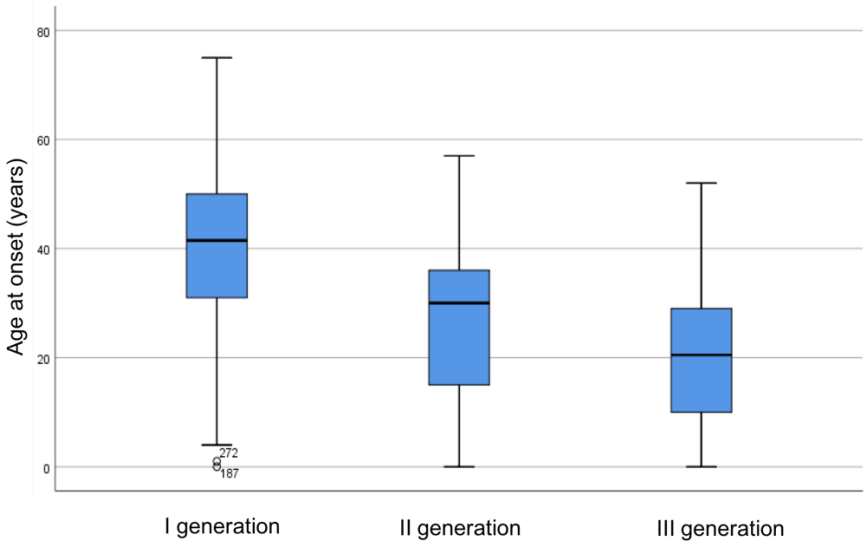
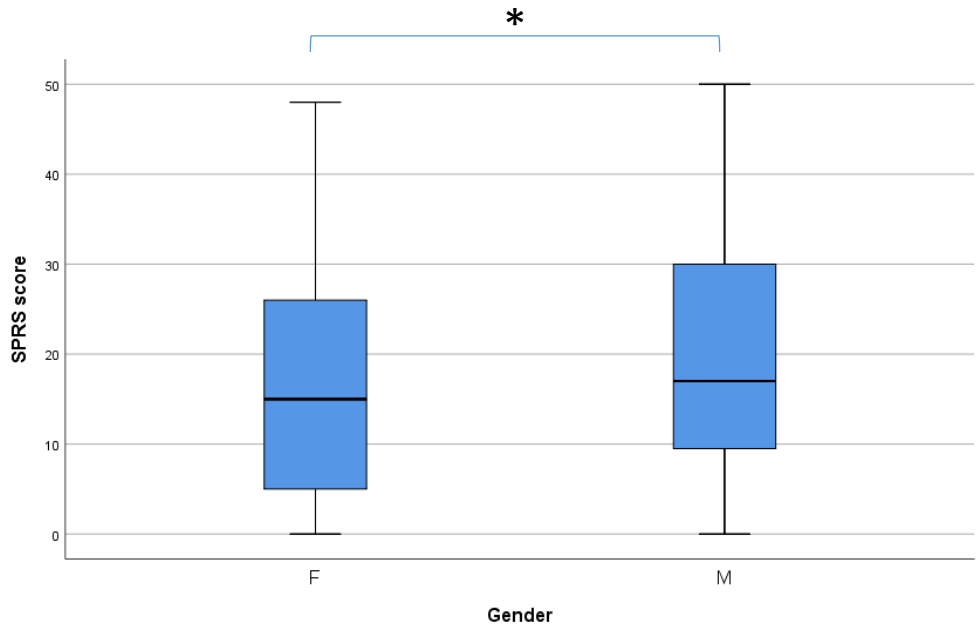


eFigure 1. Box plot showing the difference among the median age at onset in the three different subsequent generations.



*eFigure 2. Box plot showing the difference of SPRS score between male (19.67±12.58, n=199) and female (16.15±12.61, n=140) patients. *p=0.009.*

eTable 1. Comparison between probands and family members. Abbreviations: MWU, Mann-Whitney U test; SPRS, Spastic Paraplegia Rating Scale. Significant p-values are highlighted are in bold.

	Family members			**Probands**			**MWU test**
	mean	SD	n	mean	SD	n	**P**
Age at onset (years)	32.91	16.72	349	31.91	18.25	285	0.793
Age at examination (years)	44.35	19.51	366	46.51	17.48	265	**0.047**
Disease duration (years)	11.61	14.02	331	14.46	13.08	262	**<0.001**
Spatax disability scale	2.74	1.65	210	3.62	1.39	183	**<0.001**
SPRS	15.57	13.88	173	20.98	10.68	166	**<0.001**

eTable 2. Comparison between females and males. Abbreviations: LL, lover limbs; MEP: motor evoked potentials; SPRS, Spastic Paraplegia Rating Scale; SSEP, somato-sensory evoked potentials; TCC, thin corpus callosum; UL, upper limbs; WMH, white matter alterations. Significant p-values are highlighted are in bold.

