Am. J. Hum. Genet. 70:1328–1332, 2002

Report

Hereditary Spastic Paraplegia SPG13 Is Associated with a Mutation in the Gene Encoding the Mitochondrial Chaperonin Hsp60

Jens Jacob Hansen,[1] Alexandra Dürr,[2,3,4] Isabelle Cournu-Rebeix,[5] Costa Georgopoulos,[6] Debbie Ang,[6] Marit Nyholm Nielsen,[1] Claire-Sophie Davoine,[5] Alexis Brice,[2,3,4] Bertrand Fontaine,[2,5] Niels Gregersen,[1] and Peter Bross[1]

[1]Research Unit for Molecular Medicine, Århus University Hospital and Faculty of Health Sciences, Århus, Denmark; [2]Fédération de Neurologie, [3]INSERM U289, and [4]Département de Génétique, Cytogénétique et Embryologie, Groupe Hospitalier Pitié-Salpêtrière, and [5]INSERM U546, Faculté de Médecine et Groupe Hospitalier Pitié-Salpêtrière, Paris; and [6]Biochimie Médicale, Centre Médical Universitaire, Geneva

SPG13, an autosomal dominant form of pure hereditary spastic paraplegia, was recently mapped to chromosome 2q24-34 in a French family. Here we present genetic data indicating that SPG13 is associated with a mutation, in the gene encoding the human mitochondrial chaperonin Hsp60, that results in the V72I substitution. A complementation assay showed that wild-type *HSP60* (also known as "*HSPD1*"), but not *HSP60* (V72I), together with the co-chaperonin *HSP10* (also known as "*HSPE1*"), can support growth of *Escherichia coli* cells in which the homologous chromosomal *groESgroEL* chaperonin genes have been deleted. Taken together, our data strongly indicate that the V72I variation is the first disease-causing mutation that has been identified in *HSP60*.

Hereditary spastic paraplegia (HSP) represents a clinically and genetically heterogeneous group of neurodegenerative disorders that are characterized by progressive spasticity and weakness of the lower limbs. Seventeen different loci have been mapped, but only five of the corresponding genes have been cloned and identified so far (for reviews, see Tallaksen et al. 2001 and Casari and Rugarli 2001; for the recently identified fifth gene, see Zhao et al. 2001). Two of these five gene products—paraplegin (*SPG7* [MIM *602783]) (Casari et al. 1998) and spastin (*SPG4* [MIM 182601]) (Hazan et al. 1999)—feature AAA+ domains and are predicted to possess chaperone activity. Paraplegin is the human homologue of a yeast protease/chaperone that is involved in mitochondrial protein quality control (Casari et al. 1998; Langer 2000). We had previously localized the genes encoding the human mitochondrial chaperonin Hsp60 (heat-shock protein 60; also known as "Cpn60") and its co-chaperonin Hsp10 (heat-shock protein 10; also known as "Cpn10") to chromosome 2q33.1—that is, to the same region to which another locus for HSP (*SPG13* [MIM *605280]) had been mapped in a French family (Fontaine et al. 2000)—which prompted us to propose that *HSP60* and *HSP10* are candidate genes for SPG13. After determination of the *HSP60/HSP10* genomic structure (*HSPD1* [GenBank accession number AJ250915]), sequencing of the 16 exons encoding the two genes and the intervening 656-bp bidirectional promoter region in two affected members of the family with SPG13 revealed that both individuals were heterozygous for a G→A variation at position 292 of the *HSP60* cDNA, resulting in the substitution of a valine residue at position 72 in mature Hsp60 with isoleucine (V72I). Sequencing of *HSP60* exon 3 in the other members of the SAL-612 family confirmed that the variant allele segregated with the disease (fig. 1), and a peak two-point LOD score of 3.43 at recombination fraction 0, similar to that calculated for D2S2392, was obtained for this variant allele (Fontaine et al. 2000). Since the penetrance of HSP is age dependent, a possible explanation for the observation that three individuals (II:10, IV:1, and IV:2) are not clinically affected even though they carry both the disease allele and the mutation is

Received December 10, 2001; accepted for publication January 29, 2002; electronically published March 15, 2002.

