Supplementary Table 1: Clinical details of FAHN/SPG35 cases including neurophysiology and MRI findings

Family-#	F1	F2	F3-1	F3-2	F4
ethnicity	Serbian	German	Cypriot	Cypriot	Turkish
sex	m	f	m	f	m
consanguinity	-	-	+	+	+
mutation	W176* (hom) **pat. UPD**	G303V / P323L	P148L (hom)	P148L (hom)	A54Tfs*42 (hom)
age at onset [y]	3	3	4	4	3
age at wheelchair use [y]	10	6	i.m.	i.m.	15
age at exam [y]	12	23	5	10	19
cognitive deficits	moderate to severe	moderate to severe	i.m.	i.m.	severe
progression CD	+	+	i.m.	i.m.	+
optic atrophy	-	+	+	+	i.m.
saccadic smooth pursuit	+	+	+	+	+
gaze evoked nystagmus	-	+	i.m.	i.m.	+
slow horizontal prosaccades	+	+	i.m.	i.m.	+
other oculom. disturb.	exotropia	exotropia	vertical gaze palsy	i.m.	exotropia
dysarthria	pseudob.	cerebellar	+	i.m.	pseudob. & cerebellar
dysphagia	+	+	+	i.m.	+
spasticity (UL/LL)	+ / +	+ / +	i.m.	i.m. / +	+ / +
weakness (UL/LL/T)	+/+/+	-/+/+	i.m.	-/ + / -	- / + / i.m.
muscle atrophy	none	LL	i.m.	i.m.	LL
incr. DTR	UL/LL	UL/LL	i.m.	LL	UL/LL
extensor plantar response	+	+	i.m.	+	+
limb ataxia	UL/LL	UL/LL	LL	i.m.	UL / LL n.a. (weakness)
extrapyramidal involvement	rigidity	rigidity, gen. dystonia	i.m.	i.m.	i.m.
sensory deficits	none	temp. and pinprick.	i.m.	i.m.	LL distal pallhyp.
urinary dysfunction	urge	voiding	i.m.	i.m.	urge, incont.
rectal dysfunction	none	none	i.m.	i.m.	none
others	none	epilepsy	none	none	abnormal outer ears
Neurophysiology	F1	F2	F3-1	F3-2	F4
MEPs (UL/LL)	n.d. / absent	normal/normal	n.d.	n.d.	absent/absent
SEPs (UL/LL)	n.d.	normal/normal	n.d.	n.d.	n.d.
VEPs	n.d.	normal	normal	n.d.	n.d.
BAEPs	n.d.	normal	n.d.	n.d.	n.d.
EMG	normal	normal	normal	n.d.	n.d.
nerve conduction studies	sensory-motor PNP	normal	n.d.	n.d.	sensory-motor PNP
MRI	F1	F2	F3-1	F3-2	F4
year of imaging / age	2012 / 11y	2011 / 11y	no images	no images	2013 / 19y
thin corpus callosum	+ (global)	+ (global)			+ (global)
cerebral atrophy	mild parietal	global, most prom. parietal			-
cerebellar atrophy (hemispheres / vermis)	+ / mild	+ / +			+ / +
midbrain / pons atrophy (Am/Ap in mm^2)	- (115) / + (395)	- (92) / + (304)			- (92) / + (301)
white matter changes (WMC)	periventr., most prominent parieto-occ.	periventr.			periventr., most prominent parieto-occ.
globus pallidum hypointensity (T2 / T2*)	mild / n.d.	+ / -			- / n.d.

