Science in motion: common molecular pathological themes emerge in the hereditary spastic paraplegias

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The hereditary spastic paraplegias are a group of neurodegenerative conditions that all share the principal clinical feature of progressive lower limb spastic paralysis, caused by either failure of development or progressive degeneration of the corticospinal tract. The conditions are characterised by extreme genetic heterogeneity, with at least 20 genes involved. Until recently, no functional overlap was apparent in the associated molecular pathological mechanisms. However, with recent progress in hereditary spastic paraplegia gene identification, common pathological themes are now emerging.

A MOVING PROBLEM

The principal neurological pathway over which signals pass to drive a deliberate movement can be divided, in simple terms, into two stages. In the first stage, a descending connection is made from neurones (often referred to clinically as “upper motor neurones”) in the motor cortex of the brain to cells in the anterior and posterior horns of the spinal cord, while in the second stage “lower motor neurones” in the anterior horn of the spinal cord connect to the skeletal muscles at the neuromuscular junction. The axons that project from the upper motor neurone cell bodies to the spinal cord constitute the corticospinal tract.\(^1\) These axons are exceptionally long, in some cases over 1 metre, with axonal volumes that can be up to one thousand times the volume of the cell body. Corticospinal tract neurones therefore provide an extreme example of the difficulties encountered in diverse cellular processes, such as trafficking, transport, and energy metabolism.

The corticospinal tract has great clinical importance. Lesions to the tract result in spastic paralysis, a disabling clinical picture in which weakness of muscles (paralysis) is associated with increased stiffness (hypertonicity) and overactive reflexes (hyperreflexia). Depending on the site of lesions, spastic paralysis may affect both arms and both legs (tetraparesis or if more severe, tetraplegia), legs only (paraparesis or paraplegia), one limb (monoparesis or monoplegia), or the limbs on one side of the body (hemiparesis or hemiplegia). Spastic paralysis may be the result of a wide variety of insults, including common conditions such as stroke, multiple sclerosis, and cervical spinal cord injury. It may also be the result of genetic pathology and under this heading are the human hereditary spastic paraplegias (HSPs), single gene disorders in which the axons of the corticospinal tract either fail to develop normally, or show progressive degeneration after initial normal development. This review will summarise progress in identifying and characterising the function of genes that cause human HSPs.

THE CLINICAL PHENOTYPE OF HSPs

The HSPs share the principal clinical feature of progressive lower limb spastic paralysis. They are conventionally subdivided into pure and complicated forms, depending on the absence or presence of additional neurological or non-neurological features.\(^2\) Pure hereditary spastic paraplegia, in which spastic paraplegia occurs in relative isolation, is probably the single largest subgroup of HSP conditions.\(^3\) Histopathological studies of pure HSP generally show a length dependent “dying back” of the terminal ends of the corticospinal tract axons, with the longest axons being involved first. Diagnostic criteria have been suggested and the clinical and pathological features, differential diagnosis, genetic counselling, and management have been reviewed extensively elsewhere.\(^2\) The complicated forms consist of a diverse group of individually rare conditions, and again their clinical features have been reviewed previously.\(^2\)

MOLECULAR GENETICS OF HSPs

The hereditary spastic paraplegias are genetically complex. Autosomal dominant, autosomal recessive, and X linked recessive inheritance patterns have been described for pure HSP.\(^4\) Within each broad inheritance pattern group, there is further locus heterogeneity with, for example, eight known loci for autosomal dominant pure HSP. Not surprisingly for such a clinically diverse group, the complicated HSPs also show considerable genetic heterogeneity, although with a few important exceptions they tend to be inherited in an autosomal recessive pattern.\(^5\) The 20 HSP genes that have been mapped are summarised in table 1 and undoubtedly more remain to be found. Eight of these genes have now been identified and some interesting overlapping pathological mechanisms are beginning to emerge.

