

1 Introduction

Hereditary spastic paraplegias (HSP) (Mckusick number ¹ 182600) are a group of clinically and genetically diverse disorders characterized by progressive, generally severe, spasticity of lower extremity and in some cases upper extremities as well. Two groups of HSPs can be distinguished clinically by whether progressive spasticity occurs in isolation (pure HSP) or in combination with other neurologic abnormalities (complicated HSP), including optic neuropathy, retinopathy, extrapyramidal disturbance, dementia, ichthyosis, mental retardation, and deafness.^{2,3} However, there are few cases presenting intermediate clinical features. Pure HSP is more common and also genetically heterogeneous. On the other hand, Complicated HSP consists of many rare conditions which tend to be inherited as an autosomal recessive trait.⁴ Both forms can be inherited in an autosomal dominant, autosomal recessive or X-linked recessive pattern. Prevalence of HSP was estimated at 9×10^{-6} in Denmark,⁵ 140×10^{-6} in western Norway⁶ and 96×10^{-6} in northern Spain.⁷

1.1 Pure Hereditary Spastic Paraplegia (Pure HSP)

Pure HSP is the most common form of HSP. Approximately 70-80% of pure HSPs follow an autosomal dominant inheritance pattern (AD-HSP)⁸; autosomal recessive inheritance (AR-HSP)⁹ is found in 20-30% of cases and in very rare cases X-linked recessive inheritance (X-HSP)¹⁰ occurs.

1.1.1 Clinical Features of Pure HSP

The first cases of pure HSP were described in 1880 by Strümpell¹¹ who reported two brothers with an onset in middle life of progressive weakness and spasticity in the lower limbs. The clinical feature of pure HSP is the presence of a progressive spastic paraplegia mainly of the lower extremities. The disease severity ranges from completely asymptomatic (10-20%) to

wheelchairbound (10-15%). Patients develop leg stiffness and gait disturbance (stumbling and tripping) due to difficulty in dorsiflexing the foot and weakness of hip flexion. Although weakness and hypertonicity are usually restricted to the lower limbs, upper limb hyperreflexia is also common. Patients show weakness in addition to spasticity. The presence of urinary symptoms is likely. About 50% of patients are affected by urinary frequency, urgency or incontinence. Diminished sense of vibration is present in 20-65% of patients and 30-50% of patients have a Pes cavus. In addition, erectile impotence, mild upper limb incoordination, and absent ankle jerks were found in a small proportion of patients.^{2,4,8,12} The symptoms and signs of pure HSP are shown in Table 1.

Harding⁹ subclassified autosomal dominant pure HSP in Type I and Type II. Type I has an early onset (before age 35) and slow progression of symptoms. Patients show greater spasticity than weakness. On the other hand, Type II has a later onset (after age 35) and demonstrates faster progression. Dürr *et al.*¹² studied 23 families with pure autosomal dominant spastic paraplegia and found that the mean age of onset was 29, with a range between 1 and 68. Patients showed hyperreflexia in the upper limbs, sphincter disturbances, a decreased sense of vibration, and increased frequency of lower limb muscle weakness with disease duration. However, the clinical manifestations of early-onset (< age 29) and late-onset (> age 29) patients were not significantly different. Age at onset varied both between and within families. Anticipation and imprinting did not occur. These results suggested that only genetic analyses could provide accurate classification of the pure HSP. Fink *et al.*² observed that the ages of onset overlap in autosomal dominant HSP kindred and also concluded that there did not appear to be a genetic basis for HSP classification based entirely on age of symptom onset.

Table 1. Symptoms and signs in pure HSP^{2,4,8,12}

A progressive spastic paraplegia
Weakness and hypertonicity of legs
Upper limb and jaw jerk hyperreflexia
Urinary symptoms (urinary frequency, urgency, or hesitancy)
Sphincter disturbances
Decreased vibration sense
Decreased joint position sense
Absence of ankle jerks
Babinski signs
Hoffman's signs
Trömner's signs
Pes cavus
Clonus at ankles and knees
Extensor plantar responses
Mild upper limb incoordination
Scoliosis (rare)

1.1.2 Pathology

The major neuropathologic feature of pure HSP is an axonal degeneration in the terminal portions of the longest corticospinal fibers and dorsal columns.^{8,13} Loss of anterior horn cells is observed in some cases.⁴ Neuronal cell bodies of degenerating fibers are normally preserved. There is no evidence of primary demyelination reported. The dorsal root ganglia, posterior roots, and peripheral nerves are normal¹³.

