Online supplementary information

Table S1: Incidence rate of *SPAST* mutations in Hereditary spastic paraplegia (HSP)

	Number of cases	Percentage
Pure HSP	32/109	29.4%
Complex HSP	5/23	21.7%
Unknown HSP	20/68	29.4%
Familial HSP	36/99	36.4%
Sporadic HSP	3/46	6.5%
Unknown HSP	18/55	32.7%

Table S2: The distribution of all the known mutations in spastin along its structural domains.

Domain/ Region	Missense	Nonsense	Splice site	Insertions	Deletions	Small indels	Total	% of mutations	% of mutations in three major domains
N-terminus (1-87 a.a)	2	--	--	3	--	--	**5**	**2.1**	--
TM (57-79 a.a)	--	--	--	--	--	--	**0**	**0**	--
MIT (116-197 a.a)	2	5	2	3	5	--	**17**	**7.2**	**92.7**
N-terminus (228-269 a.a)	1	7	2	3	5	--	**18**	**7.6**	
MTBD (270-328 a.a)	--	--	5	1	5	1	**12**	**5.1**	
AAA (342-599 a.a)	71[a]	11	38	13	37	1	**171**	**72.7**	
C-terminus (600-616 a.a)	3	1	--	--	--	--	**4**	**1.7**	--
N-terminus* (others)	1	2	3	1	1	--	**8**	**3.4**	--
Total	**80**	**26**	**50**	**24**	**53**	**2**	**235/235**	--	--
% of mutations	**34.0**	**11.0**	**21.2**	**10.2**	**22.5**	**0.8**	--	--	--

a.a, amino acids; Indels, insertion-deletion.

[a] Percentage of missense mutations in AAA (ATPases associated with diverse cellular activities) domain over the all missense mutations in spastin: 88.7 %

* N-terminus (others) – excluding 1-87 a.a, TM (Transmembrane), MIT (Microtubule interacting and trafficking), N-terminus (228-269 a.a) and MTBD (Microtubule binding domain)

Note: Gross deletions are not included in this distribution analysis

Table S3: Classification of novel missense mutations in the AAA domain into different structural category

Active Site Motif	Consensus sequence	Disease associated mutations
Walker A	^{382}GPPGNGKTM ^{390}	G385E
Walker B	^{441}DEVD ^{444}	---
SRH	^{480}VLVMGATNRPQELDEAVLRR ^{499}	---
Pore Loop Residues		
Pore loop 1	^{408}AASLTSKYVGEGEK ^{421}	G417E
Pore loop 2	^{445}SLLCERREGEHDAS ^{458}	L447V
Key Protomer Interacting Residues	E356, I357, I359, L360, L367, F368, R372, P384, G385, K388, T412, S413, K414, T415, D441, E442, R460, L466, N487, A495, R498, R499, K502, Q525, S547, D555, A557, L558, I561, R562, L564, R578, S595, T615, V616	R460H, D555G A557V
Other mutations		P361S, P374R F427C, R514G

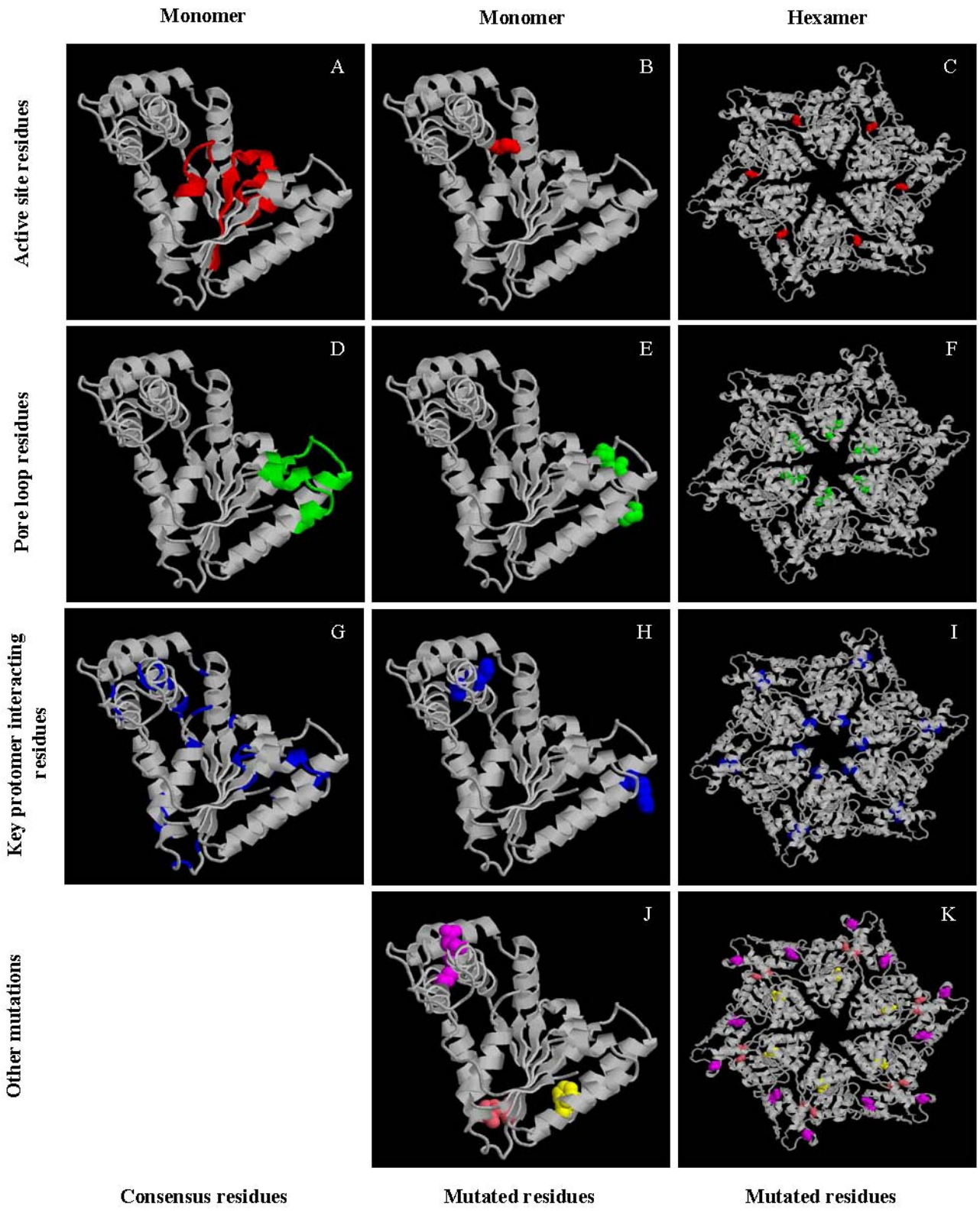
Table S4: List of recurrent mutations showing clinical heterogeneity in the HSP patients.