PATHOLOGICAL MECHANISMS ASSOCIATED WITH HSP GENES

Cell recognition and signalling: the L1-CAM gene

The cell adhesion molecule L1 (L1-CAM) is a cell surface membrane associated glycoprotein that is a member of an immunoglobulin superfamily. Mutations in L1-CAM are found in a form of X linked complicated (by mental retardation and...
absence of the extensor pollicis longus muscle) spastic paraplegia termed SPG1, in MASA (mental retardation, aphasia, shuffling gait, adducted thumbs) syndrome, and in X linked hydrocephalus. Interestingly, the same L1-CAM mutation may result in either an X linked hydrocephalus phenotype or a MASA phenotype. These conditions have been associated with over 90 different mutations, of varying forms, in the L1-CAM gene. Many of the mutations are predicted to eliminate expression of L1-CAM, although some missense mutations affect particular functions of the protein. L1-CAM is expressed throughout the nervous system on populations of developing and differentiated neurones, as well as in Schwann cells in the peripheral nervous system. In vitro studies have suggested that L1 interactions may mediate axon bundling and may be involved in the processes of neurite extension and axonal guidance, along with several other functions. L1-CAM knockout mice show phenotypes similar to the human L1-CAM associated conditions and, of particular relevance to the cause of spasticity in these cases, the normal anatomy of the mouse corticospinal tracts is disrupted, with impairment in axonal pathfinding resulting in failure of the normal crossing over of the tracts in the medulla. L1-CAM sits at the centre of a complex set of extracellular and intracellular interactions. It binds to a wide variety of extracellular ligands, including other CAMs, integrins, extracellular matrix proteins, and proteoglycans. Binding of L1-CAM to other L1-CAM molecules (that is, homophilic binding) promotes cell adhesion and neurite outgrowth. This may be influenced (positively or negatively) by interaction with other potential ligands. The molecule is linked to a number of intracellular signalling pathways. Key among these is its interaction with fibroblast growth factor receptors (FGFRs), membrane associated molecules that have intracellular tyrosine kinase signalling domains. Homophilic binding of L1-CAM stimulates FGFR tyrosine kinase activity, which in turn stimulates Ca2+ influx, resulting in modification of the axonal growth cone by a mechanism that is not yet entirely clear. L1-CAM also has important interactions via its cytoplasmic domain, notably with ankyrin, a linker protein of the spectrin based cytoskeleton, so that homophilic binding may cause reorganisation of the cytoskeleton.

### Abnormalities of myelination: the proteolipid protein gene

Mutations in the proteolipid protein gene (PLP1) can cause pure or complicated HSP (SPG2), or the much more severe Pelizaeus-Merzbacher disease (PMD). Several different missense mutations in the gene are associated with HSP, whereas PMD is associated with other missense mutations or, more commonly, with duplications of the entire gene. Complete deletions or triplication of the gene have also been described. The PLP1 gene codes for two myelin proteins, PLP and DM20. There is a correlation between PLP1 gene mutation and phenotype, with point mutations that affect highly conserved DM20 amino acid residues being associated with the most severe PMD phenotypes. The PLP and DM20 proteins are thought to play a major role in oligodendrocyte maturation and also form structural components of the myelin sheath. The precise mechanism by which certain missense mutations in the PLP1 gene cause spastic paraplegia is not clear. MRI studies in human SPG2 families show central nervous system white matter abnormalities similar to those found in demyelinating conditions. This is reminiscent of the pathological changes found in the rumpshaker natural mouse mutant model of SPG2, which shows hypomyelination with reduced levels of PLP but not DM20. It seems likely that perturbations of corticospinal tract myelination, perhaps accompanied or caused by abnormal trafficking induced toxic effects on oligodendrocytes, may contribute to spasticity in SPG2.

### Abnormalities of mitochondrial molecular chaperones: paraplegin and HSP60

Intuitively, it seems likely that the terminal ends of long axons might be especially vulnerable to defects in energy metabolism and indeed there is an emerging association between mutations in nuclear encoded mitochondrial genes and HSP. Mutations in the paraplegin gene (SPG7), at chromosome 16q24, are associated with pure or complicated (by optic, cortical, or cerebellar atrophy) autosomal recessive HSP. Paraplegin is a mitochondrial metalloprotease and a member of the AAA (ATPases associated with diverse cellular activities) protein family. Members of this large family of proteins are characterised by the presence of one or two copies of a 220–250 amino acid long sequence motif, the AAA cassette, which

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**Table 1** Genetic loci for HSP

