Hereditary spastic paraplegia: a novel mutation and expansion of the phenotype variability in SPG10

INTRODUCTION

Hereditary spastic paraplegias (HSPs) are a group of disorders characterised by slow progressive weakness and spasticity of the lower limbs. HSPs have been divided into pure and complicated forms, depending

on the absence or presence of additional neurological or non-neurological features. To date, 72 loci and 55 spastic paraplegia genes (SPGs) have been identified.[1] SPG10 is caused by mutations in the *KIF5A* gene encoding neuron-specific kinesin heavy chain 5A (NK-HC5A), a member of the kinesin-1 family of motor proteins. In mammals, NK-HC5A is necessary for the anterograde axonal transport of neurofilament subunits, and it has a role in the transport of other anterograde cargoes, such as membrane vesicles. SPG10 is an autosomal dominant HSP (ADHSP), accounting for about 10% of the complicated forms. Peripheral neuropathy and cognitive impairment are the most common additional clinical features.[1] This report describes a novel *KIF5A* genetic defect in a large Italian ADHSP family with exclusive clinical features.

METHODS

The currently living family members were examined by movement disorder specialists (TLG, GAM, RM and AO). Neurological assessments, including the Spastic Paraplegia Rating Scale (SPRS)[2] Mental Deterioration Battery (MDB)[3] electrophysiology of peripheral nerves, and neuroimaging analyses, were carried out. After informed consent was given, genomic DNA was extracted from peripheral lymphocytes. Genetic analyses were conducted as described in online supplementary data, including linkage studies at the currently known ADHSP loci, PCR-direct sequencing, PCR-restriction fragment length polymorphism (PCR-RFLP) assay, and *in silico* analysis.

RESULTS

The RM 551 family is composed of a three-generation kindred with AD inheritance. Eleven individuals were diagnosed as 'certainly affected' and classified as having complicated HSP[1] At examination, the age range of the affected individuals was 19–77 years (mean±SD=40.4±17.9 years). Spastic paraparesis was the primary symptom in the clinical course of each patient; age at onset ranged between 12 and 55 years (mean±SD=32.2±15.0 years). The decrease of age at onset between successive generations ranged from 4 to 39 years per generation. The disease was slowly progressive and urinary urgency was a common symptom. Electroneurography of both motor and sensory nerves, as well as electromyography, were normal. MDB did not show cognitive impairment. Other clinical features associated with SPG10[1] were absent.

Interestingly, this variant form of HSP was associated in all affected individuals of the family with varicose veins (VV) of the legs, as well as bilateral Dupuytren's disease (DD) at various stages, according to Clinical-Etiology-Anatomy-Pathophysiology (CEAP)[4] and Tubiana[5] classifications, respectively (table 1 and online supplementary figure S1). DD and VV were absent in all members of the family who did not have gait difficulty. The environmental associations of DD, such as alcohol consumption, tobacco exposure, manual activities, retractile capsulitis, epilepsy, diabetes, HIV and dyslipidemia,[5] were absent in all patients. Obesity and lower limb deep vein thrombosis examined by venous ultrasonography were also absent, suggesting a primary form of VV.[4]

Significant logarithm of odds (LOD) scores were obtained at the SPG10 locus only (Z_{max}=4.56 at θ=0.0 at the microsatellite marker D12S1724, online supplementary table S1) and a novel heterozygous change in exon 6 (c.484C>T) was found, resulting in amino acid substitution of arginine to tryptophan at codon 162 (p.R162W). The PCR-RFLP analysis demonstrated a complete cosegregation of the mutation with the disease and the

nucleotide change is predicted to cause the disease, as well as influence the protein function by *in silico* analysis (see online supplementary figure S2 and table S2).

DISCUSSION

To date, 21 mutations have been reported in SPG10/*KIF5A*. Most of the mutations locate within the kinesin motor domain and are missense mutations, except from one in-frame deletion mutation N256del. The residues p.R204, p.N256 and p.R280 are mutational hot spots (see online supplementary figure S3). Neither nonsense nor frameshift mutations leading to truncated proteins are reported. These data suggest that SPG10 pathogenesis is not simply due to haploinsufficiency. A dominant negative effect has been postulated in SPG10 pathogenesis. The novel missense mutation, p.R162W, would lead to altering the conformation of the kinesin-1 complex and disrupting cargo transportation along the axon.

