial infantile myoclonic	PB	PHT	still sporadic Mc, free from GTC	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

		monthly (4-12 years)	could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements					
1e/ W, 43 years	36 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental,	familial infantile myoclonic	VPA	CBZ, PB	still sporadic Mc, free from GTC	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

			precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements					
1f/ M, 59 years	16 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements	familial infantile myoclonic	none	none	Mc triggered by repetitive movements or fatigue; sporadic GTC (every 2-3 years)	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

1g/ M, 58 years	12 months	GTC, occasional (12-16 years); Mc, weekly to monthly (4-12 years)	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters, could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements	familial infantile myoclonic	none	none	Mc triggered by repetitive movements or fatigue; sporadic GTC (every 2-3 years)	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)
1h/ M, 56 years	12 months	GTC, occasional (12-16 years); Mc, weekly to	in childhood, spontaneous, erratic, bilateral, or massive, isolated or in clusters,	familial infantile myoclonic	none	VPA	Mc triggered by repetitive movements or fatigue; sporadic	chr16:2546588G>C (p.Asp147His)/ chr16:2550823C>T (p.Ala515Val)

		monthly (4-12 years)	could last for many hours, sometimes evolving into GTC; in youth, segmental, precipitated by fatigue/ drowsiness, acoustic/ light stimuli, repetitive movements				GTC (every 2-3 years)	
2/ W, 5 months	3.5 months	focal (motor), weekly; clonic, weekly; episodes of lip quivering with no EEG	not present	focal	LEV, LTG	none	drug-resistant	chr16:2547068A>G (p.Asn307Asp)/ chr16:2546994C>G (p.Pro282Arg)

		correlate, weekly						
3/ M, 2 years	3 months	GTC, rare; Mc, daily	involving peri-oral region and/or limbs, rhythmic, bilateral, lasting up to 30 minutes, no loss of consciousness	infantile myoclonic	CLB, STP, VPA	PB	with STP complete control of GTC, moderate control of Mc	chr16:2546835T>C (p.Phe229Ser)/ chr16:2550465C>T (p.Ala500Val)
4/ W, 15 years	36 hours	GTC, monthly; Mc, daily; tonic, daily	prolonged, continuous runs of spontaneous Mc, both at rest and on maintaining posture; involving tongue, peri-ocular region, lower limbs, head and trunk; not markedly worsened	progressive myoclonic	CBZ, VPA	CLZ, LEV, PIR, prednisolone, TPM	rarer GTCs (<1 / month); Mc daily; tonic seizures ceased at 7 years; progressive clinical deterioration (see Video 1)	chr16:2548334G>T (p.Arg360Leu)/ chr16:2548334G>T (p.Arg360Leu)

			by action; could result in falls, unable to feed self; precipitated by constipation, delayed medication or tiredness					
5a/ M, 46 years	2 months	focal (prominent eye blinking, facial and limb jerking), weekly; GTC, weekly	not present	focal	CBZ, CLZ, PB	none	ongoing seizures, usually due to non-compliance	chr16:2546900T>C (p.Phe251Leu)/ chr16:2546900T>C (p.Phe251Leu)
5b/ M, 40 years	2 months	focal (prominent eye blinking, facial and limb	not present	focal	PB, PHT, VPA	none	rare seizures continue despite medication	chr16:2546900T>C (p.Phe251Leu)/ chr16:2546900T>C (p.Phe251Leu)

		jerking and dyscognitive), unknown initial frequency						
5c/ W, 33 years	2 months	focal (prominent eye blinking, facial and limb jerking), weekly	not present	focal	CBZ, LTG	VPA	controlled on medication	chr16:2546900T>C (p.Phe251Leu)/ chr16:2546900T>C (p.Phe251Leu)
5d/ M, 29 years	2 months	focal (prominent eye blinking, facial and limb jerking and	not present	focal	CBZ, PHT, VPA	none	controlled on medication	chr16:2546900T>C (p.Phe251Leu)/ chr16:2546900T>C (p.Phe251Leu)

		dyscognitive), weekly						
6a/ W, deceased	5 weeks	migrating clonic, focal (lateral deviation of the head and eyes, twitches of the eyelids, flushing or cyanosis of the face; or subtle seizures with cyanosis and movement arrest), daily	initially discontinuous, then continuous lasting several hours, migrating from one limb to another; no obvious triggers	epilepsy of infancy with migrating focal seizures (EIMFS)	deceased	CBZ, CLB, CLZ, KD, PB, PHT, ZNS	drug-resistant, death as probable consequence of chronic respiratory failure at 8 years	chr16:2546617C>A (p.Cys156*)/ chr16:2546835T>C (p.Phe229Ser)

		(initial phase), almost permanent (stormy phase), clusters of several seizures per hour (late phase)						
6b/ W, deceased	4 weeks	migrating clonic, focal (lateral deviation of the head and eyes, twitches	initially discontinuous, then continuous lasting several hours, migrating from one limb to another; no obvious triggers	epilepsy of infancy with migrating focal seizures (EIMFS)	deceased	CBZ, CLB, CLZ, KD, PB, PHT, ZNS	drug-resistant, death (probable SUDEP) at 18 months	chr16:2546617C>A (p.Cys156*)/ chr16:2546835T>C (p.Phe229Ser)

		of the eyelids, flushing or cyanosis of the face; or subtle seizures with cyanosis and movement arrest), daily (initial phase) almost permanent (stormy phase), clusters of several seizures per						

		hour (late phase) (see Video 2)						
7a/ M, deceased	2 months	clonic, Mc, unknown initial frequency	erratic, segmental, multifocal, unilateral, migrating or alternating pseudo-rhythmic, affecting eye and peri-oral region, startle responses to auditory and tactile stimuli, duration increasing with age (up to 5-6 days), infrequent rhythmic clonic seizures	early-onset epileptic encephalopathy	deceased	CLB, CLZ, PB, TPM, VGB VPA	drug-resistant; death due to an infection (3.5 years)	chr16:2547714-2547715delGT (p.Ser324Thrfs*3)/ chr16:2547714-2547715delGT (p.Ser324Thrfs*3)

7b/ W, deceased	3 weeks	focal (prolonged staring episodes associated with a general increase in muscle tonus and frightened facial expression, or aversion of the head and eyes to either side), Mc, unknown	segmental, multifocal, unilateral, or generalised, pseudo-rhythmic, involving face and limbs, alternating and migrating, never occurring in sleep	early-onset epileptic encephalopathy	deceased	na	drug-resistant; death during status epilepticus associated with pulmonary infection (3.5 years)	chr16:2547714-2547715delGT (p.Ser324Thrfs*3)/ chr16:2547714-2547715delGT (p.Ser324Thrfs*3)

		initial frequency						
7c/ M, deceased	first month of life	focal (gaze deviation), Mc, spasms, unknown initial frequency	na	early-onset epileptic encephalopathy	deceased	na	drug-resistant; death due to pulmonary infection (6.5 years)	chr16:2547714-2547715delGT (p.Ser324Thrfs*3)/ chr16:2547714-2547715delGT (p.Ser324Thrfs*3)
8a/ M, 10 years	24 hours of life	Mc, daily, in clusters	involving face and eyelids, sometimes extending to the limbs or the whole body; triggers: acoustic stimuli; partially controlled with PHT	familial infantile myoclonic	CLZ, LEV, PHT, TPM	CBZ, LTG, PB, VPA	drug-resistant (Mc weekly)	chr16:2546606G>A (p.Glu153Lys)/ chr16:2546606G>A (p.Glu153Lys)

