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Title page

Loss of paraplegin drives spasticity rather than ataxia in a cohort of 241 SPG7 patients

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Abstract

Objective: We took advantage of a large multinational recruitment to delineate genotype-phenotype correlations in a large, trans-European multicenter cohort of SPG7 patients.

Methods: We analyzed clinical and genetic data from 241 SPG7 patients, integrating neurological follow-up data. One case was examined neuropathologically.

Results: SPG7 patients had a mean age of 35.5±14.3 years (n=233) at onset and presented with either spasticity (n=89), ataxia (n=74), or both (n=45). At the first visit, patients with a longer disease duration (>20 years, n=62) showed more cerebellar dysarthria ($p<0.05$), deep sensory loss ($p<0.01$), muscle wasting ($p<0.01$), ophthalmoplegia ($p<0.05$), sphincter dysfunction ($p<0.05$) than those with a shorter duration (<10 years, n=93). Progression, measured by SARA evaluations, showed a mean annual increase of 1.0±1.4 points in a subgroup of 30 patients. Patients homozygous for loss of function (LOF) variants (n=65) presented significantly more often with pyramidal signs ($p<0.05$), diminished visual acuity due to optic atrophy ($p<0.0001$), and deep sensory loss ($p<0.0001$) than those with at least one missense variant (n=176). Patients with at least one Ala510Val variant (58%) were older (37.6±13.7 *vs* 32.8±14.6, $p<0.05$) and showed ataxia at onset ($p<0.05$). Neuropathological examination revealed reduction of the pyramidal tract in the medulla oblongata and moderate loss of Purkinje cells and substantia nigra neurons.

Conclusions: This is the largest SPG7 cohort study to date, and shows a spasticity-predominant phenotype of LOF variants and more frequent cerebellar ataxia and later onset in patients carrying at least one Ala510Val variant.

Word 239/250

Abbreviations

AD: autosomal dominant

AR: autosomal recessive

LOF: loss of function

MBP-SF: myelin basic protein serum factor

MLPA: multiplex ligation-dependent probe amplification

PolyQ: polyglutamine

SARA: Scale for the Assessment and Rating of Ataxia

SCA: spinocerebellar ataxia

SPRS: Spastic Paraplegia Rating Scale

XL: X-linked

Introduction

The key feature of hereditary spastic paraplegia is the progressive degeneration of corticospinal tracts.[1] To date, 79 loci are known to be involved and are classified as spastic paraplegia genes (SPG1-79).[2] The first identified gene among autosomal recessively transmitted spastic paraparesis was SPG7[3]. Cerebellar atrophy does not always translate into cerebellar signs in patients.[4-6] However, cerebellar ataxia may be the predominant symptom, as confirmed by reports that found SPG7 to be responsible for up to 19% of undiagnosed cerebellar ataxias.[7,8] Cerebellar atrophy and peripheral neuropathy have also been reported in heterozygous relatives of SPG7 patients.[6]

SPG7 encodes paraplegin, a mitochondrial inner membrane metalloprotease.[9,10] This protein forms the hetero-oligomeric protease complexes with the homologous ATPase AFG3L2.[9] Consequences of an impaired complex include mitochondrial dysfunctions.[9] SPG7 and AFG3L2 levels are high in Purkinje neurons;[11] additionally, SPG7 is expressed in pyramidal cortical neurons and spinal motor neurons.[12]

The *SPG7* gene has 17 exons and variants of unknown significance have been frequently reported.[4,5,13,] A recurrent variant, Ala510Val, shows a minor allele frequency of 0.5% in public databases such as GnomAD. Initially considered to be a non pathological variant, its pathogenicity was established by yeast complementation assay.[14] Moreover, this variant is found more frequently in patients than controls .[8, 15-17]

Despite extensive clinical variability, few genotype-phenotype correlations have been established.[18] We aimed to delineate the progression of clinical features and define correlations between genotypes and phenotypes by exploring a large SPG7 population from several European centers that includes follow-up data for many patients.

Methods

We analyzed the clinical and genetic data of 241 patients (194 index patients, 47 affected relatives). Geographical origin was European in most (French n= 86, Netherlands n=49, German n=35, Belgium n=23, Italy n=8, Great Britain n=2, Greece n=1), some came from North Africa or Middle East (n=10), unknown for 26 patients. All SPG7 patients carried two disease-causing variants and had at least one neurological examination. Patients were followed at: the French National Reference Center for Rare Diseases "Neurogenetics" in Paris (n= 106) and Strasbourg (n= 2), the German Center for Neurodegenerative Diseases (Tubingen, Bonn, Munich, Rostock, University Hospitals) and collaborating German Hospitals (Kiel, Bochum) (n= 53), Radboud University Nijmegen Medical Centre (n= 49), Antwerp University Hospital (n= 24), the Medea Institute in Conegliano and Bosisio Parini (n= 7). Some patients (n= 90) have been previously reported: n= 8;[4] n= 7;[19] n= 16;[6] n= 49;[18] n= 1;[20] n= 9.[8] Data were collected systematically with the ataxia and spastic paraparesis databases of each center, for clinical follow up and research purposes (Spatax network), between 1995 and 2018. Clinical and imaging data were retrieved and critically reviewed. Genetic diagnosis was reached differently, depending on the local facilities and year of sampling, either by direct sequencing of SPG7 coding regions, or high throughput panel sequencing, exome sequencing, sometimes completed by multiplex ligation-dependent probe amplification (MLPA) to detect deletions or duplications within the SPG7 gene.

We compared clinical features of patients with homozygous missense variants, with homozygous LOF variants or with a nonsense variant on one allele and missense variants on the other allele. We studied the clinical consequences of the Ala510Val variant and identified genetic differences in patients with cerebellar ataxia or spasticity at onset. We analyzed the progression of the clinical signs in patients who had a follow-up and by comparing the clinical signs at first visit in three groups of patients: with disease onset at the most 10 years before the clinical examination, between 10 and 19 years, and above 19 years.

Patients were assessed with a standardized evaluation form. The categorical scale of disability was as follows: 0, no functional handicap; 1, no functional handicap but signs at examination; 2, mild, able to run, unlimited walking; 3, moderate, unable to run, limited walking without aid; 4, severe, walking with one stick; 5, walking with two sticks; 6, unable to walk, requiring a wheelchair; and 7, confined to bed. The disease progression index was calculated as the ratio between the disability stage and disease duration in years. Symptoms at onset, such as stiff legs/spastic gait or unsteadiness and gait/balance impairment were collected as reported by the patients. A subgroup of patients were evaluated using the Scale for the Assessment and Rating of Ataxia (SARA, max. value 40).[21] Transmission was classified as autosomal recessive, autosomal dominant (based on a positive first-degree familial history combined with the presence of two variants in the SPG7 gene), or sporadic (only one index in the family and absence of consanguinity). Segregation of the variants was assessed in family members for suspected autosomal dominant cases, whenever DNA was available. Variants were classified as missense or LOF (including nonsense, splicing, frameshift variants, deletions, duplications, and in-frame deletions). Pathogenicity of the variants was obtained by an *in silico* approach by five prediction programs (SIFT, PolyPhen, LRT, M-Cap, MutationTaster). Brain MRIs and electromyography were collected when available. The degree of cerebellar atrophy was evaluated by the physicians involved in this study on T1 or T2-weighted sagittal sequences.