Family-#	F5	F6	F7	F8	F9
ethnicity	Kosovar	Italian	German	German	German
sex	f	f	m	m	m
consanguinity	-	i.m.	-	-	-
mutation	Y170* (hom)	T207M / R235H	R235H / C287R	P44Q (hom) **pat. UPD**	E78K / H319R
age at onset [y]	4	i.m.	2,5	4	20
age at wheelchair use [y]	n.a.	i.m.	6	7	n.a.
age at exam [y]	7	i.m.	10	9	29
cognitive deficits	mild to moderate	i.m.	started mild	no deficits	mild
progression CD	+	i.m.	+ (at 5y)	-	-
optic atrophy	-	i.m.	i.m.	-	i.m.
saccadic smooth pursuit	+	i.m.	+	+	+
gaze evoked nystagmus	-	i.m.	-	+	-
slow horizontal prosaccades	-	i.m.	+	+	i.m.
other oculom. disturb.	none	i.m.	exotropia	exotropia	exotropia
dysarthria	cerebellar	i.m.	anarthria	pseudob.	cerebellar > pseudob.
dysphagia	-	i.m.	+	+	-
spasticity (UL/LL)	-/+	i.m.	+/+	+ / +	+ / +
weakness (UL/LL/T)	-/+/+	i.m.	-/+/+	-/+/+	-/ - / -
muscle atrophy	none	i.m.	LL	none	none
incr. DTR	UL/LL	i.m.	UL/LL	UL/LL	UL/LL
extensor plantar response	+	i.m.	+	+	+
limb ataxia	none	i.m.	UL/LL	UL/LL n.a. (weakness)	UL/LL
extrapyramidal involvement	resting tremor	i.m.	gen. dystonia	rigidity	rigidity
sensory deficits	touch	i.m.	none	temp. and pinprick.	none
urinary dysfunction	urge, incont.	i.m.	voiding	incont., voiding	urge, voiding
rectal dysfunction	none	i.m.	none	none	none
others	hyperopia (6dpt) & behavioural and psychiatric symptoms	none	epilepsy occulomotor apraxia	regular school, no formal IQ testing.	none
Neurophysiology	F5	F6	F7	F8	F9
MEPs (UL/LL)	n.d.	n.d.	normal / n.d.	normal/absent	normal/absent
SEPs (UL/LL)	n.d.	n.d.	n.d. / normal	n.d./ prol.	n.d. / normal
VEPs	n.d.	n.d.	prol.	prol.	n.d.
BAEPs	n.d.	n.d.	n.d.	normal	n.d.
EMG	n.d.	n.d.	n.d.	n.d.	n.d.
Nerve conduction studies	normal	n.d.	normal	normal	normal
MRI	F5	F6	F7	F8	F9
year of imaging / age	2014 / 7y	no images	2011 / 7y	2014 / 9y	2008 / 27y
thin corpus callosum	+ (dorsal body)		+ (dorsal body)	-	mild parietal
cerebral atrophy	-		mild parietal	-	mild parietal
cerebellar atrophy (hemispheres /vermis)	mild / mild		+ / +	mild / -	+ / +
midbrain / pons atrophy (Am/Ap in mm^2)	- (134) / + (349)		- (94) / + (339)	- (127) / -(440)	- (128) / + (380)
white matter changes	mild periventr., most prom. parieto-occ.		mild periventr., most prom. parieto-occ.	periventr., most prom. parieto-occ.	periventr., most prom. parieto-occ.
globus pallidum hypointensity (T2 / T2*)	+ / n.d.		mild / -	mild / -	- / n.d.

Family-#	F10	F11	F12-1	F12-2	F12-3
ethnicity	German	Belgian	Belgian	Belgian	Belgian
sex	f	f	f	f	f
consanguinity	-	-	-	-	-
mutation	G45W (hom) **seg. mat. UPD**	A54Tfs*42/V168Gfs*72	A8Pfs*91 (hom)	A8Pfs*91 (hom)	A8Pfs*91 (hom)
age at onset [y]	4,5	3	4,5	4,5	4,5
age at wheelchair use [y]	7	10	9	7	-
age at exam [y]	7	34	9	7	4,5
cognitive deficits	mild	moderate to severe	mild	mild	-
progression CD	+	+ (at 5y)	+	+	-
optic atrophy	-	-	-	i.m.	i.m.
saccadic smooth pursuit	+	+	i.m.	-	i.m.
gaze evoked nystagmus	+	-	-	-	i.m.
