# A founder mutation p.H701P identified as a major cause of SPG7 in Norway

Siri Lynne Rydning, MD[† 1, 2], Iselin M. Wedding, MD[† 1, 2], Jeanette Koht, MD, PhD [3], Maninder Chawla, MD [4], Ane-Marte Øye, MSc [5], Ying Sheng, MSc [5], Magnus D. Vigeland, PhD [5], Kaja K. Selmer, MD, PhD[‡ 5], Chantal M. E. Tallaksen, MD, PhD[‡ 1, 2]

[†,‡] These authors contributed equally to this study

[1] Department of Neurology, Oslo University Hospital, Norway

[2] Institute of Clinical Medicine, University of Oslo, Norway

[3] Department of Neurology, Drammen Hospital, Vestre Viken Health Trust, Norway

[4] Department of Radiology and Nuclear Medicine, Oslo University Hospital, Norway

[5] Department of Medical Genetics, Oslo University Hospital and University of Oslo, Norway

Total word count: 3425

Running title: SPG7 in Norway

Keywords: SPG7, hereditary spastic paraplegia, hereditary ataxia, spastic ataxia, p.H701P, founder mutation

Corresponding author: Siri Lynne Rydning. Oslo University Hospital, Department of Neurology, Ullevål Hospital, P.O. Box 4956 Nydalen, N-0424 Oslo, Norway. Telephone: +47 23026443. E-mail: s.l.rydning@medisin.uio.no

**Disclosure of conflicts of intererest**

The authors declare no financial or other conflicts of interest. The study was funded by Oslo University Hospital, South-Eastern Health Region grants (IMW, KKS), Vestre Viken Health Trust (JK) and grants from “Vidar Nybakks legat, slått sammen med Klara Bergs gave” (SLR).

**Abstract**

**Background and purpose:** SPG7 is one of the most common forms of autosomal recessive hereditary spastic paraplegia. The phenotype has been shown to be heterogeneous, varying from a complex spastic ataxia to pure spastic paraplegia or pure ataxia. The aim of this study was to clinically and genetically characterize patients with SPG7 in Norway.

**Methods:** Six Norwegian families with the clinical diagnosis of hereditary spastic paraplegia were diagnosed with SPG7 through Sanger sequencing and whole-exome sequencing. Haplotypes were established to identify a possible founder mutation. All patients were thoroughly examined and the clinical and molecular findings are described.

**Results:** The core phenotype was spastic paraparesis with ataxia, bladder disturbances and progressive external ophthalmoplegia. The variant p.H701P was identified in homozygous state in one family and in compound heterozygous state in three families. Haplotype analysis of seven surrounding single nucleotide polymorphisms supports that this variant resides on a founder haplotype. Four of the families were compound heterozygous for the previously well-described p.A510V variant.

**Conclusion:** SPG7 is a common subgroup of hereditary spinocerebellar disorders in Norway. The broad phenotype in the Norwegian SPG7 population illustrates that the challenges with the traditional dichotomous classification of hereditary spinocerebellar disorders into hereditary spastic paraplegia or hereditary ataxia. A Norwegian founder mutation p.H701P was identified in four out of six families, making it a major cause of SPG7 in Norway.

**Introduction**

Hereditary spastic paraplegia (HSP) and hereditary ataxia (HA) are clinically and genetically heterogeneous disorders. HSP is characterized by progressive spastic paraparesis with varying degrees of bladder involvement and mild sensory dysfunction in the lower limbs [1], while HA is characterized by progressive gait and limb ataxia, loss of coordination and disturbances of oculomotor control [2]. Classically, the disorders are divided into pure and complex forms, depending on the presence of additional neurological signs [3]. Both HSP and HA may be inherited in an autosomal dominant (AD), recessive (AR) or X-linked manner and more than 100 disease-causing genes have been identified [1, 2]. Phenotypically, HSP and HA are frequently overlapping, such as in Spastic paraplegia 7 (SPG7), caused by mutations in the *SPG7* gene. *SPG7* encodes paraplegin, a member of the AAA family of ATPases, located at the inner mitochondrial membrane. SPG7 was first described as a rare AR HSP [4]. During the last years, both dominant inheritance [5-7], and a wider phenotypic spectrum have been described. SPG7 is frequently associated with cerebellar involvement [8, 9], as well as optic neuropathy [6], supranuclear palsy [10], ptosis and progressive external ophthalmoplegia (PEO). PEO is associated with mitochondrial disorders, especially where mutations in nuclear genes lead to multiple deletions in mitochondrial DNA, which is consistent with the role paraplegin is found to play in mitochondrial DNA maintenance [11, 12].