1.2 Complicated Hereditary Spastic Paraplegia (Complicated HSP)

In complicated HSP, spastic paraplegia appears with other disorders which include amyotrophy, cerebellar signs, optic atrophy, choreoathetosis/dystonia, sensory neuropathy, disordered skin pigmentation, macular degeneration/mental retardation (Kjellin syndrome), Sjögren-Larsson syndrome, Mast syndrome, MASA syndrome (mental retardation, aphasia, shuffling gait, and adducted thumbs). Although these disorders are rare, spastic paraplegia is the common feature of them. The most common type of complicated HSP is spastic paraplegia associated with amyotrophy. This type frequently results in peroneal muscular atrophy syndrome or hereditary motor and sensory neuropathy. In addition, brisk knee jerks

and extensor plantar responses are specific signs. Distal sensory loss is observed in 50% of patients. The age of onset ranges from early childhood to 50's and some affected individuals show asymptomatic^{3,4}.

1.3 Molecular Genetics of HSP

Distinguishing between pure and complicated HSP is still a useful criteria. However, molecular genetics allow the definitive distinction of HSP due to the expanding variety of phenotypes. Now at least 15 HSP loci can be differentiated by genetic analysis, and the gene defect has been identified beyond doubt in 4 loci. These findings are a first step towards an understanding of the pathological mechanism of HSP at the molecular level.

1.3.1 X-linked HSP

Three X-linked HSP loci have been mapped to Xq28 (*SPG1*;312900), Xq22 (*SPG2*;312920), Xq11.2 (*SPG16*;300266).

SPG1

Kenwick *et al.*¹⁴ reported a complicated X-HSP and mapped the disease locus to *DXS15* and *DXS52* on the long arm of the X chromosome (Xq28). All patients examined had mental retardation. Winter *et al.*¹⁵ presented evidence showing that the family described by Kenwick *et al.* was an instance of MASA syndrome. Later, Schrander-Stumpel *et al.*¹⁶ proposed that MASA syndrome and X-linked hydrocephalus are allelic. Jouet *et al.*¹⁷ reported that the mutations in *L1CAM* gene resulted in X-HSP, MASA syndrome, and X-linked hydrocephalus and thus all three syndromes are allelic disorders. *L1CAM* is a cell surface glycoprotein which is expressed in the axon of postmitotic neurons and is involved in neuronal migration and neurite extension.

SPG2

Pure X-HSP is rare. The first genetic linkage of a pure form of spastic paraplegia was presented by Keppen *et al.*¹⁰ The mutation is linked to markers DXS17 and YNH3 located in the Xq21-q22 region. Bonneau *et al.*¹⁸ proposed that this pure X-HSP mapped to Xq21. However, this form could not be separated from the complicated variant because some members had mental retardation. This pure X-HSP was designated *SPG2*. Saugier-Verber *et al.*¹⁹ demonstrated that the gene for proteolipid protein was the closest marker mapped to Xq21 reported by Bonneau *et al.* They also found a His139Tyr mutation in exon 3B of the *PLP* gene in an affected male. *PLP* encodes two myelin proteins, PLP and DM20, and is involved in oligodendrocyte maturation and myelin sheath compaction. The His139Tyr mutation observed in this family results in production of a mutant PLP but wild-type DM20. Kobayshi *et al.*²⁰ reported the linkage results of the family with X-linked HSP whose symptoms began as pure spastic paraplegia, but later developed nystagmus, dysarthria, sensory disturbance or mental retardation. The half of patients had optic atrophy. Muscle wasting and joint contractures were observed in later symptoms. The patients needed crutches or wheelchair in their early adulthood. The disease locus was mapped to the region Xq21.3-q24 which includes the *PLP* gene.

SPG16

Another complicated X-HSP was reported by Steinmüller *et al.*²² The patients were quadriplegic and had motor aphasia, reduced vision, mild mental retardation, and dysfunction of the bowel and bladder. Onset of the symptoms occurred during the first 3 months of life. Nystagmus and dorsal flexion of the great toes were observed in the beginning development of the symptoms. Spasticity occurred first in the lower and then upper extremities, and motor

development was delayed. The patients never learned to walk and all females of the family were normal. The authors assigned the disease locus to Xq11.2-q23 by linkage and haplotype analysis. They performed mutation analysis of the *PLP* gene which was mapped to this region but no mutation was detected.

1.3.2 Autosomal Recessive HSP

Loci for autosomal recessive forms of SPG have been mapped to chromosomes 8p (*SPG5A*;270800), 16q (*SPG7*; 602783), 15q (*SPG11*; 604360), and 3q (*SPG14*; 605229).