Neuropathology

One patient was involved in a brain donation program and signed an informed consent form for brain neuropathological examination and research. The brain was removed *post-mortem* and the right hemisphere and right half of the brainstem were fixed by immersion in 4% formaldehyde (10% formalin); systematic samples from the other hemisphere were frozen at -80°C. After formalin fixation, a systematic sampling protocol was applied. The samples

involved the vermis and the cerebellar hemisphere, the whole hemi-brainstem (eight samples), the spinal cord at the upper cervical level, the basal nuclei, the hippocampus and the cerebral cortex. The samples were paraffin-embedded and cut at a thickness of 5 µm. The sections were deparaffinized in graded alcohol and stained with hematoxylin-eosin and luxol fast blue for myelin. Immunohistochemistry was performed on selected samples with primary antibodies for tau protein, Aβ, α-synuclein, TDP-43, ubiquitin, p62 and CD68 (for activated microglia). Double immunostaining for myelin (myelin basic protein) and phosphorylated neurofilaments was performed to assess myelin pallor and distinguish demyelination from degeneration.

Statistical analyses

Statistical analyses were performed with SAS software 9.4. Data are expressed as the mean ± standard deviation (SD) or frequency (n). Qualitative variables were compared between groups using Fischer's exact test and quantitative variables by ANOVA, followed by a *post-hoc* test when necessary. We compared clinical examinations at the first visit to account for disease duration (< 10 years, 10-19 years, ≥ 20 years). We used McNemar's exact test for qualitative variables to compare the clinical characteristics of the patients between two consecutive visits.

Standard Protocol Approvals, Registrations, and Patient Consents

All patients have been examined in clinical settings during their usual follow up. Informed consent form was obtained according to regulations of each European country and local ethics committee.

Data availability

Anonymized data presented in this article will be made available at the request of a qualified investigator. Requests should be made to Alexandra Durr (alexandra.durr@icm-institute.org).

Results

At first visit, 241 SPG7 patients (136 men and 105 women) were included, with a mean age at examination of 50.4± 14.1 years. The mean age of reported disease onset was 35.5± 14.3 years (n= 233) and the mean disease duration 15.1± 13 years (n=233). The patients had a mean disability stage of 3.3± 1.2 (n= 223) and a mean SARA score of 10.6 ± 6 (n= 55).

There were 167 familial cases, 64 without other affected in their families, and 10 with unknown familial history. There were 12 families (6%) with transmission of the disease from one generation to the next, despite the presence of two variants in the index case (Figure 1). These apparently dominant inherited forms were more often reported in patients who had cerebellar symptoms at onset (10 patients with cerebellar onset *vs* 3 patients with spastic onset, $p< 0.05$). Segregation of one of the two variants from the index case was confirmed for only two families (AAD-796 and FSP-554) and was reported elsewhere.[6] In these two families, the heterozygous parent showed mild cerebellar atrophy and cerebellar signs, and both carried the p.Arg485_Glu487del variant.[6] DNA was not available for the other families to analyze segregation.

Overall genotype-phenotype correlations

All variants are shown in Figure 2, with the presence of a cluster of missense variants in the peptidase domain (between the 13th and 16th exons, amino acid positions 555 to 727). New variants are listed in the supplementary table (data available from Dryad, Table 4).

Based on genotype patients homozygous for LOF variants presented significantly more often with pyramidal signs and diminished visual acuity (Table 1) than patients homozygous for missense variants.

Comparison between patients carrying the Ala510Val variant and those carrying other variants (Table 2)

The Ala510Val variant was the most frequent variant in our patients. Indeed, 141 patients carried at least one Ala510Val variant (58.5%), including 45 patients homozygous for this variant (18.7%). The presence of the Ala510Val variant, even on one allele, was associated with significantly later disease onset than non-carriers of this variant (37.6 ± 13.7 *vs* 32.8 ± 14.6, p= 0.01, n= 233), with no further differences in Ala510Val homozygous patients. Clinically, at first examination, the Ala510Val carriers did not differ in disease severity measured by disability stage (3.2 ± 1.1 *vs* 3.3 ± 1.2, p= 0.5, n= 223) or SARA score (10.8 ± 6.1 *vs* 10.1 ± 5.5, p= 0.7, n= 55) compared to patients carrying other variants. However, patients carrying the Ala510Val variant had a lower frequency of: i) pyramidal signs (86% *vs* 97%, p=0.01), including brisk reflexes (76% *vs* 92%, p=0.01) and sphincter dysfunction (38% *vs* 53%, p=0.02); ii) diminished visual acuity (6% *vs* 24%, p= <0.001); and iii) pes cavus (17% *vs* 30%, p=0.02).

Symptoms at onset: cerebellar ataxia versus spasticity

At onset, more patients reported stiff legs and spastic gait (n=89) than unsteadiness and gait or balance impairment (n= 74). A third group presented either a combination of cerebellar ataxia and spastic gait (n= 45) or other features, such as dysarthria, diplopia, diminished visual acuity, and neuropathic pain. The presence of the Ala510Val variant was significantly associated with cerebellar signs at onset (p=0.01).

Clinical progression between the first and the second clinical examination

Data of two consecutive follow-up examinations were available for 98 patients, with a mean interval between the two examinations of 5.0 ± 5.9 years (median 3 years [Q1=1; Q3=7]). The number of associated clinical signs increased significantly in the second evaluation, especially

cerebellar ataxia (66.3% *vs* 78.3%, p= 0.003, n= 92), cerebellar dysarthria (42% *vs* 57%, p< 0.001, n= 93), pyramidal syndrome (89.3% *vs* 96.8%, p< 0.05, n= 94), with increased presence of the extensor plantar reflex (71.6% *vs* 81.5%, p< 0.05, n= 81), dystonia (2.3% *vs* 11.5%, p< 0.01, n= 87), muscle wasting (10.3% *vs* 29.9%, p< 0.001, n=87), ptosis (4.7% *vs* 16.7%, p< 0.01, n= 84), dysphagia (15.3% *vs* 28.2%, p< 0.01, n= 78), decreased vibration sense at the ankles (44.3% *vs* 64.8%, p< 0.001, n= 88), cognitive impairment (8% *vs* 19.3%, p< 0.01, n= 88), and diminished visual acuity (7.4% *vs* 14.1%, p< 0.05, n= 81). In the subgroup of patients with at least two SARA scores (n= 30), the mean progression of cerebellar ataxia measured by SARA score was 4.0 ± 4.0 with a mean annual progression of 1.0 ± 1.4.

Retrospectively, there was no change in disability, measured by functional stage, between two visits for 48% (43/89) of the patients. Among the 46 patients for whom the disability stage increased, the mean annual increase of the disability progression index was 0.08/year ± 0.31. The stability in half of the patients over time indicated a slowly progressive disease.

Evolution of neurological signs based on disease duration

The clinical presentation of the SPG7 patients at first visit differed significantly, depending on disease duration (< 10 years, n= 93; 10-19 years, n= 79; ≥ 20 years, n= 62). Cerebellar dysarthria, deep sensory loss, and peripheral wasting were more predominant in the group that had the disease the longest. Ophthalmoplegia was more frequently observed with longer disease duration, as well as sphincter dysfunction (Table 3). The stage of disability increased with disease duration, but taking into consideration the disease progression index, progression was significantly faster in the group with the shortest duration of disease than the others. The rapid progression in the first stage of the disease appears to reach a *plateau* in the advanced phases; this data should be confirmed in a longitudinal study.