Other kinesin isotypes, *KIF1A* and *KIF1C*, have been reported as causative genes of SPG30 and spastic ataxia 2 (SAX2 or SPG58), respectively. This indicates that the underlying pathological mechanism in spastic paraplegia caused by

Table 1 Clinical findings in family members with the *KIF5A* mutation

	I:2	II:1	II:4	II:5	II:6	III:1	III:4	III:5	III:7	III:8	III:10
Sex	F	F	M	F	M	F	M	F	M	M	M
Age at examination	77	58	54	49	43	40	31	28	25	21	19
Age at onset*	55	49	51	39	36	32	26	18	21	16	12
Disease duration (years)	22	9	3	10	7	8	5	10	4	5	7
Disability stage†	2	2	2	3	2	3	3	4	3	3	3
SPRS‡	14	16	16	21	18	25	26	38	26	25	28
LL pyramidal weakness	+	+	+	+	+	++	++	++	++	++	++
LL hyper-reflexia	+	+	++	++	+	+	++	++	++	++	++
LL spasticity	+	+	+	+	+	++	+	++	++	+	++
Babinski reflex	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Pes cavus	−	−	−	−	−	−	+	−	−	−	−
Sensory deficits	+	−	+	−	−	+	−	+	−	−	−
VV	R/L	R/L	L	R/L	R/L	R/L	L	R	R/L	L	R/L
VV stage§ (affected or worst side)	C_2	C_2	C_{4a}	C_2	C_2	C_6	C_{4b}	C_{4a}	C_3	C_3	C_2
Bilateral DD	+	+	+	+	+	+	+	+	+	+	+
DD stage¶ (worst side)	1	1	2	N	1	3	3	2	4	3	3
Sphincter disturbances	+	+	+	−	+	+	−	+	+	−	−

*Age at onset was calculated approximately as the time when difficulty in walking first appeared in the affected individuals.
†Disability stages: 1, no mobility problems or slight stiffness of the legs; 2, moderate gait stiffness; 3, problems running, but able to walk alone; 4, problems walking; 5, wheelchair user.
‡SPRS, Spastic Paraplegia Rating Scale.[2]
§Clinical section from CEAP classification of chronic venous disease[4]: C_0, no visible or palpable signs of venous disease; C_1, telangiectasies or reticular veins; C_2, varicose veins; C_3, oedema; C_{4a}, pigmentation or eczema; C_{4b}, lipodermatosclerosis or atrophie blanche; C_5, healed venous ulcer; C_6, active venous ulcer.
¶Tubiana classification[5]: 0, no lesion; N, palmar nodule without the presence of contracture; 1, TFD between 0° and 45°; 2, TFD between 45° and 90°; 3, TFD between 90° and 135°; 4, TFD greater than 135°.
+ and −, indicate the presence and absence of a feature, respectively; DD, Dupuytren's disease; F, female; L, left leg; LL, lower limbs; M, male; R, right leg; TFD, total flexion deformity; VV, varicose veins.

mutations in the *kinesin* gene family varies depending on the basis of the isotype. Moreover, HSP-related proteins interact with each other, including myelin proteolipid protein 1, atlastin-1, kinesin-HC5A, kinesin-3, REEP1, KIF1C and reticulon proteins. Mutations in *KIF5A* might further affect the biological pathway revealed by such interactions leading to motoneuron degeneration, as described in the *Drosophila* model.[6]

Additional clinical features observed in the family, such as VV and DD, might be explained by two potential pathological mechanisms. The genetic factor(s) for developing VV and/or DD might exist within the approximately 7.5 cM interval encompassed by the two microsatellite markers, D12S270 and D12S1601. Developmental abnormalities, including vessel malformation, were observed in an individual with chromosomal deletion of 1.76-Mb comprising the SPG10 locus[7] suggesting that there might be genetic predisposing factor(s) for fragility of the blood vessel within the region. Considering the histopathology in DD, genes involved in myofibroblast regulation, extracellular matrix or collagen might be a candidate for developing the disease. The *OS-9* gene, encoding osteosarcoma amplified 9, has been mapped 143-kb away from *KIF5A* [National Center for Biotechnology Information (NCBI) website at http://www.ncbi.nlm.nih.gov/]. The gene expression was coamplified with the cyclin-dependent kinase 4 (*CDK4*) gene in sarcoma tissues.[8] Dysregulation of the gene might be involved in DD observed in the patients. The other possibility is that the combined phenotypic features might be due to the coexistence of the *KIF5A* mutation and other genetic variation(s) mapped to region(s) away from the SPG10 locus. There is a growing body of evidence that expression of the transforming growth factor β (TGFβ) is elevated in Dupuytren's fibroblasts.[9] Further investigation of genetic variations at the promoter region of TGFβ is needed to confirm the genetic model.

