

Supporting Information

S.1. ALS2 Known Mutations

S.1.1. IAHSP

Table 1. Mutations discovered in various studies.

Family	Origin	N.patients	Mutation cDNA	Mutation protein	Onset
1	Kuwait (1,2)	3	c.1425_1426delAG	p.E476Gfs*71	11 mo
2	Algeria (3,4)	3	c.3619delA	p.M1207*	1 yr
3	France (3,4)	1	c.1472_1481del	p.V491Gfs*3	1.5yr
4	Italy (3,4)	1	c.2537_2538delAT	p.N846Ifs*13	16 mo
5	Italy (3,4)	1	c.1007_1008delTA	p.I336Tfs*5	1.5 yr
6	Pakistan (5)	1	c.4721delT	p.V1574Afs*44	1.5 yr
7	Buchari Jewish (6)	2	c.2992C > T	p.R998*	14 mo
8	Turkey (7)	2	c.470G > A	p.C157Y	< 1 yr
9	The Netherlands (8)	2	c.2143C > T	p.Q715*	1 yr
10	Hungary (9)	2	[c.1821_1825dup]; [c.3529G > T]	[p.E609Afs*9]; [p.G1177*]	< 1 yr
11	Germany (10)	1	c.1999-2A > T	p.E724fs*32	1.5 yr
12	Italy (11)	1	c.3836 + 1G > T	p.K1234 fs*3	1 yr
13	Portugal (12)	1	[c.1427_1428del]; [c.145G > A]	[p.G477Afs*19]; [p.G49R]	3 yr
14	Saudi Arabia (13)	2	c.2761C > T	p.R921*	2 yr
15	Turkey (14)	4	c.2351 + 2C > A	Splicing site	1.5 yr
16	Cina (15)	2	[c.2351_2351 +17del]; [c.1310_1313del]	[p.G437Vfs*9]; [p.E724Gfs*26]	<1 yr
17	Pakistan (16)	6	c.2998delA	p.I1000*	1-2 yr
18	Pakistan (16)	5	c.194 T > C	p.F65S	1-2 yr
19	Pakistan (17)	2	c.1918C > T	p.R640*	1-2 yr
20	Iran (18)	11	c.1640+1G>A	Splicing Site	<1.5 yr
21	Turkey (19)	1	c.1718C>A	p.A573E	19 mo
22	Turkey (19)	1	c.1044C>G	p.Y348*	15 mo
23	Turkey (19)	2	c.3161T>C	p.L1054P	15 mo
24	Turkey (19)	1	c.4573dupG	p.V1525Gfs*17	16 mo
25	Turkey (19)	1	c.470G>A	p.C157Y	14 mo
26	Turkey (19)	2	c.1471+1G>A	Splicing site	12 mo
27	NA (20)	NA	c.4831C>T	p.R1611W	NA

yr, year(s); mo, months; NA, Not Available.

S1.2. JPLS

Table S2: Mutations discovered in various studies.

Family	Origin	N.patients	Mutation cDNA	Mutation protein	Onset
1	Italy (21)	1	c.1619G>A	p.G540E	2 yr
2	Cyprus (22)	3	c. 2980-2A>G	p.993fs*7	2 yr
3	Yemen (19)	3	c.275_276delAT	p.Y92Cfs*11	17 mo

yr, year(s); mo, months.

S1.3. JALS

Table S3: Mutations discovered in various studies.

Family	Origin	N.patients	Mutation cDNA	Mutation protein	Onset
1	Tunisia (2)	12	c.138delA	p.A46Afs*5	3-10 yr
2	Turkey (23)	1	c.553delA	p.T185Lfs*5	<2 yr
3	Japan (24)	2	c.3565delG	p.V1189Wfs*19	13 mo - 3 yr
4	Italy (25)	2	[c.299 G>T] [c.2580-2A>G]	p.S100I Splicing Site	3-6 yr
5	Bangladesh (26)	2	c.2002T>G	p.G668*	1-2 yr
6	Turkey (26)	1	c.4573dupG	p.V1525Gfs*17	2-3 yr
7	Pakistan (27)	4	c.3512+1C>A	splicing site	1 yr
8	Japan (28)	1	c.575C>T	p.P192L	<1 yr

yr, year(s); mo, months.

S2. IAHSP Clinical Features

S2.1. IAHSP

Table S4: Clinical features of family number 1, 2, 3.