8b/ M, 3.5 years	2.5 months	Mc, in clusters, seizure-free intervals<15 days	long-lasting, segmental or generalized, variable topography, involving eyelids, peri-oral region, or the whole face, without loss of consciousness; triggers: feeding (facial Mc), febrile episodes; partially controlled with PHT	familial infantile myoclonic	CLZ, PHT	na	drug-resistant	chr16:2546606G>A (p.Glu153Lys)/ chr16:2546606G>A (p.Glu153Lys)
9/ W, 3 years	first day of life	focal, Mc, tonic, daily, in clusters	segmental or generalised, variable topography, involving eyelids, peri-oral	early-onset epileptic encephalopathy	CLZ, KD, PIR, ZNS	CLB, CBZ, LEV, LTG, PB, PHT, TPM, VPA	drug-resistant (Mc daily)	chr16:2546426C>T (p.Pro93Ser)/ chr16:2550823C>T (p.Ala515Val)

			region, or the whole face					
10/ W, 1 year	2 months	clonic, Mc, sGTC, monthly	triggered by fever	early-onset epileptic encephalopathy	hydrocortisone, LEV, PB, VPA	none	drug-resistant (Mc daily, clonic and sGTCs monthly)	chr16:2546181A>G (p.Asp11Gly)/ chr16:2546181A>G (p.Asp11Gly)
11/ M, 8.5 years	7 months	Mc, twice a month	alternatively affecting eyelids, either the right or left limbs, sometimes all four limbs or the trunk, lasting from several hours to up to 2 weeks, mostly disappearing during sleep, no loss of consciousness,	infantile myoclonic	TPM	BDZs, CBZ, LEV, PB, VPA, ZNS	good response to TPM (3 month seizure-free period and now minor Mc triggered by fever)	chr16:2546958G>A (p.Arg270His)/ chr16:2546958G>A (p.Arg270His)

			triggered by fever or fatigue					
12a/ W, 7 years	15 months	focal (motor), sGTC, weekly	not present	focal	LEV, OXC, ZNS	none	>90% reduction in frequency on ZNS	chr16:2546264G>C (p.Ala39Pro)/ chr16:2550940-2550946del (p.Gln554Leufs*12)
12b/ W, 13 years	8 months	focal (motor), sGTC, weekly	not present	focal	LEV, ZNS	OXC	>90% reduction in frequency on ZNS	chr16:2546264G>C (p.Ala39Pro)/ chr16:2550940-2550946del (p.Gln554Leufs*12)
13/ W, 12 years	2 months	focal (eye deviation to the left, staring gaze, limb	not present	early-onset epileptic encephalopathy	CZP, FBM, PB	CBZ, KD, LEV, OXC, PHT, PRM, TPM	drug-resistant	chr16:2546880C>T (p.Ala244Val)/ chr16:2546880C>T (p.Ala244Val)

		posturing), GTC, daily						
14/ M, 14 years	3 months	clonic, focal (multiple types including dyscognitive), sGTC, daily	na	multifocal	CLZ, TPM, VPA	CLB, LTG	drug-resistant, clinical deterioration	chr16:2546828C>T (p.Arg227Trp)/ chr16:2550823C>T (p.Ala515Val)
15/ M, 8 years	7 months	focal, Mc, sGTC, monthly	segmental, involving right or left hand, tongue or other facial parts	focal	OXC, SUL	AZM, CBZ, LEV, LTG, TPM VPA, ZNS	free of sGTC, occasional Mc	chr16:2546682C>G (p.Ser178Trp)/ chr16:2546829G>A (p.Arg227Gln)
16/ M, 9.5 years	5 months	GTC, weekly	not present	unclassified	VPA, CLB	LTG	drug-resistant, daily GTC	chr16:2547106G>C (p.Lys319Asn)/ chr16:2547106G>C (p.Lys319Asn)

17a/ W, deceased	45 minutes after birth	clonic, Mc (mostly), spasms, tonic, daily, longest remission for 2 months	multifocal Mc, involving peri-oral region (and other facial parts) and limbs	early-onset epileptic encephalopathy	deceased	CLB, ESM, LEV, LTG, OXC, PB, PHT, TPM, VGB, VPA, ZNS, VPA	drug-resistant epilepsy, predominant Mc; died at the age of 20 months due to respiratory failure following respiratory infection	chr16:2548263delT (p.His336Glnfs*12)/ chr16:2546181A>G (p.Asp11Gly)
17b/ M, deceased	20 minutes after birth	clonic, Mc (mostly), tonic, daily after neonatal period until 13 months	multifocal, erratic, Mc, involving face (prominent eye twitching) and limbs	early-onset epileptic encephalopathy	deceased	CLB, ESM, LEV, LTG, OXC, PB, PHT, TPM	on continuous thiopental infusion after 13 months; died at the age of 24 months due to respiratory failure following	chr16:2548263delT (p.His336Glnfs*12)/ chr16:2546181A>G (p.Asp11Gly)

							respiratory infection	
18/ M, 11 years	7 months	Mc, sGTC, weekly	triggered by fever and fatigue, sometimes evolving into prolonged GTC	infantile myoclonic	CLZ, PIR, VPA	CLB, LEV, LTG, TPM	drug-resistant (frequent Mc, sometimes evolving into sGTCs, usually prolonged)	chr16:2546768C>T (p.Gln207*)/ chr16:2550809A>G (splice-site)
19/ M, 21 years	2 months	focal, sGTC, weekly	not present	focal	CBZ, CLZ	CLB, PB, TPM, STP VPA, ZNS	drug-resistant	chr16:2546873C>T (p.Arg242Cys)/ chr16:2546267C>T (p.Arg40Cys)
20a/ M, 15 years	6 months	focal, unknown	not present	unclassified	na	na	good control with LAC, LTG, TPM	chr16:2546873C>T (p.Arg242Cys)/

		initial frequency						chr16:2546873C>T (p.Arg242Cys)
20b/ M, 8 years	4 months	focal, unknown initial frequency	not present	unclassified	na	na	good control with CZP, LAC, TPM	chr16:2546873C>T (p.Arg242Cys)/ chr16:2546873C>T (p.Arg242Cys)
21/ W, 3 years	6 weeks	focal, sGTC, daily	not present	focal	na	CLB, cortisone pulses, CLZ, DZP, LEV, LTG, MDZ, OXC, PB, SUL,VPA, ZNS	drug-resistant	chr16:2548263delT (p.His336Glnfs*12)/ chr16:2549421+5G>A (splice-site)
22/ M, 6 years	7 months	clonic, focal, sGTC, weekly	na	focal	CBZ, CLB, VPA	none	drug-resistant	chr16:2546873C>T (p.Arg242Cys)/ chr16:2546873C>T (p.Arg242Cys)