Group Number	DNA Number	Exon	cDNA	Protein	Age at Onset	Familial	Symptoms
1	25910	Exon 1 + Exon12	[c.131C>T] + [c.1417C>T]	[S44L] + [p.Q473X]	<10	Yes	Abnormal autonomic nervous system, increased sweating of hands and feet
	19598	Exon 1 + Exon13	[c.131C>T] + [c.1507C>T]	[S44L] + [p.R503W]	<60	?	
2	21938	Exon 9	c.1196C>T	p.S399L	>10	Yes	Pure
	24224	Exon 9	c.1196C>T	p.S399L	<60	Yes	?
3	21214	Exon 11	c.1378C>T	p.R460C	<60	Yes	Pure
	24222	Exon 11	c.1378C>T	p.R460C	<60	Yes	Polyneuropathy
	24228	Exon 11	c.1379G>A	p.R460H	<60	Yes	Pure
	24285	Exon 11	c.1378C>T	p.R460C	<35	Yes	Pure
4	21929	Exon 13	c.1496G>A	p.R499H	<10	Yes	Trunk-ataxia
	25923	Exon 13	c.1495C>T	p.R499C	<10	Yes	Pure
5	21900	Exon 14	1665A>G	p.R514G	<60	Yes	?
	21985	Exon 14	1665A>G	p.R514G	?	?	?
6	21920	Exon 15	c. 1684C>T	p.R562X	?	?	?
	21976	Exon 15	c. 1684C>T	p.R562X	?	?	?
	21974	Exon 15	c. 1684C>T	p.R562X	<60	Yes	Pure
	24201	Exon 15	c. 1684C>T	p.R562X	<35	Yes	Pure
	25912	Exon 15	c. 1684C>T	p.R562X	<60	Yes	Pure
7	19597	Exon17	c.1821G>C	p.W607C	?	?	?
	25936	Exon 17	c.1821G>C	p.W607C	<35	Yes	Pure

Legend for figure

Figure S1: Structural categorization of novel missense mutations identified in our HSP cohort. The missense mutations identified were categorized into four classes based upon the model of the AAA domain of spastin. The first category consists of the active site mutations (A-C), the consensus amino acid residues are highlighted in red in the tertiary structure of spastin (A), the position of the mutation indicated as a red ball and stick in the tertiary and quaternary structure of spastin (B-C). The conserved pore loop residues are marked as green in the spastin structure (D), the HSP mutated residues categorized as pore loop mutations are depicted as a green ball and stick in the spastin monomer and hexamer (E-F). The key interacting residues between protomers required for oligomerization are shown in blue color in the tertiary structure of spastin (G). Furthermore, the identified HSP mutations in this group are also shown in the spastin monomer and hexamer (H-I). The HSP mutations which could not be classified in one of the above groups were designated as other class of mutations. Labeling of these mutated residues in the modeled spastin structure revealed that they can further be grouped in three clusters which are labeled in magenta, orange and yellow (J-K).

Figure S1


Monomer
Monomer
Hexamer
Active site residues
Pore loop residues
Key protomer interacting residues
Other mutations
A
B
C
D
E
F
G
H
I
J
K
Consensus residues
Mutated residues
Mutated residues