SPG5A

Hentati *et al.*²³ first reported a pure AR-HSP locus and the patients examined showed a mild loss of vibratory and positiona sense of the toes and dysfunction of the bladder sphincter. The age of onset ranged from one to 20 years. This locus was mapped to a 32.2 cM (centimorgen) interval in the pericentromeric region of chromosome 8 between 8p12 and 8q13.

SPG7

De Michele *et al.*²⁴ described a large consanguineous family with complicated AR-HSP. All cases had abnormal gait and vibration sense was frequently decreased in legs. Hypernasal, slowed speech, dysphagia, urinary urgency, scoliosis, pes cavus and pale optic disk appeared in some cases. There were no cerebellar or extrapyramidal signs in any of 6 affected individuals. The mean age of onset was 30±8 years in this family. This locus was linked to markers *D16S413* and *D16S303* on 16q24.3. Soon after the mapping, the same team²⁵ identified a gene within this region that encodes a novel protein, paraplegin that is highly homologous to yeast mitochondrial ATPases, AFG3, RCA1, and YME1, which have both proteolytic and chaperone-like activities at the inner mitochondrial membrane.

Immunofluorescence analysis and import experiments showed that paraplegin localizes to mitochondria. Analysis of muscle biopsies showed typical signs of OXPHOS defects that include cytochrome c oxidase deficient fibers and the presence of subsarcolemmal accumulation of mitochondria of bizarre shape. These data indicated that a mitochondria protein defect in HSP. *Paraplegin* is composed of 17 exons and spans approximately 52 kb. The cDNA length of this gene is approximately 3.2kb and it encodes a 795 amino acid protein. A 9.5kb deletion and two frameshift mutations which were found in one pure and in one complicated HSP family have been reported.

Paraplegin and yeast mitochondrial ATPases (AFG3, RCA1, and YME1) are the members of AAA protein family (ATPase associated with a variety of cellular activities). AAA proteins play essential roles in peroxisome biogenesis, the assembly of mitochondrial membrane proteins, cell cycle control, mitotic spindle formation, cytoskeletal interactions, vesicle secretion, signal transduction, and transcription. These proteins are characterized by a highly conserved AAA motif. This motif forms a 230-250 amino acids domain that includes the Walker homology sequences of P-loop ATPases and other regions of similarity unique to AAA family such as Walker B and the minimal AAA consensus sequence. The motif confers ATPase activity. Evolutionary conservation of the AAA domain ranges from eubacteria and archaeobacteria to modern eukaryotes and tends to be homologous in regions outside the AAA domain. All members of the AAA family are Mg^{2+} -dependent ATPases and include metalloproteases, proteins involved in vesicle and organelle biogenesis, cell-cycle regulators, components of the 26S proteasome, and chaperone and chaperone-like protein families (AAA⁺ class).^{26,27,28}

SPG11

The third AR-HSP locus (*SPG11*) was described by Martínez Murillo *et al.*²⁹ The patients in eight examined AR-HSP families had the common features of spasticity and weakness in the lower limbs, hyperreflexia, extensor plantar reflexes. Mental retardation was present in two families. The age of onset ranged from one to 50 years. In seven of eight AR-HSP families, the disease locus was mapped to chromosome 15q13-q15, a 6.41 cM region between markers *D15S971* and *D15S118*. The other family showed significant linkage to the previously characterized 8q locus.

SPG14

Recently, Vazza *et al.*³⁰ described a consanguineous Italian family with HSP. The clinical features were spastic gait, hyperreflexia, mild lower limb hypertonicity. Bilateral pes cavus, extensor plantar responses, and mild mental retardation were present in all cases. The average age of onset was 30. All three patients were able to walk without aid. The authors performed a whole genome scan in another AR-HSP family and reported a new AR-HSP locus. The disease locus assigned to 3q27-q28.

1.3.3 Autosomal Dominant HSP

To date, 8 AD-HSP loci have been mapped to chromosome 14q (*SPG3*;182600), 2p (*SPG4*;182601), 15q (*SPG6*;600363), 8q (*SPG8*;603563), 10q (*SPG9*;601162), 12p (*SPG10*;604187), 19q (*SPG12*;604805), 2q (*SPG13*;605280).