In 2008 the prevalence of AR HSP was estimated to 0.6/100 000 and the total prevalence of HSP to 7.4/100 000 in southeast Norway [13]. As expected from other populations, mutation in the *SPG4* gene was reported as the most common cause of AD HSP, and mutations in the *SPG11* gene as the most common cause of AR HSP in Norway [14]. At the time, the *SPG7*-gene was not included in the diagnostic work-up, and thus not identified. The first Norwegian family with mutations in *SPG7* was described in 2008 [15]. Two additional families were reported in 2014 [11].

Due to the genetic and clinical heterogeneity in HSP, the traditional targeted genetic testing represents a major diagnostic challenge. With the development of high-throughput sequencing (HTS), whole-exome sequencing (WES) has made it possible to look for disease-causing variants in all

protein-coding genes, comprising about 1.5% of the entire genome. This genetic screening is of particular value in genetically heterogeneous monogenic diseases, although it introduces interpretation challenges. These new methods have led to identification of more SPG7 patients, making SPG7 a frequent cause of AR HSP in Norway, consistent with reports from other European populations [6, 16]. The aim of this study was to clinically and genetically characterize patients with SPG7 in Norway. We describe six Norwegian families with SPG7 and provide evidence of a novel founder mutation p.H701P which is hitherto only found in Norwegian patients.

## Material and methods

### Patients

All patients with hereditary spinocerebellar disorders referred to the Department of Neurology, Oslo University Hospital, Ullevaal are systematically registered in a research database. Most patients are recruited from southeast Norway. At the time of this study, 530 probands with HSP or HA were included. Based on phenotype and pedigree information, 215 were classified as HSP and 28 as recessive HSP. After genetic testing six of the 28 AR probands were diagnosed with SPG7. The families were of Norwegian origin, with consanguinity in one family, otherwise no known common ancestors (Fig. 1). All patients underwent neurological examination according to a standard protocol that included Spastic Paraplegia Rating Scale (SPRS) and Scale for the Assessment and Rating of Ataxia (SARA) [17, 18]. Written informed consent was obtained from all study participants. The project is approved by the Regional Committee for Medical and Health Research Ethics, South East Norway (ethical agreement nº REK 2010/1579a).

### Genetic analyses

Families A-C were diagnosed by Sanger sequencing as previously described [11, 15], whereas families D-F were diagnosed through WES. We performed targeted enrichment of the exome using the Agilent SureSelect Human All Exon kit v4. Sequencing was performed on an Illumina HiSeq2000 with 100 bp paired-end reads. Reads were aligned to the reference human genome (hg19) using Novoalign (V2.07.17) (http://www.novocraft.com/products/novoalign) and the Genome Analysis

Toolkit (GATK, v2.4-9) [19, 20]. Variant calling was performed using GATK (v2.4-9). Variants were annotated by ANNOVAR (2012 May 25) [21]. Downstream filtering and analysis was done in Filtus (http://folk.uio.no/magnusv/filtus.html), using allele frequencies, conservation scores (GERP) and various *in silico* prediction tools [22-25] to search for pathogenic variants compatible with recessive inheritance. Validation of the *SPG7* variants was performed by Sanger sequencing in the patients and first degree relatives when available (primer sequences available on request). Haplotyping was performed by genotyping all available members of families A, B, D, E and F with seven SNPs, covering a 1.3 Mb region surrounding the *SPG7* gene. The results were analysed in MERLIN with the "--best" option [26].

**Results**

**Clinical description**

Clinical findings are reported in detail in Table 1. The core findings were spasticity and hyperreflexia in the lower limbs (11/11), urge incontinence (11/11), ataxia (10/11), hyperreflexia in the upper limbs (9/11), ptosis (9/11) and progressive external ophthalmoplegia (PEO) (8/11). Cerebellar atrophy was present on MRI in all patients (Fig. 2). Patient FIII-1 had complicating features with epilepsy, possibly due to birth asphyxia, as well as cervical myelopathy due to disc herniation with medullar impression in C3/C4. These features were not present in the sibling FIII-2. Serum creatinine kinase (CK) levels were available in seven patients and all were within normal range. Nutritional status was in general reported as good, without evidence of wasting or dysphagia. The symptoms were progressive in all patients. In patient EIII-1, examinations with a ten-year interval showed an increase in SPRS from 7 to 29, and in SARA from 12 to 26.5. In a two-year interval, patient FIII-2 showed an SPRS increase of 16.5 to 21 and SARA increase of 9.5 to 13. IQ assessments were previously reported in three patients (AIV-2, BIII-2, BIII-4) [11], with total IQ ranging from 78 to 79 and a more pronounced verbal than executive IQ impairment in all [27]. Only one of the patients was full-time employed at the time of examination and only one had an academic education. Psychiatric problems were reported in 5/6 families, with symptoms of depression and anxiety as the most frequent.

