table e-1. Clinical information

Group 1 - severe cerebral atrophy									
Patient	**LBSL285**	**LBSL281**	**LBSL292**	**LBSL287**	**LBSL294**	**LBSL286**	**LBSL293**	**LBSL295**	**LBSL284**
Sex	f	f	f	f	f	m	f	f	f
Year of birth	2013	2007	2012	2013	2016	2017	2017	2009	2010
Consanguinity parents	-	-	-	-	-	-	-	-	-
Affected/unaffected siblings	0/1	0/2	1/0	0/0	0/0	0/3	1/1 (LBSL293)	1/1 (LBSL295)	0/2
Pregnancy/delivery	microcephaly, meconium-stained amniotic fluid	microcephaly	microcephaly	nl	microcephaly	reduced fetal movements	microcephaly	microcephaly	nl
Head circumference / height / weight at birth	<-2SD / nl / nl	<-2SD / nl / nl	<-2SD / <-2SD / <-2SD	? / ? / nl	<-2SD / nl / nl	nl / nl / nl	<-2SD / nl / nl	<-2SD / nl / nl	nl / nl / nl
Neonatal period	tachypnea due to metabolic acidosis	mild hypotonia	hypotonia, poor feeding	hypotonia	hypotonia, poor feeding	nl	hypotonia, irritability	hypotonia	nl
Initial motor development	none	delayed	none	delayed	none	none	none	none	delayed
Maximum motor milestone	trying to roll	rolling over	none	none	none	none	none	none	supported steps
Start of unsupported walking	never	never	never	never	never	never	never	never	never
Age at first signs at or after birth, other than microcephaly	at birth	3 we	at birth	3 mo	3 mo	1 mo	1 mo	at birth	1 mo
First signs at or after birth, other than microcephaly	irritability, exaggerated startle reflex	hypotonia	hypotonia, jitteriness, poor feeding	arrested development	hypotonia, poor feeding	no development	hypotonia, irritability	hypotonia, poor eye contact	poor eye contact, developmental delay
Course over time	deterioration	deterioration	stable	deterioration	deterioration	partial recovery	stable	deterioration	stable
Speed of overall deterioration	rapid	rapid	na	na	rapid	?	na	rapid	na
Factors provoking decline	-	-	-	-	-	-	-	-	-
Epileptic seizures	**+**	**+**	**+**	**+**	-	-	**+**	**+**	**+**
Tube feeding, since	-	**+,** 6 y	**+,** 10 mo	**+**, age?	**+,** 10 mo	-	-	**+,** 2y 6 mo	**+,** 5 y 9 mo
Age at latest examination	3 y	11 y	7 y	2 y 7 mo	2 y 8 mo	18 mo	2 y	10 y	8 y
Head circumference / height / weight	<-2SD / <-2SD / nl	<-2SD / <-2SD / <-2SD	<-2SD / <-2SD / <-2SD	<-2SD / nl / nl	<-2SD / nl / nl	<-2SD / nl / nl	<-2SD / <-2SD / nl	<-2SD / <-2SD / <-2SD	<-2SD / nl / nl
Eye contact	-	-	-	-	-	-	-	-	-
Language	no speech	no speech	no speech	no speech	no speech	no speech	no speech	no speech	no speech
Truncal hypotonia	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Spasticity	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**	**+**
Ataxia	ne	ne	ne	ne	ne	ne	ne	ne	ne
Walking, age at loss	-	-	-	-	-	-	-	-	steps with support
Death, if so age and cause	3 y 9 mo, resp. failure	alive	alive	alive	alive	alive	alive	alive	alive
Other observations					severe leukopenia, requiring periodic transfusions				