Address for correspondence and reprints: Dr. Peter Bross, Research Unit for Molecular Medicine, Skejby Sygehus, Brendstrupgaardsvej, 8200 Århus N, Denmark. E-mail: Peter.Bross@MMF.au.dk

0002-9297/2002/7005-0023$15.00

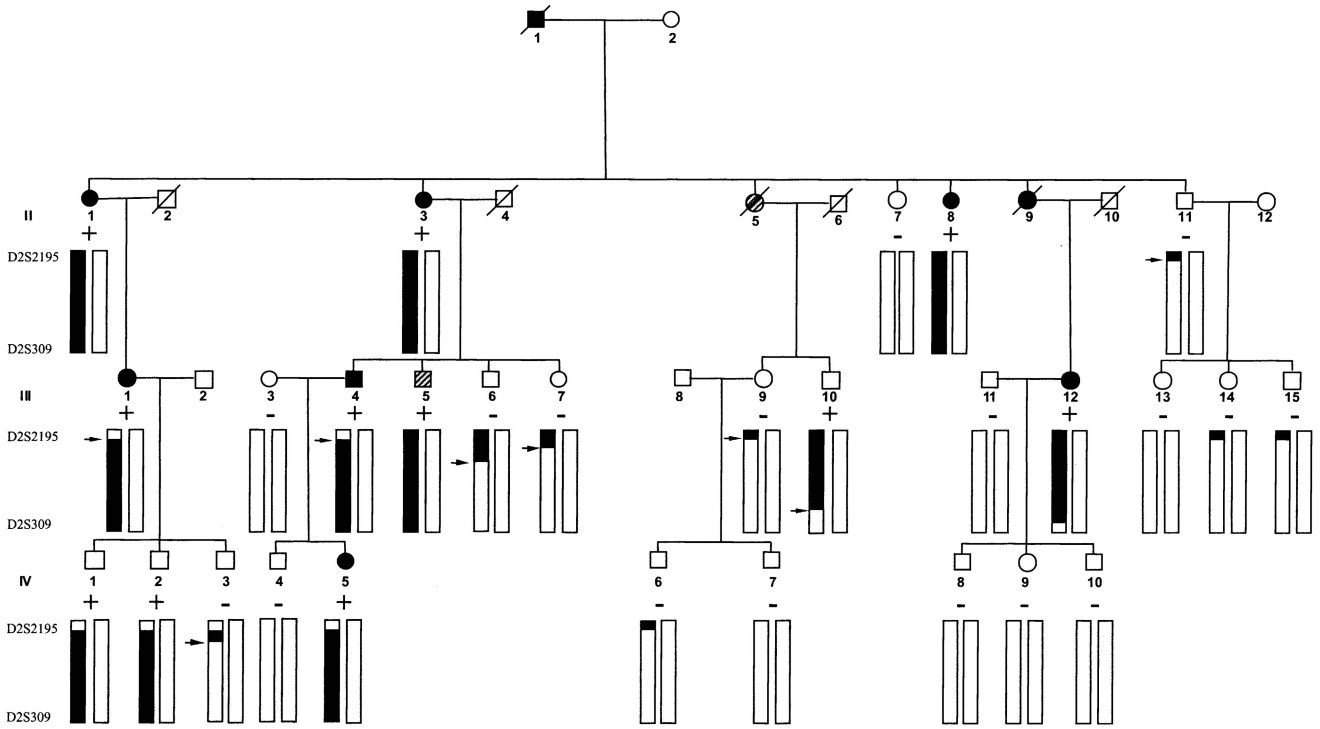

Figure 1 Pedigree of family SAL-612. SPG13 is flanked by D2S2195 and D2S309. The haplotype segregating with HSP is indicated, and recombinants are noted by an arrow. The plus sign identifies the individuals in whom the *HSP60* 292 G→A variant was found. A perfect segregation is observed between the disease haplotype and the *HSP60* 292 G→A variant.

that they were younger than the mean age at onset of SPG13 at the time of clinical examination. Analysis of genomic DNA from unrelated control individuals of European descent showed that none of 800 control chromosomes carried the 292G→A variation.