slow horizontal prosaccades	-	n.a. at 34y	+	-	-
other oculom. disturb.	exotropia	exotropia	none	i.m.	i.m.
dysarthria	pseudobub.+ cerebellar	cereb. 14y, anarth. 34y	+ (slow speech)	+ (slow speech)	-
dysphagia	+	+ (PEG at 29y)	+	-	-
spasticity (UL/LL)	+ /+	+/+	+/+	+/+	-/+
weakness (UL/LL/T)	+/+/+	+/+/+	+/+/+	+/+/+	i.m.
muscle atrophy	none	UL/LL	none	none	none
incr. DTR	UL/LL	UL/LL	LL	LL	LL
extensor plantar response	+	+	+	+	+
limb ataxia	UL/LL	UL/LL n.a.	none	none	none
extrapyramidal involvement	none	dystonic posturing feet at 9y	none	none	none
sensory deficits	none	none as child	none	none	none
urinary dysfunction	urge, voiding	incont.	none	none	none
rectal dysfunction	none	incont. at age 34y	none	none	none
others	hyperopia & regular school, problems with typography and slow working pace	febrile seizures (3y), episodes of generalized myoclonus and nocturnal agitation (31y), EEG normal	none	none	none
Neurophysiology	F10	F11	F12-1	F12-2	F12-3
MEPs (UL/LL)	n.d.	n.d.	n.d.	n.d.	n.d.
SEPs (UL/LL)	n.d.	prol.(c.c.)/ absent	normal/normal	n.d.	n.d.
VEPs	n.d.	prol.	n.d.	n.d.	n.d.
BAEPs	n.d.	normal	n.d.	n.d.	n.d.
EMG	n.d.	normal	normal	n.d.	n.d.
Nerve conduction studies	n.d.	normal	normal	n.d.	n.d.
MRI	F10	F11	F12-1	F12-2	F12-3
year of imaging / age	2015 / 7y	2014 / 33y	2015 / 9y	no images	no images
thin corpus callosum	-	fronto-temp., mild parietal	-		
cerebral atrophy	-	fronto-temp., mild parietal	-		
cerebellar atrophy (hemispheres /vermis)	- / -	+ / +	mild / mild		
midbrain / pons atrophy (Am/Ap in mm^2)	- (137) / + (354)	+ (81) / + (203)	- (135) / +(305)		
white matter changes	periventr., most prominent parietal	periventr.	periventr., most prominent parieto-occ.		
globus pallidum hypointensity (T2 / T2*)	+ / +	mild / mild	+ / mild		

Family-#	F13	F14	F15	F16
ethnicity	Spanish	Belgian	Turkish	Italian
sex	f	f	f	m
consanguinity	-	-	-	-
mutation	K262T (hom) **pat. UPD**	E78K / A8Pfs*91	H69Y (hom)	E88* / P154C *de novo*
age at onset [y]	4	10	5,83	3,5
age at wheelchair use [y]	11	29	-	5,5
age at exam [y]	18	37	6,83	4,66
cognitive deficits	mild	moderate	IQ 85 (7;6y)	mild
progression CD	+	+	+	+
optic atrophy	+	-	-	-
saccadic smooth pursuit	+	+	-	-
gaze evoked nystagmus	-	-	-	-
slow horizontal prosaccades	+	+	-	-
other oculom. disturb.	exotropia	-	-	-
dysarthria	pseudobulb. later anarthria	cerebellar, later pseudobulbar	-	moderate dysarthria
dysphagia	+ (PEG at 19y)	+	-	+
spasticity (UL/LL)	+/+	+/++	-/+	+/++
weakness (UL/LL/T)	+/+/head drop	-/-/+	-/+/-	+/++/n.a.