23a/ M, 9 years	3 months	focal, Mc, weekly	na	myoclonic	CLB, LTG, VPA	CLZ, LEV, PB, TPM	after initial control on PB and CLB, at the age of 11.5 years clinical deterioration with daily seizures and encephalopathy; very frequent Mc persisted after recovery (see Video 3)	chr16:2546207C>T (p.Gln20*)/ chr16:2546873C>T (p.Arg242Cys)
23b/ M, 1 year	3 months	focal and Mc, frequently; sGTC, two-three times per year	na	myoclonic	CLB, LEV, TPM	none	drug-resistant (see Video 4)	chr16:2546207C>T (p.Gln20*)/ chr16:2546873C>T (p.Arg242Cys)

24/ W, 5 years	3 months	clonic and Mc, weekly; focal and GTC, monthly	prolonged (several hours), erratic, involving one segment of a limb; no alteration of consciousness; triggered by fever	familial infantile myoclonic	CLZ, VPA	CBZ, LEV, STP, TPM	drug-resistant (prolonged Mc daily; clonic seizures monthly, requiring BDZ injection, see Video 5)	chr16:2548263delT (p.His336Glnfs*12)/ ?
25/ M, 22 years	7 months	absences, GTC, spasms, unknown initial frequency	not present	generalised	CLB, PHT	CBZ	seizure-free >12 months on CLB and PHT	chr16:2546873C>T (p.Arg242Cys)/ chr16:2546873C>T (p.Arg242Cys)
26/ W, deceased	first day of life	focal, Mc, daily	na	early-onset epileptic encephalopathy	deceased	PB	drug-resistant, died at age of 6 months	chr16:2546268G>T (p.Arg40Leu)/ chr16:2546268G>T (p.Arg40Leu)

27/ M, 4 years	9 weeks	focal, GTC, Mc, tonic, daily	spontaneous, no obvious triggers	infantile myoclonic	LTG, PB, TPM	LEV, prednisolone, VPA	drug-resistant, currently Mc and tonic seizures weekly	chr16:2546477G>A (p.Gly110Ser)/ chr16:2548254G>T (p.Leu333Phe)
28/ W, deceased	first day of life	focal (multiple types), spasms, frequency ranged from daily to monthly	not present	early-onset epileptic encephalopathy	deceased	CLZ, KD, LAC, LEV, MDZ, OXC, PB, PHT, steroids, TPM, VGB	drug-resistant epilepsy; died at 10 months	chr16:2550426dupA (p.His487Glnfs*71)/ chr16:2546462T>C (p.Cys105Arg)
29/ W, 27 years	8 years	GTC, initially sporadically, since the age of 15 years monthly	not present	generalised	LTG, OXC, PRM	LEV, TPM, VPA	drug-resistant	chr16:2546768C>T (p.Gln207*)/ chr16:2548381G>C (p.Gly376Arg)

30/ M, 7 years	1 week	focal (prolonged stiffening with eye and head deviation to one side), Mc, sGTC, daily	na	focal	na	CLB, PB, TPM, VPA	drug-resistant	chr16:2550350del (p.Glu462Serfs*61)/ chr16:2550350del (p.Glu462Serfs*61)
31/ W, 9 years	6 years	focal (gazing, cyanosis, cramping of left hand, tremor, atonia), GTC, sGTC, daily,	not present	unclassified	LTG, VPA	dexamethasone pulses, LEV, OXC, TPM, VGB	drug-resistant (weekly seizure frequency at last follow-up)	chr16:2546328G>A (p.Arg60Gln)/ chr16:2546851G>A (p.=); *in cis*

		initially in clusters						
32/ M, 13 years	3 years	absence, atonic, GTC, Mc, daily	spontaneous, unprovoked by triggers, previous reports of trigger by light but not documented	epilepsy with myoclonic atonic seizures	FBM, RFM, VNS	AZM, CLB, CLZ, ESM, KD, LEV, LTG, prednisolone, VPA	drug-resistant	16p13.3 duplication of 407 Kb including, but not interrupting *TBC1D24* (2,481,289-2,888,632)x3

The patients are ordered as follows: patients with normal cognitive function (#1a-b-2); patients with intellectual disability but no other DOORS features (#3-13); patients with intellectual disability and acral manifestations (#14-16); patients with intellectual disability and hearing impairment (#17a-b-18); patients with DOORS syndrome (#19-30); patients where a clear association of the clinical phenotype with changes in *TBC1D24* could not be established (#31-32).

*Age is on January 2015.

Videos 1-5 are in Supplemental data.

All mutations were validated by Sanger sequencing.

*The histidine (His) at position 336 is substituted by a glutamine (Gln), which is followed by a frameshifted protein sequence (fs) ending with a termination codon (Ter) after 12 non-native aminoacids.

? In patient #24, no mutation was identified in the second allele.

The following patients have been reported to some degree previously: #1a-h (de Falco *et al*., 2001), #4 (Muona *et al*., 2014), #5a-d (Corbett *et al*., 2010), 6a-b (Milh *et al*., 2010), #7a-c (Güven et al., 2013), #8a-b (Poulat et al., 2015), #11(Doummar et al, 2015), #12a-b(Cardon & Holder, 2015), #17a-b(Gnidovec Stražišar *et al*., 2015), #19-27 (Campeau *et al*., 2014a), #29 (Bilo *et al*., 2014).

AEDs=anti-epileptic drugs; na=data not available; AZM=acetazolamide; BDZ=benzodiazepine; CBZ=carbamazepine; CLB=clobazam; CLZ=clonazepam; CZP= clorazepate; DZP=diazepam; ESM=ethosuximide; FBM=felbamate; GBP=gabapentin; KD=ketogenic diet; LEV=levetiracetam; LTG=lamotrigine; MDZ=midazolam; OXC=oxcarbazepine; PB=phenobarbital; PGB=pregabalin; PIR=piracetam; PHT=phenytoin; PRM=primidone; RFM=rufinamide; STP=stiripentol; SUL=sulthiame; TPM=topiramate; VGB=vigabatrin; VNS= vagus nerve stimulator; VPA=sodium valproate; ZNS=zonisamide; W=woman; M=man; GTC= tonic-clonic seizures without apparent focal onset; sGTC=tonic-clonic seizures with apparent focal onset; Mc=myoclonic seizures; SE=status epilepticus.

Table e-2. EEG and neuroimaging results.