To further investigate the frequency of *HSP60* variations in a population of patients with HSP, we sequenced the coding region and the promoter of the *HSP10* and *HSP60* genes in four affected individuals belonging to two French families in which linkage to chromosome 2q24-34 was suggested (Fontaine et al. 2000) and in 20 index cases with autosomal dominant HSP from French families, which were not informative enough to be investigated by linkage analysis, and in which mutations in the gene encoding spastin (i.e., *SPG4*) had been excluded. In only one of these patients, we found a base pair change that resulted in an amino acid substitution—an A→G variation at position 551 of the *HSP60* cDNA, replacing asparagine-158 with serine (N158S). However, the absence of this variation in another affected family member, as well as its presence in 1 of 800 alleles from control individuals of European descent, suggested that it represents a rare variation and not a disease-causing mutation.

In an alignment of human Hsp60 with the *Escherichia coli* Hsp60 homologue GroEL, valine-72 and asparagine-158 correspond to valine-74 and lysine-160 in *E. coli* GroEL, respectively. Inspection of the crystal structure of *E. coli* GroEL (Xu et al. 1997) shows that valine-74 is located in helix C of the equatorial domain, buried inside a conserved substructure of the GroEL monomer, whereas lysine-160 is situated in the intermediate domain and its side chain protrudes from the outer surface of the barrel structure (fig. 2). This suggests that replacement of the conserved valine may have more-severe consequences than replacement at the other position. To experimentally determine the function of the *HSP60* (*V72I*) and *HSP60* (*N158S*) variants, we turned our attention to the *E. coli* genetic system. The *groESgroEL* operon encoding the *E. coli* chaperonins is essential for bacterial growth under a variety of growth conditions (Fayet et al. 1989). We have recently shown that the entire *E. coli groESgroEL* chromosomal operon can be deleted when the human *HSP60* and *HSP10* genes are supplied in *trans* (Richardson et al. 2001). However, the resulting *E. coli*–derivative strain grows slowly, at 37°C, or not at all, at 42°C. Thus, we reasoned that the ability of the *HSP60* and *HSP10* human chaperone genes to

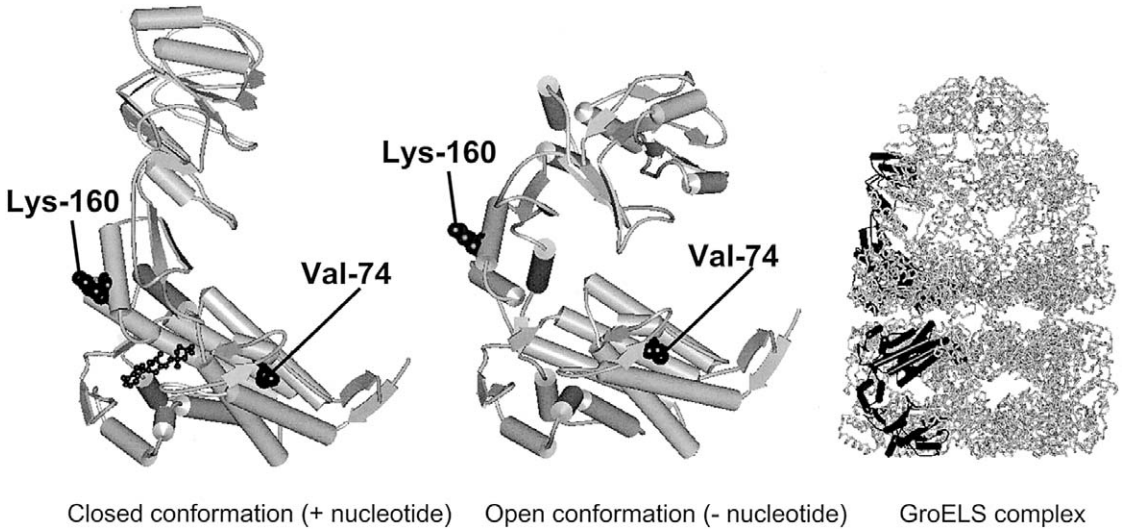

Figure 2 Schematic representation of the structure of the Hsp60 chaperonin homologue GroEL from *E. coli* (Protein Data Bank entry 1AON). The architecture of a subunit in the closed and open conformations and an overview of the GroEL/GroES chaperonin complex with one subunit in the closed (upper ring) and open (lower ring) conformations highlighted in black are shown. The side chains of valine-74 and lysine-160, which correspond to valine-72 and asparagine-158, respectively, in human Hsp60, are shown in space-filling representation. The figure was produced with WebLab ViewerLite software (Molecular Simulations).

replace the endogenous *groESgroEL* operon may represent a very sensitive system to test our two *HSP60* mutant alleles for their relative in vivo function.