In conclusion, this study demonstrates further allelic heterogeneity, thus expanding the clinical-genetic spectrum of SPG10.

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SUPPLEMENTARY DATA

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METHODS

Genetic study

The microsatellite markers for the currently known ADHSP loci listed in the supplementary table 1 were used for linkage study. The computer program FASTLINK was used to calculate two-point LOD scores[s1]. For initial genetic linkage analysis, an AD monogenic mode of inheritance was used, assuming a disease allele frequency of 0.001, and assigning a genetic penetrance equal to 0.90. When the LOD score > 1.5 was obtained, the LOD scores were re-calculated using allele frequencies obtained from spouses or controls recruited in Italy. We also extended the analysis using other microsatellite markers to define the recombination sites. Constructing haplotype at each locus was performed using the computer program Genehunter Plus[s2].

Sequence analyses of 11 exons (including flanking sequences) of *KIF5A* were conducted in the proband, as previously described[s3]. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed by the restriction enzyme *Msp*I to prove whether the mutation segregates with the disease phenotype. The assay was also applied for mutation surveillance in 200 control chromosomes.

Missense variants were analyzed with PolyPhen-2, MutationTaster, SIFT, and PROVEAN to predict the pathological features of single amino acid mutation[s4-s7].

RESULTS

Significant LOD scores > 3.0 were obtained at the SPG10 locus only (supplementary table 1). The disease locus was refined to the 7.5 cM region between markers D12S270 and D12S1601 by the obligate recombination events observed in this family (members

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II:1, III:1, and III:4). The region includes SPG10 locus. Sequence analysis of coding region in *KIF5A* demonstrated a novel heterozygote variant in exon 6, c.484C>T, which results in missense substitution of arginine to tryptophan at codon 162 (p.R162W) (supplementary figure 2). The mutation was identified in all affected members and absent in unaffected members, as well as in 200 normal control chromosomes (supplementary figure 2). The mutation locates at the presumed kinesin motor domain and the amino acid R162 is highly conserved in evolution (supplementary figure 3). The four prediction programs used demonstrated the disease-causing effect. The summary of *in silico* analyses was described in supplementary table 2.

Supplementary Table 1.

Two-point LOD score for ADHSP loci.

ADHSP locus	Microsatellite markers	Zmax at $\theta = 0.0$	Reference
SPG3A	D14S259	$-\infty$	Zhao X, et al. 2001[s8]
	D14S978	$-\infty$	
SPG4	D2S352	$-\infty$	Hazan J, et al. 1999[s9]
	D2S2347	$-\infty$	
SPG6	D15S128	$-\infty$	Rainier S, et al. 2003[s10]
	D15S122	$-\infty$	
SPG8	D8S1804	$-\infty$	Valdmanis PN, et al. 2007[s11]
	D8S1774	$-\infty$	
SPG9	D10S583	$-\infty$	Panza E, et al. 2008[s12]
	D10S1736	$-\infty$	
SPG10	D12S270	-3.86	Reid E, et al. 2002[s13]
	D12S359	3.37	
	D12S1586	3.89	
	D12S1724	4.56	
	D12S90	4.19	
	D12S1691	3.44	
	D12S355	3.22	
	D12S1601	-2.97	
SPG12	D19S416	$-\infty$	Orlacchio A, et al. 2002[s14]
	D19S220	$-\infty$	