muscle atrophy (UL/LL)	+/+	none	-/(+) triceps surae	-/+
incr. DTR	UL/LL	UL/LL	LL	UL/LL
extensor plantar response	+	+	+	+
limb ataxia	UL/LL	UL/LL	none	UL/LL
extrapyramidal involvement	dystonic posturing in upper limbs	none	none	none
sensory deficits	none	none	none	none
urinary dysfunction	incont.	none	none	none
rectal dysfunction	incont.	none	none	none
others	none	none	pes cavus	none
Neurophysiology	F13	F14	F15	F16
MEPs (UL/LL)	normal	n.d.	n.d.	normal
SEPs (UL/LL)	normal	normal/normal	n.d.	normal
VEPs	normal	normal	n.d.	normal
BAEPs	normal	n.d.	n.d.	normal
EMG	normal	n.d.	n.d.	normal
Nerve conduction studies	sensory conduction delayed	n.d.	n.d.	normal
MRI	F13	F14	F15	F16
year of imaging / age	2015 / 21y	2015 / 37	2015/ 6;10	2015/4;10
thin corpus callosum	global	+ (mild global)	-	n.a.
cerebral atrophy	global	parietal	+ (dorsal body)	n.a.
cerebellar atrophy (hemispheres /vermis)	+ / +	mild / -	- / -	n.a.
midbrain / pons atrophy (Am/Ap in mm^2)	+ (97) / -(297)	- (114) / + (338)	- (144) / + (343)	n.a.
white matter changes	periventr., most prominent parietal	periventricular, most prominent parieto-occipital	mild periventricular, most prominent parietal	periventricular posterior and semioval centers
globus pallidum hypointensity (T2 / T2*)	mild / mild	+ / n.d.	- / n.d.	n.a./n.d.

Abbreviations: '+' – sign/symptom present; '-' – sign/symptom absent; absent: not measurable , Am: midbrain area, anarth.: anarthria, Ap: pons area, BAEPs: brainstem auditory evoked potentials, cc: central conduction, CD: cognitive deficit, cereb.: cerebellar, discrim.: discrimination, disturb.: disturbance, DTR: deep tendon reflexes, EMG: electromyography, fronto-temp.: fronto-temporal, gen: generalized., i.m. – information missing; incr.: increased, incont.: incontinence, LL: lower limb, mat.: maternal, MEPs: motor evoked potentials, most prom.: most prominent, n.a. – not applicable, NC: nerve conduction, oculom.: oculomotor, pallhyp: pallhypaesthesia, parieto-occ.: parieto-occipital, pat.: paternal, PNP: peripheral

neuropathy, periventr.: periventricular, prol.: prolonged latency, pseudob.: pseudobulbar, seg.: segmental, SEPs: sensory evoked potentials, temp.: temperature: T: trunc, T2 / T2*: imaging contrast., UL: upper limb, UPD: uniparental disomy; VEP: visually evoked potentials; [y] years, age at onset = begin gait disturbance

Supplementary Table 2: Overview of FA2H/SPG35 mutations with associated phenotypes

Allele 1	Number of occurrences among 38 families (# affected)	Allele 2	Number of occurrences among 38 families (# affected)	Phenotype	age of onset (gait problems)	Reference