We found that when either the *E. coli* wild-type (wt) *groESgroEL* operon or the human *HSP60* (*wt*)–*HSP10* (*wt*) or *Hsp60* (*N158S*)–*HSP10* (*wt*) set of genes was expressed from a plasmid, they allowed the deletion of the chromosomally encoded *groESgroEL* operon—that is, ~50% of the tetracycline-resistance (Tet^R) transductants simultaneously inherited the ω-chloramphenicol-resistance (Cam^R)–encoding cassette, replacing the *groESgroEL* operon (for details, see the note for table 1). In contrast, the presence of the *HSP60* (*V72I*)–*HSP10* (*wt*) set of genes on a plasmid did not allow the deletion of the $groES^+groEL^+$ chromosomally encoded operon—that is, none of the Tet^R transductants (0/206) inherited the Cam^R-encoding DNA cassette. The *E. coli* transductants maintained alive by either the *HSP60* (*wt*)–*HSP10* (*wt*) or *HSP60* (*N158S*)–*HSP10* (*wt*) operon could not be distinguished from each other by a variety of physiological and genetic tests. For example, the colony sizes of the two *E. coli* strains were indistinguishable from each other, and both strains failed to grow at ⩾40°C. In addition, both strains supported equally well the growth of bacteriophage T5, which requires both the GroES and GroEL proteins for its morphogenesis (Richardson et al. 2001). In contrast, neither strain supported the growth, at 37°C, of bacteriophage λ, T4, RB43, and RB49, in agreement with our previous results (Richardson et al. 2001). The following control experiments were performed to ensure the validity of our results and conclusions (fig. 3): first, western blot analysis indicated that all plasmid constructs expressed equal levels of Hsp60; second, western blot analysis with GroEL-specific antibody showed that Cam^R *E. coli* transductants did not express any GroEL, as expected; and, third, the three *HSP60* plasmid constructs introduced into the *E. coli* B178 bacteria (table 1) were shown by DNA sequencing to possess the correct *HSP60* allele (data not shown).

The inevitable conclusion of all our genetic and protein-expression experiments is that, whereas the Hsp60 (N158S) mutant protein functions as well as its Hsp60 wt counterpart in the promotion of *E. coli* growth, the Hsp60 (V72I) mutant protein fails to do so. Dominant inheritance of the *HSP60* (V72I) allele in spastic paraplegia may result from haploinsufficiency through the formation of mixed chaperonin rings consisting of active wt and functionally incapacitated Hsp60 (V72I) subunits. This would lead to a lowering of overall chaperonin activity, especially under stress conditions in which the up-regulation of the wt allele cannot fully compensate for the nonfunctional allele. Alternatively, Hsp60 (V72I) chaperonin subunits may perturb allosteric cou-

pling between the subunits in the ring structure and thus exert a genuine dominant negative effect.

Taken together, our studies strongly suggest that the V72I amino acid change that is caused by the 292G→A mutation in *HSP60* is responsible for SPG13. That three (*SPG4, SPG7,* and *SPG13*) of the six genes for HSP that have been identified so far encode proteins with putative chaperone activity supports the hypothesis that a significant fraction of the HSPs are "chaperonopathies." The detection of the first disease-causing mutation in the biochemically well-characterized Hsp60 chaperonin enables the investigation of the mechanisms by which mutations in the various chaperone genes predominantly manifest in the distal regions of the very long axons of the corticospinal tract. A role of chaperones is increasingly recognized in neurodegenerative diseases (Slavotinek and Biesecker 2001), and our findings lend further weight to the notion that chaperones play an important role in neurodegenerative diseases in general.