Group 2 - severe white matter abnormalities						
Patient	**LBSL288**	**LBSL291**	**LBSL289**	**LBSL290**	**LBSL297**	**LBSL280**
Sex	m	f	f	f	f	f
Year of birth	2009	2010	2011	2013	2012	2016
Consanguinity parents	-	?	-	-	-	-
Affected/unaffected siblings	0/2	0/0	0/1	1/4 (LBSL297)	1/4 (LBSL290)	0/1
Pregnancy/delivery	nl	nl	nl	nl	nl	premature, birth at 29 we gestation
Head circumference /	nl /	nl /	nl /	nl /	nl /	? /
height / weight at birth	nl / nl	nl / <-2SD	? / nl	nl / nl	nl / nl	? / nl
Neonatal period	nl	nl	nl	nl	nl	nl
Initial motor development	nl	nl	nl	nl	delayed	delayed
Maximum motor milestone	walking without support	walking without support	walking without support	supported sitting, grasping objects	supported sitting	hands to feet
Start of unsupported walking	23 mo	14 mo	11 mo	never	never	never
Age at first signs at or after birth, other than microcephaly	6 mo	3 mo	2 y 2 mo	9 mo	4 mo	2-3 mo
First signs at or after birth, other than microcephaly	mild spasticity, developmental delay	hypotonia, weakness	developmental delay	hypotonia	developmental delay	nystagmus, arrested development
Course over time	episodic with partial recovery	episodic deterioration	improvement	deterioration	deterioration	deterioration
Speed of overall deterioration	slow	?	slow	rapid	rapid	rapid
Factors provoking decline	head injury	fever	flu	fall from 1 m	fever	
Epileptic seizures	-	?	-	-	**+**, controlled	**+**, West syndrome
Tube feeding, since	-	?	-	-	-	+, 16 mo
Age at latest examination	8 y	22 mo	7 y 3 mo	2 y 1 mo	7 y 3 mo	1 y 11 mo
Head circumference /	>2SD /	nl /	nl /	nl /	<-2SD /	nl /
height / weight	nl / nl	nl / <-2SD	nl / nl	>2SD / <-2SD	<-2SD / <-2SD	<-2SD / >2SD
Eye contact	**+**	?	**+**	**+**	+	-
Language	nl	?	nl	minimal (max 15 words)	no speech	no speech
Truncal hypotonia	-	?	-	**+**	**+**	**+**
Spasticity	**+**	?	-	**+**	**+**	**+**
Ataxia	**+**	?	**+**	-	-	ne
Walking, age at loss	unsupported	-, 16 mo	unsupported	-	-	-
Death, if so age and cause	alive	?	alive	alive	alive	2 y, rapid neurological decline

f, female; m, male; nl, normal; we, weeks; mo, months; y, years; -, no/not present; +, yes/present; ?, unknown; na, not applicable; ne, not evaluable

