Exome Sequencing Links Corticospinal Motor Neuron Disease to Common Neurodegenerative Disorders
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Exome Sequencing Links Corticospinal Motor Neuron Disease to Common Neurodegenerative Disorders

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Hereditary spastic paraplegias (HSPs) are neurodegenerative motor neuron diseases characterized by progressive age-dependent loss of corticospinal motor tract function. Although the genetic basis is partly understood, only a fraction of cases can receive a genetic diagnosis, and a global view of HSP is lacking. By using whole-exome sequencing in combination with network analysis, we identified 18 previously unknown putative HSP genes and validated nearly all of these genes functionally or genetically. The pathways highlighted by these mutations link HSP to cellular transport, nucleotide metabolism, and synapse and axon development. Network analysis revealed a host of further candidate genes, of which three were mutated in our cohort. Our analysis links HSP to other neurodegenerative disorders and can facilitate gene discovery and mechanistic understanding of disease.

Heredity spastic paraplegias (HSPs) are a group of genetically heterogeneous neurodegenerative disorders with prevalence between 3 and 10 per 100,000 individuals (1). Hallmark features are axonal degeneration and progressive lower limb spasticity resulting from a loss of corticospinal tract (CST) function. HSP is classified into two broad categories, uncomplicated and complicated, on the basis of the presence of additional clinical features such as intellectual disability, seizures, ataxia, peripheral neuropathy, skin abnormalities, and visual defects. The condition displays several distinct modes of inheritance, including autosomal dominant, autosomal recessive, and X-linked. Several loci have been linked to autosomal recessive HSP (AR-HSP), from which 22 genes with mutations have been cloned. However, most of the underlying causes of HSP remain unidentified.

We analyzed 55 families displaying AR-HSP by whole-exome sequencing (WES). We identified the genetic basis in about 75% of the cases, greatly increasing the number of mutated genes in HSP; functionally validated many of these genes in zebrafish; defined new biological processes underlying HSP; and created an “HSPome” interaction map to help guide future studies.

Multiple Genes Are Implicated in HSP

We used WES to identify the genetic causes of AR-HSP in families with documented consanguinity. Selecting from these families without congenital malformations referred for features of either complicated or uncomplicated HSP (table S1), we performed WES on 93 individuals typically from two affected siblings or cousins where possible, for multiplex families, or one affected and one unaffected sibling or both parents, for simplex families. We prioritized predicted protein frame shift, stop codon, splice defects, and conserved nonsynonymous amino acid substitution mutations [Genomic Evolutionary Rate Profile (GERP) score > 4 or phastCons (genome conservation) score > 0.9]. We excluded variants with an allele frequency of greater than 0.2% in our in-house exome database of over 2000 individuals.

We genotyped each informative member from the majority of families with a 5000 single-nucleotide polymorphism (SNP) panel and generated genome-wide parametric multipoint linkage plots or used WES data to generate homozygosity plots (2). We excluded variants falling outside of homozygous intervals <2.0 Mb threshold (fig. S1).

We tested segregation of every variant meeting these criteria (table S2). We report a candidate HSP gene only if there was a single deleterious variant that segregated in the family or if the gene was identified as mutated in multiple families (3). For 15 families, a single genetic cause could not be identified. We identified mutations in 13 genes known to be mutated in HSP (33% of the cases in our cohort) (table S3 and fig. S2), supporting the methodology. These include EIF2B5, associated with vanishing white-matter disease [Online Mendelian Inheritance in Man (OMIM) no. 603896]; CLN9, associated with ceroid lipofuscinosis (OMIM 600143); and ARG1, which causes arginase deficiency (OMIM 207800). The diversity of genes identified speaks to the heterogeneity of HSP presentations. ALS2 (OMIM 205100) was mutated in four different families presenting with uncomplicated HSP, and ATL1 (OMIM 182600) was mutated in three different families, some displaying partial penetrance (4).

We identified 14 candidate genes not previously implicated in disease (Table 1), accounting for 42% of the cases in our cohort. We also evaluated five non-consanguineous families by WES, implicating one additional candidate gene. We estimated, on the basis of our false discovery rate (FDR), that fewer than 0.1 alleles per family should pass this threshold randomly, dependent on the number of informative meioses, suggesting that fewer than 1:10 genes identified with this method should prove false positive (i.e., identify by chance) (3).