Patient number	EEG	Photoparoxysmal response	Neuroimaging
1a	normal (age 12 years)	not present	na
1b	generalized spike and wave; (age 14 years)	yes, Waltz type IV	normal (age 39 years)
1c	normal (age 28 years)	not present	normal (age 32 years)
1d	normal (age 18 years)	not present	normal (age 20 years)
1e	normal (age 37 years)	not present	normal (age 38 years)
1f	normal (age 18 years)	not present	na
1g	left temporal abnormalities (age 19 years)	not present	na
1h	normal background activity (age 15 years)	yes, Waltz type II-III	na
2	frontal abnormalities; one focal seizure without alteration of consciousness was recorded, with probable left hemispheric onset	not present	normal
3	normal	not present	normal

4	focal interictal epileptiform activity (at 5 months); focal epileptiform activity and bilaterally synchronous posterior quadrant fast spike and slow wave discharges, myoclonic jerks without correlated epileptiform discharges (at 16 months); generalised slowing and focal epileptiform discharges (at 2 years); focal slowing (at 3 and 5 years); intermittent bilaterally synchronous and independent spike-slow wave and polyspike-slow wave discharges, most prominent during early sleep (at 8 years); generalised epileptiform discharges increasing in sleep (at 9 years); generalised slowing and epileptiform discharges and frequent focal discharges, more frequent in sleep, no obvious EEG correlate with myoclonic	not present	cerebellar atrophy, right hippocampal sclerosis (at 14 years)

	jerks (at 11 years); interictal generalised spike wave and polyspike wave activity (at 15 years)		
5a	na	not present	na
5b	6-7 Hz low amplitude background; no paroxysmal activity (at 31 years)	not present	na
5c	irregular background 7-11 Hz; bitemporal high amplitude theta; no epileptiform activity (at 24 years)	not present	right hippocampal sclerosis; cerebellar atrophy (at 28 years)
5d	irregular background 6-9 Hz; some paroxysmal slow and sharp; no definite epileptiform activity (at 20 years)	not present	cerebellar atrophy (at 21 years)
6a	first interictal: slow background activity, with slow waves, rare paroxysmal activity; interictal stormy phase: multifocal spikes, slow background activity; ictal: focal theta discharge followed by delta large amplitude hemispheric discharge; interictal late	na	birth: no structural brain abnormality; six months old: moderate brain atrophy sparing the posterior fossa

	phase: absence of any organization, rare spikes in both temporal regions; myoclonic seizures associated with EEG abnormalities (frequency range 0.25-1 Hz)		
6b	first interictal: slow background activity, with slow waves, rare paroxysmal activity; interictal stormy phase: multifocal spikes low background activity, rare spindles; ictal: focal migrating discharges; interictal late phase: absence of any organization, rare spikes in both temporal regions; myoclonic seizures associated with EEG abnormalities (frequency range 0.25-1 Hz)	na	one month old: no structural brain abnormality; nine months old: global brain atrophy (grey matter) sparing the posterior fossa
7a	despite frequent seizures, several waking-sleep EEGs were within normal limits in the early months of the disease; a progressive slowing of the background activity and a gradual regression in the	not present	MRI at 6 months of age showed a diffuse delay in myelination and a thin corpus callosum; brain CT at 2 years revealed diffuse atrophy with dilatation of the cerebral ventricles, subarachnoid space, and brain sulci

	phasic elements of sleep became evident in later records, as periods of waking and sleep became less distinctive, as well as rare and isolated small spikes and multiple spikes that were predominantly in the frontal and central regions; myoclonic activity on EMG present throughout all EEGs, more prominent during the waking state, becoming more abundant over time		
7b	monotonous background activity composed of medium voltage and irregular slow waves within theta and delta ranges; amplitude was lower on the right hemisphere (left hemiplegia); there was ongoing phasic activity on the EMG channel, but no spikes were visible on the EEG channels; EMG was normal	not present	brain MRIs at the ages of 5 and 7 months normal; at 31 months, shortly following left-sided hemiplegia, diffuse atrophy with right predominance, especially of right hippocampus; brain SPECT at that age disclosed areas of hypoperfusion in right frontal lower and middle, right mesial and lateral temporal, and left mesial temporal areas

7c	early EEGs were reported to have generalized and multifocal multiple spikes as well as spike-waves discharges	not present	brain MRI at the ages of 14 and 37 months revealed progressive, diffuse cerebral and cerebellar atrophy with dilatation of the ventricles, sulci, and subarachnoid space
8a	always subnormal; at last follow-up (10 years), moderate and bilateral slowing of the background rhythm, 6-Hz theta and slow spikes at the vertex, as well as spike and wave activity in central regions	not present	at 11 months, normal brain MRI scan; at 6 years, after cardiac arrest, signal hyperintensity of the lentiform nuclei, moderate ventricular dilatation, and white matter rarefaction, on the T2 and FLAIR sequences
8b	mostly normal; 4 months: one recorded episode of myoclonia with very low amplitude spikes, mostly at the vertex, followed by hemispheric slow waves, predominating in central regions, intermixed with rare spikes and waves; 3.5 years: rare posterior slow spikes and increased amplitude of the posterior regions, during drowsiness	not present	at the age of 3 and 23 months, normal brain MRI

9	at 1.5 months, slow rhythms with transient burst-suppression pattern; later abnormal background activity with delta slow waves, associated with spike and wave activity, more often with occipital localisation; last record showed very attenuated background activity	not present	global brain atrophy, in particular atrophy of the caudate and lenticular nuclei (8 months)
10	delta rhythm with multifocal paroxysms, no clear correlate of myoclonus (at 8 months)	na	brain atrophy (at 8 and 14 months)
11	first EEG normal (7 months); later, interictal: isolated or bursts of spikes over bifrontal regions and slow posterior waves; ictal: no paroxysmal anomalies during myoclonic jerks, but video recording with EEG jerk-locked back-averaging was suggestive of cortical origin of myoclonus	na	at 3 and 7 years: progressive hemispheric (but not vermian) cerebellar atrophy with hyperintense signal of the cerebellar cortex and white matter, on T2 and FLAIR sequences

12a	no ictal activity captured; at 4 years, slightly slow occipital dominant rhythm of 7.5 Hz without focal findings	not present	normal (23 months)
12b	no ictal activity captured; at 10 years, dominant slow (7Hz) occipital rhythm, bilateral frontocentral rhythmic slow high-voltage activity	not present	normal (7 years)
13	normal at 4 months; very abnormal EEG at 6 months with paroxysmal epileptiform discharges, bouts of intense crying considered ictal on EEG	na	elevated glutamine peak (MRI); cerebellar atrophy, volume loss in left frontal lobe, enlargement of temporal horns suggestive of bilateral hippocampal atrophy (CT)
14	interictal multifocal independent spike waves (at 13 years)	na	right hippocampal sclerosis, bilateral cerebellar atrophy, hyperintense signal of the cerebellar cortex (at 9 years)
15	mostly normal ictal and interictal EEG, rare interictal epileptic discharges fronto-centrally; in focal status epilepticus, rare seizures patterns with repetitive fronto-central epileptic discharges	not present	normal