	Females		***Males***		***χ2 (*Fisher's exact test)***	***Mann-Whitney U test***
	% (n)	mean±SD (n)	% (n)	mean±SD (n)	p	p
Age at onset		32.04±17.87 (256)		32.75±17.11 (378)		0.855
Age at examination		44.26±19.27 (263)		45.96±18.28 (368)		0.372
Disease duration		12.70±14.15 (237)		12.98±13.38 (356)		0.307
SPATAX score		3.01±1.69 (162)		3.25±1.52 (162)		0.199
SPRS score		16.15±12.61 (140)		19.67±12.58 (199)		**0.009**
Symptomatic	96.6 (280/290)		98.8% (396/401)		0.064	
Probands	41.8 (127/304)		45.1 (189/419)		0.373	
Family history positive	87.6 (262/299)		86.4 (357/413)		0.643	
LL hyperreflexia	98.1 (264/269)		98.9 (372/376)		0.501*	
Babinski sign	67.3 (179/266)		75.2 (282/375)		**0.028**	
Spastic gait	90.1 (246/273)		96.6 (373/386)		**0.001**	
Spasticity at rest	53.2 (142/267)		59.9 (224/374)		0.091	
LL proximal weakness	53.2 (142/267)		54.9 (203/370)		0.674	
Pes cavus	30.6 (79/258)		28.8 (102/354)		0.629	
Hypopallesthesia	29.4 (74/252)		29.8 (105/352)		0.902	
UL hyperreflexia	22.8 (61/267)		20.2 (74/367)		0.415	
Hoffman sign	9.4 (24/256)		9.7 (33/339)		0.883	
Saccadic intrusions	2.9 (7/245)		1.1 (4/351)		0.136*	
Nystagmus	2.7 (7/255)		5.5 (20/362)		0.096	
Urinary disturbances	40.7 (105/258)		45.9 (166/362)		0.202	
Urgency	28.3 (73/258)		29.0 (105/362)		0.847	
Frequency	1.9 (5/258)		5.0 (18/362)		0.054*	

Incontinence	13.6 (35/258)		12.7 (46/362)		0.754	
Dysuria	1.6 (4/258)		1.9 (7/362)		1.00*	
Brain MRI alteration	15.9 (33/208)		22.9 (69/301)		0.051	
Cerebral atrophy	5.3 (11/208)		10.0 (30/301)		0.057	
Cerebellar atrophy	1.4 (3/208)		1.3 (4/301)		1.00*	
TCC	1.4 (3/208)		1.3 (4/301)		1.00*	
WMH	6.3 (13/208)		6.3 (19/301)		0.97	
MRI spinal cord atrophy	4.5 (8/178)		2.1 (5/242)		0.167*	
Therapy	59.9 (121/202)		71.6 (204/285)		**0.007**	
Baclofen	51.5 (104/202)		58.2 (166/285)		0.139	
Eperisone	2.0 (4/202)		1.1 (3/285)		0.456*	
Cannabinoids	1.5 (3/202)		1.4 (4/285)		1.00*	
botulinum toxin	4.5 (9/202)		12.3 (35/285)		**0.003**	
Gabapentin/pregabalin	1.0 (2/202)		0.7 (2/285)		1.00*	
Benzodiazepines	0 (0/202)		0.7 (2/285)		0.514*	
Tizanidine	7.4 (15/202)		8.1 (23/285)		0.794	
MEP central	74.1 (140/189)		73.8 (200/271)		0.948	
SEP central	17.2 (27/157)		19.0 (38/200)		0.661	
Polineuropathy	4.6 (9/195)		8.1 (22/271)		0.134	
Complicated	25.3 (70/277)		27.6 (107/388)		0.507	
Intellectual disability	8.4 (21/251)		7.7 (27/349)		0.779	
Cognitive decline	5.2 (13/251)		7.4 (26/350)		0.270	

eTable 3. Comparison between early-onset (age at onset≤10 years) and late-onset cases (age at onset>10 years). Abbreviations: LL, lover limbs; MEP: motor evoked potentials; SPRS, Spastic Paraplegia Rating Scale; SSEP, somato-sensory evoked potentials; TCC, thin corpus callosum; UL, upper limbs; WMH, white matter alterations. Significant p-values are highlighted are in bold.