Brain MRI and electromyography

During follow-up, we collected 137 individual brain MRIs. Cerebellar atrophy of varying severity was present in 80 patients (58.4%) (Figure 3). There was no correlation between severity of atrophy and specific variants. Electromyography data were available for a small group of patients (n= 23) with a mean age at examination of 55 ± 13.9 years and a mean disability stage of 3.7± 1.1 and showed the main peripheral involvement to be sensorimotor axonal neuropathy (n= 20).

Neuropathological findings in SPG7

Individual AAR-247-004 died from pancreatic cancer at the age of 56. The parents were not related and had a normal neurological examination at 68 and 66 years of age. The onset of SPG7 disease occurred at 30 years of age, with gait instability. She subsequently suffered from stiff legs. She needed walking aids by age 45 and a wheelchair by age 50. She was dysarthric without swallowing difficulties. Clinical evaluation at the age of 55 showed pyramidal syndrome with spasticity of the lower limbs, bilateral extensor plantar reflex, and a mild proximal weakness of the lower limbs. Deep sensation was impaired and she had a cerebellar syndrome (SARA score 16.5/40). Oculomotor examination showed asymmetric ptosis, saccadic pursuit, and a limitation of vertical gaze. Brain MRI was performed at ages 40 and 55 and revealed cerebellar atrophy with vermis predominance. Nerve conduction studies were normal, both at 43 and 55 years of age. Neuropsychological assessment was performed at 55 years of age, showing normal cognitive efficiency but apathy and depressive signs. Muscular biopsy revealed mitochondrial abnormalities with COX negative fibers. The genetic test, at 56 years of age, confirmed the presence of compound heterozygote variants c.1749G>C (p.Trp583Cys) in exon 13 and c.2181+2dup (p.?) in exon 16.

Neuropathological examination

The brain weight was 1,256 g. The vermis of the cerebellum was atrophic (Figure 4 A). The pyramids of the medulla oblongata were small. The spinal cord at the cervical level was slightly altered: there was no myelin pallor in the lateral, anterior, or posterior columns. The anterior horns were normal. The pyramids of the medulla oblongata were small, but without myelin pallor. The vermis of the cerebellum was severely atrophic: there was a loss of Purkinje cells, with empty baskets, torpedoes, and Bergmann glia (Figure 4 B-D). The cerebellar hemispheres, the dentate nucleus, the inferior, middle, and superior cerebellar peduncles were normal, as well as the pontine nuclei and the locus coeruleus. There was some degree of neuronal loss in the pars compacta of the substantia nigra, as shown by the presence of extrapyramidal pigments (Figure 4 E). The pars reticulata was also affected and appeared gliotic. The subthalamic nucleus, striatum, and pallidum were normal. The hippocampus was remarkable with a thin dentate gyrus (Figure 4 F). There were some Betz cells in the motor cortex; one of them appeared chromatolytic. There was unusual thinning of the granular layer of the dentate gyrus. The CD68 antibody did not show inflammation foci: the pyramidal tract did not contain an abnormal number of microglia. Tau, Aβ, TDP-43, and α-synuclein immuno-staining were negative. The anti-ubiquitin and p62 staining did not label any inclusion.

Discussion

We report data from 241 European SPG7 patients, including 98 with neurological follow-up, in a collaborative effort that allowed us to study the largest SPG7 cohort available to date. Clinical features matched previous work that defined SPG7 as a relevant cause of late onset spastic ataxia.[5,6,18,22,23] Lower limb spasticity or cerebellar ataxia, both affecting gait stability, were present at onset making difficult to consider *SPG7* as primarily an HSP or an ataxia gene.[24] We were able to show that, among patients with a disease duration > 20 years, 88%

had pyramidal syndrome and 72% cerebellar ataxia. The predominance of pyramidal signs and symptoms was significantly associated with the presence of homozygous LOF variants rather than missense variants, suggesting that the loss of paraplegin function drives spasticity. We could speculate that loss of function of paraplegin still allows AFG3L2 to form functional oligomeric m-AAA protease. This could compensate for loss of paraplegin in the cerebellum, because of the high AFG3L2 cerebellar expression, while results insufficient in the spinal cord (low AFG3L2 expression). Missense variants on the other hand may form dysfunctional heteromeric complexes with AFG3L2 in the cerebellum and may disturb AFG3L2 function. Nonsense variants on both alleles predisposed patients to a more severe and complicated phenotype, with more frequent ophthalmological involvement. Diminished visual acuity occurred in 31% of patients carrying homozygous LOF variants. Paraplegin is one of the metallopeptidases involved in the OPA1 cleavage, protein which regulates mitochondrial fission/fusion process and mitochondrial cristae structure.[25] The impaired balance of the different forms of OPA1 could be more severe in the presence of LOF variants leading to optic atrophy even in the absence of OPA1 mutations.[26] Furthermore, we confirm that the Ala510Val variant is the most common variant in patients (58.5%), showing a minor allele frequency of 0.5% in public databases, in agreement with other reports.[7,15,17,23,27] This variant was associated with a delayed onset of disease and a less complicated phenotype, *i.e*. fewer pyramidal signs. This finding is similar to that for the Canadian population, for which Ala510Val was the most frequent variant and cerebellar features, including ataxia, were more pronounced than spasticity.[27] In contrast with the recent report on the English cohort,[23] we did not found significant difference in age at onset of the disease between Ala510Val homozygous and Ala510Val compound heterozygous patients. Since Ala510Val variants represented 73% of the missense variant group, the cerebellar phenotype is biased towards

one single variant. A link between predominant cerebellar presentation and null alleles has been reported,[18] but this difference could be explained by a possible recruitment bias.

We were able to study the progression in 30 cerebellar SPG7 patients, as the mean annual progression of SARA score was 1.0 ± 1.4. Compared to autosomal dominant spinocerebellar ataxias (SCAs), which show an annual SARA progression of 2.1 for SCA1, 1.5 for SCA2, 1.6 for SCA3, and 0.8 for SCA6,[28] SPG7 is comparable to SCA6 evolution. As for SCAs, SPG7 is not restricted to cerebellar features and the SARA score may not reflect the entirety of disease progression.[29] The slow evolution is also reflected by the fact that SPG7 patients were still able to walk with a cane after 20 years of the disease, which is, for example, not the case for SCA1 patients.[30] Obviously, SARA does not quantify spasticity and the lack of the Spastic Paraplegia Rating Scale (SPRS) scores[31] limits our estimation of overall disease progression since scoring was not done for the patients in this study, explained only partially by the presence of the cerebellar phenotype. The clinical picture became increasingly complex, with an increased frequency of cerebellar dysarthria, sphincter dysfunction, deep sensory loss, and muscle wasting over the years. Horizontal eye gaze limitation, a possible sign of mitochondriopathy, significantly increased in frequency during disease progression. Intriguingly, this was uncoupled form optic atrophy, which appeared to be present from disease onset, without decreased visual acuity for most patients.[6]

The presence of cerebellar atrophy by MRI was consistently associated with SPG7 but its degree did not correlate with severity of cerebellar signs (Figure 3). When available, for a small subgroup of patients, nerve conduction studies confirmed the presence of axonal sensorimotor neuropathy.