Table 1

Replacement of the *E. coli* Chromosomally Encoded *groESgroEL* Operon by Plasmids That Express Various *HSP60* Alleles and *HSP10* (*wt*)

Plasmid	Tet^{Ra}	Cam^{Ra}	Linkage (%)
None	105	0	0
pOFX *groES*$^+$*groEL*$^+$	114	59	49
pOFX *HSP60* (*wt*)–*HSP10* (*wt*)	218	100	46
pOFX *HSP60* (*N158S*)–*HSP10* (*wt*)	216	108	50
pOFX *HSP60* (*V72I*)–*HSP10* (*wt*)	206	0	0

NOTE.—A bacteriophage P1 lysate was first prepared on the donor strain AR189, a derivative of OF3465 (a kind gift from Drs. Marie-Pierre Castanié and Olivier Fayet, Centre National de Recherche Scientifiq, Toulouse), in which the *groESgroEL* operon has been deleted and replaced by a Cam^R DNA cassette (Richardson et al. 2001). The *groESgroEL*-deleted strain is maintained alive with plasmid pOFX *groES*$^+$*groEL*$^+$ (Castanie et al. 1997). In addition, AR189 carries a Tn*10* insertion, *zid*1::*Tn*10, that encodes Tet^R and is ~50% cotransferable with the *groESgroEL* operon by bacteriophage P1 transduction (Fayet et al. 1989). The resulting P1 lysate was used to infect the bacterial strain B178 transformed with derivatives of plasmid pOFX-tac1 (Castanie et al. 1997) carrying the cDNA for human *HSP10* and wt or variant *HSP60* (without mitochondrial transit peptide) as an operon under control of the tac promoter. Nontransformed cells or cells that were transformed with a plasmid that carried the *E. coli groESgroEL* operon were used as controls. After removal of unadsorbed P1 bacteriophage by centrifugation, cells were spread on plates that were supplemented with 10^{-3}M isopropyl-β-D-thiogalactopyranoside (IPTG), 12.5 μg/ml of tetracycline and 5×10^{-3} M sodium citrate (to prevent bacteriophage P1 re-adsorption). After a 48-h incubation at 30°C, Tet^R colonies were replica plated onto LB-agar plates supplemented with 10 μg/ml chloramphenicol and IPTG, tetracycline, and sodium citrate, as described above, and were scored after a 48-h incubation at 30°C, for acquisition of the Cam^R marker. Inheritance of the Cam^R phenotype indicates that the endogenous, chromosomally encoded *groESgroEL* operon has been successfully deleted and replaced by the Cam^R-encoding DNA cassette.

[a] The values represent the number of transductants and are the result of a single experiment, for recipient bacteria carrying no plasmid or pOFX *groES*$^+$*groEL*$^+$, and the sum of two independent experiments, for the rest of the constructs.