	Early-onset (≤10 years)		***Late-onset (>10 years)***		***χ2 (*Fisher's exact test)***	***Mann-Whitney U test***
	% (n)	mean±SD (n)	% (n)	mean±SD (n)	p	p
Age at examination		23.22±18.30 (93)		49.84±15.65 (500)		**<0.001**
Disease duration		19.59±18.12 (93)		11.62±12.31 (93)		**<0.001**
SPATAX score		3.75±1.50 (63)		3.31±1.44 (286)		0.055
SPRS		21.11±11.94 (57)		19.74±11.93 (241)		0.438
SPATAX progression index		0.26±0.27 (63)		0.38±0.49 (273)		**0.014**
SPRS progression index		1.18±1.18 (55)		1.87±2.13 (223)		**<0.001**
Male	53.0 (53/100)		60.9 (325/534)		0.141	
Family history	73.0 (73/100)		90.9 (482/530)		**<0.001**	
Probands	57.0 (57/100)		42.7 (228/534)		**0.008**	
LL hyperreflexia	100.0 (92/92)		99.0 (504/509)		0.34*	
Babinski sign	78.3 (72/92)		73.4 (372/507)		0.325	
Spastic gait	96.8 (92/95)		97.7 (507/519)		0.623	
Spasticity at rest	65.2 (60/92)		58.3 (295/506)		0.214	
LL proximal weakness	54.3 (50/92)		56.8 (285/502)		0.666	
Pes cavus	30.3 (27/89)		30.8 (149/484)		0.933	
Hypopallesthesia	26.6 (21/79)		31.7 (153/483)		0.364	
UL hyperreflexia	26.4 (24/91)		21.0 (105/499)		0.258	
Hoffman sign	12.0 (10/83)		9.8 (46/471)		0.525	
Saccadic intrusions	1.2 (1/85)		2.1 (10/473)		0.567*	
Nystagmus	5.7 (5/87)		4.5 (22/487)		0.618	
Urinary disturbances	36.4 (32/88)		46.0 (226/491)		0.093	
Cerebral atrophy	1.3 (1/79)		9.8 (39/396)		**0.012***	
Cerebellar atrophy	2.5 (2/79)		1.3 (5/196)		0.329*	

TCC	5.1 (4/79)		0.8 (3/396)		**0.004***	
WMH	10.1 (8/79)		5.6 (22/396)		0.127	
MRI spinal cord atrophy	5.6 (4/71)		2.8 (9/317)		0.237*	
Therapy	39.4 (28/71)		72.2 (291/403)		**<0.001**	
Baclofen	26.8 (19/71)		61.5 (248/403)		**<0.001**	
Eperisone	1.4 (1/71)		1.2 (5/403)		0.624*	
Cannabinoids	1.4 (1/71)		1.2 (5/403)		0.907*	
botulinum toxin	14.1 (10/71)		7.9 (32/403)		0.093	
Gabapentin/pregabalin	1.4 (1/71)		0.7 (3/403)		0.573*	
Benzodiazepines	1.4 (1/71)		0.2 (1/403)		0.164*	
Tizanidine	7.0 (5/71)		7.9 (32/403)		0.795	
MEP central	73.4 (47/64)		79.3 (288/363)		0.290	
SEP central	22.0 (13/59)		18.2 (49/269)		0.498	
Polineuropathy	4.7 (3/64)		7.6 (28/368)		0.403*	
Complicated	46.0 (46/100)		24.8 (131/529)		**<0.001**	
Intellectual disability	35.6 (32/90)		3.4 (16/469)		**<0.001**	
Cognitive decline	1.1 (1/87)		7.8 (37/473)		**0.023***	
Mutation type (missense)	56.7 (51/90)		40.7 (188/462)		**0.005**	
Mutation type (truncating)	43.3 (39/90)		59.3 (274/462)		**0.005**	

eTable 4. Comparison between short-disease duration (≤7 years) and long-disease duration cases (>7 years). Abbreviations: LL, lover limbs; MEP: motor evoked potentials; SPRS, Spastic Paraplegia Rating Scale; SSEP, somato-sensory evoked potentials; TCC, thin corpus callosum; UL, upper limbs; WMH, white matter alterations. Significant p-values are highlighted are in bold.