table e-2. MRI characteristics

		Group 1 - severe cerebral atrophy															Group 2 - severe white matter abnormalities								
Patient, LBSL number		**285**		**281**		**292**	**287**		**294**	**286**	**293**		**295**		**284**		**288**		**291**	**289**	**290**	**297**	**280**		
Age at MRI (years)		0.01	0.4	0.6	1.2	0.01	0.6	1.2	0.3	0.6	ante	0.2	0.2	1.6	3.9		1.2	8.2	1.5	2.5	1.1	2.3	0.7	2.0	
Subdural effusions		+	+	-	-	+	-	-	+	+	-	+	-	-	-	5/9	-	-	-	-	-	-	-	-	0/6
Increased number of tortuous vessels		+	-	-	-	+	-	-	+	+	+	+	+	-	-	6/9	-	-	-	-	-	-	-	-	0/6
Cerebrum	Atrophy	+	+	+	+	+	+	+	+	+	+	+	+	+	+	9/9	-	-	-	-	-	-	-	-	0/6
	Hypoplasia	+	+	+	+	+	+	+	+	+	+	+	+	+	-	8/9	-	-	-	-	-	-	-	-	0/6
Cerebral cortex	SA	+	+	+	+	+	+	+	+	+	+	+	+	+	-	8/9	-	-	-	-	-	-	-	-	0/6
	Restricted diffusion	+	-	+	-	-	-	-	+	-	na	-	na	-	-	3/9	-	-	na	-	-	na	-	-	0/4
Cerebral WM	SA Periventricular rim	-	ne	-	ne	ne	-	-	+	+	ne	-	ne	ne	-	2/7	-	-	-	-	-	+	-	+	2/6
	SA Deep WM	-	ne	-	ne	ne	+	+	+	+	ne	+	ne	ne	-	4/7	+	+	+	+	+	+	+	+	6/6
	SA Subcortical rim	-	ne	-	ne	ne	-	+	+	+	ne	-	ne	ne	-	3/7	-	-	-	-	-	+	+	+	2/6
	Rarefied WM	-	ne	-	ne	-	na	-	-	+	-	-	-	-	na	1/8	+	+	+	+	+	+	+	+	6/6
	Cystic WM	-	ne	-	-	-	-	-	-	+	-	-	-	-	-	1/9	-	+	-	-	+	+	-	+	4/6
	Restricted diffusion	-	+	+	+	-	+	+	+	+	na	+	na	+	-	7/9	+	-	na	+	+	na	+	-	4/4
	Contrast enhancement	na	na	na	na	na	-	na	na	-	na	na	na	-	na	0/3	+	-	-	-	-	-	-	-	1/6
	Lactate in WM MRS	na	ne	na	ne	-	+	na	na	na	na	na	na	na	na	1/2	+	-	na	na	na	na	+	+	3/3
Corpus callosum	Atrophy	+	+	+	+	+	+	+	+	+	ne	+	+	+	+	9/9	-	-	-	-	-	+	-	-	1/6
	SA Splenium	ne	ne	ne	ne	ne	+	ne	ne	ne	ne	ne	ne	ne	-	1/2	+	+	+	+	+	ne	+	+	5/5
	SA Body	ne	ne	ne	ne	ne	-	ne	ne	ne	ne	ne	ne	ne	-	0/2	+	-	-	-	+	ne	+	+	3/5
	SA Genu	ne	ne	ne	ne	ne	-	ne	ne	ne	ne	ne	ne	ne	-	0/2	+	-	+	+	+	ne	+	+	5/5
	SA Inner blade	ne	ne	ne	ne	ne	-	ne	ne	ne	ne	ne	ne	ne	-	0/2	-	-	-	-	-	ne	-	+	1/5
	SA Middle blade	ne	ne	ne	ne	ne	+	ne	ne	ne	ne	ne	ne	ne	-	1/2	+	+	+	+	+	ne	+	+	5/5
	SA Outer blade	ne	ne	ne	ne	ne	-	ne	ne	ne	ne	ne	ne	ne	-	0/2	-	-	-	-	-	ne	-	+	1/5
Internal Capsule	SA Posterior limb	+	+	+	+	-	+	+	-	+	-	-	-	+	-	5/9	+	-	+	+	+	+	+	+	6/6
	SA Anterior limb	-	+	-	-	-	+	+	-	+	-	-	-	-	-	3/9	-	-	-	-	-	-	+	+	1/6
Globus pallidus	SA	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1/9	-	-	-	-	-	-	+	+	1/6
Thalamus	SA	-	-	-	-	-	-	-	+	-	-	-	-	-	-	1/9	-	-	-	-	-	-	-	+	1/6
Cerebellum	SA Hilum dentate n.	-	+	-	-	+	-	+	+	+	-	+	-	+	-	7/9	-	-	-	-	-	-	+	+	1/6
	SA Central WM	-	+	-	+	+	+	+	+	+	-	+	+	+	-	8/9	+	+	+	-	+	+	+	+	5/6
	SA Subcortical WM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/9	-	-	-	-	-	-	-	+	1/6
	SA Superior peduncle	-	+	-	-	-	-	+	+	+	-	-	-	-	-	4/9	-	-	-	-	-	-	+	+	1/6
	SA Middle peduncle	-	+	-	-	+	+	+	+	+	-	+	+	+	-	7/9	+	+	+	-	+	+	+	+	5/6
	SA Inferior peduncle	+	+	-	-	-	+	+	+	+	-	-	-	-	-	4/9	-	-	-	-	-	+	+	+	2/6
Pons	Atrophy	-	-	-	-	+	-	-	-	-	-	-	-	-	-	1/9	-	-	-	-	-	-	-	-	0/6
Pyramidal tract	SA Midbrain	+	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	+	+	1/6
	SA Pons	+	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	+	+	1/6
	SA Medulla	+	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	+	+	1/6
Medial lemniscus	SA Midbrain	-	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	-	+	1/6
	SA Pons	-	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	-	+	1/6
	SA Medulla	-	+	-	-	-	-	-	+	-	-	-	-	-	-	2/9	-	-	-	-	-	-	+	+	1/6
Brain stem	Restricted diffusion	+	+	-	-	-	+	+	+	+	na	-	na	-	-	4/9	-	-	na	-	-	na	-	-	0/4
Spinal cord	SA Dorsal columns	+	+	-	-	-	+	+	+	+	-	-	-	na	-	4/9	-	-	+	+	-	na	+	+	3/5
	SA Lat. corticosp. tracts	na	+	-	-	-	na	+	+	+	-	-	-	na	-	4/9	-	-	+	na	-	na	+	+	2/4