c.21delC, p.A8Pfs*91	3 (5)	c.21delC, p.A8Pfs*91	2 (4)	cHSP with initially only spasticity; in eldest patient at age 9 complicated by mild symptoms of dysarthria, altered eye motility and cognitive impairment; cerebellar atrophy on imaging	4,5	F12 this study
c.21delC, p.A8Pfs*91	3 (5)	c.232G>A, p.E78K	2 (2)	cHSP presenting at age 9 with progressive spasticity and ataxia as well as progressive cognitive decline of moderate severity at age 37, wheelchair dependence since age 29.	10	F14 this study
c.21delC, p.A8Pfs*91	3 (5)	c.554G>A, p.T185*	1 (1)	cHSP with additional ataxia and hypomimia. No iron accumulation in MRI.	4	(van de Warrenburg *et al.*, 2016)
c.101A>G, p.Y34C	1 (2)	c.620C>T, p.T207M	2 (3)	cHSP with progressive leg spasticity at age 32 and progressive dementia at age 40.	32	(Pensato *et al.*, 2014)
c.103G>T, p.D35Y	2 (3)	c.103G>T, p.D35Y	2 (3)	cHSP with spastic paraplegia progressing to spastic tetraparesis, dystonia, mild cognitive detoriation, dysmetria, dysdiadochokinesis	4&6	(Edvardson *et al.*, 2008)
c.103G>T, p.D35Y	2 (3)	c.193C>T, p.P65S	1 (1)	cHSP with spastic paraplegia, cerebellar eye signs, EEG abnormalities without epilepsy, MEP lower limbs prolonged, normal SEP	6	(Mari *et al.*, 2018)
c.131C>A, p.P44Q	1 (1)	c.131C>A, p.P44Q	1 (1)	cHSP with spastic paraplegia progressing to spastic tetraparesis, with cerebellar ataxia, exotropia, dysphagia, rigor and no cognitive decline.	4	F8 - (Soehn* *et al.*, 2016))
c.133G>T, p.G45W	1 (1)	c.133G>T, p.G45W	1 (1)	cHSP with spastic paraplegia and mild upper limb spasticity, mild cognitive progressive impairment.	4,5	F10 this study
c.137G>A, p.G46D	1 (2)	c.137G>A, p.G46D	1 (2)	cHSP with moderate cognitive impairment (IQ = 51), mild ophthalmoparesis in the vertical gaze, spastic gait and mild paraspasticity.	4	(Pensato *et al.*, 2014)
c.157_174del, p.R53_I58del	1 (1)	c.157_174del, p.R53_I58del	1 (1)	cHSP with initial presentation being progressive balance problems and toe walking. Spastic quadriplegia was present on examination, along with early bladder problems, dysarthria, dysphagia and limb ataxia.	6	(Kara *et al.*, 2016)

c.159_176del, p.R53_I58del	1 (4)	c.159_176del, p.R53_I58del	1 (4)	cHSP with spastic paraplegia, possible dystonia, dysarthria progressing to anarthria, generalized seizures, mild cognitive decline, no cerebellar signs, bilateral optic atrophy, opthalmoplegia with markedly reduced upgaze, dystonic upper limb movements	4	(Dick *et al.*, 2010)
c.160_169del, p.A54Tfs*42	2 (2)	c.160_169del, p.A54Tfs*42	2 (2)	cHSP with spastic paraplegia progressing to spastic tetraparesis, loss of ambulation, exotropia, dysphagia and severe cognitive decline.	3	F4