	Short-disease duration (≤7ys)		***Long-disease duration (>7ys)***		***χ2 (*Fisher's exact test)***	***Mann-Whitney U test***
	% (n)	mean±SD (n)	% (n)	mean±SD (n)	p	p
Age of onset		36.15±17.04 (299)		29.39±17.32 (294)		**<0.001**
Age at examination		38.98±17.20 (299)		52.47±17.87 (294)		**<0.001**
SPATAX score		2.71±1.20 (91)		3.61±1.46 (252)		**<0.001**
SPRS		12.40±8.83 (73)		22.63±11.81 (211)		**<0.001**
Male	58.2 (174/299)		61.9 (182/294)		0.356	
Family history	89.6 (266/297)		85.7 (251/293)		0.151	
Probands	34.8 (104/299)		53.7 (158/294)		**<0.001**	
LL hyperreflexia	99.7 (298/299)		98.6 (288/292)		0.212*	
Babinski sign	56.4 (168/298)		91.4 (267/292)		**<0.001**	
Spastic gait	97.0 (290/299)		98.0 (287/293)		0.456	
Spasticity at rest	38.9 (116/298)		79.0 (230/291)		**<0.001**	
LL proximal weakness	40.4 (120/297)		73.0 (211/289)		**<0.001**	
Pes cavus	26.8 (78/291)		33.6 (91/271)		0.080	
Hypopallesthesia	14.2 (41/289)		48.7 (129/265)		**<0.001**	
UL hyperreflexia	10.2 (30/295)		33.0 (95/288)		**<0.001**	
Hoffman sign	3.8 (11/289)		17.1 (44/258)		**<0.001**	
Saccadic intrusions	3.1 (9/290)		0.8 (2/260)		0.067*	
Nystagmus	4.5 (13/292)		5.1 (14/274)		0.714	
Urinary disturbances	28.9 (85/294)		60.9 (168/276)		**<0.001**	
Brain MRI alteration	10.6 (24/226)		30.2 (71/235)		**<0.001**	
Cerebral atrophy	2.2 (5/226)		14.0 (33/235)		**<0.001***	
Cerebellar atrophy	0 (0/226)		2.6 (6/235)		**0.030***	
TCC	0 (0/226)		2.6 (6/235)		**0.030***	

WMH	0.9 (2/226)		10.6 (25/235)		**<0.001***	
MRI spinal cord atrophy	0.5 (1/198)		6.1 (11/181)		**0.002**	
Therapy	64.7 (176/272)		70.7 (140/198)		0.171	
Baclofen	54.8 (149/272)		58.1 (115/198)		0.476	
Eperisone	0.7 (2/272)		2.0 (4/198)		0.245	
Cannabinoids	0 (0/272)		3.0 (6/198)		**0.005**	
botulinum toxin	2.2 (6/272)		18.2 (36/198)		**<0.001**	
Gabapentin/pregabalin	0.4 (1/272)		1.5 (3/198)		0.315*	
Benzodiazepines	0 (0/272)		1.0 (2/198)		0.177	
Tizanidine	8.1 (22/272)		7.6 (15/198)		0.839	
MEP central	92.3 (227/246)		59.9 (106/177)		**<0.001**	
SEP central	9.6 (18/187)		31.2 (43/138)		**<0.001**	
Polineuropathy	5.1 (11/217)		8.3 (17/204)		0.179	
Complicated	30.1 (90/299)		27.1 (79/291)		0.428	
Intellectual disability	7.6 (22/290)		9.9 (26/263)		0.367	
Cognitive decline	2.1 (6/290)		12.2 (32/262)		**<0.001**	
Mutation type (missense)	48.8 (137/281)		36.9 (90/244)		**0.006**	
Mutation type (truncating)	51.2 (144/281)		63.1 (154/244)		**0.006**	

eTable 5. Comparison between slowly, typically and rapidly-progressing cases based on tertiles of disease progression index values (lower and upper tertile 0.83 and 1.7 points/year, respectively). Abbreviations: LL, lover limbs; MEP: motor evoked potentials; SD, standard deviation; SPRS, Spastic Paraplegia Rating Scale; SSEP, somato-sensory evoked potentials; TCC, thin corpus callosum; UL, upper limbs; WMH, white matter alterations. Significant p-values are highlighted are in bold.

	Slowly-progressing cases (disease progression index ≤0.83, N=87)		**Typically-progressing cases (0.83<disease progression index ≤1.7, N=99)**		**Rapidly-progressing cases (disease progression index >1.7, N=92)**				
	mean±SD	n (%)	mean±SD	n (%)	mean±SD	n (%)	**Kruskal-Wallis test (p-value)**	**χ^2 (p-value) (*Fisher's exact test)**	**Significant pairwise comparisons (groups whose proportions differ significantly from each other at the 0.05 level)**
Age at onset	23.09 ± 17.52		32.94 ± 16.61		38,91 ± 18,88		**<0.0005**		slowly vs typically p<0.0005, slowly vs rapidly p<0.0005, typically vs rapidly p=0.023
Disease duration	51.01 ± 16.80		53.70 ± 18.57		46.93 ± 19.08		**<0.0005**		rapidly vs typically p=0.023
Spatax disability scale score	2.91 ± 1.33		3.86 ± 1.44		3.57 ± 1.48		**<0.0005**		slowly vs rapidly p=0.009, slowly vs typically p<0.0005
SPRS score	14.21 ± 9.84		24.08 ± 11.71		21.87 ± 11.73		**<0.0005**		slowly vs rapidly p<0.0005, slowly vs typically p<0.0005
Male		50 (57.5)		68 (68.7)		58 (63.0)		0,285	
Positive family history		78 (89.7)		83 (83.8)		74 (81.3)		0,283	
Probands		41 (47.1)		56 (56.6)		53 (57.6)		0,301	
LL hyperreflexia		85 (98.8)		98 (99.0)		92 (100.0)		0,601	
Babinski sign		75 (87.2)		93 (93.9)		85 (93.4)		0,195	
Spastic gait		85 (97.7)		97 (99.0)		90 (97.8)		0,767	
Spasticity at rest		74 (86.0)		90 (90.9)		74 (81.3)		0,159	
LL proximal weakness		51 (60.0)		78 (78.8)		58 (63.7)		**0,014**	slowly vs typically
Pes cavus		14 (17.7)		27 (29.0)		20 (22.5)		0,211	
Hypopallesthesia		27 (33.3)		43 (49.4)		42 (52.5)		**0,031**	rapidly vs slowly
UL hyperreflexia		23 (26.7)		32 (33.0)		31 (34.8)		0,483	