Clinical features	fam 1	Pt 1	Pt2	Pt3	fam 2	Pt 1	Pt2	Pt3	fam 3	Pt 1
Loss of ambulation	2yr	NW	NW		1yr	1yr	1yr		4yr	
Upper limb involvement	+	+	+		<7yr	<7yr	<7yr		6yr	
Dysphagia	NA	NA	NA		+	+	+		13yr	
Dysarthria	3-7yr	5-6yr	+		13yr	13yr	13yr		4yr	
Ocular movements	N	N	N		N	N	N		Abn	

yr, year; N, normal; NT, never talking; NW, never walking; +, present; -, abstent; Abn, abnormal; NA, not available. (1,2), (3,4).

Table S5: Clinical features of family number 4, 5, 6, 7.

Clinical features	fam 4	Pt 1	fam 5	Pt 1	fam 6	Pt 1	fam 7	Pt 1	Pt2
Loss of ambulation	5yr		4yr		+		NW	NW	
Upper limb involvement	10yr		9yr		+		2yr	6yr	
Dysphagia	18yr		+		NA		NA	NA	
Dysarthria	10yr		9yr		+		3yr	3yr	
Ocular movements	Abn		Abn		NA		NA	NA	

yr, year; NW, never walking; +, present; Abn, abnormal; NA, not available. (3,4), (5), (6).

Table S6: Clinical features of family number 8, 9, 10.

Clinical features	fam 8	Pt 1	Pt2	fam 9	Pt 1	Pt2	fam 10	Pt 1	Pt 2
Loss of ambulation	NW	NW		NW	NW		4yr	NW	
Upper limb involvement	12yr	10yr		<4yr	+		6yr	-	
Dysphagia	-	+		5yr	4yr		13yr	-	
Dysarthria	+	+		5yr	4yr		4yr	5yr	
Ocular movements	NA	NA		NA	NA		Abn	NA	

yr, year; NW, never walking; +, present; -, abstent; Abn, abnormal; NA, not available. (7), (8), (9).

Table S7 Clinical features of family number 11, 12, 13, 14.

Clinical features	fam 11	Pt 1	fam 12	Pt 1	fam 13	Pt 1	fam 14	Pt 1	Pt 2
Loss of ambulation		NW		NW		NW		4yr	4yr
Upper limb involvement		<7yr		8yr		6yr		+	NA
Dysphagia		NA		8yr		14yr		<4yr	4yr
Dysarthria		<7yr		8yr		8yr		+	4yr
Ocular movements		N		N		Abn		NA	NA

yr, year; N, normal; NW, never walking; +, present; Abn, abnormal; NA, not available. (10), (11), (12), (13).

Table S8 Clinical features of family number 15, 16.

Clinical features	fam 15	Pt 1	Pt2	Pt3	Pt4	fam 16	Pt 1	Pt2
Loss of ambulation		+	NW	NW	NW		NW	NW
Upper limb involvement		+	+	+	+		4yr	+
Dysphagia		+	+	+	+		5yr	+
Dysarthria		+	NT	+	+		5yr	+
Ocular movements		NA	NA	N	N		NA	NA

yr, year; N, normal; NT, never talking; NW, never walking; +, present; NA, not available. (14), (15).

Table S9 Clinical features of family number 17.

Clinical features	fam 17	Pt 1	Pt2	Pt3	Pt4	Pt5	Pt6
Loss of ambulation		NW	NW	NW	NW	NW	NW
Upper limb involvement		+	+	+	+	+	+
Dysphagia		NA	NA	NA	NA	NA	NA
Dysarthria		+	+	+	+	+	+
Ocular movements		NA	NA	NA	NA	NA	NA

N, normal; NW, never walking; +, present; NA, not available. (16).

Table S10 Clinical features of family number 18, 19.

Clinical features	fam 18	Pt 1	Pt2	Pt3	Pt4	Pt5	fam 19	Pt 1	Pt2
Loss of ambulation		NW	NW	NW	NW	NW		NW	NW
Upper limb involvement		+	+	+	+	+		-	-
Dysphagia		5yr	5yr	5yr	5yr	5yr		-	-
Dysarthria		5yr	5yr	5yr	5yr	5yr		+	+
Ocular movements		N	N	N	N	N		N	N

yr, year; N, normal; NW, never walking; +, present; -, abstent. (16), (17).

Table S11 Clinical features of family number 20.