16	excessively rhythmic background activity over parasagittal electrodes and bursts of high-amplitude slow waves anteriorly	na	normal
17a	first interictal EEG recording (on first day of life) unremarkable despite frequent seizures; later ictal EEG showed generalized spike-wave and poly-spike discharges with fronto-central predominance; on several video-EEGs with myoclonic jerks, no clear epileptiform discharges were recorded; progression to burst-suppression before death	not present	unremarkable (age 1 month and 3 months)
17b	first interictal EEG recording (on first day of life) unremarkable despite frequent seizures; later generalized spike-wave and multiple spike-wave discharges with fronto-central predominance, slowing of the baseline activity and multifocal spikes	not present	prominent fronto-temporal atrophy with widening of the subarachnoid spaces and Sylvian fissures (1 month)

18	no paroxysmal abnormalities until the age of 7 years, then rare bilateral sharp-waves prevalent in the right temporo-occipital regions	na	wide cisterna magna, thin corpus callosum, mild signal hyperintensity in tegmental area (at 6 years)
19	left frontal dominant spike and wave	na	thin cerebellar cortex, hyperintense signal on T2-imaging, and myelination delay - white matter damage
20a	na	na	normal
20b	na	na	punctate foci of increased T2 signal in right frontal region; increased FLAIR signal around occipital horn
21	normal	not present	delayed myelination (at 33 months)
22	normal	not present	normal (at 2.5 years)
23a	normal EEG at the age of 10 years 7 months; poor organization, generalized slowing, frequent multifocal epileptiform activity at the age of 11 years	na	normal
23b	at the age of 10 months, severely abnormal background EEG, no evident epileptiform activity	na	normal initially; at the age of 17 months, after acute hypoxic episode, marked increase in ventricular and

			subarachnoid space, with decrease in grey and white matter, diffuse but with occipital predominance
24	normal interictal	na	normal
25	poorly organised background with slow waves (age 18 and 19 years)	na	hyperintense T2 signals in the cerebellar hemispheres, especially on the left
26	na	na	normal cranial ultrasound after birth
27	excess of irregular slow wave activity, often of higher amplitude on the right, with occasional runs of more prominent slow wave activity over the right hemisphere, more marked posteriorly; no epileptiform activity recorded (age 7 months)	not present	bilateral cerebral atrophy, particularly of the frontal lobes with widening of the Sylvian fissures, significantly delayed myelination and thin corpus callosum (age 7 months)
28	multifocal interictal epileptiform discharges (sharp waves, fast activity, spikes, polyspikes), more frequent in sleep, disorganised and slow	na	day 7: normal; day 56: increased T2 signal in left hippocampus, prominent extra-axial cerebrospinal fluid spaces

	background, between 6 weeks and 8 months of age; ictal: spasms and multiple types of focal seizures (right temporal, left temporal, midline-left central) were recorded		
29	dominant symmetrical monomorphic theta activity associated with rare symmetrical alpha activity and bilateral occipital paroxysmal spike-and-wave activity on eye closure (eye closure sensitivity)[e2] (age 23 years)	not present	mild hypoplasia of the cerebellar vermis (age 23 years)
30	interictal: widespread slow activity with frequent sharp waves over mid-parietal and right parietal regions and independent brief episodes of repetitive spikes over both fronto-central regions; ictal: repetitive spikes over the left temporal leads	na	normal antenatal brain MRI; evidence of trigonocephaly due to premature fusion of the metopic suture; mild lack of white matter bulk with delayed myelination (age 4 months)
31	at onset (6years) generalized slowing of background activity, reminiscent of encephalitis;	not present	normal (age 6 years and 7 years)

	follow-up: multifocal spike-waves, bi-frontal sharp-waves, generalized sharp-waves		
32	frequent epileptiform discharges, both hemispheres, frequently associated with eyelid jerks	photic stimulation between 4 and 8Hz provoked increasing duration of generalised spike and slow waves without clinical accompaniments	normal

na=data not available

Table e-3. Family history, facial and cranial features, acral manifestations and neurological examination.

Patient number	Family history of epilepsy*	DOORS syndrome	Facial and cranial features	Acral manifestations	Other findings on neurological examination
1a-h	all siblings or double first cousins	no	none	none	none
2	older brother with epilepsy	no	none	none	none
3	none	no	thick lips, everted lower lip	none	hypotonia, nystagmus, tongue fasciculations
4	consanguineous parents	no	none	none	ataxia, bilateral spasticity, supranuclear gaze palsy, dystonia, superimposed tremor, progressive deterioration of gait (see Supplementary Video 1)
5a	consanguineous parents, three	no	none	none	dysarthria and ataxia

	siblings (5b,c,d) and three first cousins				
5b	consanguineous parents, three siblings (5a,c,d) and three first cousins	no	none	none	dysarthria and ataxia, jerky pursuit eye movement
5c	consanguineous parents, three siblings (5a,b,d) and three first cousins	no	none	none	jerky pursuit eye movement and mild dysarthria
5d	consanguineous parents, three siblings (5a,b,c)	no	none	none	ataxia, jerky pursuit eye movement and increased jaw jerk

	and three first cousins				
6a	sister (6b)	no	acquired microcephaly	na	normal at the initial phase; loss of eye contact at 4 months, severe axial hypotonia, dystonic movements
6b	sister (6a)	no	acquired microcephaly	na	normal at the initial phase; axial hypotonia from 3 months, loss of eye contact at 4 months, severe hypotonia at 4.5 months old
7a	four similarly affected relatives, all born to consanguineous parents	no	na	na	dystonic episodes (both focal and axial) from the second year of life; right-sided spastic hemiparesis as a consequence of a prolonged unilateral seizure evident at 18 months; axial hypotonia and spasticity in the extremities with asymmetrical pyramidal signs were detected at the age of 21 months; at the age of

					23 months opisthotonic posturing was predominant, and repetitive startle responses occurred, without habituation
7b	four similarly affected relatives, all born to consanguineous parents	no	na	na	at the age of 31 months, left- sided epilepsia partialis continua started, associated with a permanent hemiplegia; two months later, she was inattentive and only partly responsive to verbal stimuli; there was spastic hemiparesis and dystonia on the left side
7c	four similarly affected relatives, all born to consanguineous parents	no	na	na	at the age of 3 years, totally nonreactive to all environmental stimuli; generalized hypotonia with pyramidal signs and minor erratic myoclonia; pupils dilated, with no light response

