

ONLINE MUTATION REPORT

Meta-analysis of age at onset in spastin-associated hereditary spastic paraplegia provides no evidence for a correlation with mutational class

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The hereditary spastic paraplegias (HSPs) are a group of single gene disorders in which the corticospinal tracts fail to develop normally, or degenerate after initially normal development. The HSPs all share the principal clinical feature of progressive lower limb spastic paralysis, and are subdivided into pure and complicated forms, depending on the presence of additional neurological or non-neurological features.^{1,2}

The pure HSPs tend to be associated with neurodegeneration, rather than abnormal development, and histopathological studies in pure HSP show a length-dependent “dying back” of the terminal ends of the corticospinal tract axons, with the longest axons being involved first.¹ The *SPG4* gene, spastin, is the most important pure HSP gene, being responsible for approximately 40% of definite autosomal dominant pure HSP and a smaller proportion of sporadic cases and cases with uncertain family history.^{3,4} The 616 amino acid spastin protein is a widely expressed AAA (ATPases associated with diverse cellular activities) protein.⁵ More than 100 different spastin mutations have been described, including numerous missense, nonsense, frameshift, and splice site mutations, as well as less frequent whole exon deletions. With only a few possible exceptions, the missense mutations are located in the AAA cassette, from amino acids 342–599. Splice site mutations almost exclusively involve exons 5–16. Nonsense and frameshift mutations are scattered across the gene, with the smallest predicted protein consisting of fewer than 40 amino acids, the largest 562 amino acids.²

It is likely that the molecular pathological mechanism of truncating and splice site spastin mutations is loss of function. The associated abnormal transcripts may be unstable, and recent data show that mutant spastin protein is absent in fibroblasts from patients with nonsense and frameshift spastin mutations.^{6,7} These classes of spastin mutation are probably associated with haploinsufficiency, with disease occurring once functioning spastin levels fall below a critical threshold level. Tolerance for reduced dosage of functioning spastin may be very low, as 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.⁸ On the other hand, spastin missense mutations may act via a different mechanism. It has been suggested that spastin has a microtubule severing function and that spastin missense mutants bind constitutively to microtubules, perhaps acting in a dominant negative fashion to block the normal function of spastin or unidentified spastin-related proteins.⁹

One approach to resolve the issue of whether spastin missense mutations have a different molecular pathological mechanism to other mutational types is to examine whether mutational class is correlated with clinical features. The main quantifiable clinical variable in autosomal dominant pure HSP families is age at onset of symptoms, and this shows

Key points

- The hereditary spastic paraplegias (HSPs) are a group of single gene disorders in which the corticospinal tracts fail to develop normally, or degenerate after initial normal development.
- Mutations in the *SPG4* gene, spastin, are responsible for circa 40% of definite autosomal dominant pure HSP.
- The range of spastin mutational classes found in HSP families is very broad. It is likely that nonsense, frameshift and splice site mutations of spastin act via a loss of function mechanism. However, it has been suggested that spastin missense mutations may act via a dominant negative mechanism.
- One approach to resolve the issue of whether spastin missense mutations have a different molecular pathological mechanism to other mutational types is to examine whether mutational class is correlated with clinical features. We therefore carried out a meta-analysis of age at onset v mutational class correlations in HSP caused by spastin mutations, in order to test the null hypothesis that there is no difference in age at onset between groups of families with different classes of spastin mutation.
- Data gathered on 75 families revealed no significant difference in age at onset between HSP patients with missense v other spastin mutational classes, providing no evidence for a genotype-phenotype correlation.

striking variability within and between families with spastin mutations. If all spastin mutational classes act via the same molecular mechanism, then no difference in age at onset would be expected when families are compared by mutational class. On the other hand, if missense mutations act via a dominant negative mechanism and other mutational types act via a loss of function mechanism, then these different pathological processes might be reflected clinically in discernible differences in age at onset. Such a correlation has not previously been identified.³ We therefore carried out a meta-analysis of age at onset v mutational class correlations in HSP caused by spastin mutations, in order to test the null hypothesis that there is no difference in age at onset between groups of families with different classes of spastin mutation.

We analysed all studies (n = 4) reporting five or more HSP families with spastin mutations that were published before December 2001.^{3,8,10,11} We obtained age at onset of symptoms

Abbreviations: HSP, hereditary spastic paraplegia

data for individual affected patients directly from the relevant papers, or from unpublished data. Asymptomatic individuals were excluded from the analysis.

Within each study, we compared age at onset across different mutational classes and then estimated, as the summary “effect”, a hazard ratio of non-missense relative to missense mutation families using conditional Cox regression.¹² We then pooled the resultant hazard ratios and variances using standard methods of meta-analysis as implemented in Stata 7.0 (Stata Corp., TX, USA).^{13,14}

In total, we obtained data from 75 families with known spastin mutations. Age at onset data were available from 356 subjects from these families (details of ages at onset and mutations are available on request). The mutations present were divided into four broad groups; 18 of the families had missense mutations, 23 had presumed splice site mutations, 30 had mutations that resulted in premature truncation (either frameshift or nonsense mutations), and 4 families had mutations that were predicted to cause both a splice mutation and a frameshift. Table 1 shows the numbers of patients with each class of mutation, unweighted mean age at onset and the mean age at onset weighted by family membership. Individual study and pooled hazard ratios for missense mutations relative to all other classes of mutations are shown in table 2. The hazard ratio was homogeneous across studies and there was no significant difference in age at onset in families with missense mutations *v* families with other classes of mutation.

The pooled data from 75 families confirmed several previously reported observations. The mutational spectrum associated with spastin is wide, and most mutations involve truncation of the molecule or abnormal splicing. Nearly all missense mutations are located within the AAA cassette. Although the age at onset of spastin associated HSP is variable, the mean age at onset is in the late third decade of life.

We looked to see whether any clinical difference in age at onset was apparent between HSP patients with missense *v* other spastin mutational classes. Although patients with missense mutations had a slightly younger mean age at onset (tables 1 and 2), the standard deviations overlapped widely and this difference was not statistically significant. We cannot therefore reject the null hypothesis that there is no correlation between age at onset and mutational class. On the other hand, our data do not exclude the possibility that different mutational classes may act by different molecular pathological mechanisms, as firstly, our sample size may not have been large enough to detect a small difference in age at onset between groups, and secondly, it is feasible that similar ages at onset may arise from different molecular pathological mechanisms. It is likely that direct experimental work or

Table 1 Unweighted and weighted mean age at onset data for each mutational class, for families described in the four studies included in the meta-analysis

Mutational class	No of families	No of subjects	Mean age at onset	
			Unweighted years (SD) Range (years)	Weighted by family years (SD)
Frameshift+ presumed exon skip	4	25	28.91 (4.98) (<1–62)	28.44 (5.02)
Missense	18	69	24.88 (13.96) (<1–60)	25.26 (11.31)
Splice site	23	115	29.02 (9.63) (<1–60)	29.56 (8.12)
Truncating	30	147	29.98 (10.63) (<1–74)	30.37 (10.63)

Table 2 Individual study and pooled hazard ratios for missense mutations relative to all other classes of mutations

Study	Hazard ratio	95% confidence interval
Fontnechten ³	1.33	0.86 to 2.04
Hentati ¹¹	0.99	0.60 to 1.64
Lindsey ¹⁰	1.19	0.29 to 4.96
Svenson ⁸	1.11	0.66 to 1.77
Pooled	1.14	0.78 to 1.50

Test for heterogeneity: $Q=0.79$, $p=0.85$.

further meta-analyses with very large sample sizes will be required to resolve this issue.

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