**Genetic analyses**

The variants identified in *SPG7* are listed in Table 1. In silico predictions and conservation scores of the missense variants were analysed in SIFT, PolyPhen2 and GERP and are listed in Table 3. Segregation analysis confirmed that the variants were *in trans*, except in family D where no additional family members were available for analysis. In the families who underwent WES, we did not identify additional variants in other genes related to HSP or HA which were considered pathogenic.

Haplotype analysis was performed using genotypes from seven SNPs surrounding the *SPG7* gene. In families A and F phasing was unique, assuming no recombination or mutation. In particular, both patients in family A were homozygous for all markers, thus determining the haplotype carrying p.H701P. Furthermore, family F gave haplotypes surrounding the p.A510V and p.K588T variants, respectively. In families B, D and E phasing was not unique, due to too few available family members. However, in all three cases the genotypes matched the haplotypes identified in families A and F (Fig. 1). As each of these haplotypes contains several low frequency alleles, this is unlikely to occur by chance. Thus, we identified a shared haplotype co-segregating with the p.H701P mutation in all four families with this mutation, supporting that this is a founder mutation in the Norwegian population.

**Discussion**

By using both the traditional phenotype-guided sequencing of single candidate genes, and the HTS parallell sequencing approach, we have identified 13 SPG7 patients in the Norwegian population, making SPG7 the second most common recessive paraplegia in Norway. In our cohort of 28 probands with autosomal recessive HSP, 21% (6) are now diagnosed as SPG7, 25% (7) as SPG11 and 7% (2) as SPG5. Among these, only *SPG11* has been available for Sanger sequencing in a diagnostic laboratory Norway, which probably biases our results. To the best of our knowledge, these are all the identified families with SPG7 in southeast and west Norway, covering about 4/5 of the Norwegian population. SPG7 is traditionally classified as HSP. However, as ataxia is increasingly recognized as a major feature, SPG7 could also be classified as a spastic ataxia [28]. Interestingly, paraplegin is the binding

partner of AFG3L2 on the inner mitochondrial membrane. Mutations in *AFG3L2* causes the dominant ataxia SCA28 [29], and this may explain the association between ataxia and spastic paraparesis in SPG7. A recently published study even showed that SPG7 is a common cause of undiagnosed ataxia presenting in mid-adult life [16]. SPG7 is a good example of a phenotype which is difficult to fit into the dichotomy of the traditional classification system. This is important to remember when chosing molecular work-up for patients.

There appears to be a core phenotype for SPG7 in the Norwegian population, which includes spastic paraparesis with ataxia, PEO, bladder disturbances and slight cognitive impairment. Thus, these symptoms and a possible AR inheritance pattern should raise the suspicion of SPG7. The only patient without clinical ataxia had cervical myopathy due to cervical disc herniation, which may have skewed the clinical picture towards spastic paraparesis; however MRI revealed mild cerebellar atrophy. Signs of cognitive deficit, more pronounced verbal than executive, were present in the patients tested. In addition, psychiatric symptoms were observed in most families, and the clinical impression of mild anosognosia was noted in several patients. All these features are present in the clinical description of the Cerebellar Cognitive Affective Syndrome (CCAS), which is increasingly recognized in cerebellar disorders [30]. Bladder symptoms were reported as one of the patients' main problems and should be carefully addressed in the clinic, as it may have significant impact on the patients' quality of life. As bladder problems are seldom reported in hereditary ataxias, urge incontinence could be a diagnostic clue to differentiate SPG7 from other hereditary ataxias.