All index patients carried two variants, of which six percent were of apparently dominant transmission, as already suggested by others.[6,15,18,32] The p.Arg485_Glu487del variant (the

second most common mutation present in 9.3% of our cohort) was the most frequent in this group (three families, four patients) and its link with dominant transmission has already been suggested.[18] Segregation has been confirmed and reported for only two autosomal dominant families.[6] The p.Leu78* variant[15] was not found in our patients. The other variants detected in the dominant group were: p.A286fs*, p.E320*, p.G349fs*, p.P350Qfs*36, p.A510V, p.A658T, p.N739fs*, p.P750L, and c.861+2dup (p.?). The possibility that variants in other HSP or SCA genes are also present cannot be ruled out in the affected heterozygous relatives, but we previously excluded the presence of a second variant in SCA28 in a subgroup of SPG7 patients, because of the interaction of its gene product with paraplegin.[6] The potential dominant transmission in SPG7, the possibility to present neurological signs when carrying only one variant (as for parents in autosomal dominant families), and the high frequency of the A510V variant make genetic counseling challenging.

The neuropathological data from our *post-mortem* study showed clear involvement of the cerebellum, with the loss of Purkinje cells and gliosis in the dentate nucleus. Despite the conserved number of Betz cells, the pyramidal tract diameter appeared to be reduced at the level of the medulla oblongata. This shows that axonopathy is the hallmark of HSPs. Until now, only three *post-mortem* studies have been performed in SPG7: one patient carrying a homozygous p.Arg470Gln variants,[18] one patient homozygous for the p.Ala510Val variant,[20] and our case carrying p.Trp583Cys and c.2181+2dup (p.?). For the second patient the authors mentioned the presence of tau-pathology, which we did not find in our patient, possibly explained by the different variants and the greater age (i.e. 70 years old *vs* 56 years old of our patient).

This study assembled an unprecedented cohort of SPG7 patients, showing cerebellar ataxia to be the most notable element after the pyramidal syndrome. Genetic counseling will remain difficult in families with seemingly dominant transmission and requires functional

experiments to be developed and/or the identification of biomarker(s) to prove variant pathogenicity.

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Table 1. Clinical comparison at the first visit between patients harboring different genotypes.

	Homozygous missense (n = 77)	**Heterozygous missense-LOF (n = 99)**	**Homozygous LOF (n = 65)**	**p-value**
Age at onset of the disease, y, (Mean ± SD, n = 233)	38.2 ± 14.3 (n = 73)	35.4 ± 13.6 (n = 96)	32.7 ± 14.7 (n = 64)	0.08
Disease duration, y, (Mean ± SD, n = 233)	16.2 ± 12.4 (n= 73)	15.2 ± 15.3 (n = 96)	13.6 ± 9.8 (n = 64)	0.49
SARA (Mean ± SD, n=65)	10 ± 5.2 (n= 20)	10.7 ± 6.9 (n = 25)	11.6 ± 5.2 (n = 10)	0.80
Disability stage (max value 7) (Mean ± SD, n = 223)	3.3 ± 1.1 (n = 73)	3.1 ± 1.2 (n = 91)	3.4 ± 1.2 (n = 59)	0.25
Women/Men, % (n)	40%/60% (31/46)	42%/58% (42/57)	49%/51% (32/33)	0.54
Sporadic case, n=64 (%)	17 (26%)	29 (45%)	18 (28%)	0.70
Autosomal recessive n=154 (%)	51 (33%)	60 (39%)	43 (28%)	
Autosomal dominant n=13 (%)	4 (31%)	7 (54%)	2 (15%)	
Cerebellar ataxia, % (n)	66% (48/73)	61% (57/93)	60% (38/63)	0.80
Cerebellar dysarthria, %	40% (29/72)	37% (34/92)	36% (23/63)	0.89

(n)				
Brisk reflexes, % (n)	82% (55/67)	80% (70/88)	88% (51/58)	0.44
Babinski sign, % (n)	63% (42/67)	80% (70/87)	75% (43/57)	**0.04**
Pyramidal syndrome, % (n)	82% (60/73)	95% (89/94)	95% (60/63)	**0.01**
Pes cavus, % (n)	14% (10/69)	23% (21/91)	31% (19/61)	0.07
Parkinsonism, % (n)	6% (4/69)	4% (4/94)	3% (2/62)	0.84
Dystonia, % (n)	1% (1/71)	4% (4/95)	3% (2/62)	0.63
Muscle wasting, % (n)	18% (13/71)	17% (16/93)	13% (8/61)	0.74
Abnormal vibration at ankles, % (n)	59% (43/73)	29% (27/92)	57% (35/61)	**<0.0001**
Ophthalmoplegia, % (n)	14% (9/65)	15% (13/88)	19% (10/54)	0.78
Ptosis, % (n)	6% (3/54)	2% (2/77)	6% (3/50)	0.60
Diminished visual acuity, % (n)	7% (4/55)	6% (5/84)	31% (19/62)	**<0.0001**
Dysphagia, % (n)	16% (9/58)	10% (8/79)	17% (8/48)	0.51
Sphincter dysfunction, % (n)	39% (27/69)	41% (38/92)	55% (32/58)	0.15
Cognitive impairment, % (n)	6% (4/71)	8% (8/95)	8% (5/63)	0.81

Abbreviations: LOF = loss of function; SD = standard deviation. Significant differences are indicated in bold.

Table 2. Clinical comparison at the first visit between patients harboring at least one Ala510Val variant and patients with other variants.

	p. Ala510Val (n = 141)	**Other variants (n = 100)**	**p**
Age at onset of the disease, y, (Mean ± SD, n = 233)	37.6 ± 13.7 (n = 134)	32.8 ± 14.6 (n = 99)	**0.01**
Age at examination, y, (Mean ± SD, n = 241)	52.6 ± 12.7 (n = 141)	47.4 ± 15.4 (n =100)	**0.03**
Disease duration, y, (Mean ± SD, n = 234)	15.2 ± 13.6 (n = 135)	14.9 ± 12.3 (n =99)	0.82
SARA (Mean ± SD, n = 55)	10.8 ± 6.2 (n = 40)	10.1 ± 5.5 (n = 15)	0.73
Disability stage (max value 7) (Mean ± SD, n = 223)	3.2 ± 1.1 (n = 130)	3.3 ± 1.2 (n = 93)	0.46
Disease progression index (fct/disease duration) (Mean ± SD, n = 221)	0.4 ± 0.4 (n = 128)	0.4 ± 0.5 (n = 93)	0.74
Women/Men, % (n)	41%/59% (58/83)	47%/53% (47/53)	0.42
Cerebellar ataxia, % (n)	66% (87/132)	58% (56/97)	0.2
Cerebellar dysarthria, % (n)	36% (51/131)	36% (35/93)	0.7
Brisk reflexes, % (n)	76% (96/126)	92% (80/87)	**0.003**
Babinski sign, % (n)	71% (89/125)	77% (66/86)	0.4
Pyramidal syndrome, % (n)	86% (116/134)	97% (93/96)	**0.01**
Pes cavus, % (n)	17% (22/129)	30% (28/92)	**0.02**
Parkinsonism, % (n)	4% (5/131)	5% (5/94)	0.74

Dystonia, % (n)	4% (5/134)	2% (2/94)	0.70
Muscle wasting, % (n)	19% (25/133)	13% (12/92)	0.27
Abnormal vibration at ankles, % (n)	47% (62/133)	46% (43/93)	1
Ophthalmoplegia, % (n)	15% (18/122)	16% (14/85)	0.84
Ptosis, % (n)	3% (3/103)	6% (5/78)	0.29
Diminished visual acuity, % (n)	6% (6/108)	24% (22/93)	**<0.001**
Dysphagia, % (n)	15% (17/111)	11% (8/74)	0.51
Sphincter, % (n)	38% (49/129)	53% (48/90)	**0.02**
Mental retardation, % (n)	2% (3/134)	3% (3/95)	0.69
Cognitive impairment, % (n)	7% (10/134)	7% (7/95)	1

Abbreviations: SD = standard deviation. Significant changes are indicated in bold.