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>MIM number</th>
<th>Chromosomal location</th>
<th>Gene product</th>
<th>Phenotype</th>
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<td>L1-CAM</td>
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<td>Dominant</td>
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<td>13q12.3</td>
<td>Spastin</td>
<td>CHSP (Troyer syndrome)</td>
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CHSP=complicated HSP, PHSP=pure HSP, XLH=X linked hydrocephalus, PMD=Pelizaeus-Merzbacher disease.
Hereditary spastic paraplegias

consists of Walker type ATPase motifs A and B, along with other sequence specific to the AAA class of proteins. They have been subclassified according to the number of AAA cassettes contained and by amino acid sequence similarity within the cassette. Members of the AAA family of proteins are proven or putative ATPases, which take part in many cellular functions, including cell cycle regulation, protein degradation in the 26S proteasome, organelle biosynthesis, and vesicle mediated protein transport. HSP patients with paraplegin mutations have defects in mitochondrial oxidative phosphorylation, as evidenced by the finding of characteristic structural and functional abnormalities in muscle biopsies. The precise mechanism of action of paraplegin has not yet been characterised. However, it has a high degree of homology to a mitochondrial subclass of yeast and fungal ATPases, members of which have proteolytic and chaperone-like activities at the inner mitochondrial membrane. These proteins may participate in protein “quality control”, degrading misfolded proteins in the intermembrane space. Yeast and fungi lacking the respective paraplegin homologues Yme1 or sip-1 have defective respiration under certain conditions, with yeast accumulating abnormally formed mitochondria. By analogy, it has been suggested that defects in paraplegin may cause an accumulation of abnormally synthesised mitochondrial proteins, leading to mitochondrial respiratory dysfunction and eventual axonal degeneration.

As well as paraplegin, mutation in at least one other nuclear encoded mitochondrial protein has been associated with HSP. A missense mutation in the mitochondrial chaperone heat shock protein HSP60 has been found in the original family used to map the chromosome 2q autosomal dominant pure HSP (SPG13) locus. Elegant complementation studies showed that the mutant has a functional effect and so it is highly likely that abnormalities of this gene can cause HSP. The pathological mechanism of the mutation is not yet clear and may involve loss of function or a dominant negative effect on the multimeric chaperone complex in which HSP60 participates.

Defects of molecular trafficking and transport? KIF5A, spartin, spastin, and atlastin

The close map in 1999 of the chromosome 2p24 HSP gene spastin (SPG4) was a major advance in HSP research. This was the first gene associated with autosomal dominant pure HSP to be identified and, in epidemiological terms, it is undoubtedly the most important HSP gene. Mutations in it are responsible for approximately 40% of definite autosomal dominant pure HSP. The prevalence of spastin mutations in sporadic cases and cases with uncertain family history is probably much lower.

The 616 amino acid spastin protein is a widely expressed AAA protein, but from a different subgroup to paraplegin. More than 100 spastin mutations have been described, including numerous missense, nonsense, frameshift, and splice site mutations, as well as less frequent large scale deletions. From a diagnostic point of view there are unfortunately no common mutations, with most families having private mutations. With only a few possible exceptions, the missense mutations are located in the part of the gene that codes for the AAA cassette, from amino acids 342-599. Splice site mutations involve exons 5-16, but curiously not so far exons 1-4. Nonsense and frameshift mutations have been found in all exons of the gene except for exon 4, with the smallest predicted protein consisting of fewer than 40 amino acids and the largest 562 amino acids. Some of the abnormal transcripts may be unstable. Exon 4 is alternatively spliced in brain and blood, with the main transcript in adult spinal cord lacking exon 4. Since no mutations of any kind have been found in exon 4, it is possible that it is the spinal cord isoform that is pathologically relevant.

The broad mutational spectrum observed in spastin associated HSP initially suggested that the molecular pathological mechanism is likely to be loss of function, with disease occurring once functioning spastin levels fall below a critical threshold level. Indeed, it has been argued that tolerance for reduced dosage of functioning spastin is very low, since some “leaky” (that is, creating both wild type and aberrant splice variants) splice site mutations result in only slight reductions in wild type mRNA expression. However, the loss of function viewpoint has been challenged by recent work describing the cellular expression of wild type and missense mutant spastin. This has suggested that wild type spastin associates with microtubules in vivo, that dissociation of spastin from microtubules occurs in an ATPase dependent manner and that spastin may function as a microtubule severing enzyme, similar to some related AAA proteins such as katanin. Spastin mutants containing missense mutations in the AAA cassette appeared to bind to microtubules in a constitutive fashion, perhaps acting in a dominant negative fashion to block the normal function of spastin or unidentified spastin related proteins.