The families have no known common ancestors, though four share the same missense mutation c.2102A>C; p.H701P which has only been described in Norwegian patients. The variant co-segregates with disease in these families and is believed to be pathogenic. It was absent in 384 Norwegian control alleles, and the mutation changes a highly conserved histidine to proline in the catalytic Pfam metallopeptidase domain of paraplegin [11], where most of the known pathogenic mutations in paraplegin occur [31].The variant is not present in neither the esp6500, nor the 1000G database, and

has only been reported four times in heterozygous state in the ExAC database (www.exac.broadinstitute.org), resulting in the low frequency of 3.4E-05.

Haplotype analysis revealed that all patients having the p.H701P variant also share a common haplotype covering 1.3 Mb region surrounding the variant (see Fig. 1), which indeed supports that this variant represents a founder mutation. This variant is involved in four of the six identified Norwegian families with SPG7 and is thus a major cause of SPG7 in Norway.

The mutation c.1672A>T in family F causes a premature stop codon, p.K588X. It has been reported once before in a homozygous state in a man with late onset spastic paraparesis [8] and is believed to be disease causing. The variant c.1529C>T; p.A510V present in families D-F is the most common cause of SPG7 and has been shown to co-segregate with disease in several studies from different countries [32]. It was originally considered a polymorphism due to high carrier frequency [33], but both *in silico* analysis and functional studies in yeast suggest a pathogenic effect [34] and more recent studies conclude that this is a disease-causing mutation [7, 32].

**Conclusions**

SPG7 causes at least 21% of the AR HSP cases in a large Norwegian cohort. Core phenotype includes PEO, bladder disturbances and spastic ataxia, the latter emphasizing the prominent role of ataxia in SPG7. Moreover, a variant p.H701P appears to be unique for Norwegian patients and resides on a founder haplotype.

**Acknowledgements**

We thank the families for participating in this study and C. Tzoulis and L.A. Bindoff at Haukeland University Hospital, Bergen, Norway for collaboration and patients.

**References**

1. Fink JK. Hereditary spastic paraplegia: clinical principles and genetic advances. *Seminars in neurology*. 2014;34(3):293-305.
2. Synofzik M, Schöls L, Riess O. Hereditäre Ataxien. *medizinische genetik*. 2013;25(2):235-48.

3. Harding AE. Classification of the hereditary ataxias and paraplegias. *Lancet*. 1983;1(8334):1151-5.

4. Casari G, De FM, Ciarmatori S, et al. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell*. 1998;93(6):973-83.

5. Casari G, Marconi R. Spastic Paraplegia 7. In: Pagon R, Adam M, Bird T, Dolan C, Fong C, Smith R, editors. GeneReviews. GeneReviews. Seattle (WA)1993.

6. Klebe S, Depienne C, Gerber S, et al. Spastic paraplegia gene 7 in patients with spasticity and/or optic neuropathy. *Brain*. 2012;135(10):2980-93.

7. 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;83(3):257-62.

8. 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;135(10):2994-3004.

9. Elleuch N, Depienne C, Benomar A, et al. Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia. *Neurology*. 2006;66(5):654-9.

10. Warnecke T, Duning T, Schwan A, Lohmann H, Epplen JT, Young P. A novel form of autosomal recessive hereditary spastic paraplegia caused by a new SPG7 mutation. *Neurology*. 2007;69(4):368-75.

11. Wedding IM, Koht J, Tran GT, et al. Spastic paraplegia type 7 is associated with multiple mitochondrial DNA deletions. *PloS one*. 2014;9(1):e86340.

12. 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;137(5):1323-36.

13. Erichsen AK, Koht J, Stray-Pedersen A, Abdelnoor M, Tallaksen CM. Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: a population-based study. *Brain*. 2009;132(6):1577-88.

14. Erichsen AK, Stevanin G, Denora P, Brice A, Tallaksen CM. SPG11--the most common type of recessive spastic paraplegia in Norway? *Acta Neurol Scand Suppl*. 2008;188:46-50.

15. Tzoulis C, Denora PS, Santorelli FM, Bindoff LA. Hereditary spastic paraplegia caused by the novel mutation 1047insC in the SPG7 gene. *JNeurol*. 2008;255(8):1142-4.

16. Pfeffer G, Pyle A, Griffin H, et al. SPG7 mutations are a common cause of undiagnosed ataxia. *Neurology*. 2015;84(11):1174-6.

17. Schule R, Holland-Letz T, Klimpe S, et al. The Spastic Paraplegia Rating Scale (SPRS): a reliable and valid measure of disease severity. *Neurology*. 2006;67(3):430-4.