8a	brother (8b)	no	acquired microcephaly; bulbous nasal tip, flat nasal root	none	at seizure onset (24 hours of life), global hypertonia and incessant crying; at last follow-up (10 years), spastic tetraparesis, no verbal language
8b	brother (8a)	no	bulbous nasal tip, flat nasal root, down-slanting palpebral fissures	none	at 3.5 years, he could walk with help and say 10 words
9	none	no	bulbar nose, flat nasal root	none	pyramidal tract signs
10	no	no	microcephaly	none	none
11	none	no	narrow forehead, thin upper lip, marked philtrum	none	mild ataxia, clumsiness and tremor

12a	sister (12b)	no	upslanting palpebral fissures, increased intercanthal distance	none	none
12b	sister (12a)	no	none	none	none
13	none	no	microcephaly, widely spaced teeth	none	choreoatethoid movement, dystonia, hypotonia, spastic quadriplegia
14	none	no	triangular face	onychodystrophy in 4th and 5^{th} toenail	ataxia, hand tremor, progressive gait deterioration, no verbal language
15	paternal great uncle	no	relatively large round head, smaller right eye	small hands and short fat fingers	fine motor deficits, slight balance problems
16	none	no	small head, large ears, narrow palpebral fissures, prominent nasal bridge, protruding	clinodactyly of fourth and fifth toes	unable to walk

			nasal tip, prominent incisors, microcephaly		
17a	paternal grandmother, father's half-sister and brother (17b)	no	none	none	axial hypotonia, dyskinetic movements with upper limb dystonia
17b	paternal grandmother, father's half-sister and sister (17a)	no	acquired microcephaly	none	axial hypotonia, dyskinetic movements with upper limb dystonia
18	two cousins in paternal line with epilepsy	no	none	none	mild ataxia, severe hyperactivity

19	none	yes	prominent eyelashes, thick eyebrows, narrow palpebral fissures, thick lower vermilion	small nails all fingers and toes bilaterally, hand brachydactyly, triphalangeal thumb, long thumbs and halluces	none
20a	brother with DOORS syndrome (20b)	yes	large central incisors, widely spaced teeth, delayed eruption of permanent teeth, sagittal craniosynostosis	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly, calcaneal deformity	na
20b	brother with DOORS syndrome (20a)	yes	na	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly	na

21	na	yes	microcephaly	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly	at 5 years, could say a few words, use sign language, broad-based gait
22	one sibling with epilepsy	yes	low anterior hairline, thick hair, narrow forehead, prominent eyelashes, synophrys, thick eyebrows, broad nasal bridge, stained teeth	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly, triphalangeal thumb, long thumbs and halluces, hypoplastic distal phalanx, fetal finger pads	none
23a	brother with DOORS syndrome (23b)	yes	prominent nose and ears, widely spaced teeth, brachycephaly	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly	none

23b	brother with DOORS syndrome (23a)	yes	prominent nose and ears, widely spaced teeth	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly	hypotonia, absent eye contact
24	mother	yes	microcephaly, bilateral epicanthus, thin upper lip, reverse dental articulation, moderate mid face retraction	hand and foot abnormalities (involving nails and fingers/toes), hand brachydactyly	hypotonia, ataxia
25	none	yes	thick eyebrows, downward slant palpebral fissures, wide nasal bridge,	hand brachydactyly, small 5^{th} finger with hypoplastic distal phalanx, small nails all fingers and toes	none

			thick alae nasi, broad nasal bridge		
26	similarly affected brother, who died at the age of 9 months; consanguineous parents	yes	broad nasal tip, narrow palate, prominent and broad alveolar ridge, sparse eyebrows, long and prominent philtrum, thin upper lip, short lingual frenulum causing furrowing of the tongue, capillary hemangioma (glabella and at nose), mild bitemporal	hand brachydactyly, triphalangeal thumb	hypotonia

			narrowing, flat glabella, prominent occiput, parietal prominence, frontal bossing wide fontanelles		
27	none	yes	broad nasal tip, thick alae nasi, broad nasal bridge, broad and longish philtrum; thick lower vermillion, drooping lower lip, thick hair at 3 years and 7 months, high arched	hand brachydactyly, long halluces, small 5th finger, absent distal phalanx 5th finger; fetal finger pads; bilateral small nails all fingers/toes	hypotonia, congenital

			palate, asymmetric brachycephaly		
28	father	yes	premature closure of fontanelle, coarse facial features	absent toenail and end of the right second toe	hypotonia, nystagmoid eye movements
29	none	yes	frontal bossing, sunken nasal bridge, low-implanted ears	anonychia on the first and fifth finger of both hands and on all toes, presence of small dystrophic nails on the remaining fingers, absence of the distal phalanx of the fifth finger of both hands and hypoplasia of the distal	parkinsonism with onset at 21 years (mixed resting-postural tremor and rigidity of the right arm and right-side bradykinesia; mild dysdiadochokinesia and reduced tendon reflexes

				phalanxes of the remaining fingers	
30	one sibling affected prenatal (pregnancy terminated because of increased nuchal of 5.1mm at 12/40)	yes	arched, fine eyebrows; bilateral pre-auricular tags, narrow ear canals; broad nasal bridge; cleft lip and alveolus; gum hypertrophy, bifid uvula; metopic ridging, leading to appearance of trigonocephaly, microcephaly	absent distal phalanges of fingers from index to little in both hands, with hypoplastic distal phalanx of thumbs; in feet, absent distal phalanges all toes, with hypoplastic middle phalanges of little toes; stippling of tarsal bones; absent nails of all digits on hands and feet	initially hypotonia, then developed hypertonia; no speech; unable to walk

31	none	no	broad nasal bridge, high arched palate	small toenails	mild hypotonia, no focal neurological abnormality
32	none	no	none	na	none

na=data not available

*There were seven similarly affected siblings (one in family 2, three in family 5, one in family 7, one in family 22 and one in family 27) that did not have DNA analysis and were not included in our analysis, but we presume they had the same mutation(s) as their siblings.

We thus identified two more patients (one affected patient in family 7, and a sibling of patient #27) with myoclonic epilepsy and presumed *TBC1D24* mutation. Overall, with these additional patients, clonic or myoclonic seizures were present in 32/55 (58%) of patients.

S2. Multi-organ involvement.

The following non-DOORS patients had hearing loss: #17a (bilateral sensorineural hearing loss of 50 dB threshold), #17b (profound sensorineural deafness), #18 (bilateral hypoacusia). There was family history of hearing loss in patients #16 (brother) and #31 (hearing loss in maternal grandmother from childhood onwards, now nearly deaf).

There was visual impairment in patients: #3 (nystagmus), #4 (nystagmus, visual decline), #6a (pre-terminal cortical blindness), #7c (bilateral optic atrophy and macular degeneration), #10 (no ocular pursuit, severe encephalopathy), #15 (+2 dioptres in the left eye), #20a-b and 21 (all with myopia), #23a, #23b (post-asphyxia), #28 (cortical visual impairment), #30.