c.160_169del, p.A54Tfs*42	2 (2)	c.503_506del, p.V168Gfs*70	1 (1)	cHSP with spastic ataxia and LL dystonia progressing to spastic tetraparesis leading to loss of ambulation at 10y, progressive cognitive decline, anarthria and PEG-fed at 34y, state of prolonged survival	3	F11 this study
c.205C>T, p.H69Y	1 (1)	c.205C>T, p.H69Y		cHSP with spastic paraplegia and cognitive deficit (IQ 85 at 7;6 yrs)	5	F15 this study
c.209C>T, p.S70L	1 (1)	c.968C>T, p.P323L	2 (2)	cHSP with spastic paraplegia, loss of oromotor control with mild dysphagia and progressive hypophonia and bradylalia, mild cognitive decline, mild upper limb spasticity	3	(Rupps *et al.*, 2013)
c.230T>G, p.L77R	1 (1)	c.230T>G, p.L77R	1 (1)	cHSP with spastic paraplegia progressing to tetraspasticity, dysarthria, cerebellar signs, exotropia, abnormal ocular motility, epilepsy, ataxia, MCI	4	(Liao *et al.*, 2014)
c.232G>A, p.E78K	2 (2)	c.956A>G, p.H319R	1 (1)	cHSP withspastic paraplegia progressing to spastic tetraparesis, MCI, exotropia and dysarthria	20	F9 this study
c.262G>T, p.E88*	1 (1)	c.460C>T, p.R154C	3 (5)	cHSP with spastic tetraplegia, dysphagia, dysarthria, limb ataxia, dysphagia, cognitive deficite with progressive cognitive decline	3	F16 this study
c.270+3A>T, del ex 2-7	1 (2)	c.270+3A>T, del ex 2-7	1 (2)	cHSP with spastic paraplegia leading to tetraspasticity, cerebellar signs, optic atrophy, dysarthria leading to anarthria, cognitive decline, generalized seizures	4&7	(Garone *et al.*, 2011)
c.340_363del24, p.F114_K121del and c.363+1_8del8	1 (1)	c.1055C>T, p.T352I	1 (1)	cHSP with progressive spastic paraplegia, strabism with surgery, mild intellectual disability (Full Scale IQ, 70), EMG with chronic denervation in the distal muscles oft he lower limbs (*3 mutations described in one patient)*	9	(Mari *et al.*, 2018)
c.388C>T, p.L130F	1 (2)	c.506+6C>G, del ex3	1 (2)	cHSP with spastic paraperesis, horizontal nystagmus, behavioral disturbances, mild dysarthria, dysphagia, urinary dysfunction, hearing loss, extrapyramidal	10 & 17	(Liao *et al.*, 2014)

c.443C>T, p.P148L	1 (2)	c.443C>T, p.P148L	1 (2)	cHSP with with spastic paraparesis, gait ataxia, cognitive decline, optic atrophy and vertical gaze palsy	4	F3 this study
c.460C>T, p.R154C	3 (5)	c.460C>T, p.R154C	3 (5)	cHSP with spastic paraplegia progressing to spastic tetraparesis, dysmetria, exotropia, progr, dysarthria leading up to anarthria, xeroderma, ataxia, asymmetric optic atrophy, lateral-beating nystagmus, acquired epilepsy	4-5	(Kruer *et al.*, 2010)