SPG3

The first AD-HSP locus was reported by Hazan *et al.*³¹ In one of three AD-HSP families the disease was linked to locus AFM267zd5 on chromosome 14q. The clinical feature was very similar in three families. Spastic gait was the prominent sign in all patients, with a variable

degree of severity. Some affected individuals (18.75%) showed asymptomatic. The age of onset ranged from 2 to 50 years. Another two studies demonstrated that anticipation and early age of onset in two SPG3 linked families.^{32,33}

SPG4

Hazan *et al.*³⁴ reported the second AD-HSP locus in one Dutch and five French families. The average age of onset ranged from 20 to 39 years in these families. Wide variations of age of onset were observed within families. The symptoms occurs relatively nonprogressive. The authors mapped this disease locus to a 4 cM region flanked by loci *D2S400* and *D2S367*. Fink *et al.*² reviewed seven publications which included 67 pure AD-HSP families. Of the families, 34 were indeterminate and the disease loci of 33 kindreds were mapped to chromosomes 2p, 14q, and 15q. Of these 33 kindreds, the disease loci of 15 kindreds (45%) were linked to chromosome 2p, two (6%) were associated with chromosome 14q, and only one (3%) was associated with chromosome 15q. Scott *et al.*³⁵ studied 11 Caucasian pedigrees with pure AD-HSP to determine the linkage to the previously identified loci on 2p, 14q, and 15q. Chromosome 15q was excluded in all families. There was evidence of linkage to 2p in five families and linkage to 14q in one family. The other five families remained indeterminate. In pure AD-HSP families, a high proportion have linkage to *SPG4*.

In 1999, Hazan *et al.*^{36,37} identified the *SPG4* gene (*SPAST*) by using positional cloning. *SPAST* is composed of 17 exons spanning a region of approximately 90 kb. The cDNA of *SPAST* is 3263 bp containing a 1848 bp ORF preceded by a 125 bp 5' UTR and followed by a 1290 bp 3' UTR. The promoter is predicted to be located at 43 bp upstream from the first base of *SPAST* exon 1. The ORF encodes a 616 amino acid protein which is named spastin. The spastin carboxy terminus has striking homology with several members of AAA family

and contains three conserved ATPase domains, including Walker motifs A and B and the AAA minimal consensus, found at amino acid positions 382-389, 437-442 and 480-498 of spastin, respectively. Walker motif A 'GPPFNFKT' corresponds to the ATP-binding domain. Two leucine-zipper domains are detected at amino acid positions 50-78 and 508-529 and a helix-loop-helix dimerization domain is located between amino acid positions 478 and 486. RT-PCR analysis reveals that human *SPAST* and its mouse homologue are expressed early and ubiquitously in fetal and adult tissues.

SPG6

Fink *et al.*³⁸ demonstrated the third AD-HSP locus in a large kindred extensively affected with pure AD-HSP. Neurologic examination of patients showed hyperreflexia, spasticity in the lower limbs, weakness of hip flexion and ankle dorsiflexion, extensor plantar responses, diminished vibratory sense in the feet, and pes cavus. Bladder disturbance was present in some cases. The patients developed progressive gait disturbance at age 12 to 35 years and the average age of onset was 22 ± 5.3 years. The authors mapped this disease locus to chromosome 15q11.1.

SPG8

Hedera *et al.*³⁹ examined a Caucasian kindred which developed insidiously progressive gait disturbance at age 22 to 60 years. Neurological examination demonstrated spastic diplegic gait disturbance, frank corticospinal -tract deficits in legs, weakness of hip flexion and ankle dorsiflexion, diminished vibratory sensation in the feet, and often, pes cavus. The patients in wheelchairs had mild muscle atrophy and bladder disturbance was present in some cases. The disease locus mapped to chromosome 8q23-q24, a 6.2 cM region between *D8S1804* and *D8S1774*.

SPG9

Seri *et al.*⁴⁰ studied a large pedigree with complicated AD-HSP. The patients presented with bilateral cataracts, gastroesophageal reflux with persistent vomiting, and spastic paraparesis with amyotrophy. Severity of spastic paraparesis varied. Pes cavus and the Babinski signs, along with different degrees of muscle wasting localized in the hands and “forelegs” (*sic.*) were present in some cases. The authors performed a genome wide scan and assigned this disease locus to a 12-cM region in 10q23.3-q24.2.

SPG10

Reid *et al.*⁴¹ reported a large family with pure AD-HSP. All but one affected members had a spastic gait abnormality, in addition to lower-limb reflexia. The asymptomatic affected individual was a carrier who had bilateral lower-limb hyperreflexia, bilateral extensor-plantar responses, and unilateral sustained ankle clonus. The development of asymptomatic to symptomatic history was observed in one patient. The age of onset ranged from 8 to 40 years and the average age of onset was 10.8 ± 9.6 years. The authors performed a genome wide scan data and mapped this pure ADHSP locus to chromosome 12q13, a 9.2 cM interval between markers *D12S368* and *D12S8334*. Later, this interval was narrowed to a 6.95 cM region flanked by *D12S270* proximally and *D12S355* distally by further genetic analysis.