Clinical features	fam 20	Pt 1	Pt2	Pt3	Pt4	Pt5	Pt6	Pt7	Pt8	Pt9	Pt10	Pt11
Loss of ambulation		NW	NW	NW	1yr	NW	NW	NW	NW	NW	NW	NW
Upper limb involvement		+	+	+	+	+	+	+	+	+	+	+
Dysphagia		-	-	7yr	8yr	<2yr	6yr	6yr	6yr	6yr	6yr	6yr
Dysarthria		+	+	NT	7yr	NT	6yr	+	+	+	+	+
Ocular movements		N	N	N	N	N	N	N	N	N	N	N

yr, year; N, normal; NT, never talking; NW, never walking; +, present; -, abstent. (18)

Table S12 Clinical features of family number 21, 22, 23, 24.

Clinical features	fam 21	Pt 1	fam 22	Pt 1	Fam 23	Pt1	Pt 1	fam 24	Pt 1
Loss of ambulation		-		-		-	8yr		NW
Upper limb involvement		-		-		+	-		+
Dysphagia		-		-		-	-		+
Dysarthria		6yr		9yr		+	+		+
Ocular movements		NA		NA		NA	NA		+

yr, year; NW, never walking; +, present; -, absent; NA, not available. (19)

Table S13 Clinical features of family number 25, 26, 27.

Clinical features	fam 25	Pt 1	fam 26	Pt1	Pt 2
Loss of ambulation		-		NW	NW
Upper limb involvement		-		NA	NA
Dysphagia		+		NA	NA
Dysarthria		+		+	+
Ocular movements		NA		NA	NA

yr, year; NW, never walking; +, present; -, absent; NA, not available. (19), (12)

S2.2. JPLS

Table S14 Clinical features of family number 1, 2, 3.

Clinical features	fam 1	Pt1	fam 2	Pt 1	Pt2	Pt3	fam 3	Pt1	Pt2	Pt3
Loss of ambulation		19 yr		50 yr	2yr	-		10yr	3yr	NW
Upper limb involvement		<21yr		+	+	+		+	+	+
EMG		Abn		Abn	Abn	Abn		NA	NA	NA
Dysarthria		6 yr		NA	NA	NA		+	+	+
Ocular movements		<21yr		3 yr	2yr	2yr		NA	NA	NA

yr, year; NW, never walking; +, present; -, absent; Abn, abnormal; NA, not available. (21), (19), (22)

S2.3. JALS

Table S15 Clinical features of family number 1.

Clinical features	fam 1	Pt 1	Pt2	Pt3	Pt4	Pt5	Pt6	Pt7	Pt8	Pt9	Pt10	Pt11	Pt12
Loss of ambulation		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Upper limb involvement		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EMG		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dysarthria		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ocular movements		10yr	6yr	+	6yr	9yr	6yr	6yr	+	+	+	+	+

yr, year; +, present; NA, not available. (2)

Table S16 Clinical features of family number 2, 3, 4.

Clinical features	fam 2	Pt 1	fam 3	Pt1	Pt 2	fam 4	Pt1	Pt 2
Loss of ambulation		16yr		-	-		NA	NA
Upper limb involvement		12yr		NA	NA		+	+
EMG		Abn		N	N		NA	NA
Dysarthria		18yr		11yr	11yr		7yr	7yr
Ocular movements		15yr		11yr	11yr		+	+

yr, year; +, present; -, absent; Abn, abnormal; NA, not available. (23), (24), (25)

Table S17 Clinical features of family number 5, 6.

<i>Clinical features</i>	<i>fam 5</i>	Pt1	Pt 2	<i>fam 6</i>	Pt 2
<i>Loss of ambulation</i>		+	+		8yr
<i>Upper limb involvement</i>		+	+		+
<i>EMG</i>		NA	NA		NA
<i>Dysarthria</i>		+	+		4yr
<i>Ocular movements</i>		+	+		+

yr, year; +, present; NA, not available. (26)

Table S18 Clinical features of family number 7, 8.