18. Schmitz-Hubsch T, Tezenas du MS, Baliko L, et al. Reliability and validity of the International Cooperative Ataxia Rating Scale: a study in 156 spinocerebellar ataxia patients. *Mov Disord*. 2006;21(5):699-704.

19. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491-8.

20. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-303.

21. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164.

22. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003;31(13):3812-4.

23. Sunyaev S, Ramensky V, Koch I, Lathe W, III, Kondrashov AS, Bork P. Prediction of deleterious human alleles. *HumMolGenet*. 2001;10(6):591-7.

24. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res*. 2002;30(17):3894-900.

25. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Meth*. 2014;11(4):361-2.

26. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nature genetics*. 2002;30(1):97-101.

27. Wechsler DA. Wechsler Adult Intelligence Scale. San Antonio, TX: Psychological Corporation; 2008.

28. de Bot ST, Willemsen MA, Vermeer S, Kremer HP, van de Warrenburg BP. Reviewing the genetic causes of spastic-ataxias. *Neurology*. 2012;79(14):1507-14.

29. Di Bella D, Lazzaro F, Brusco A, et al. Mutations in the mitochondrial protease gene AFG3L2 cause dominant hereditary ataxia SCA28. *Nature genetics*. 2010;42(4):313-21.

30. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain*. 1998;121 (4):561-79.

31. Pierson TM, Adams D, Bonn F, et al. Whole-exome sequencing identifies homozygous AFG3L2 mutations in a spastic ataxia-neuropathy syndrome linked to mitochondrial m-AAA proteases. *PLoS genetics*. 2011;7(10):e1002325.

32. 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;260(5):1286-94.

33. McDermott CJ, Dayaratne RK, Tomkins J, et al. Paraplegin gene analysis in hereditary spastic paraparesis (HSP) pedigrees in northeast England. *Neurology*. 2001;56(4):467-71.

34. 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):617-21.

**Figure legends**

**Figure 1.** Schematic illustration of the pedigrees. Square = male; circle = female; diamond = masked gender (for anonymisation purposes); filled symbol = affected; diagonal line = deceased. Phased

genotypes are displayed below each genotyped individual, with colours highlighting the haplotypes carrying mutations. Details about the SNPs are given in the lower right corner of the figure. SNP number four resides within the *SPG7*-gene.

**Figure 2.** Cerebral MRI from six SPG7 patients in sagittal, coronal and axial view. DD = disease duration in years (y). 1: DD; 60 y. Atrophy of the vermis, widening of cerebellar folias. Cerebral hemisphere atrophy with widening of sulci, thinning of the corpus callosum, and ventricular dilatation. 2: DD; 48 y. Mild thinning of the vermis, mild widening of cerebellar folias including primary fissure. 3: DD; 15 y. Moderate atrophy of the vermis, mild widening of cerebellar folias. 4: DD; 15 y. Mild thinning of the vermis, mild widening of cerebellar folias. 5: DD; 25 y. Normal vermis, mild widening of cerebellar folias. 6: DD; 11 y. Mild thinning of the vermis, mild widening of cerebellar folias.

Table 1. Summary of clinical features at the time of examination and results of investigations. Core phenotypic features are marked in bold.