Hoffman sign		10 (13.5)		17 (18.7)		13 (16.5)		0,672	
Saccadic intrusions		0 (0.0)		1 (1.1)		2 (2.4)		0,381*	
Nystagmus		0 (0.0)		6 (6.3)		8 (9.2)		**0,026***	rapidly vs slowly
Urinary disturbances		47 (58.8)		72 (74.2)		53(59.6)		0,05	
Urgency		39 (48.8)		56 (57.7)		46 (51.7)		0,469	
Frequency		1 (1.3)		0 (0.0)		3 (3.4)		0,164*	
Incontinence		9 (11.3)		20 (20.6)		10 (11.2)		0,115	
Dysuria		0 (0.0)		2 (2.1)		3 (3.4)		0,269*	
Brain MRI alterations (at least one)		10 (14.7)		17 (21.8)		16 (25.0)		0,32	
Cerebral atrophy		3 (4.4)		7 (9.0)		3 (4.7)		0,436*	
Cerebellar atrophy		1 (1.5)		1 (1.3)		3 (4.7)		0,348*	
TCC		1 (1.5)		3 (3.8)		2 (3.1)		0,683*	
WMH		5 (7.4)		9 (11.5)		6 (9.4)		0,69*	
MRI spinal cord atrophy		5 (7.7)		1 (1.6)		3 (5.6)		0,265*	
Therapy (at least one)		21 (53.8)		45 (69.2)		45 (61.6)		0,283	
Baclofen		15 (38.5)		32 (49.2)		39 (53.4)		0,317	
Eperisone		1 (2.6)		3 (4.6)		2 (2.7)		0,789*	
Cannabinoids		4 (10.3)		1 (1.5)		0 (0.0)		**0,006***	rapidly vs slowly
Gabapentin/pregabalin		1 (2.6)		0 (0.0)		3 (4.1)		0,266*	
Benzodiazepines		1 (2.6)		0 (0.0)		1 (1.4)		0,473*	
Tizanidine		4 (10.3)		4 (6.2)		4 (5.5)		0,612*	
MEP central impaired		18 (34.0)		24 (43.6)		33 (62.3)		**0,012**	rapidly vs slowly
SEP central impaired		11 (22.4)		19 (35.8)		21 (43.8)		0,081	
Polineuropathy		3 (4.5)		4 (5.6)		7 (11.9)		0,235*	
Complicated forms		11 (12.6)		16 (16.2)		33 (36.7)		**<0.0005**	rapidly vs typically, rapidly vs slowly
Intellectual disability		8 (10.1)		5 (5.5)		9 (10.3)		0,429*	
Cognitive decline		4 (5.1)		4 (4.3)		7 (8.1)		0,531*	
Mutation type (missense)		22 (31.0)		26 (33.3)		35 (45.5)		0,141	
Mutation type (truncating)		49 (69.0)		52 (66.7)		42 (54.5)			

eTable 6. *SPAST* variants detected in our cohort. Novel variants are highlighted in bold.

Mutation	Amino acid change	Consequence	gnomAD	CADD
c.143G>A	**p.R48Q**	missense	0.00001077	23.2
c.167C>T	**p.P56L**		-	24.6
c.326C>G	p.P109R		0.000006517	13.77
c.404 A>G	p.E135G		0.00003230	24.4
c.583C>G	p.L195V		-	31
c.782C>T	**p.S261L**		0.00003580	22.8
c.870G>A	p.K290K		-	24.7
c.1031T>A	**p.I344K**		-	26.6
c.1039C>A	p.Q347K		-	26.4
c.1055A>C	p.Q352P		-	24.3
c.1082C>G	p.P361R		-	25.6
c.1092G>T	p.R364S		-	22.06
c.1094C>G	p.P365R		-	23.8
c.1105A>C	p.T369P		-	26.9
c.1108G>A	p.G370R		-	33
c.1121C>T	p.P374L		0.00003187	27.9
c.1130G>A	p.G377E		-	29.1
c.1139T>C	p.L380P		-	29.1
c.1151 C>G	p.P384R		-	28
c.1163A>G	p.K388R		-	32
c.1165A>G	p.T389A		-	31
c.1166C>A	**p.T389K**		-	28.8
c.1168A>G	p.M390V		-	25.7
c.1172T>C	p.L391P		-	32
c.1180G>C	**p.A394P**		-	29.3
c.1196C>T	p.S399L		-	31
c.1208T>C	p.F403S		-	29.7
c.1217 T>G	p.I406R		-	26.7
c.1216A>G	p.I406V		-	32
c.1219A>C	p.S407R		-	27.7
c.1223 C>G	p.A408G		-	29.8
c.1238C>T	p.S413L		-	33
c.1240A>G	**p.K414E**		-	28.6
c.1243T>C	**p.Y415H**		0.000003982	32
c.1246G>T	**p.V416L**		-	25.9
c.1276C>G	p.L426V		-	28.5
c.1280T>C	**p.F427S**		-	29.2
c. 1307 C>T	P.S436F		-	32
c.1322A>G	p.D441G		-	32
c.1330G>C	**p.D444H**		-	29.2