Continue supplementary table 1			
SPG13	D2S2196	$-\infty$	Hansen JJ, et al. 2002[s15]
	D2S309	$-\infty$	
SPG17	D11S1765	$-\infty$	Windpassinger C, et al. 2004[s16]
	D11S987	$-\infty$	
SPG19	D9S934	$-\infty$	Valente EM, et al. 2002[s17]
	D9S1818	$-\infty$	
SPG29	D1S2865	$-\infty$	Orlacchio A, et al. 2005[s18]
	D1S2626	$-\infty$	
SPG31	D2S2951	$-\infty$	Züchner S, et al. 2006[s19]
	D2S2181	$-\infty$	
SPG36	D12S78	$-\infty$	Schüle R, et al. 2009[s20]
	D12S338	$-\infty$	
SPG37	D8S601	$-\infty$	Hanein S, et al. 2007[s21]
	D8S1718	$-\infty$	
SPG38	D4S2935	$-\infty$	Orlacchio A, et al. 2008[s22]
	D4S394	$-\infty$	
SPG40	D10S1174	$-\infty$	Subramony SH, et al. 2009[s23]
	D10S579	$-\infty$	
SPG41	D11S1751	$-\infty$	Zhao GH, et al. 2008[s24]
	D11S935	$-\infty$	
SPG42	D3S1744	$-\infty$	Lin P, et al. 2008[s25]
	D3S1746	$-\infty$	
	D3S1545	$-\infty$	

Continue supplementary table 1			
SPG72	D5S476	$-\infty$	Esteves T, et al. 2014[s26]
	D5S500	$-\infty$	

Supplementary Table 2.

Bioinformatic analyses

Variant	PolyPhen-2		Mutation Taster		SIFT		PROVEAN	
	Prediction[a]	Score[b]	Prediction[c]	Probability[d]	Prediction[e]	Score[f]	Prediction[g]	Score[h]
p.Arg162Trp	Probably damaging	1.000	Disease causing	0.999	Damaging	0	Deleterious	-7.745

[a]PolyPhen-2 qualitatively classifies the results into 4 groups ("benign", "possibly damaging", "probably damaging", or "damaging")[s4];

[b]Profile scores are logarithmic ratios of the likelihood of a given amino acid occurring at a particular position to the likelihood of this amino acid occurring at any position[s4];

[c]MutationTaster classifies the results as "disease-causing" or "polymorphism"[s5];

[d]Probability of prediction ranges from 0 to 1. A value close to 1 indicates a high security of the prediction[s5];

[e]SIFT classifies the results as "tolerated", "damaging" or "not applicable"[s6];

[f]SIFT scores range from 0 to 1, and the threshold of 0.05 is given between "tolerated" and "damaging". An amino acid substitution is predicted to be "damaging" if the score is 0.05, and "tolerated" if the score is > 0.05[s6];

[g]PROVEAN classifies the results as "deleterious" or "neutral"[s7];

[h]The default score of thresholds of -2.500 is given in PROVEAN. If the PROVEAN score is equal to or lower than a predefined threshold, the protein variant is predicted to have a "deleterious" effect[s7].
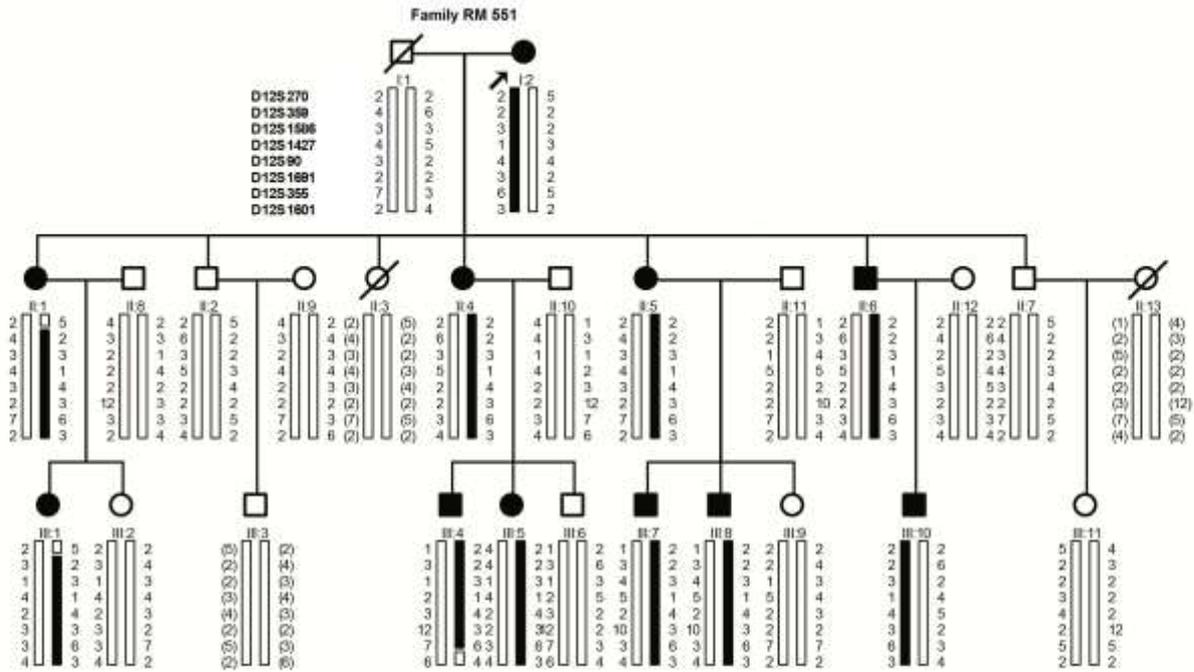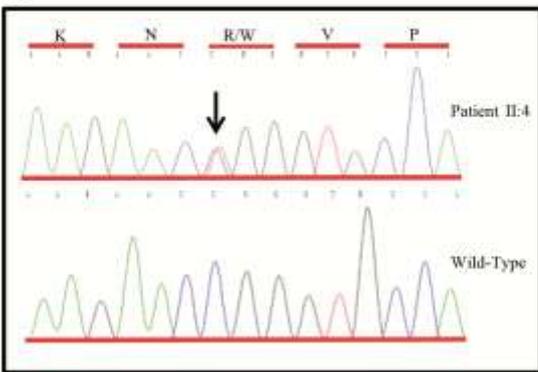
Supplementary figure 1