The suggestion of a potential dominant negative pathological mechanism for spastin missense mutations is intriguing. None of the truncating or splice mutants that are common in spastin associated HSP were examined and so it is not clear whether these classes of mutation might exert pathological effects by the same mechanism. A model could be suggested whereby mutations of any type result in a dominant negative effect, by rendering the molecule non-functional (for example ablation its ATPase function in the case of missense, truncating, or splice mutants affecting the AAA cassette), but leaving the N-terminal microtubule binding region intact. The mutant protein would be constitutively bound to microtubules via this region, and directly or indirectly block the action of wild type spastin or some other critical microtubule associated protein.

With this interpretation, a possible explanation for the pathological effects of leaky splice site mutations would be provided by the dominant negative effect of mutant spastin produced by the leaky allele. The dominant negative model does give rise to some testable hypotheses, not least that truncating or splice mutants should express truncated spastin protein and that these truncated proteins (including that which was smaller than 40 amino acids) should be able to bind microtubules. These different models for the pathological mode of action need to be carefully resolved, since they clearly have different implications for experimental approaches to exploring the function of mutant and normal spastin and for the likely success of replacement gene therapy in spastin associated HSP.

The neuronal specific kinesin gene KIF5A is the most recent gene to have been associated with HSP. It is a member of the kinesin superfamily of molecular motors that transport cargoes along tracks of microtubules, in an ATP dependent fashion. More than 40 human kinesin motors have been identified. KIF5A has an exclusively neuronal expression pattern, enriched in motor neurones. It is a component of conventional kinesin, which is a heterotrimer of two kinesin heavy chain (KHC) motor subunits (KIF5A, KIF5B, or KIF5F in mammals) and two kinesin light chain (KLC) subunits (KLC1, KLC2, or KLC3 in mammals) and acts as a plus ended motor, moving cargoes towards the cell periphery. In neurones, it is involved with fast anterograde axonal transport.

KIF5A is located within the chromosome 12q autosomal dominant pure HSP (SPG10) candidate region and a mutation in this gene, N256S, was identified in the original British family used to map the locus. The mutated asparagine residue is invariant within all known kinesins, and occurs within a highly conserved block, NINK(R)S. Residue 256 is contained within the switch 2 helix motif of the motor domain of KIF5A and work on orthologous kinesins mutated at the corresponding amino acid suggests that this residue is crucial for kinesin

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function, participating in the activation of ATPase via hydrogen bonding and side chain interactions with the switch 1 and switch 2 regions. Deletion of the orthologous kinesin heavy chain gene of several species cause movement defects reminiscent of human spastic paraplegia. These data strongly suggest that the mutation identified has a significant detrimental effect on kinesin driven motility, and that this is likely to have a pathological effect. Along with the finding that another kinesin, KIF1Bbeta, is responsible for an axonal form of hereditary motor and sensory neuropathy, they add to the growing body of evidence that kinesins may be involved in human neurological disease. A full account of the cargo specificity of individual kinesins is not yet available, but they have been suggested to be involved in transport of mitochondria, lysosomes, endosomes, protein complexes, and mRNAs, and in Golgi to ER membrane trafficking. KIF5B, a molecule closely related to KIF5A, is also expressed in the central nervous system, but predominately in glial cells rather than neurons. Mice lacking KIF5B die in utero, but cultured null mutant extra-embryonic cells had abnormal dispersion of mitochondria and lysosomes, so it is possible that KIF5A may be responsible for transport of these organelles in neuronal cells.

The chromosome 14q autosomal dominant pure HSP gene, atlastin (SPG3A), was identified in late 2001. This gene is a novel GTPase that has sequence homology to members of the dynamin family of large GTPases, particularly guanylate binding protein-1. It is widely expressed, but is most abundant in brain and spinal cord, and codes for a protein of 558 amino acids. It is likely that mutations in atlastin are responsible for approximately 10% of autosomal dominant pure HSP (E Reid, unpublished data). All of the atlastin gene mutations described so far have occurred in autosomal dominant pure HSP families showing childhood onset and it is possible that mutations in this gene are not a cause of adult onset HSP. To date, four atlastin gene mutations have been published, all missense mutations and clustered in exons 7 and 8 of the gene. One of these mutations, R239C, was present in three separate families. Three of the mutations are predicated to alter amino acids at the surface of a globular N-terminal region containing the conserved GTPase domain, without affecting GTPase motifs directly. The other mutation alters a conserved element of the GTPase domain itself. It is not yet clear how these mutations affect the function of the protein and how they act in causing HSP. The dynamins, the group of proteins to which atlastin shows strongest homology, are involved in vesicle trafficking events, including recycling of synaptic vesicles and in the dispersion of mitochondria. They are capable of associating with microtubules.