WM, white matter; SA, signal abnormality; MRS, Magnetic Resonance Spectroscopy; +, present; -, absent; ne, not evaluable; na, not assessed; ante, antenatal

table e-3. *DARS2* variants and mtAspRS domains and functional effects

Group 1 - severe cerebral atrophy													
LBSL nr	*DARS2* variant	Amino acid change	Protein Polyphen[a]	SIFT[b]	Russell[c]	ΔΔG[d]	Bacterial variant	ΔΔG[d]	Functional region[e]	Predicted effect	Yeast position	residue	functional effects[f]
285	c.172C>G[g]	p.(Arg58Gly)	0.992	0.30	-2	-0.10	Arg11Gly	1.22	tRNA^{Asp} binding	May impair tRNA binding	yLys42	non-conserved	mild
	c.742C>T	p.(Gln248*)	na	na	na	ne	ne	ne	ne	-	ne		ne
281	c.748C>G	p.(Leu250Val)	0.996	0	1	3.22	Leu207Val	2.99	Asp-AMP binding, homo-dimerization	Destabilizes structure near the catalytic and dimerization regions	yLeu232	conserved	severe
	c.1452C>G	p.(Phe484Leu)	1	0	0	2.09	Phe426Leu	0.92	Asp-AMP binding	Alters multiple interactions with surrounding residues near the catalytic region	yPhe487	conserved	severe
292	c.90C>A	p.(Tyr30*)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.785C>T	p.(Ala262Val)	0.997	0	0	-2.11	Ala219Val	-1.15	Asp-AMP binding, homo-dimerization	Causes important structural reorganization and effects on catalysis and homo-dimerization	yAla244	conserved	moderate
287	c.90C>A	p.(Tyr30*)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.469T>A	p.(Phe157Ile)	0.995	0.15	0	1.74	Phe108Ile	2.78	tRNA^{Asp} binding	May alter protein structure and interaction with tRNA	yAla136	non-conserved	ne
294	c.228-20_-12delinsCCCCCCCCG[g]	p.(Arg76Serfs*5)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.407C>A	p.(Thr136Lys)	1	0.01	-1	5.15	Thr87Lys	6.39	tRNA^{Asp} binding	May alter protein structure and interaction with tRNA	yAla115	region non-conserved	ne
286	c.492+2T>C[g]	p.(Met134_Lys165del)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.172C>G[g]	p.(Arg58Gly)	0.992	0.30	-2	-0.10	Arg11Gly	1.22	tRNA^{Asp} binding	May alter tRNA binding	yLys42	non-conserved	mild
293& 295	c.535C>T	p.(Arg179Cys)	1	0	-3	3.54	Arg136Cys	4.93	tRNA^{Asp} binding, homo-dimerization	Destabilizes homo-dimerization and tRNA binding regions	yArg157	conserved	severe
	c.1789A>G	p.(Ser597Gly)	1	0.08	0	1.56	Ser541Gly	3.37	tRNA^{Asp} binding, Asp-AMP binding	Significant structural changes at catalytic and tRNA binding regions	ySer617	conserved	severe
284	c.382G>C	p.(Gly128Arg)	1	0.02	-2	-0.32	ne	ne	tRNA^{Asp} binding	Alters interactions with tRNA	y-	region non-conserved	ne
	c.1441G>A	p.(Val481Met)	1	0.01	1	3.69	Val423Met	2.06	Asp-AMP binding	Causes significant structural reorganizations affecting the catalytic region	yIle484	non-conserved	severe