Supplementary fig. 1. **(A)** Truncal varicosity of the great saphenous vein of the left leg, in the proband; **(B)** Axial and T1-weighted **(I)** and T2 fat-saturated **(II)** images show low signal thickening (white arrows) within the palmar aponeurosis of the hand resulting in Dupuytren's contracture, in the same patient.
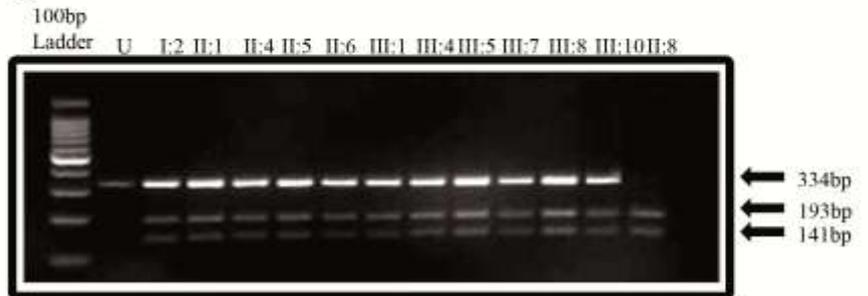
Supplementary figure 2

Supplementary fig. 2. (A) Pedigree chart. The marker order, from top to bottom, is D12S270, D12S359, D12S1586, D12S1427, D12S90, D12S1691, D12S355, and D12S1601. The sex-averaged genetic distance between markers is described at http://research.marshfieldclinic.org/genetics/GeneticResearch/data/Maps/Map12.txt. D12S270-(0.53 cM)-D12S359-(1.07 cM)-D12S1586-D12S1427-D12S90-(0.59 cM)-D12S1691-(2.38 cM)-D12S355-(0.59 cM)-D12S1601. The black bar indicates the haplotype segregating with the disease in the family. Reconstructed genotypes are in parentheses. Solid symbols designate affected individuals, circles = females, squares =