The mutations in the 15 novel genes were identified in patients presenting with a spectrum of HSP phenotypes. Three of these genes, ERLIN1,
of HSP. ADP, adenosine diphosphate; IMP, inosine monophosphate; ATP, adenosine triphosphate; DDHD, Asp-Asp-His domain; GPI, glycosyl phosphatidylinositol; GTPase, guanosine triphosphatase; tRNA, transfer RNA. N/A, not applicable. Single-letter amino acid abbreviations are as follows: C, Cys; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and X, termination.

Table 1. Novel candidate HSP genes. List of novel candidate HSP genes identified through WES, divided into major functional modules (ERAD, etc.). OMIM nomenclature refers to established or new (beginning with SPG58) locus. Positions are given in the subnetwork containing all seed genes plus private genes.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Locus Nomenclature</th>
<th>Entrez Gene ID</th>
<th>Gene Name</th>
<th>Putative Biological Function</th>
<th>Family Position (h19)</th>
<th>Nucleotide Change</th>
<th>Deduced Protein Change</th>
<th>Effect on Protein</th>
<th>Phenotype</th>
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<tr>
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<td>ARL6IP1</td>
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<td>23304</td>
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<td>Protein transport</td>
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<td>c.763T&gt;A</td>
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<td>KIF1C</td>
<td>SPG58</td>
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<td>Kinesin family member 1C</td>
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<td>chr17:4904143 G&gt;A</td>
<td>c.1879_1881delAAG</td>
<td>p.del628E</td>
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<td>SPG59</td>
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<td>Deubiquitinating enzyme</td>
<td>chr5:150679124 C&gt;A</td>
<td>c.22978_23001del24 C</td>
<td>p.G760DfsX3</td>
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<td>ARSI</td>
<td>SPG66</td>
<td>340075</td>
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<td>chr1:19717620 C&gt;T</td>
<td>c.1151+2T&gt;CA</td>
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<td>SPG64</td>
<td>32359</td>
<td>DDHD domain-containing protein 2</td>
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<td>SPG67</td>
<td>80055</td>
<td>Post-GR attachment to proteins 1</td>
<td></td>
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<td>FLRT1</td>
<td>SPG68</td>
<td>23769</td>
<td>Fibronectin leucine rich transmembrane protein 1</td>
<td></td>
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<td>Stop-loss</td>
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<td>RAB3 GTPase activating protein subunit 2</td>
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<td>MARS</td>
<td>SPG70</td>
<td>4141</td>
<td>Methionyl-tRNA synthetase</td>
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We next expanded the HSP seed + candidate network to derive the HSPome (i.e., HSP seeds + candidates + proximal interactors network), allowing a global view of HSP and flagging other potential genes that may be mutated in HSP patients. The HSPome contains 589 proteins (i.e., potential HSP candidates) (supplementary data 2 and table S5).

**Implicated Causal Genes Suggest Modules of HSP Pathology**

Studies in HSP consistently report an ascending axonal CST degeneration ([12]), but the processes modulating this degeneration are not well defined. Supporting the hypothesis that individual rare mutations in distinct genes may converge on specific biological pathways, we identified major modules involved in the pathophysiology of HSP. Several HSP genes have previously implicated endoplasmic reticulum (ER) biology (i.e., *ATL1*, *REEP1*, *RTN2*, and *SPAST*) and the ER-associated degradation (ERAD) pathway (i.e., *ERLIN2*) ([13–15]). From the HSPome, we focused on this ER subnetwork containing the newly identified genes *ARL6IP1* and *ERLIN1* (fig. S7). *ARL6IP1* encodes a tetraspan membrane protein localized to the ER, composed of highly conserved hydrophobic hairpin domains implicated in the formation of ER tubules ([16]). We overexpressed *ARL6IP1* in cells and noted dramatically altered ER shape (fig. S7). The ERAD system controls protein quality control, critical for cellular adaptation to stress and survival. *ERLIN1* encodes a prohibitin-domain-containing protein localized to the ER that forms a ring-shaped complex with *ERLIN2*, further implicating defective ERAD in HSP etiology.