Renal anomalies were present in two patients: #21 (nephrocalcinosis) and #27 (hydronephrosis left kidney, prenatal diagnosis).

Skeletal anomalies, other than acral manifestation, were found in patients: #4 (bilateral cavovarus deformities; significant external tibial torsion; kyphotic posture), #6a-b, 13 and 30 (all with scoliosis).

Cardiac anomalies were present in patients: #8a (at 6 years and 10 months, cardiac arrest of unknown cause), #20b (double outlet right ventricle), #23b (at 10 months, cardiac arrest of unknown cause), #28 (patent foramen ovale).

Feeding difficulties were reported in patients: #4 (due to myoclonus), #6a-b (both with gastrostomy), #7a-c, #9 (enteral feeding), #10, #11 (only during episodes of facial myoclonus), #13 (naso-gastric tube from 3 years), #16, #17a (gastrostomy), #17b (naso-gastric tube), #20a-b, #23a-b, #28, #30 (naso-gastric tube from 4 months).

Other co-morbidities were described in patients; #4(severe iron deficiency, severe constipation, drooling, cataplexy, neonatal jaundice), #7a-c (repeated episodes of

common infections), #8a (self-injurious behaviour and sleep disturbance), #13 (paracentric inversion (1)(q42.13q44) on karyotype; Raynaud phenomenon in lower extremities), #16 (motor neuropathy), #19 (autism spectrum disorder), #24 (hyperactivity, sleep disorder), #25 (hypothyroidism), #27 (cryptorchidism, frequent respiratory tract infections), #28 (periods of irregular breathing in wake; irritability; poor sleep), #29 (supernumerary nipple; asymptomatic peripheral polyneuropathy, mixed axonal-demyelinating sensorimotor; psychotic symptoms).

Urine 2-oxoglutarate was elevated in patients #4, #15, #20b, #25, #26, #27, #28, #29.

Table e-4. Coding DNA position and ExAC allele count of the mutations identified in *TBC1D24*. Longest isoform, harbouring exon 3, is used (isoform 1, NM_001199107.1, hg19).

Patient number	**Origin**	**Alleles, DNA (protein)**	**ExAC allele count (version 0.3, accessed 18.10.15)**	**Reference**
1a-h	Italy	c.439G>C (p.Asp147His)/ c.1544C>T(Ala515Val)	not present/ 3/103786	de Falco et al., 2001
2	USA	c.919A>G(p.Asn307Asp)/ c.845C>G(p.Pro282Arg)	not present/ 21/120442	unreported
3	Germany	c.686T>C(p.Phe229Ser)/ c.1499C>T(p.Ala500Val)	not present/ not present	unreported
4	Afghanistan	c.1079G>T(p.Arg360Leu)/ c.1079G>T(p.Arg360Leu)	1 / 84934	Muona et al., 2014

5a-d	Arab-Israeli family	c.751T>C(p.Phe251Leu)/ c.751T>C(p.Phe251Leu)	not present	Corbett et al., 2010
6a-b	France	c.468C>A(p.Cys156*)/ c.686T>C (p.Phe229Ser)	not present/ not present	Milh et al., 2013
7a-c	Turkey	c.969_970delGT (p. Ser324Thrfs*3)/ c.969_970delGT (p. Ser324Thrfs*3)	not present	Güven et al., 2013
8a-b	France	c.457G>A p.Glu153Lys)/ c.457G>A p.Glu153Lys)	10 / 118188	Poulat et al., 2015
9	France	c.277C>T(p.Pro93Ser)/ c.1544C>T(p.Ala515Val)	not present/ 3 / 103786	unreported
10	Italy	c.32A>G(p.Asp11Gly)/ c.32A>G(p.Asp11Gly)	not present	unreported
11	France	c.809G>A(p.Arg270His)/ c.809G>A(p.Arg270His)	2 / 120532	Doummar et al, 2015

12a-b	USA	c.115G>C(p.Ala39Pro)/ c.1661_1667del(p.Gln 554Leu fs*12)	not present/ not present	Cardon & Holder, 2015
13	USA	c.731C>T(p.Ala244Val)/ c.731C>T(p.Ala244Val)	2 / 120538	unreported
14	Chile	c.679C>T(p.Arg227Trp)/ c.1544C>T(p.Ala515Val)	2 / 120400/ 3 / 103786	unreported
15	Germany	c.533C>G(p.Ser178Trp)/ c.680G>A(p.Arg227Gln)	not present/ 3 / 120392	unreported
16	Pakistan	c.957G>C(p.Lys319Asn)/ c.957G>C(p.Lys319Asn)	not present	unreported
17a-b	Slovenia	c.1008delT(p.His336Glnfs*12)/ c.32A>G(p.Asp11Gly)	not present/ not present	Gnidovec Stražišar et al., 2015
18	Italy	c.619C>T(p.Gln207*)/ c.1530A>G(splice-site)	3 / 120282/ 2 / 95480	unreported

19	Japan	c.724C>T(p.Arg242Cys)/ c.118C>T(p.Arg40Cys)	1 / 120504/ 2 / 119952	Campeau et al., 2014a
20a-b	USA	c.724C>T(p.Arg242Cys)/ c.724C>T(p.Arg242Cys)	1 / 120504	Campeau et al., 2014a
21	Germany	c.1008delT(p.His336Glnfs*12)/ c.1206+5G>A(spice-site)	not present/ not present	Campeau et al., 2014a
22	India	c.724C>T(p.Arg242Cys)/ c.724C>T(p.Arg242Cys)	1 / 120504	Campeau et al., 2014a
23a-b	Chile	c.58C>T(p.Gln20*)/ c.724C>T(p.Arg242Cys)	10 / 120240/ 1 / 120504	Campeau et al., 2014a
24	France	c.1008delT(p.His336Glnfs*12)/ not identified	not present	Campeau et al., 2014a
25	Brazil	c.724C>T(p.Arg242Cys)/ c.724C>T(p.Arg242Cys)	1 / 120504	Campeau et al., 2014a

26	Turkey	c.119G>T(p.Arg40Leu)/ c.119G>T(p.Arg40Leu)	not present	Campeau et al., 2014a
27	UK	c.328G>A(p.Gly110Ser)/ c.999G>T(p.Leu333Phe)	1 / 118678/ not present	Campeau et al., 2014a
28	Australia	c.1460dupA(p.His487Glnfs*71)/c.313T>C(p.Cys105Arg)	not present/ not present	unreported
29	Italy	c.619C>T(p.Gln207*)/ c.1126G>C(p.Gly376Arg)	3 / 120282/ not present	Bilo et al., 2014
30	Afghanistan	c.1384del(p.Glu462Serfs*61)/ c.1384del(p.Glu462Serfs*61)	not present	unreported
31	Germany	c.179G>A(p.Arg60Gln)/ c.702G>A(p.=); *in cis*	26 / 119704/ 23 / 120454	unreported

32	UK	16p13.3 duplication of 407 Kb including, but not interrupting, *TBC1D24* (2,481,289-2,888,632)x3	/	unreported

All mutations were validated by Sanger sequencing. Individuals with letters a-h after the number are part of families.