Table 3. Clinical presentation at first visit grouped by disease duration.

Disease duration (y)	**< 10 y (n = 93)**	**10-19 y (n = 79)**	**≥ 20 y (n =6 2)**	**p-value**
SARA (Mean ± SD, n)	7.9 ± 3.6 (n = 20)	11.3 ± 7.7 (n = 16)	12.7 ± 5.5 (n = 19)	**0.03***
Disability stage (max value 7) (Mean ± SD, n =221)	2.6 ± 0.8 (n = 88)	3.5 ± 1.1 (n = 75)	3.9 ± 1.2 (n = 58)	**<0.0001****
Disease progression index (fct/disease duration) (Mean± SD, n = 221)	0.74 ± 0.6 (n = 88)	0.26 ± 0.09 (n = 75)	0.13 ± 0.04 (n = 58)	**<0.0001****
Cerebellar ataxia, % (n)	56% (50/89)	64% (49/76)	72% (43/60)	0.16
Cerebellar dysarthria, % (n)	29% (26/89)	36% (27/74)	55% (33/60)	**0.01**
Brisk reflexes, % (n)	80% (70/87)	91%) (59/65)	77% (44/57)	0.09
Babinski sign, % (n)	73% (63/86)	73% (48/66)	76% (42/55)	0.91
Pyramidal syndrome, % (n)	91% (84/92)	93% (70/75)	88% (51/58)	0.58
Pes cavus, % (n)	27% (24/88)	18% (13/71)	19% (11/57)	0.35
Parkinsonism, % (n)	7%	4%	2%	0.42

	(6/90)	(3/73)	(1/58)	
Dystonia, % (n)	1% (1/91)	3% (2/74)	7% (4/59)	0.15
Muscle wasting, % (n)	10% (9/90)	11% (8/74)	32% (18/57)	**0.001**
Abnormal vibration at ankles, % (n)	33% (30/90)	55% (40/73)	53% (31/58)	**0.008**
Ophthalmoplegia, % (n)	11% (9/82)	13% (9/70)	27% (14/52)	**0.04**
Ptosis, % (n)	3% (2/70)	3% (2/63)	9% (4/46)	0.31
Diminished visual acuity, % (n)	11% (9/81)	10% (7/67)	24% (12/51)	0.09
Dysphagia, % (n)	12% (9/72)	11% (7/63)	19% (9/48)	0.53
Sphincter dysfunction, % (n)	33% (28/84)	48% (35/73)	56% (33/59)	**0.02**
Cognitive impairment, % (n)	7% (6/90)	8% (6/76)	8% (5/59)	0.90

Abbreviations: SD = standard deviation; y = years. Significant changes are indicated in bold.

p-value: ANOVA for quantitative data and Fisher's exact test for qualitative variables

*Significant difference for the SARA score between the first group (evolution < 10 y) and the third group (evolution ≥ 20) (*post-hoc* comparisons)

**Significant difference for the stage of disability between the first group (evolution < 10 y) and the second group (evolution 10-19 y) and between the first and the third groups (evolution ≥ 20) (*post-hoc* comparisons)

Figure 1. Pedigrees of the 12 families with a dominant inheritance pattern.

The index case is indicated by an arrow.

Figure 2. Representation of all detected variants of this study.

Representation of the SPG7 gene with functional domains (FtsH family, ATPases associated with diverse cellular activities, peptidase M41 family) and all detected variants. In grey, previously reported variants; in bold, missense variants.

Figure 3. Brain MRI of SPG7 patients carrying different variants.

Sagittal T1 weighted and T2 weighted (indicated by *) brain MRI showing different degrees of cerebellar atrophy. The age at brain MRI, disease duration (DD) and variants are reported for each patient and no evident correlation with the degree of atrophy can be seen.

Figure 4. **Cerebellum of SPG7 patient AAR-247-004.** **A.** Macroscopical aspect of the cerebellum. Sagittal section of the cerebellar vermis on the left and of the hemisphere on the right. Note the contrast between the severe atrophy of the vermis and the relatively preserved size of the hemisphere. **B.** A closer view of a folium of the vermis showing pallor of the album and an almost complete loss of Purkinje cells. **C.** Preserved Purkinje cell marked with a black arrow, loss of a Purkinje cell shown by an empty basket (red arrow). **D.** Preserved Purkinje cell marked by a black arrow, and a torpedo, evidence of axonal alteration, marked by a red arrow. **E.** Substantia nigra pars compacta with the presence of extracellular pigments as an evidence of moderate neuronal loss (marked by red arrows). The black arrow shows one normal, pigmented neuron. **F.** Hippocampus with a thin dentate gyrus marked by red arrows and two neurons (CA4 sector) indicated by black arrows. **A-D.** Double labeling (histochemistry) of myelin (myelin basic protein in brown) and axons (neurofilament in red). Scale bar 5 mm (A), 2 mm (B), 50 µm (C and D). **E and F.** Haematein-eosin stain with additional luxol for myelin in E, scale bar: 100µm.

Table 4. New SPG7 variants found in the analyzed cohort (data available from Dryad**).**

Abbreviations: *SIFT (D: deleterious), PolyPhen (D: probably damaging), LRT (D: deleterious), M-Cap (D:deleterious), MutationTaster (A: disease causing automatic ; D: disease causing)