We identified an endosomal and membrane-trafficking subnetwork composed of seeds and candidates *KIF1C*, *USP8*, and *WDR48*, implicating the endosomal sorting complexes required for transport (ESCRT) pathway (fig. S8). *USP8*
encodes a deubiquitinating enzyme (DUB) in the ESCRT pathway (17). The WDR48-encoded protein forms stable complexes with multiple DUBs, such as USP1, USP12, and USP46, and is required for enzymatic activity and linked to lysosomal trafficking (18, 19). KIF1C encodes a motor protein localized to the ER/Golgi complex, suggesting a role in trafficking (20). To validate the effect of the putative splicing mutation in family 789, we obtained fibroblasts and confirmed skipping of exon 4 (fig. S9). Defects in ESCRT are linked to neurodegenerative disorders such as frontotemporal dementia, Charcot Marie Tooth disease, and recently AR-HSP (21–23). Additionally, the HSP gene products SPG20, SPAST, and ZFYVE26 interact with components of this complex (24–26). Taken together, this suggests that disruptions in ESCRT and endosomal function can lead to HSP and other forms of neurodegeneration.

AMPD2, ENTPD1, and NTSC2 are involved in purine nucleotide metabolism (fig. S10). Nucleotide metabolism is linked to the neurological disorder Lesch-Nyhan disease, among others (27), but was not previously implicated in HSP. AMPD2 encodes one of three adenosine monophosphate (AMP) deaminase enzymes involved in balancing purine levels (28). Mutations in AMPD2 have been recently linked to a neurodegenerative brainstem disorder (28). In addition, the deletion we have identified in this study affects just the longest of the three AMPD2 isoforms, indicating that the most N-terminal domain of AMPD2 is important to prevent motor neuron degeneration. ENTPD1 encodes an extracellular ectonuclease hydrolyzing adenosine nucleotides in the synaptic cleft (29). NTSC2 encodes a downstream cytosolic purine nucleotide 5′ phosphatase. Purine nucleotides are neuroprotective and play a critical role in the ischemic and developing brain (29); thus, alterations in their levels could sensitize neurons to stress and insult. ENTPD1 was recently identified as a candidate gene in a family with nonsyndromic intellectual disability, but HSP was not evaluated (30).

**Candidate HSP Genes Identified by Network Analysis**

For families that were not included in our initial analysis, we interrogated our exome database for variants in genes emerging from the extended HSPome network. By using this method, we identified potentially pathogenic variants in MAG, BICD2, and REEP2, found in homozygous intervals in three families (Fig. 3), validating the usefulness of the HSPome to identify new HSP genes. Interacting with KIF1C in the HSPome is CCDC64, encoding a member of the Bicaudal family (31), a paralog of the BIC2 gene that emerged in the HSPome (FDR < 0.05, table S5). Family 1370 displays a homozygous Ser608→Leu608 missense change in the BIC2 gene within a homozygous haplotype. The Drosophila bicaudal-D protein is associated with Golgi-to-ER transport and potentially regulates the rate of synaptic vesicle recycling (32). Coimmunoprecipitation confirmed that BICD2 physically interacts with KIF1C (fig. S11).

MAG was identified as a significant potential candidate (FDR < 0.05) from the HSPome, interacting with PLP1, the gene product mutated in SPG2. MAG is a membrane-bound adhesion protein implicated in myelin function, and knockout

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**Fig. 2. Hereditary spastic paraplegia interactome.** (A) HSP seeds + candidate network (edge-weighted force-directed layout), demonstrating many of the genes known to be mutated in HSP (seeds, blue) and new HSP candidates (red), along with others (circles) constituting the network. (B and C) Comparison of statistical strength of HSP subnetworks with 10,000 permutations of randomly selected proteins. Dots denote the value of the metric on the true set (i.e., seeds or seeds + candidates). Box and whisker plots denote matched null distributions (i.e., 10,000 permutations). (B) Seed (known mutated in HSP) versus random proteins drawn with the same degree distribution. (C) Seed plus candidate HSP versus a matching set of proteins. (Left) Within group edge count (i.e., number of edges between members of the query set). (Middle) Interaction neighborhood overlap (i.e., Jaccard similarity). (Right) Network random walk similarity.
mice display defects of the periaxonal cytoplasmic collar in the spinal cord with later oligodendrocyte degeneration (34). MAG was found mutated in family 1226, displaying a homozygous Cys^{330}→Gly^{330} missense mutation.

**REEP2** encodes the receptor expression-enhancing protein 2, a paralog of **REEP1**, mutated in **SPG31** (35). Family 1967 displays a homozygous Met^{1}→Thr^{1} mutation in **REEP2** removing the canonical start codon and is mutated in a second recessive HSP family in an independent cohort (36). All of these gene mutations segregated with the phenotype in the family according to recessive inheritance and were not encountered in our exome database, consistent with pathogenicity. Although further validation of these three candidates is necessary in larger cohorts, the data suggest the HSPome can be useful to identify HSP-relevant pathways and genes.