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males, slashes = deceased, and arrow = proband. **(B)** Electropherogram showing the p.R162W mutation in *KIF15A*. **(C)** RFLP analysis using *Msp*I. The wild-type sequence results in the cleavage of the PCR product into two fragments, 193-bp and 141-bp. The 334-bp PCR product remains uncleaved in the case of the c.484C>T mutant product. Undigested fragment (U), wild-type (II:8), and patients (I:2-III:10)**.**
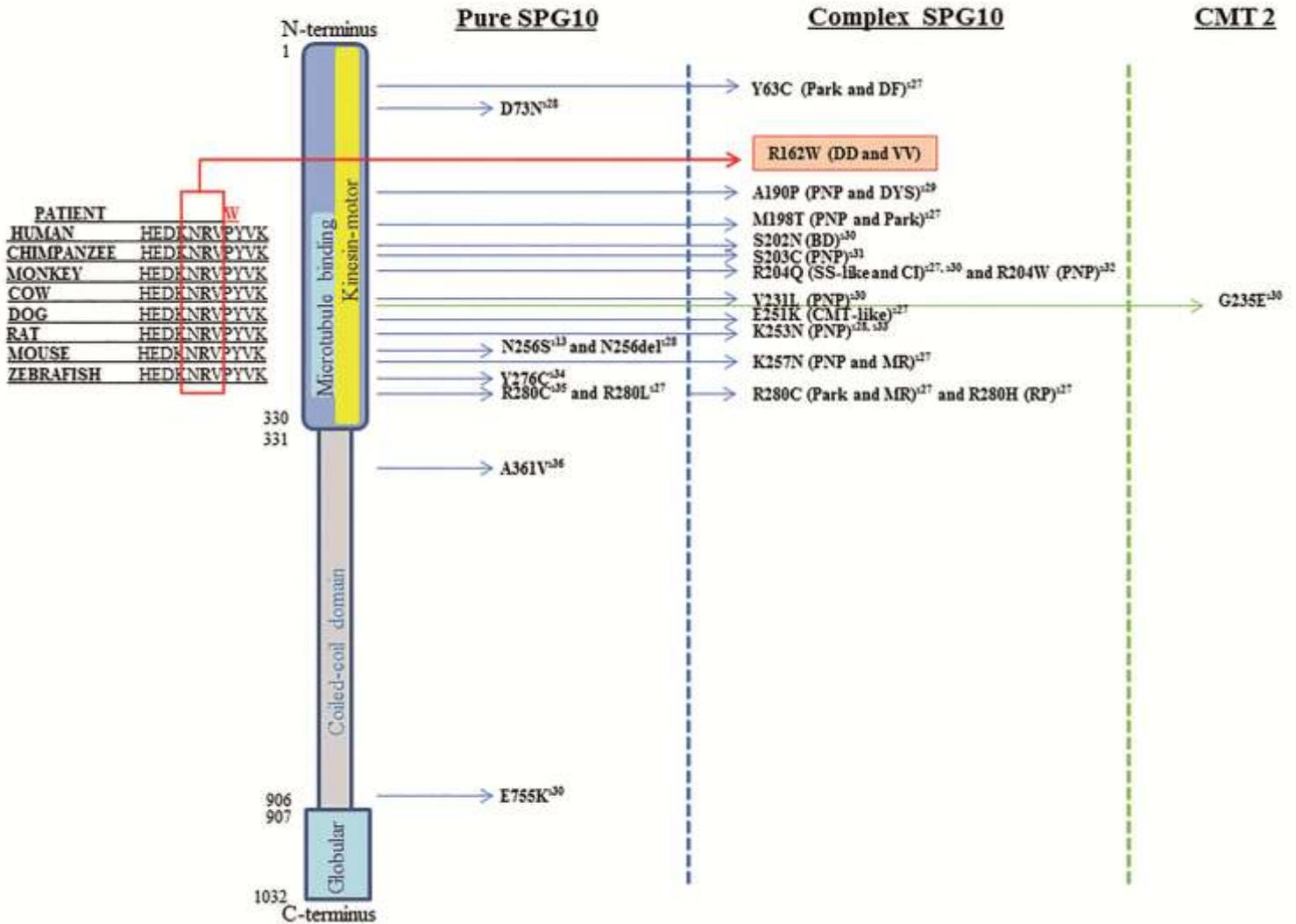
Supplementary fig. 3. Schematic representation of KIF5A protein, its functional domains, and the position of the previously reported mutations with clinical spectrum. In the left box, alignment of *KIF5A* orthologues: the p.R162 is completely conserved amongst *KIF5A* orthologues. Data are based on NCBI Protein ID, available at http://www.ncbi.nlm.nih.gov/protein. They are the following: human (NP_004975), chimpanzee (XP_509167), monkey (XP_002798698), cow (NP_001192623), dog (XP_003431493), rat (NP_997688), mouse (NP_001034089), and zebrafish (NP_001186705). Amino acid numbering is based on the human protein.

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BD = behavioral disturbance; CI = cognitive impairment; CMT = Charcot-Marie-Tooth disease; DD = Dupuytren's disease; DF = deafness; DYS = dysautonomia; MR = mental retardation; Park = parkinsonism; PNP = peripheral neuropathy; RP = retinitis pigmentosa; SS-like = Silver syndrome-like; VV = varicose veins.

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