References

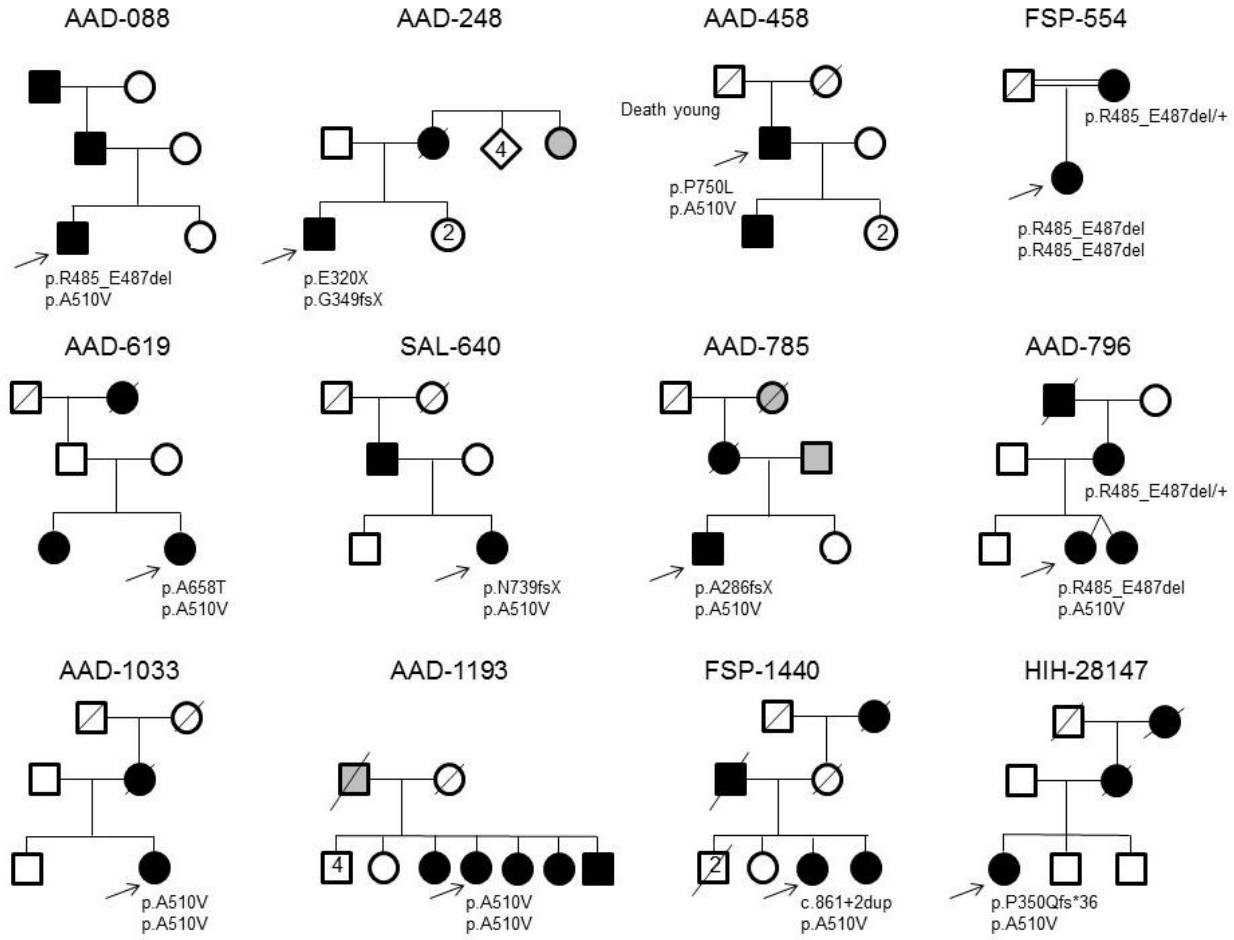
1. Harding AE. 1983. Classification of the hereditary ataxias and paraplegias. Lancet 1151–1154.
2. Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), {date}. World Wide Web URL: https://omim.org/.
3. Casari G, De Fusco M, Ciarmatori S, et al. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. Cell 1998; 93: 973–983.
4. Elleuch N, Depienne C, Benomar A, et al. Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia. Neurology. 2006 Mar 14;66(5):654-659.
5. Brugman F, Scheffer H, Wokke JH, et al. Paraplegin mutations in sporadic adult-onset upper motor neuron syndromes. Neurology. 2008 Nov 4;71(19):1500-1505.
6. Klebe S, Depienne C, Gerber S, et al. Spastic paraplegia gene 7 in patients with spasticity and/or optic neuropathy. Brain 2012 Oct;135 (Pt 10):2980-2993.
7. Pfeffer G, Pyle A, Griffin H, et al. SPG7 mutations are a common cause of undiagnosed ataxia. Neurology. 2015 Mar 17;84(11):1174-1176.
8. Coutelier M, Coarelli G, Monin ML, et al. A panel study on patients with dominant cerebellar ataxia highlights the frequency of channelopathies. Brain. 2017 Jun 1;140(6):1579-1594.
9. Atorino L, Silvestri L, Koppen M, et al. Loss of m-AAA protease in mitochondria causes complex I deficiency and increased sensitivity to oxidative stress in hereditary spastic paraplegia. J Cell Biol 2003; 163: 777–787.

10. Koppen M, Metodiev MD, Casari G, Rugarli EI, Langer T. Variable and tissue-specific subunit composition of mitochondrial m-AAA protease complexes linked to hereditary spastic paraplegia. Mol Cell Biol 2007;27: 758–767.
11. Sacco T, Boda E, Hoxha E, et al. Mouse brain expression patterns of Spg7, Afg3l1, and Afg3l2 transcripts, encoding for the mitochondrial m-AAA protease. BMC Neurosci 2010;11:55.
12. Patron M, Sprenger HG, Langer T. m-AAA proteases, mitochondrial calcium homeostasis and neurodegeneration. Cell Res. 2018 Mar;28(3):296-306.
13. Arnoldi A, Tonelli A, Crippa F, et al. A clinical, genetic, and biochemical characterization of SPG7 mutations in a large cohort of patients with hereditary spastic paraplegia. Hum Mutat. 2008 Apr;29(4):522-531.
14. Bonn F, Pantakani K, Shoukier M, Langer T, Mannan AU. Functional evaluation of paraplegin mutations by a yeast complementation assay. Hum Mutat 2010: 31 (5): 617–621.
15. Sanchez-Ferrero E, Coto E, Beetz C, et al. SPG7 mutational screening in spastic paraplegia patients supports a dominant effect for some mutations and a pathogenic role for p.A510V. Clin Genet. 2013 Mar;83(3):257-262.
16. Mancini C, Giorgio E, Rubegni A et al. Prevalence and phenotype of the c.1529C>T SPG7 variant in adult-onset cerebellar ataxia in Italy. Eur J Neurol. 2018 Aug 11.
17. Roxburgh RH, Marquis-Nicholson R, Ashton F, et al. The p.Ala510Val mutation in the SPG7 (paraplegin) gene is the most common mutation causing adult onset neurogenetic disease in patients of British ancestry. J Neurol. 2013 May;260(5):1286-94.

18. van Gassen KL, van der Heijden CD, de Bot ST, et al. Genotype-phenotype correlations in spastic paraplegia type 7: a study in a large Dutch cohort. Brain. 2012 Oct;135(Pt 10):2994-3004.
19. Schlipf NA, Schüle R, Klimpe S, et al. Amplicon-based high-throughput pooled sequencing identifies mutations in CYP7B1 and SPG7 in sporadic spastic paraplegia patients. Clin Genet. 2011 Aug;80(2):148-160.
20. Thal DR, Züchner S, Gierer S, et al. Abnormal Paraplegin Expression in Swollen Neurites, τ- and α-Synuclein Pathology in a Case of Hereditary Spastic Paraplegia SPG7 with an Ala510Val Mutation. Int J Mol Sci. 2015 Oct 21;16(10):25050-66.
21. Schmitz-Hübsch T, du Montcel ST, Baliko L, et al. Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology. 2006 Jun 13;66(11):1717-1720.
22. Pfeffer G, Gorman GS, Griffin H, et al. Mutations in the SPG7 gene cause chronic progressive external ophthalmoplegia through disordered mitochondrial DNA maintenance. Brain. 2014 May;137(Pt 5):1323-36.
23. Hewamadduma CA, Hoggard N, O'Malley R, et al. Novel genotype-phenotype and MRI correlations in a large cohort of patients with SPG7 mutations. Neurol Genet. 2018 Oct 24;4(6):e279.
24. Synofzik M, Schüle R. Overcoming the divide between ataxias and spastic paraplegias: Shared phenotypes, genes, and pathways. Mov Disord. 2017 Mar;32(3):332-345.
25. Ehses S, Raschke I, Mancuso G, et al. Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. J Cell Biol. 2009 Dec 28;187(7):1023-36.