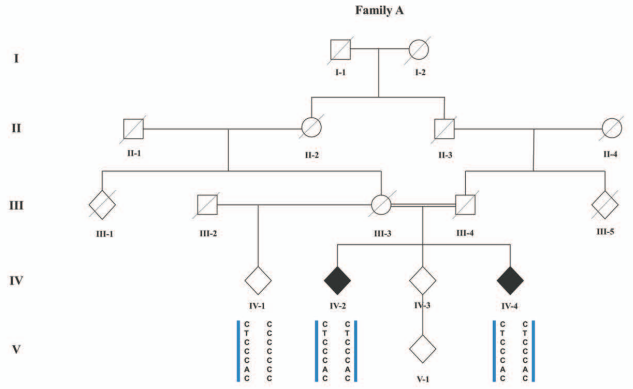
| **Patient** | **AIV-2** | **AIV-4** | **BIII-2** | **BIII-4** | **C** | **DIII-3** | **DIII-4** | **EIII-1** | **EIII-2** | **FIII-1** | **FIII-2** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age at onset (years) | 7 | 8 | 15 | 27 | 43 | 20 | 24 | 36 | 22 | 20 | 36 |
| Disease duration at examination | 60 | 55 | 54 | 40 | 10 | 23 | 22 | 10 | 15 | 25 | 17 |
| Disability stage[a] | 6 | 6 | 5 | 5 | 2 | 3 | 4 | 3 | 3 | 5 | 3 |
| **Ptosis** | + | + | + | + | + | + | + | + | - | - | + |
| **Progressive eksternal ophthalmoplegia** | + | + | + | + | + | - | - | + | - | + | + |
| **Urge incontinence** | + | + | + | + | + | + | + | + | + | + | + |
| **Lower limb spasticity** | + | + | + | + | + | + | + | + | + | + | + |
| **Limb ataxia** | + | ++ | + | + | - | + | ++ | + | + | - | + |
| First symptom | Unsteady | Unsteady | Leg paresis | Unsteady | Stiffness | Unsteady, stiffness | Unsteady, stiffness | Stiffness, unsteady | Unsteady | Stiffness | Stiffness |
| Dysarthria | + | + | + | + | - | - | - | + | + | - | - |
| Upper limb hyperreflexia | + | + | + | + | - | + | + | + | + | + | - |
| Upper limb spasticity | + | + | + | - | - | - | - | + | - | - | - |
| Upper limb muscle strength[b] | 1 | 1 | 0 | 0 | 0 | 1 | na | 0 | 0 | 0 | 1 |
| Lower limb hyperreflexia | + | + | + | + | + | + | + | + | + | + | + |
| Lower limb muscle strength[b] | 3 | 3 | 2 | 2 | 0 | 1 | na | 1 | 0 | 2 | 1 |
| Plantar response inversion | Bilateral | Bilateral | Bilateral | Bilateral | Indifferent | Bilateral | Bilateral | Bilateral | Unilateral | - | Bilateral |
| Truncal ataxia | + | + | + | + | + | + | + | + | + | - | + |
| Decreased vibratory sense in lower limbs | + | + | + | + | + | - | na | - | - | - | na |
| Nystagmus | - | - | + | + | + | - | + | + | + | na | - |
| Saccadic pursuit | nt | nt | + | + | + | + | na | + | + | na | + |
| Cognitive impairment | + | na | + | + | na | - | na | - | - | + | na |
| Dysphagia | - | na | - | - | - | + | - | + | - | na | - |
| Amyotrophy in lower limbs | + | + | - | - | - | + | na | - | - | - | - |
| SARA[c] | 29 | 25 | 26 | na | na | 6.5 | na | 12 | 13 | na | 13 |
| SPRS[d] | 43 | 41 | 45 | na | na | na | na | 7 | na | 12 | 21 |
| Cerebral MRI (disease duration at MRI in years) | Cbl.atr., TCC (60) | na | Cbl.atr. (48) | na | na | na | na | Cbl.atr. (15) | Cbl.atr. (15) | Cbl.atr (25) | Cbl.atr. (11) |
| Nerve Conduction Velocity Test (NCV) | Axonal sensorimotor neuropathy | na | na | na | na | na | na | Normal | Demyelinating neuropathy | Normal | Normal |
| Electromyography (EMG) | Decreased recruitment pattern | na | na | na | na | na | na | Normal | Normal | Normal | Normal |
| Other | - | - | - | Ca. coli, depression | Optic atrophy, bipolar disorder | Psoriasis | Anxiety | Rigidity | Anxiety, depression | Epilepsy, schizophrenia, diabetes | - |
| **Mutation allele 1**[e] | **c.2102A>C; p.H701P** | | **c.2102A>C; p.H701P** | | **c.1047insC** | **c.2102A>C; p.H701P** | | **c.2102A>C; p.H701P** | | **c.1672A>T; p.K558X** | |
| Mutation type | missense | | missense | | frameshift insertion | missense | | missense | | nonsense | |
| Prediction and concervation scores (SIFT/PP2/GERP)[f] | T/Da/4.86 | | T/Da/4.86 | | na | T/Da/4.86 | | T/Da/4.86 | | na | |
| **Mutation allele 2** | **c.2102A>C; p.H701P** | | **c.1454_1462del; p.A485_E487del** | | **c.1047insC** | **c.1529C>T; p.A510V** | | **c.1529C>T; p.A510V** | | **c.1529C>T; p.A510V** | |
| Mutation type | missense | | frameshift deletion | | frameshift insertion | missense | | missense | | missense | |
| Prediction and concervation scores (SIFT/PP2/GERP) | T/Da/4.86 | | na | | na | De/Da/4.56 | | De/Da/4.56 | | De/Da/4.56 | |