c.1361 A>G	p.E454G		-	32
c.1360G>A	p.E454K		-	30
c.1378C>T	p.R460C		-	32
c.1382T>C	p.L461P		-	28.9
c.1384 A>C	**p.K462Q**		-	27.2
c.1391A>T	**p.E464V**		-	32
c.1412G>A	p.G471D		-	28.8
c.1412 G>T	p.G471V		-	31
c.1445T>G	**p.V482G**		-	26.8
c.1454C>T	p.A485V		-	25.5
c.1490T>C	**p.L497P**		-	23.2
c.1494G>C	p.R498S		-	24.4
c.1495C>T	p.R499C		-	33
c.1496G>A	p.R499H		-	31
c.1508G>T	p.R503L		-	31
c.1507C>T	p.R503W		0.000003992	32
c.1536G>C	p.E512D		-	33
c.1540A>G	p.R514G		-	28.5
c.1550T>G	p.L517W		-	29.3
c.1601 T>C	p.L534P		-	30
c.1610 T>C	p.L537P		-	29.4
c.1613C>T	**p.A538V**		-	25.8
c.1625A>G	p.D542G		0.0004130	23.3
c.1637G>T	**p.G546V**		-	32
c.1640G>A	**p.S547N**		-	26.7
c.1640G>T	**p.S547I**		-	28.7
c.1642G>A	p.D548N		-	32
c.1649C>T	p.T550I		-	24.4
c.1675G>C	p.G559R		-	29.2
c.1679C>T	**p.P560L**		-	28.6
c.1685G>T	**p.R562L**		-	33
c.1685 G>A	p.R562Q		-	28.9
c.1709A>G	p.K570R		-	24.7
c.1763C>A	p.S588Y		-	25.8
c.1821G>T	p.W607C		-	26.2
c.1831T>G	**p.F611V**		-	24.5
c.1841C>T	p.T614I		-	22.6
c.*2G>T	undefined splicing effect	splicing	-	-
c.683-2A>G	undefined splicing effect		-	34
c.870+1G>T	undefined splicing effect		-	33
c.1004 +3A>C	p.G290Wfs*5		-	-
c.1173+1 G>A	undefined splicing effect		-	26.7
c.1245+1G>A	undefined splicing effect		-	34

c.1245+3insT	undefined splicing effect		-	-
c.1322-2A>G	undefined splicing effect		-	34
c.1322-2A>C	undefined splicing effect		-	34
c.1413+3delAAGT	undefined splicing effect		-	-
c.1413+1G>A	undefined splicing effect		-	34
c.1493+1G>A	undefined splicing effect		-	32
c.1536+1G>T	undefined splicing effect		-	34
c.1688-3C>G	undefined splicing effect		-	27
c.1728+1G>A	undefined splicing effect		-	34
c.308C>A	**p.S103***	nonsense	-	35
c.334G>T	p.E112*		-	36
c.364C>T	p.Q122*		-	37
c.373G>T	p. E125*		-	37
c.421C>T	p.Q141*		-	38
c.439G>T	p.E147*		-	38
c.499C>T	p.Q167*		-	37
c.513_514delTG	**p.C171***		-	-
c.577C>T	p.Q193*		-	41
c.734C>G	p.S245*		-	37
c.746C>G	p.S249*		-	37
c.766G>T	p.G256*		-	37
c.868A>T	p.K290*		-	46
c.910_914delCCTACinsTAGG	**p.P304***		-	-
c.1054C>T	p.Q352*		-	37
c.1244dupA	p.Y415*		-	-
c.1245C>G	p.Y415*		-	35
c.1291 C>T	p.R431*		-	40
c.1417C>T	p.Q473*		-	39
c.1555A>T	p.L519*		-	41
c.1634C>G	p.S545*		-	43
c.1684C>T	p.R562*		-	37
c.1702C>T	p.Q568*		-	46
c.1741C>T	p.R581*		-	42
c.1850A>C	p.*617Sext*46		-	6.496
c.78_126del49	p.P26Pfs*16	frameshift	-	-
c.164delA;	p.Y55fs*5		-	-
c.280delG	**p.A95Pfs***		-	-
c.316_335delinsCACC	**p.A106Hfs*49**		-	-
c.322_334delGTGCCGGGCGGCG	p.V108Rfs*49		-	-
c.349dupC	**p.R117Pfs*19**		-	-
c.450_453delGAAA	p.K150fs*8		-	-
c.458delT	p.I153Mfs*		-	-
c.557delT	**p.I153Mfs*8**		-	-
c.523dupA	**p.R175Kfs*5**		-	-