26. Magri S, Fracasso V, Plumari M, et al. Concurrent AFG3L2 and SPG7 mutations associated with syndromic parkinsonism and optic atrophy with aberrant OPA1 processing and mitochondrial network fragmentation. Hum Mutat. 2018 Dec;39(12):2060-2071.
27. Choquet K, Tétreault M, Yang S, et al. SPG7 mutations explain a significant proportion of French Canadian spastic ataxia cases. Eur J Hum Genet. 2016 Jul;24(7):1016-1021.
28. Jacobi H, du Montcel ST, Bauer P, et al. Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. Lancet Neurol. 2015 Nov;14(11):1101-1108.
29. Schmitz-Hübsch T, Fimmers R, Rakowicz M et al. Responsiveness of different rating instruments in spinocerebellar ataxia patients. Neurology. 2010 Feb 23;74(8):678-684.
30. Jacobi H, du Montcel ST, Bauer P, et al. Long-term evolution of patient-reported outcome measures in spinocerebellar ataxias. J Neurol. 2018 Sep;265(9):2040-2051.
31. Schüle R, Holland-Letz T, Klimpe S, et al. The Spastic Paraplegia Rating Scale (SPRS): a reliable and valid measure of disease severity. Neurology. 2006 Aug 8;67(3):430-434.
32. McDermott CJ, Dayaratne RK, Tomkins J, et al. Paraplegin gene analysis in hereditary spastic paraparesis (HSP) pedigrees in northeast England. Neurology. 2001 Feb 27;56(4):467-471.

FIGURE 1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AAD-1193
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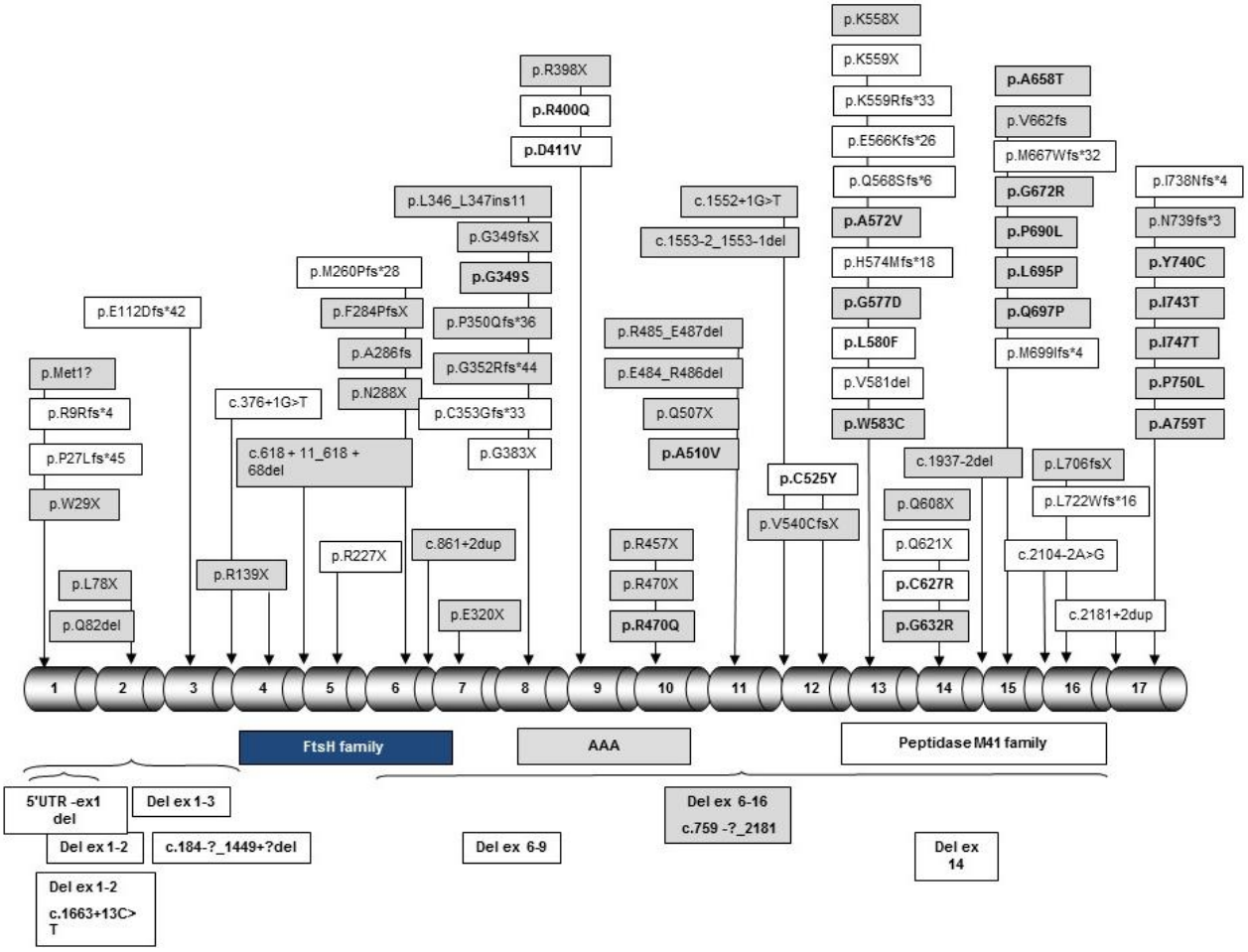
p.A510V