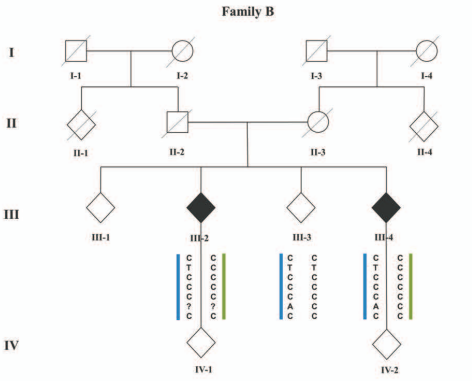
**+ = present; - = not present; na = not available; nt = not testable due to ophthalmoplegia; Cbl.atr = cerebellar atrophy; TCC = Thin Corpus Callosum**

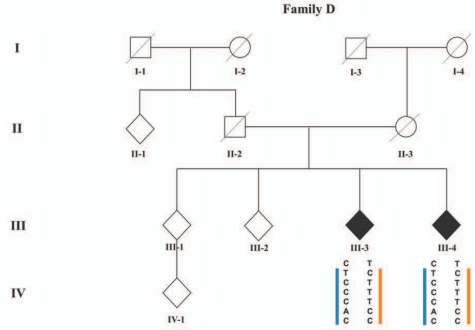
**[a]Disability stage 1-6; 1:Signs at examination; 2:Mild, able to run; 3:Moderate, limited walking without aid; 4:Severe, walking with one stick, 5:Walking with two sticks; 6:Requiring wheelchair**

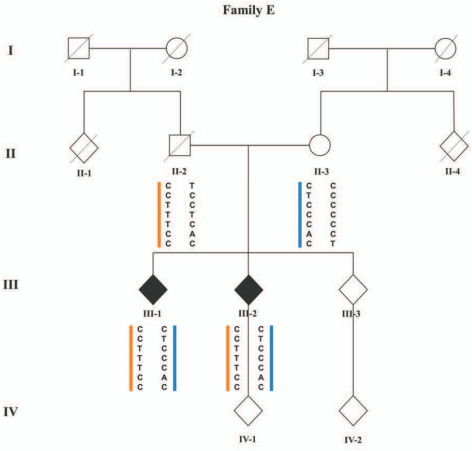
**[b]Graded 0-3: 0:Normal, 1:Mild, 2:Moderate, 3:Severe. [c]Standardized Rating Scale of Ataxia.[d]Spastic Paraplegia Rating Scale.**

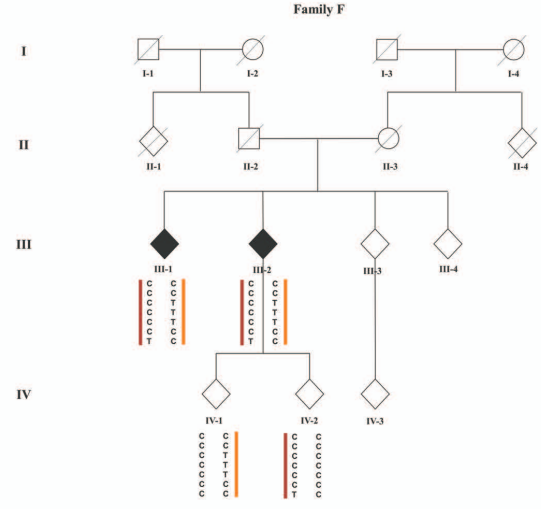
**[e]Transcript: NM_003119. [f]De=deleterious, Da=damaging, T=tolerated.**




## Haplotypes

- = with c.2102A>C; p.H701P
- = with c.1454_1462del; p.R485_E487
- = with c.1529C>T; p.A510V
- = with c.1672A>T; p.K588X

| SNP name | Position | Minor allele frequency (minor allele) |
|---|---|---|
| rs148564095 | 88798635 | 0.01 (T) |
| rs8182228 | 89180695 | 0.11 (T) |
| rs118065221 | 89199522 | 0.01 (T) |
| rs80324518 | 89614534 | 0.04 (T) |
| rs17719249 | 89793731 | 0.01 (T) |
| rs2159116 | 89831510 | 0.10 (A) |
| rs74583214 | 90110798 | 0.03 (T) |

**Figure 2**