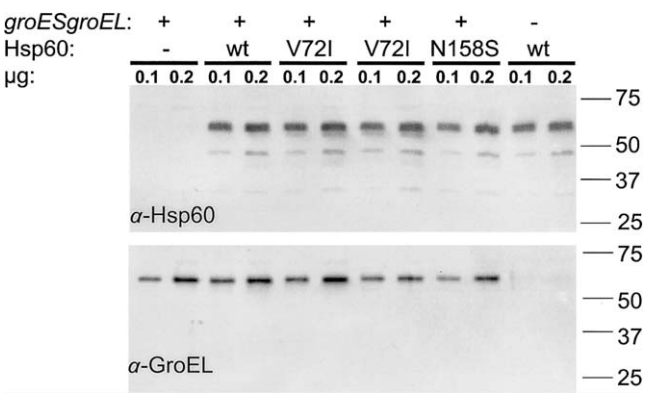

Figure 3 Western blot analysis of Hsp60 and GroEL protein expression. *E. coli* B178 cells transformed with the plasmids carrying the cDNA for human *HSP10* and wt or variant *HSP60* (see table 1) or cells transformed with the vector plasmid were grown in dYT medium to midlog phase. Expression of plasmid-encoded chaperonins was induced for 3 h by addition of 1 mM IPTG. One of the *groESgroEL* deleted colonies that was maintained alive with plasmid pOFX *HSP60* (*wt*)–*HSP10* (*wt*) was grown overnight in dYT medium that was supplemented with 1 mM IPTG. Soluble extracts prepared as described elsewhere (Bross et al. 1995) were subjected to SDS-PAGE and western blot analysis through use of monoclonal antibodies that were directed against Hsp60 (H-3524; Sigma) and GroEL (SPA 870; StressGen), respectively. Blots were developed using ECL$^+$ (Amersham Biosciences) and were scanned using a STORM molecular imager. The strain genotypes with respect to chromosomal *groESgroEL* and plasmid-expressed human Hsp60 variant and the amount of total protein loaded per lane are indicated at the top. The position and molecular mass (in kD) of co-electrophoresed marker proteins are indicated at the right margin.

Acknowledgments

We thank patients and their families, for their kind participation; the Association Strümpell-Lorrain and the physicians, for referring patients to us; and the DNA and cell bank from the Institut Fédératif de Recherche des Neurosciences, for sample preparations. Patients provided informed consent in accordance with the Declaration of Helsinki and European bioethics laws. This work was financially supported by the Karen Elise Jensen Foundation, Association Française contre les Myopathies, and INSERM, the Danish Centre for Human Genome Research, the Danish Research Council, and the Swiss National Foundation.

Electronic-Database Information

Accession numbers and URLs for data in this report are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for human *HSP60* (*HSPD1*) genome structure [accession number AJ250915])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for SPG4 [MIM 182601], SPG7 [MIM *602783], and SPG13 [MIM *605280])

Protein Data Bank (PDB), http://pdb.ccdc.cam.ac.uk/pdb/ (for GroESL structure coordinates [AOL])

References

Bross P, Jespersen C, Jensen TG, Andresen BS, Kristensen MJ, Winter V, Nandy A, Kräutle F, Ghisla S, Bolund L, Kim JJP, Gregersen N (1995) Effects of two mutations detected in medium chain acyl-CoA dehydrogenase (MCAD)-deficient patients on folding, oligomer assembly, and stability of MCAD enzyme. J Biol Chem 270:10284–10290

Casari G, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, De Michele G, Filla A, Cocozza S, Marconi R, Durr A, Fontaine B, Ballabio A (1998) Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. Cell 93:973–983

Casari G, Rugarli E (2001) Molecular basis of inherited spastic paraplegias. Curr Opin Genet Dev 11:336–342

Castanie MP, Berges H, Oreglia J, Prere MF, Fayet O (1997) A set of pBR322-compatible plasmids allowing the testing of chaperone-assisted folding of proteins overexpressed in *Escherichia coli*. Anal Biochem 254:150–152

Fayet O, Ziegelhoffer T, Georgopoulos C (1989) The groES and groEL heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. J Bacteriol 171:1379–1385

Fontaine B, Davoine CS, Durr A, Paternotte C, Feki I, Weissenbach J, Hazan J, Brice A (2000) A new locus for autosomal dominant pure spastic paraplegia, on chromosome 2q24-q34. Am J Hum Genet 66:702–707

Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Cattolico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig B, Weissenbach J (1999) Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nat Genet 23: 296–303

Langer T (2000) AAA proteases: cellular machines for degrading membrane proteins. Trends Biochem Sci 25:247–251

Richardson A, Schwager F, Landry SJ, Georgopoulos C (2001) The importance of a mobile loop in regulating chaperonin/co-chaperonin interaction: humans versus *Escherichia coli*. J Biol Chem 276:4981–4987

Slavotinek AM, Biesecker LG (2001) Unfolding the role of chaperones and chaperonins in human disease. Trends Genet 17:528–535

Tallaksen CM, Durr A, Brice A (2001) Recent advances in hereditary spastic paraplegia. Curr Opin Neurol 14:457–463

Xu ZH, Horwich AL, Sigler PB (1997) The crystal structure of the asymmetric GroEL-GroES-$(ADP)_7$ chaperonin complex. Nature 388:741–750

Zhao X, Alvarado D, Rainier S, Lemons R, Hedera P, Weber CH, Tukel T, Apak M, Heiman-Patterson T, Ming L, Bui M, Fink JK (2001) Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. Nat Genet 29:326–331