FIGURE 2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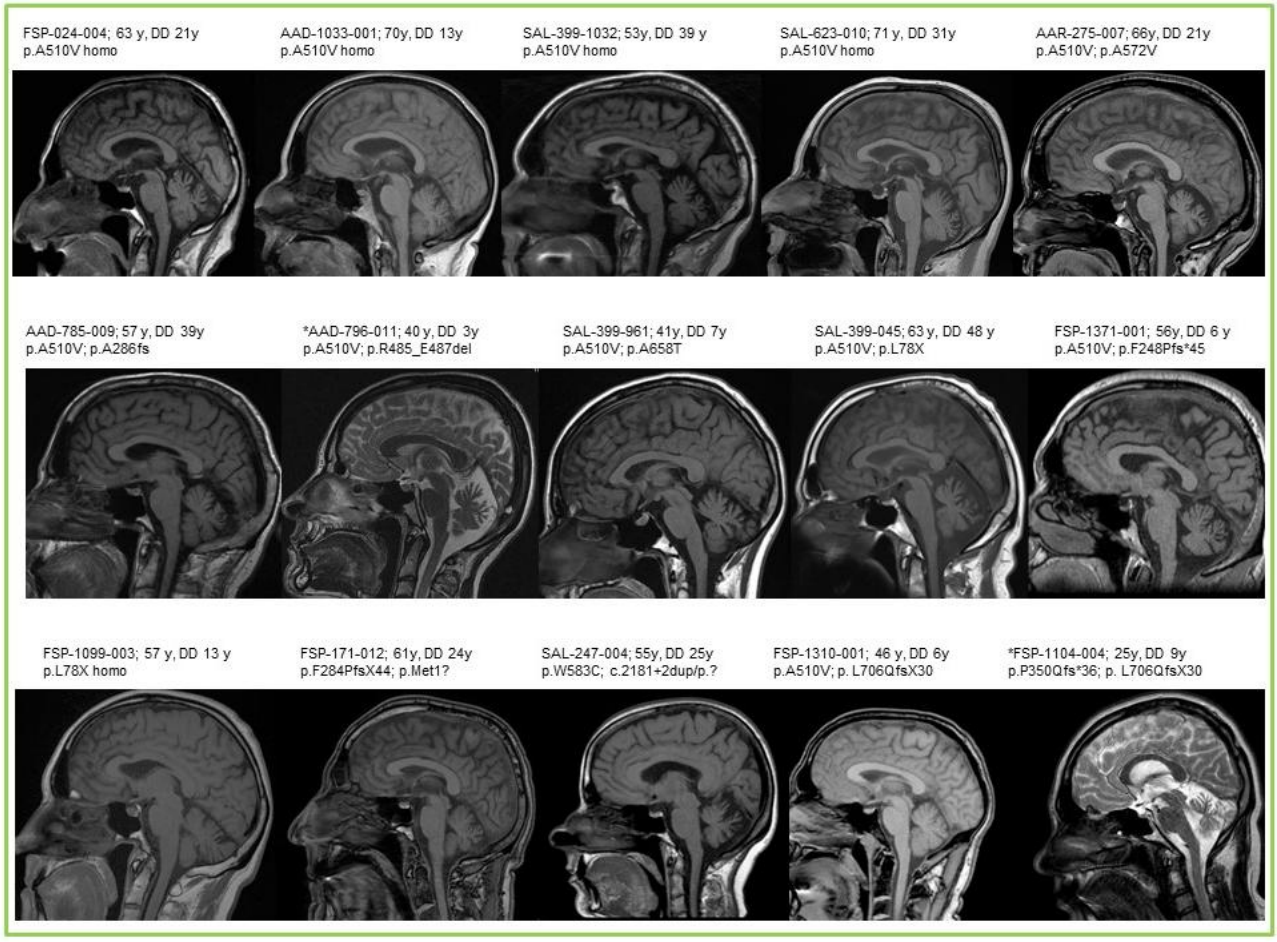
p.L695P
p.Q697P
p.M699Ifs*4
p.I738Nfs*4
p.N739fs*3
p.Y740C
p.I743T
p.I747T
p.P750L
p.A759T
p.R398X
p.R400Q
p.D411V
p.L346_L347ins11
p.G349fsX
p.G349S
p.M260Pfs*28
p.F284PfsX
p.P350Qfs*36
p.A286fs
p.G352Rfs*44
p.N288X
p.C353Gfs*33
p.G383X
p.E112Dfs*42
p.Met1?
p.R9Rfs*4
p.P27Lfs*45
p.W29X
c.376+1G>T
c.618 + 11_618 + 68del
p.L78X
p.Q82del
p.R139X
p.R227X
c.861+2dup
p.E320X
c.1552+1G>T
c.1553-2_1553-1del
p.R485_E487del
p.E484_R486del
p.Q507X
p.A510V
p.R457X
p.R470X
p.R470Q
p.C525Y
p.V540CfsX
c.1937-2del
p.L706fsX
p.L722Wfs*16
p.Q608X
p.Q621X
p.C627R
p.G632R
c.2104-2A>G
c.2181+2dup
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
FtsH family
AAA
Peptidase M41 family
5'UTR -ex1 del
Del ex 1-3
Del ex 1-2
c.184-?_1449+?del
Del ex 1-2
c.1663+13C>T
Del ex 6-9
Del ex 6-16
c.759 -?_2181
Del ex 14

FIGURE 3


FSP-024-004; 63 y, DD 21y
p.A510V homo
AAD-1033-001; 70y, DD 13y
p.A510V homo
SAL-399-1032; 53y, DD 39 y
p.A510V homo
SAL-623-010; 71 y, DD 31y
p.A510V homo
AAR-275-007; 66y, DD 21y
p.A510V; p.A572V
AAD-785-009; 57 y, DD 39y
p.A510V; p.A286fs
*AAD-796-011; 40 y, DD 3y
p.A510V; p.R485_E487del
SAL-399-961; 41y, DD 7y
p.A510V; p.A658T
SAL-399-045; 63 y, DD 48 y
p.A510V; p.L78X
FSP-1371-001; 56y, DD 6 y
p.A510V; p.F248Pfs*45
FSP-1099-003; 57 y, DD 13 y
p.L78X homo
FSP-171-012; 61y, DD 24y
p.F284PfsX44; p.Met1?
SAL-247-004; 55y, DD 25y
p.W583C; c.2181+2dup/p.?
FSP-1310-001; 46 y, DD 6y
p.A510V; p. L706QfsX30
*FSP-1104-004; 25y, DD 9y
p.P350Qfs*36; p. L706QfsX30

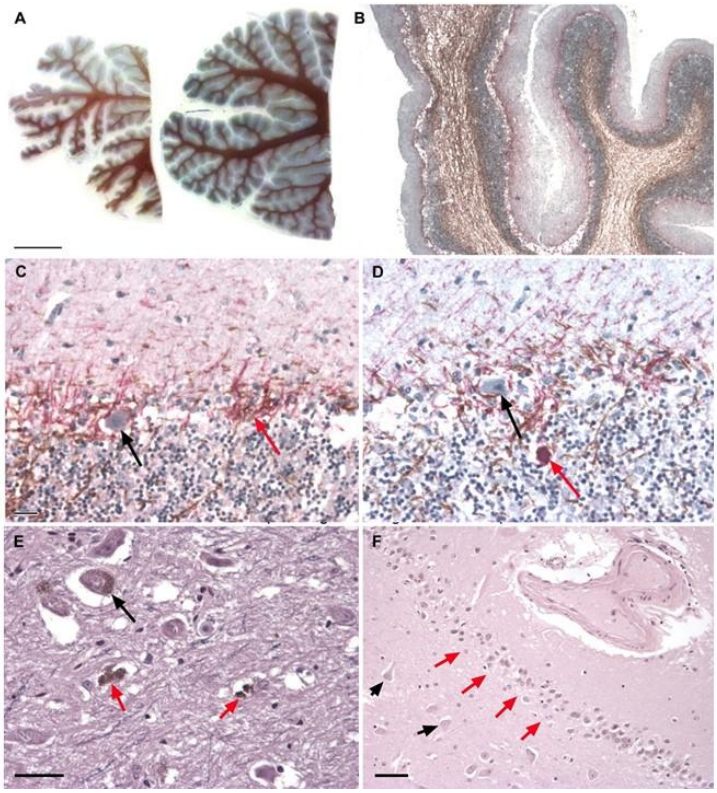



FIGURE 4