c.584_585delTA	p.L195Rfs*17		-	-
c.751_752insA	p.T251Nfs*14		-	-
c.864_865dupTC	**p.H289Lfs*26**		-	-
c.872_875delGTACnsCCA	p.G291Afs*24		-	-
c.906delT	p.T303Pfs*12		-	-
c.907delA	**p.T303Pfs*12**		-	-
c.908dupC	**p.T305Yfs***		-	-
c.959insG	**p.V320Gfs*11**		-	-
c.961dupG	p.D321Gfs*6		-	-
c.1034delC	**p.A345Vfs*18**		-	-
c.1053_1054insA	**p.Q352Tfs*15**		-	-
c.1082delC	**p.P361Lfs*2**		-	-
c.1215_1219delTATAA	p.N405Kfs*35		-	-
c.1264delT	**p.L422Wfs*1**		-	-
c.1271insGA	p.R424fs		-	-
c.1276_1277delCT	p.L426fs*		-	-
c.1308_1312delTATAA	**p.S436Sfs*4**		-	-
c.1340dupT	**p.L447Ffs*3**		-	-
c.1341insTC	**p.C448Sfs*14**		-	-
c.1352_1356delGAGAA	p.R451fs*		-	-
c.1457_1460delCTAA	p.T486Ifs*43		-	-
c.1535delA	p.E512Gfs*17		-	-
c.1591delC	**p.Q531Kfs*4**		-	-
c.1602delA (esone 4)	**p.A535Hfs*6**		-	-
c.1716delG	**p.M572Ifs*6**		-	-
c.1714_1715delAT	p.M572Vfs*3		-	-
c.1850A>T	**p.*617Lextfs46**		-	11.14
c.1-?_415+?del (exon1)	p.M1_A139del	deletion	-	-
c.1-?_682+?del (ex1-4)	p.M1_S227del		-	-
c.1-?_1851+?del (spg4)	p.M1_V616del		-	-
c.318_323del	**p.V108_P109del**		-	-
c.416-?_1851+?del (ex2-17)	p.A139_V616del		-	-
c.587-?_1851+?del (ex 4-17)	p.E196_V616del		-	-
c.683-?_1004+?del (ex5-6)	p.E228_N335del		-	-
c.1099_1170del	**p.L367_M400del**		-	-
c.1099−?_1245+?del (ex8-9)	p.L367_Y415del		-	-
c.1099-?_1493+?del (ex8-12)	p.L367_R499del		-	-
c.1099-?_1728+?del (ex8-16)	p.L367_E576del		-	-
c.1099-?_1851+?del (ex 8-17)	p.L367_V616del		-	-
c.1137_1142delACTCCT	p.L379_L380del		-	-
c.1174-?_1245+?del (ex 9-10)	**p.A392_T415del**		-	-
c.1246-?_1321+?del (ex 10)	p.V416_D441del		-	-
c.1246-?_1493+?del (ex 10-12)	p.V416_R498del		-	-
c.1246-?_1728+?del (ex 10-16)	p.V416_E576del		-	-

c.1246-?_1851+'del (ex 10-17)	p.V416_V616del		-	-
c.1251_1253delAGA	p.E418del		-	-
c.1354_1356delGAA	p.E452del		-	-
c.1543_1545delCTA	p.L515del		-	-
c.1688-?_1728+?del (ex 6)	p.E563_E576del		-	-
c.1620_1623DelGACT/InsC	p.M540_T541delinsI		-	-
c.1729-?_1851+?del (ex 17)	p.M577_V616del		-	-
c.1494-?_1851+?del (ex13-17)	p.R498_V616del		-	-
c.1492_1494delAGG	p.R498del		-	-
c.1617-?_1851+?del (ex 15-17)	p.R539_V616del		-	-
c.1_1851dup (spg4)	p.M1_V616dup	duplication	-	-
c.1246_1728dup (ex 10-16)	p.V416_E576dup		-	-
c.1617_1728dup (ex 15-16)	p.R539_E576dup		-	-

eTable 7. Comparison between missense mutations' and truncating mutations' (frameshift, nonsense, splice site, and small deletions) carriers. Abbreviations: LL, lover limbs; MEP: motor evoked potentials; SPRS, Spastic Paraplegia Rating Scale; SSEP, somato-sensory evoked potentials; TCC, thin corpus callosum; UL, upper limbs; WMH, white matter alterations. Significant p-values are highlighted are in bold.