SPG12

Recently, Reid *et al.*⁴² performed a genome wide linkage screen of a large Welsh family with AD-HSP which had previously reported⁴³ but no linkage with known pure AD-HSP loci. The authors assigned this novel AD-HSP locus to 19q13, a 16.1 cM interval between markers

D19S868 and *D19S902*. The candidate region was narrowed to a 5 cM region between markers *D19S868* and *D19S220* by additional linkage analysis.

SPG13

Fontaine *et al.*⁴⁴ excluded all known AD-HSP loci in a large family of French descent with pure AD-HSP. A genomewide scan demonstrated location of the disease locus in the 5 cM region flanked by *D2S294* and *D2S2195* on 2q24-q34. The authors compared the SPG13 family with 12 SPG4 families. They found SPG13 family had significantly more patients without Babinski signs, with increased reflexes in arms and more severe handicaps than the patients of those 12 SPG4 families they examined. The severe handicaps score was obtained by dividing the disability score by disease duration (in years) and multiplying by 100. The authors defined a three point scale: 1 = normal gait or very light stiffness of the legs, 2 = inability to run, and 3 = either inability to walk without help or confinement to a wheelchair. The currently known HSP loci are summarized in Table 2.

1.4 The purpose of this dissertation

This dissertation began with study of a pure AD-HSP German family of three generations (see Figure 1). The family consists of 66 living members and 5 were diagnosed as symptomatic. The purpose of this dissertation is to determine the disease locus of this family and identify the mutation of this locus. The AD-HSP family was first tested for linkage with the 3 known AD-HSP loci at 2p, 14q, and 15q. The linkage results revealed that the disease locus of this family was linked to chromosome 2p (*SPG4*). Therefore, this dissertation focuses on *SPG4* which is also the most common form of pure AD-HSP. Since there was no AD-HSP gene identified during the proceeding of this study, a physical map including YACs, BACs, and PACs was established to facilitate the isolation of gene. In addition, the proposed CAG

trinucleotide repeat expansion was investigated in this dissertation. Finally, using the sequence data of the recently cloned *SPAST* gene, the SPG4 family and members from nine pure HSP families were screened by direct sequencing.

Table 2. Classification of hereditary spastic paraplegias

HSP loci	Gene location	Gene product	inheritance mode	HSP forms	References
<i>SPG1</i>	Xq28	L1CAM	X-linked	complicated	Saugier-Veber ¹⁴ <i>et al.</i> (1994)
<i>SPG2</i>	Xq21	PLP	X-linked	complicated or pure (rare)	Jouet ¹⁷ <i>et al.</i> (1994)
<i>SPG3</i>	14q11.2-q24.3	unknown	AD	pure	Hazan ³¹ <i>et al.</i> (1993)
<i>SPG4</i>	2p21-p24	Spastin	AD	pure or complicated	Hazan ³⁴ <i>et al.</i> (1994)
<i>SPG5A</i>	8p12-q13	unknown	AR	pure	Hentati ²³ <i>et al.</i> (1994)
<i>SPG6</i>	15q11.1	unknown	AD	pure	Fink ³⁸ <i>et al.</i> (1995)
<i>SPG7</i>	16q24.3	Paraplegin	AR	complicated or pure	Casari ²⁴ <i>et al.</i> (1998)
<i>SPG8</i>	8q23-q24	unknown	AD	pure	Hedera ³⁹ <i>et al.</i> (1999)
<i>SPG9</i>	10q23.1-q24.1	unknown	AD	complicated	Seri ⁴⁰ <i>et al.</i> (1999)
<i>SPG10</i>	12q13	unknown	AD	pure	Reid ⁴¹ <i>et al.</i> (1999)
<i>SPG11</i>	15q13-q15	unknown	AR	complicated or pure	Martinez ²⁹ <i>et al.</i> (1999)
<i>SPG12</i>	19q13	unknown	AD	pure	Reid ⁴² <i>et al.</i> (2000)
<i>SPG13</i>	2q24-q34	unknown	AD	pure	Fontaine ⁴⁴ <i>et al.</i> (2000)
<i>SPG14</i>	3q27-q28	unknown	AR	complicated	Vazza ³⁰ <i>et al.</i> (2000)
<i>SPG16</i>	Xq11.2	unknown	X-linked	complicated	Steinmüller ²² <i>et al.</i> (1997)

AD: autosomal dominant inheritance

AR: autosomal recessive inheritance