*The histidine (His) at position 336 is substituted by a glutamine (Gln), which is followed by a frameshifted protein sequence (fs) ending with a termination codon (Ter) after 12 non-native aminoacids.

? In patient #24, no mutation was identified in the second allele.

The ExAC frequency was not matched for ethnicity.

Patients #31 and #32 were not included in the final analysis because a clear association of the clinical phenotype with changes in *TBC1D24* could not be established.

S3. Genotype-phenotype correlation

Below, details are provided about the most recurrent mutations, presented in order of frequency.

The most common recurrent mutation was chr16:2546873C>T transition (hg19 numbering used), resulting in an arginine to cysteine (p.Arg242Cys) substitution in the TBC domain, present in seven individuals in five unrelated families of different national origins (Japan, USA, India, Chile and Brazil), all with DOORS syndrome.[e3] Four individuals were homozygous (#20a-b; #22; #25) for this missense variant, and three were compound heterozygous (#19; #23a-b). Three patients had different epilepsy types and 'adequate' seizure control; one patient (#25) was seizure-free on phenytoin and clobazam. Patients #19, #22 and #23b were drug-resistant. Patient #23a, after initial satisfactory seizure control on phenobarbital and clobazam, at the age of 11.5 years showed clinical deterioration with daily seizures and encephalopathy. He was treated with a combination of clobazam, lamotrigine and sodium valproate, with gradual resolution of the encephalopathy, after which he continued to have very frequent myoclonic movements (not rhythmic, rather erratic, sometimes ameliorating with postural changes) of the arms, sometimes also affecting the legs, without impairment of awareness (see Supplemental Video 2). The degree of intellectual disability varied from mild to severe. The interictal EEG showed different patterns: focal abnormalities (#19), normal (#22 and initially for #23a), poor organization, generalised slowing and multifocal abnormalities (later #23a), poor organization without epileptiform activity (#23b), and poor organization and slow waves (#25).

The frameshift mutation chr16:2548263delT (p.His336Glnfs*12) was identified as part of compound heterozygosity in four patients (#17a-b; #21 and #24; in the last patient no mutation was detected in the other allele). This mutation was not in a known functional domain. All these four patients had severe drug-resistant epilepsy with early seizure onset (ranging from 45 minutes after birth to three months of age) and multiple seizure types. Three have microcephaly and/or hypotonia. All have profound bilateral sensorineural hearing loss. These patients are two unrelated patients with DOORS syndrome and one sibling pair with early-onset epileptic encephalopathy and early death.

The heterozygous missense chr16:2550823C>T (p.Ala515Val) mutation, in the TLDc domain, was present in the eight members (#1a-h) of the Italian family with familial infantile myoclonic epilepsy, in a French patient with early-onset epileptic encephalopathy (#9) and in a Chilean patient (#14) with multifocal epilepsy. The epilepsy type and outcome were quite different, varying from myoclonic or tonic-clonic seizures, well-controlled on one or no antiepileptic medication (patients #1a-h), to myoclonic, clonic, focal or tonic-clonic seizures with focal onset, not responsive to antiepileptic treatment (patients #9 and #14). Patients #1a-h had no signs of cognitive impairment and a normal neurological examination, while patients #9 and #14 had severe to profound intellectual disability and neurological abnormalities. Functional experiments suggest that this variant causes a loss of function of TBC1D24 protein.[e4]

The heterozygous missense chr16:2546835T>C (p.Phe229Ser) mutation, in the TBC domain, was found in three individuals (#3; #6a-b), one with infantile myoclonic epilepsy and two siblings with familial epilepsy of infancy with migrating focal seizures (EIMFS). Functional

assay revealed loss of ARF6 binding.[e5] Clonic or myoclonic seizures, and hypotonia, were reported in all three patients. Neuroimaging was initially unremarkable, but in the two siblings later showed supratentorial brain atrophy in the two siblings. The two siblings had a more severe phenotype and early death.

The missense mutation chr16:2546181A>G (p.Asp11Gly) is not in a known functional domain, and was detected in three individuals (#10; #17a-b), all with early-onset epileptic encephalopathy. Of these, two were a sibling pair who had the mutation as part of compound heterozygosity in combination with the above-described frameshift mutation chr16:2548263delT, while the other individual was homozygous. All three patients had drug-resistant epilepsy, predominantly myoclonic seizures, and severe intellectual disability. EEG showed multifocal epileptiform activity. Brain MRI scan (all performed between 1 to 16 months of age) revealed brain atrophy in two patients (#10, #17b) and was normal in one other (#17a). Microcephaly was reported in patients #10 and #17b. The two siblings #17a and #17b both had profound hearing loss and died early.

Another recurrent heterozygous mutation in the TBC domain was the transition chr16:2546768C>T, leading to a premature termination codon, p.Gln207*. This mutation was found in two unrelated patients (#18; #29), one with generalised epilepsy, DOORS syndrome and parkinsonism[e6] and the other with infantile myoclonic epilepsy. Both patients were drug-resistant, with tonic-clonic seizures. Both had bilateral hypoacusia/deafness, but patient #18 did not have onychodystrophy or osteodystrophy and was therefore not classified as DOORS syndrome.

We report two additional patients (#31, #32) with variants in *TBC1D24* of uncertain significance for the clinical phenotype (Tables e1-4). Patient #31 has drug-resistant unclassified epilepsy and some facial features (broad nasal bridge, high arched palate): two single nucleotide variants of uncertain significance of *TBC1D24* were identified on the maternal allele (chr16:2546328G>A; chr16:2546851G>A, non-coding). Patient #32 has severe epilepsy with myoclonic atonic seizures and a heterozygous 16p13.3 duplication of 407 Kb including *TBC1D24,* identified by array-CGH. The clinical relevance of this copy number variant is uncertain, as 22 genes, including *TBC1D24*, reside in this interval. Nonetheless, it is worth noting here that overexpression of *TBC1D24* resulted in a marked increase in neurite length and arborisation in vitro,[e4,e7] indicating a possible adverse effect of gene duplication.

The homozygous mutation p.Glu153Lys, in the two siblings with familial infantile myoclonic epilepsy without evidence of hearing impairment (#8a-b), was recently identified as part of compound heterozygosity in a Moroccan family with recessive non-syndromic hearing loss.[e8] None of the other six *TBC1D24* mutations previously identified in families with non-syndromic hearing loss (p.Asp70Tyr; p.Ser178Leu; p.Arg214His; p.Lys266Asn; p.Arg293Pro; p.Val445Val.fs32)[e8-11] was present in our cohort. We note that two individuals in one of the families with recessive non-syndromic hearing loss due to p.Asp70Tyr mutation have a history of seizures, though the authors suggest that this association is coincidental[e10].

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