Troyer syndrome is an autosomal recessive HSP in which spastic paraparesis is complicated by dysarthria, distal amyotrophy, mild developmental delay, and short stature. The gene responsible for this condition has been identified within the last few months. It codes for a 666 residue protein termed spartin which is expressed ubiquitously. This protein has sequence homology to proteins SNX15, VPS5, and Skd1, involved in endosome membrane dynamics and protein trafficking of late endosomal components. Interestingly, it also shows sequence homology to the N-terminal region of spastin that has been implicated in binding microtubules.

In summary, there are some common features that might tentatively link spastin, KIF5A, atlastin, and spartin. With varying degrees of certainty, all four may all bind microtubules; for KIF5A the evidence for this is incontrovertible, for spastin it is strong but needing confirmation, and for atlastin and spartin it is weak and based on sequence homology to known microtubule binding proteins. KIF5A is clearly involved in intracellular trafficking and, again by sequence homology, atlastin and spartin have been suggested to be involved in this. Finally, spastin and spartin show a degree of sequence homology at their N terminals. It will be interesting to see whether further investigation of the biology of these proteins confirms a functional overlap in their cellular roles and a common mechanism for the development of corticospinal tract degeneration.

CONCLUSION: THE END OF THE BEGINNING?
The last few years have seen a quickening of the pace of identification of hereditary spastic paraplegia genes. Some common pathological themes for HSP may be emerging and it is even possible that some of these themes may eventually link, with, for example, altered function of KIF5A potentially deranging mitochondrial trafficking. Gene identification has also led to immediate practical benefits for patients; spastin and atlastin gene testing can be expected to yield positive results in 50% of patients with autosomal dominant “pure” HSP the single most common HSP phenotype presenting in northern Europe and North America. This has opened up the possibility of predictive testing for a substantial number of at risk family members. Prenatal diagnosis may also be feasible for such families, although this must be weighed in the context of a disease that is not usually associated with reduced life expectancy and which may have very variable (and often unpredictable) age at onset and severity.

The advances in HSP molecular genetics hold the promise of uncovering cellular processes that are involved in corticospinal tract neurodegeneration, in the case of early onset/congenital HSPs, or in corticospinal tract maintenance, in the case of the late onset HSPs. Further, many of the molecules involved in HSPs are widely expressed outside the nervous system. The axons of the corticospinal tract, with their peculiarities of size and distance, represent a challenging cellular environment in which the processes involved may be working close to the limits of tolerance. Their failure in the HSPs may act as a key to understanding cellular systems that have broad biological relevance.

In the final reckoning of our current knowledge of the HSPs, many questions remain. What specific factors make corticospinal tract axons particularly vulnerable to abnormalities of proteins that are often widely expressed? How can theaxon length dependence of the disease pathology be explained? What underlies the age dependent penetrance of pure HSP and how can the variability of the condition within families be explained? In late onset cases, what processes are involved in triggering the disease? Over the next few years, these questions will start to be answered by systematic study of the cellular pathological mechanisms involved with the gene products discussed here, and of other genes that have yet to be identified.

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REFERENCES
Hereditary spastic paraplegias


Transient transfection permits analysis of mismatch repair genes

Transfections to establish the role of the MMR genes in apoptosis and cell proliferation were also investigated. They showed that the wt hMSH2 gene controlled apoptosis and that wt hMLH1, with hPMS2 and hMSH6, increased expression of hPMS2 protein. Similarly, in one or other gene. These cell lines were derived from patients with hereditary non-polyposis colorectal cancer (HNPCC), and their gene mutations were matched by the constructs.

For mismatch repair to occur hMLH1 and hMSH2 proteins must interact with other specific proteins—hMLH1 with hPMS2 and hMSH2 with hMSH6. Transient transfection of cell line HCT-116 gene controlled apoptosis and that wt hMLH1, with hPMS2 and hMSH2, increased expression of hPMS2 protein. Similarly, in one or other gene. These cell lines were derived from patients with hereditary non-polyposis colorectal cancer (HNPCC), and their gene mutations were matched by the constructs.

They used a transient transfection model between wild type (wt) and two mutant DNA constructs of the major human mismatch repair (MMR) genes hMLH1 and hMSH2 and two cell lines, each deficient in one or other gene. These cell lines were derived from patients with hereditary non-polyposis colorectal cancer (HNPCC), and their gene mutations were matched by the constructs.