c.460C>T, p.R154C	3 (5)	c.620C>T, p.T207M	3 (4)	cHSP with initial presentation being progressive balance problems and toe walking. Spastic quadriplegia was present on examination, along with early bladder problems, dysarthria, dysphagia and limb ataxia.	22	(Kara *et al.*, 2016)
c.486G>C, p.E162D	1 (1)	c.1051A>C, p.S351R	1 (1)	cHSP with initial presentation being progressive balance problems and toe walking. Spastic quadriplegia was present on examination, along with early bladder problems, dysarthria, dysphagia and limb ataxia.	3	(Kara *et al.*, 2016)
c.509A>G, p.Y170C	1 (2)	c.509A>G, p.Y170C	1 (2)	cHSP with spastic paraplegia, dysarthria, lateral beating nystagmus, dysmetria, mild cognitive decline, behavioral disturbances	38 & 40	(Tonelli *et al.*, 2012)
c.509_510delAC, p.Y170*	2 (4)	c.509_510delAC, p.Y170*	2 (4)	cHSP, seizures at age 2, spastic tetraparesis at age 4, progressive ataxia, and dystonia, bradylalia and dysarthria, MCI, bladder incontinence, exotropia; brother with no seizures	3&4	(Kruer *et al.*, 2010)
c.509_510delAC, p.Y170*	2 (4)	c.509_510delAC, p.Y170*	2 (4)	cHSP with spastic paraplegia. #1: dysarthric speech and head and hand tremor and difficulty chewing, head drop and bilateral hand muscle atrophy + gastrocnemius atrophy. #2: dropped head, truncal hypotonia, bilateral atrophy of the lower extremity muscles, and tremor.	3&4	(Donkervoort *et al.*, 2014)
c.510_511delCA, p.Y170*	1 (1)	c.510_511delCA, p.Y170*	1 (1)	cHSP with spastic paraplegia progressing to spastic tetraparesis, cerebellar dysarthria, progressive MCI, reduced touch sensation, resting tremor.	4	F5 this study
c.527G>A, p.W176*	1 (1)	c.527G>A, p.W176*	1 (1)	cHSP with spastic paraplegia progressing to spastic tetraparesis. Almost loss of ambulation. Moderate cognitive decline, exotropia, pseudobulbar dysarthria, truncal hypotonia.	3	F1 - (Soehn* *et al.*, 2016)
c.620C>T, p.T207M	4 (6)	c.704G>A, p.R235H	3 (4)	cHSP with progressive paraplegia	child-hood	F6 this study

c.620C>T, p.T207M	4 (6)	c.704G>A, p.R235H	3 (4)	cHSP with progressive paraplegia, bipolar II disorder, progressive dysarthria, progressive long term temporal disorientation and memory impairments, cerebellar ataxia, and dysphagia. & cHSP with progressive paraplegia, with progressive dysphagia and dysarthria, sphincter disturbances, cerebellar ataxia, and short- and long-term visuo-spatial and verbal memory impairment along with mild anxiety and depression.	9&15	(Magariello *et al.*, 2017)
c.688G>A, p.E230K	1 (2)	c.968C>A, p.P323Q (c.976G>A, p.G326S: VUS)	1 (2)	cHSP with spastic paraplegia leading to tetraspasticity, mild cerebellar dysfunction, cognitive decline, dysarthria progressive to anarthria, dystonia, epileptic seizures, neck weakness, urinary and fecal incontinence, nystagmus (*3 mutations described in two patients)*	4&5	(Cao *et al.*, 2013)