<i>Clinical features</i>	<i>fam 7</i>	Pt1	Pt2	Pt3	Pt 4	<i>fam 8</i>	Pt1
<i>Loss of ambulation</i>		5yr	NW	NA	NA		NA
<i>Upper limb involvement</i>		+	+	NA	NA		NA
<i>EMG</i>		NA	NA	NA	NA		NA
<i>Dysarthria</i>		+	7yr	NA	NA		NA
<i>Ocular movements</i>		+	+	NA	NA		NA

yr, year; +, present; NA, not available; NW, never walking. (27), (28)

Bibliography

1. Lerman-Sagie T, Filiano J, Warwick Smith D, Korson M. Infantile Onset of Hereditary Ascending Spastic Paralysis With Bulbar Involvement. *J Child Neurol.* 1996 Jan;11(1):54–7.
2. Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, Devon RS, et al. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat Genet [Internet].* 2001 Oct;29(2):166–73. Available from: <http://www.nature.com/articles/ng1001-166>
3. Lesca G, Eymard-Pierre E, Santorelli FM, Cusmai R, Di Capua M, Valente EM, et al. Infantile ascending hereditary spastic paralysis (IAHSP). *Neurology [Internet].* 2003 Feb 25;60(4):674–82. Available from: <http://www.neurology.org/lookup/doi/10.1212/01.WNL.0000048207.28790.25>
4. Eymard-Pierre E, Lesca G, Dollet S, Santorelli FM, di Capua M, Bertini E, et al. Infantile-Onset Ascending Hereditary Spastic Paralysis Is Associated with Mutations in the Alsin Gene. *Am J Hum Genet.* 2002 Sep;71(3):518–27.
5. Gros-Louis F, Meijer IA, Hand CK, Dubé M-P, MacGregor DL, Seni M-H, et al. An ALS2 gene mutation causes hereditary spastic paraparesis in a Pakistani kindred. *Ann Neurol.* 2003 Jan;53(1):144–5.
6. Devon R, Helm J, Rouleau G, Leitner Y, Lerman-Sagie T, Lev D, et al. The first nonsense mutation in alsin results in a homogeneous phenotype of infantile-onset ascending spastic paraparesis with bulbar involvement in two siblings. *Clin Genet.* 2003 Sep;64(3):210–5.
7. Eymard-Pierre E, Yamanaka K, Haeussler M, Kress W, Gauthier-Barichard F, Combes P, et al. Novel missense mutation in ALS2 gene results in infantile ascending hereditary spastic paraparesis. *Ann Neurol.* 2006 Jun;59(6):976–80.
8. Verschuuren-Bemelmans CC, Winter P, Sival DA, Elting J-W, Brouwer OF, Müller U. Novel homozygous ALS2 nonsense mutation (p.Gln715X) in sibs with infantile-onset ascending

- spastic paralysis: the first cases from northwestern Europe. *Eur J Hum Genet.* 2008 Nov;16(11):1407–11.
9. Sztriha L, Panzeri C, Kálmánchey R, Szabó N, Endreffy E, Túri S, et al. First case of compound heterozygosity in *ALS2* gene in infantile-onset ascending spastic paraparesis with bulbar involvement. *Clin Genet.* 2008 Apr;73(6):591–3.
 10. Herzfeld T, Wolf N, Winter P, Hackstein H, Vater D, Müller U. Maternal uniparental heterodisomy with partial isodisomy of a chromosome 2 carrying a splice acceptor site mutation (IVS9–2A>T) in *ALS2* causes infantile-onset ascending spastic paraparesis (IAHSP). *Neurogenetics [Internet].* 2009 Feb 23;10(1):59. Available from: <http://link.springer.com/10.1007/s10048-008-0148-y>
 11. Racis L, Tessa A, Pugliatti M, Storti E, Agnelli V, Santorelli FM. Infantile-onset ascending hereditary spastic paraparesis: A case report and brief literature review. *Eur J Paediatr Neurol [Internet].* 2014 Mar;18(2):235–9. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1090379813001463>
 12. Flor-de-Lima F, Sampaio M, Nahavandi N, Fernandes S, Leão M. Alsin Related Disorders: Literature Review and Case Study with Novel Mutations. *Case Rep Genet [Internet].* 2014;2014:1–5. Available from: <http://www.hindawi.com/journals/crig/2014/691515/>
 13. Wakil SM, Ramzan K, Abuthuraya R, Hagos S, Al-Dossari H, Al-Omar R, et al. Infantile-onset ascending hereditary spastic paraparesis with bulbar involvement due to the novel *ALS2* mutation c.2761C > T. *Gene.* 2014 Feb;536(1):217–20.
 14. Koçak Eker H, Ünlü SE, Al-Salmi F, Crosby AH. A novel homozygous mutation in *ALS2* gene in four siblings with infantile-onset ascending hereditary spastic paraparesis. *Eur J Med Genet.* 2014 May;57(6):275–8.
 15. Xie F, Cen Z, Xiao J, Luo W. Novel compound heterozygous *ALS2* mutations in two Chinese siblings with infantile ascending hereditary spastic paraparesis. *Neurol Sci.* 2015 Jul;36(7):1279–80.
 16. Daud S, Kakar N, Goebel I, Hashmi AS, Yaqub T, Nürnberg G, et al. Identification of two novel *ALS2* mutations in infantile-onset ascending hereditary spastic paraparesis. *Amyotroph Lateral Scler Front Degener [Internet].* 2016 May 18;17(3–4):260–5. Available from: <https://www.tandfonline.com/doi/full/10.3109/21678421.2015.1125501>
 17. Tariq H, Mukhtar S, Naz S. A novel mutation in *ALS2* associated with severe and progressive infantile onset of spastic paraparesis. *J Neurogenet.* 2017 Apr;31(1–2):26–9.
 18. Helal M, Mazaheri N, Shalbafan B, Malamiri RA, Dilaver N, Buchert R, et al. Clinical presentation and natural history of infantile-onset ascending spastic paraparesis from three families with an *ALS2* founder variant. *Neurol Sci.* 2018;39(11):1917–25.
 19. Sprute R, Jergas H, Ölmez A, Alawbathani S, Karasoy H, Salimi Dafsari H, et al. Genotype–phenotype correlation in seven motor neuron disease families with novel <sc> *ALS2* </sc> mutations. *Am J Med Genet Part A.* 2020 Nov;ajmg.a.61951.