	Missense mutations' carriers		***Truncating mutations' carriers***		***χ^2 (*Fisher's exact test)***	***Mann-Whitney U test***
	% (n)	mean±SD (n)	% (n)	mean±SD (n)	p	p
Age of onset		30.98±18.60 (239)		33.38±16.70 (313)		0.317
Age at examination		41.26±19.18 (237)		47.13±18.30 (315)		**0.001**
Disease duration		10.16±12.34 (227)		14.31±14.54 (298)		**<0.001**
SPATAX score		3.56±1.69 (114)		3.05±1.49 (204)		**0.009**
SPRS		20.04±13.39 (97)		18.14±12.11 (169)		0.335
Male	58.3 (151/259)		59.4 (211/355)		0.778	
Family history	85.8 (219/256)		87.1 (304/349)		0.580	
Symptomatic	98.0 (251/256)		98.8 (331/335)		0.511*	
Probands	44.4 (115/259)		42.8 (152/355)		0.696	
LL hyperreflexia	100 (239/239)		98.1 (317/323)		**0.041***	
Babinski sign	59.1 (140/237)		78.6 (253/322)		**<0.001**	
Spastic gait	95.6 (237/248)		92.6 (302/326)		0.147	
Spasticity at rest	44.5 (106/238)		61.5 (198/322)		**<0.001**	
LL proximal weakness	44.1 (105/238)		59.6 (190/319)		**<0.001**	
Pes cavus	23.1 (54/234)		36.1 (110/305)		**0.001**	
Hypopallesthesia	24.4 (54/221)		32.1 (99/308)		0.054	
UL hyperreflexia	19.4 (46/237)		21.2 (67/316)		0.605	
Hoffman sign	7.0 (16/228)		8.2 (25/305)		0.613	
Saccadic intrusions	1.3 (3/229)		2.0 (6/296)		0.738*	
Nystagmus	2.6 (6/233)		5.3 (16/303)		0.118	
Urinary disturbances	31.3 (73/233)		50.0 (157/314)		**<0.001**	
Urgency	20.6 (48/233)		33.1 (104/314)		**0.001**	

Frequency	2.1 (5/233)		5.7 (18/314)		0.051*	
Incontinence	9.4 (22/233)		14.3 (45/314)		0.085	
Dysuria	1.3 (3/233)		1.9 (6/314)		0.739*	
Brain MRI alteration	19.7 (35/178)		20.5 (53/259)		0.838	
Cerebral atrophy	3.4 (6/178)		11.2 (29/259)		**0.003**	
Cerebellar atrophy	1.1 (2/178)		0.8 (2/259)		1.00*	
TCC	2.2 (4/178)		0.8 (2/259)		0.230*	
WMH	4.5 (8/178)		6.2 (16/259)		0.448	
MRI spinal cord atrophy	1.9 (3/162)		3.0 (6/202)		0.737*	
Therapy	71.3 (144/202)		66.0 (157/238)		0.232	
Baclofen	57.9 (117/202)		57.1 (136/238)		0.869	
Eperisone	1.5 (3/202)		1.3 (3/238)		1.00*	
Cannabinoids	0 (0/202)		2.9 (7/238)		**0.017***	
botulinum toxin	7.4 (15/202)		10.1 (24/238)		0.328	
Gabapentin/pregabalin	1.0 (2/202)		0.4 (1/238)		0.596*	
Benzodiazepines	1.0 (2/202)		0 (0/238)		0.210*	
Tizanidine	9.4 (19/202)		6.7 (16/238)		0.377	
MEP central	81.7 (152/186)		75.6 (167/221)		0.148	
SEP central	16.2 (22/136)		18.8 (32/170)		0.651	
Polineuropathy	1.1 (2/177)		11.1 (25/226)		**<0.001***	
Complicated	25.9 (64/247)		31.6 (102/323)		0.140	
Intellectual disability	14.5 (34/234)		4.4 (13/297)		**<0.001**	
Cognitive decline	2.6 (6/232)		9.7 (29/299)		**0.001**	

eTable 8. Binomial logistic regression to identify variables associated with Spastic Paraplegia Rating Scale (SPRS) score≥26.

	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I. for EXP (B) Lower	95% C.I. for EXP (B) Upper
Disease duration	0.065	0.010	39.779	1	**0.000**	1.067	1.046	1.089
Constant	-2.139	0.262	66.848	1	0.000	0.118		