c.703C>T, p.R235C	1 (8)	c.703C>T, p.R235C	1 (8)	cHSP with paraspasticity (other family members) tetraspasticity, dysarthria, cognitive descline, some with epilepsy	6-11	(Dick *et al.*, 2010)
c.704G>A, p.R235H	2 (2)	c.859T>C, p.C287R	1 (1)	cHSP with tetraspasticity, generalized dystonia, MCI, focal epilepsy, exotropia, anarthria and dysphagia.	2,5	F7 this study
c.782_783insA, p.H261Qfs*52	1 (1)	c.798C>G, p.D266E	1 (1)	cHSP with initial presentation being progressive balance problems and toe walking. Spastic quadriplegia was present on examination, along with early bladder problems, dysarthria, dysphagia and limb ataxia.	4	(Kara *et al.*, 2016)
c.785A>C, p.K262T	2 (3)	c.785A>C, p.K262T	1 (3)	cHSP with spastic paraplegia leading to tetraspasticity. Progressive dysarthria to anarthria. Optic atrophy & teeth disposition alteration. Early cerebellar dysfunction, upper limb dystonia and MCI.	4	F13 - (Soehn* *et al.*, 2016)
c.786+1G>A, del ex 5-6	2 (5)	c.786+1G>A, del ex 5-6	2 (5)	cHSP with spastic paraplegia progressing to spastic tetraparesis, dystonia, mild cognitive decline, dysmetria, dysdiadochokinesis, some with seizures	4,5-6	(Edvardson *et al.*, 2008)
c.805C>T, p.R269C	1 (1)	c.1501A>G, p.S351G	1 (1)	cHSP with progressive spastic gait, diffuse muscular hyptonia, club foot, EEG abnormalities without epilepsy, normal electrophysiology.	4	(Mari *et al.*, 2018)
c.806G>A, p.R269H	1 (2)	c.806G>A, p.R269H	1 (2)	cHSP with spastic paraplegia, ataxia, cognitive decline, incontinence, bilateral optic atrophy.	12-16	(Marelli *et al.*, 2015)

c.908G>T, p.G303V	1 (1)	c.968C>T, p.P323L	2 (2)	cHSP with spastic paraplegia leading to tetraspascitcity with loss of ambulation, progressive moderate to severe cognitive impairment, exotropia, cerebellar dysarthria, dysphagia, rigor, generalized dystonia, sensory involvement and focal epilepsy.	3	F2 this study
c.1006C>A, p.H336N	1 (3)	c.1006C>A, p.H336N	1 (3)	cHSP with progressive spastic paraplegia and ataxia without cognitive decline.	4	(Marelli *et al.*, 2015)
28kb del, del ex 3-7	1 (1)	c.707T>C, p.F236S	1 (1)	cHSP with spastic paraplegia dysarthria, neck weakness, no seizures, mild dysmetria and ataxia, no dystonia, mild cognitive decline	3	(Pierson *et al.*, 2012)

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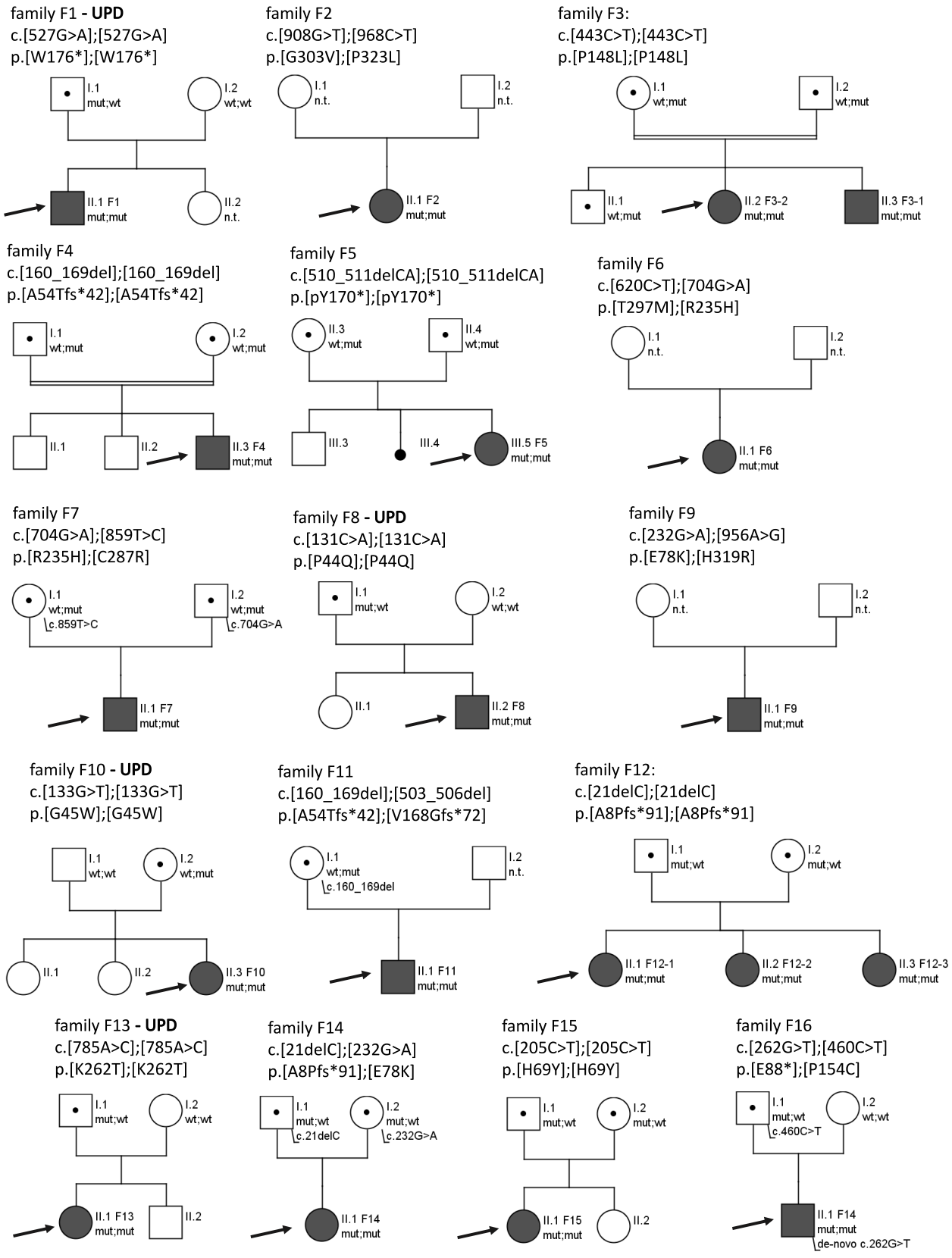
Pedigrees of all SPG35 cases with segregation analysis and mutations identified



Supplementary Figure 1: Pedigrees and segregation analysis of all 16 families