20. Sato K, Otomo A, Ueda MT, Hiratsuka Y, Suzuki-Utsunomiya K, Sugiyama J, et al. Altered oligomeric states in pathogenic ALS2 variants associated with juvenile motor neuron diseases cause loss of ALS2-mediated endosomal function. *J Biol Chem* [Internet]. 2018 Nov 2;293(44):17135–53. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.RA118.003849>
21. Panzeri C. The first ALS2 missense mutation associated with JPLS reveals new aspects of alsin biological function. *Brain*. 2006 Jul;129(7):1710–9.
22. Mintchev N, Zamba-Papanicolaou E, Kleopa KA, Christodoulou K. A novel ALS2 splice-site mutation in a Cypriot juvenile-onset primary lateral sclerosis family. *Neurology*. 2009 Jan;72(1):28–32.
23. Kress JA, Kühnlein P, Winter P, Ludolph AC, Kassubek J, Müller U, et al. Novel mutation in theALS2 gene in juvenile amyotrophic lateral sclerosis. *Ann Neurol*. 2005 Nov;58(5):800–3.
24. Shirakawa K, Suzuki H, Ito M, Kono S, Uchiyama T, Ohashi T, et al. NOVEL COMPOUND HETEROZYGOUS ALS2 MUTATIONS CAUSE JUVENILE AMYOTROPHIC LATERAL SCLEROSIS IN JAPAN. *Neurology*. 2009 Dec;73(24):2124–6.
25. Luigetti M, Lattante S, Conte A, Romano A, Zollino M, Marangi G, et al. A novel compound heterozygous ALS2 mutation in two Italian siblings with juvenile amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Front Degener* [Internet]. 2013 Sep 3;14(5–6):470–2. Available from: <http://www.tandfonline.com/doi/full/10.3109/21678421.2012.756036>
26. Sheerin U-M, Schneider SA, Carr L, Deuschl G, Hopfner F, Stamelou M, et al. ALS2 mutations: Juvenile amyotrophic lateral sclerosis and generalized dystonia. *Neurology*. 2014 Mar;82(12):1065–7.
27. Siddiqi S, Foo JN, Vu A, Azim S, Silver DL, Mansoor A, et al. A Novel Splice-Site Mutation in ALS2 Establishes the Diagnosis of Juvenile Amyotrophic Lateral Sclerosis in a Family with Early Onset Anarthria and Generalized Dystonias. Raoul C, editor. *PLoS One*. 2014 Dec;9(12):e113258.
28. Nishiyama A, Niihori T, Warita H, Izumi R, Akiyama T, Kato M, et al. Comprehensive targeted next-generation sequencing in Japanese familial amyotrophic lateral sclerosis. *Neurobiol Aging*. 2017 May;53:194.e1-194.e8.