Group 2 - severe white matter abnormalities													
LBSL nr	*DARS2* variant	Amino acid change	Protein Polyphen[a]	SIFT[b]	Russell[c]	ΔΔG[d]	Bacterial variant	ΔΔG[d]	Functional region[e]	Predicted effect	Yeast position	residue	functional effects[f]
288	c.1762C>G	p.(Leu588Val)	1	0.04	1	3.56	Leu532Val	1.55	Asp-AMP binding	May affect the Asp-AMP binding without affecting main protein structure	yMet608	non-conserved	severe
	c.562C>T	p.(Arg188*)	na	na	na	ne	ne	ne	ne	-	ne		ne
291	c.228-20_21delTTinsC[g]	p.(Arg76Serfs*5)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.1817A>T	p.(Lys606Met)	1	0	-1	-0.35	Lys550Met	-1.00	homo-dimerization	May stabilize the homodimer	yLys626	conserved	mild
289	c.228-15C>A[g]	p.(Arg76Serfs*5)	na	na	na	ne	ne	ne	ne		ne		ne
	c.492+2T>C[g]	p.(Met134_Lys165del)	na	na	na	ne	ne	ne	ne		ne		ne
290& 297	c.1762C>G	p.(Leu588Val)	1	0.04	1	3.56	Leu532Val	1.55	Asp-AMP binding	May affect the binding of the ligand without affecting main protein structure	yMet608	non-conserved	severe
	c.1A>C	p.?	na	na	na	ne	ne	ne	ne	-	ne		ne
280	c.228-20_-16delinsCCCCG[g]	p.(Arg76Serfs*5)	na	na	na	ne	ne	ne	ne	-	ne		ne
	c.1273G>T (paternal)[g]	p.(Glu425*)	na	na	na	ne	ne	ne	ne		ne		ne
	c.536G>A (paternal)[g]	p.(Arg179His)	1[h]	0[h]	0[h]	6.23[h]	Arg136His	6.98[h]	tRNA^{Asp} binding, homo-dimerization[h]	Destabilizes homo-dimerization and tRNA binding region[h]	yArg157	conserved	ne

nr, number; na, not applicable; ne, not evaluated; [a] Scores indicate probably damaging.[31] [b] Score < 0.05 is predicted deleterious.[32] [c] A lower Russell score indicates a more disfavored amino acid change.[9] [d] Positive and negative ΔΔG values indicate, respectively, destabilization and stabilization of the local protein structure. [e] Functional regions: tRNA^{Asp} binding, Asp-AMP = aspartyl-adenylate, homodimerization.[f] Severe, oxidative growth and respiratory activity are both strongly reduced; moderate, oxidative growth and respiratory activity are both partially reduced; mild, respiratory activity only is mildly reduced. [g] Variants seen before in different combination, Van Berge et al.[4]
[h] Predictions for the missense variant of LBSL280 are not taken into account when comparing groups 1 and 2, as it is present on a null-allele. Conserved, residue is conserved between species; non-conserved, non-conserved residue in a conserved stretch; stretch non-conserved, the stretch is non-conserved;