**Link Between HSP and Neurodegenerative Disease Genes**

Some of the genes we identified in this cohort have been previously associated with other neurodegenerative disorders (e.g., **CLN8**, **EIF2B5**, and **AMPD2**) primarily affecting areas of the nervous system other than the corticospinal tract. Prompted by this observation, we used the network to examine the similarity of HSP genes (seed + candidates) to other common neurological disorders. By using the random walk distance, we found that the set of HSP seeds plus candidates is significantly overlapping with sets of genes previously implicated in three neurodegenerative disorders, amyotrophic lateral sclerosis (ALS), Alzheimer’s disease, and Parkinson’s disease ($P = 1.1 \times 10^{-02}, P = 7.6 \times 10^{-03}, P = 1.6 \times 10^{-02}$, respectively) (Fig. 4). In contrast, we did not find a similar association with sets for representative neurodevelopmental disorders such as autism spectrum disorders and epilepsy ($P = 0.49$ and $P = 0.51$, respectively; fig. S12), nor with nonneurological disorders represented by heart and pulmonary disorders.

**Discussion**

By using WES, we identified 18 previously unknown candidates for AR-HSP (fig. S13), three of which (**ERLIN1**, **KIF1C**, and **NT5C2**) alone explain almost 20% of this cohort. These new candidates are predicted to display near 100% risk of HSP when mutated (37). All mutations were predicted as damaging to protein function, probably resulting in null or severely reduced function, consistent with the recessive mode of inheritance. In about 25% of the families a single candidate gene mutation could not be identified, probably a result of two factors: (i) Some mutations are in noncoding regions. (ii) Some causative mutations within the exome do not stand out more than other variants.

Four of our candidate HSP genes are located within previously identified loci for AR-HSP for which genes were not known: **ENTPD1**, **NTSC2**, **ERLIN1**, and **MARS**. Both **ERLIN1** and **NTSC2** are in the **SPG45** locus (38) and **ENTPD1** resides in **SPG27** (39). Recently, the **MARS2** gene, encoding a methionyl-tRNA synthetase, was implicated in the spastic ataxia 3 (**SPAX3**) phenotype (40). **KIF1C** is within the spastic ataxia 2 (**SAX2**) locus (41). On the basis of our findings, we returned to the original **SPAX2** family and identified a homozygous deletion of exons 14 to 18, confirming **KIF1C** as the **SPAX2** gene (fig. S14).

Our data support the idea that rare genetic mutations may converge on a few key biological processes, and our HSP interactome demonstrates that many of the known and candidate HSP genes are highly connected. This highlights important biological processes, such as cellular transport, nucleotide metabolism, and synapse and axon development. Some of the HSP gene modules suggest potential

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**Fig. 3. Genes from HSP networks found mutated in HSP.** (A) HSP candidate genes predicted from the HSPome found mutated in the HSP cohort. **BICD2**, **MAP2**, and **REEP2** were subsequently found mutated in HSP families 1370 (B), 1226 (D), and 1967 (F), respectively. (C) Homozygosity plot from family 1370. Red bars, regions of homozygosity; arrow, homozygous block containing **BICD2**. (E) Linkage plot of family 1226; arrow, **MAG** locus. (F) Homozygosity plot; arrow, **REEP2** locus. (H to J) Zoom in from HSPome for specific interaction identifying candidates **CCDC64** (a paralog of **BICD2**), **MAG**, and **REEP2** (yellow) with previously published (blue) and newly identified (red) genes mutated in HSP. Blue lines denote manually curated interactions.
Fig. 4. Functional link between HSP genes and genes of other neurodegenerative conditions. (A) Density distribution representing random walk distances of OMIM-derived neurodegeneration gene networks along with 10,000 permutations of randomly selected protein pools compared with the HSP seeds plus candidates pool. The top 5% distance is shaded. Only for Parkinson’s, Alzheimer’s, and ALS do the HSP seeds plus candidates fall within this 5%, whereas epilepsy and autism spectrum disorder show no statistical overlap. (B) Bipartite network showing the top links between the set of HSP and ALS proteins. Clear circles, HSP seeds; yellow circles, HSP candidates; boxes, ALS genes (VCP and ALS2 are implicated as causative of both HSP and ALS); line thickness, diffusion similarity between the two proteins.

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References and Notes
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