SPG20 is mutated in Troyer syndrome, an hereditary spastic paraplegia

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Troyer syndrome (TRS) is an autosomal recessive complicated hereditary spastic paraplegia (HSP) that occurs with high frequency in the Old Order Amish. We report mapping of the TRS locus to chromosome 13q12.3 and identify a frameshift mutation in *SPG20,* encoding spartin. Comparative sequence analysis indicates that spartin shares similarity with molecules involved in endosomal trafficking and with spastin, a molecule implicated in microtubule interaction that is commonly mutated in HSP.

Although a large number of loci have been mapped for HSP, only a few forms of the disease have been attributed to specific genes, and these all have divergent proposed mechanisms of action[1]. Although aberrations in several different cellular processes may underlie HSP and related phenotypes, the identification of additional genes is clearly essential to delineate the mechanisms responsible for more common forms of the disease. TRS, which occurs with high incidence in the Old Order Amish, is characterized by spastic paraparesis complicated by dysarthria, distal amyotrophy, mild developmental delay and short stature[2].

After excluding the known autosomal recessive loci associated with HSP (at 3q27–q28, 8p12–q13, 14q22–q24, 15q13–q15 and 16q24.3) by linkage analysis (data not shown), we used the ABI Linkage Mapping Set Version 2 marker set to pinpoint the gene for TRS by homozygosity mapping. The gene is located in an interval of 731 kb of chromosome 13q12.3 flanked by markers *D13S1840* and *D13S1845*, and all affected individuals that we analyzed were homozygous for markers *D13S1841–D13S1844* (Table 1 and Web Fig. A online). Multipoint linkage analysis using GENEHUNTER v2.1 to conduct an affected-only analysis, assuming autosomal recessive inheritance, 100% penetrance and a trait-allele frequency of 0.039, conclusively confirmed linkage to the interval *D13S1840–D13S1845* and yielded a maximum location score of 19.6 between markers *D13S1841* and *D13S1842* (Web Fig. B online).

Genomic contig construction and DNA sequence analysis in this region showed that this interval encompasses 8 PACs and 16 nonoverlapping transcriptional units (Web Fig. C online). Sequence analysis of all coding exons and adjacent splice signals (primer sequences are available from the authors on request) in the interval identified a single-nucleotide deletion (1110delA) of transcript *bA251J8.2* (Fig. 1*a*). Single-strand conformation polymorphism (SSCP) analysis confirmed complete cosegregation of the 1110delA mutation with the disease phenotype (Web Fig. A online), which was verified subsequently by direct sequence analysis.

To determine whether the mutation was present in the message of individuals with TRS, we extracted total RNA from lymphocytes taken from an affected individual, carrier parents and normal controls. Sequence analysis of the resulting products (Fig. 1*b*) confirmed the homozygous inheritance of the 1110delA mutation in the RNA transcripts in individuals with TRS (data not shown). Although we did not quantify expression of the mutant transcript, its presence indicates that it may not be degraded significantly. To confirm that this mutation does not represent a polymorphism, we screened 760 normal control chromosomes of mixed Caucasian (80%), Asian (10%) and African (10%) descent by SSCP. We also examined 100 chromosomes from individuals that originated from the same and neighboring Amish communities but were not from close relatives of the individuals with TRS. The 1110delA mutation was not detected in any of these chromosomes.

SPG20 comprises nine exons spanning a genomic distance of 43.3 kb, which produces a transcript of 3,402 bp encoding a protein with 666 amino-acid residues (72.7 kDa). The 1110delA frameshift mutation is located in exon 4 and results in a predicted substitution of 29 aa after the mutation and a protein truncated by 268 residues (fs369-398X399). Northern dot-blot analysis showed that *SPG20* is expressed ubiquitously, with highest levels of expression detected in adipose tissue (Fig. 1*c*).

Database searches using spartin detected partial sequence similarity to animal proteins SNX15, VPS4, Skd1 and to the amino-terminal region of spastin. Studies have suggested that SNX15 affects endosome morphology and is involved in protein trafficking[3]. The yeast protein Vps4 is essential for controlling membrane trafficking through endosomes and is required for successful transport of carboxypeptidase Y from the Golgi network to the vacuole[4]. Consistent with this, experimental evidence indicates that the two mammalian orthologs of VPS4 (VPS4A and VPS4B) may be involved in intracellular protein trafficking of late endosomal components[5]. Mouse Skd1 protein, which is closely related to VPS4, also regulates the morphology and membrane trafficking of endosomes[6].

Several studies have shown that one mechanism for endosomal transportation involves microtubules[7,8]. Spastin, which is commonly mutated in autosomal dominant HSP, associates with the microtubule cytoskeleton[9]. Cells overexpressing spastin mutants show constitutive binding of abnormal spastin to microtubules, which leads to redistribution of the microtubule array[9]. Studies using artificial spastin mutants have indicated that the microtubule-binding capability of this

Table 1 • Haplotypes derived from the analysis of 21 affected individuals

Marker	Derived haplotypes					
	A	B	C	D	E	F
D13S127	34	34	33	23	33	22
CAGR1	26	26	22	29	22	22
AFMb316wb5	12	12	11	12	11	11
D13S1840	22	22	22	12	22	22
D13S1841	22	22	22	22	22	22
D13S1842	11	11	11	11	11	11
D13S1851	44	44	44	44	44	44
D13S1843	11	11	11	11	11	11
D13S305	33	33	33	33	33	33
D13S219	33	33	33	33	33	33
D13S1844	22	22	22	22	22	22
D13S1845	55	55	55	55	15	55
D13S1846	11	11	11	11	12	11
D13S1847	22	22	22	22	26	22
D13S1848	44	46	44	44	14	44
D13S1849	33	34	33	33	23	33
D13S1850	22	22	22	22	22	22
D13S218	33	23	33	33	33	33

The putative ancestral haplotype for markers *D13S1841–D13S1844* is 2-1-4-1-3-3-2 (in blue). Six distinct recombinant haplotypes (in red; nonrecombinants are in black) were identified across the wider interval encompassed by markers *D13S127–D13S218* in the individuals affected with TRS. A, patients 15, 17, 18, 21, 22 and 23; B, patient 19; C, patients 24, 26, 27, 28, 30, 35, 36, 39, 40 and 41; D, patient 29; E, patient 34; and F, patients 45 and 46.

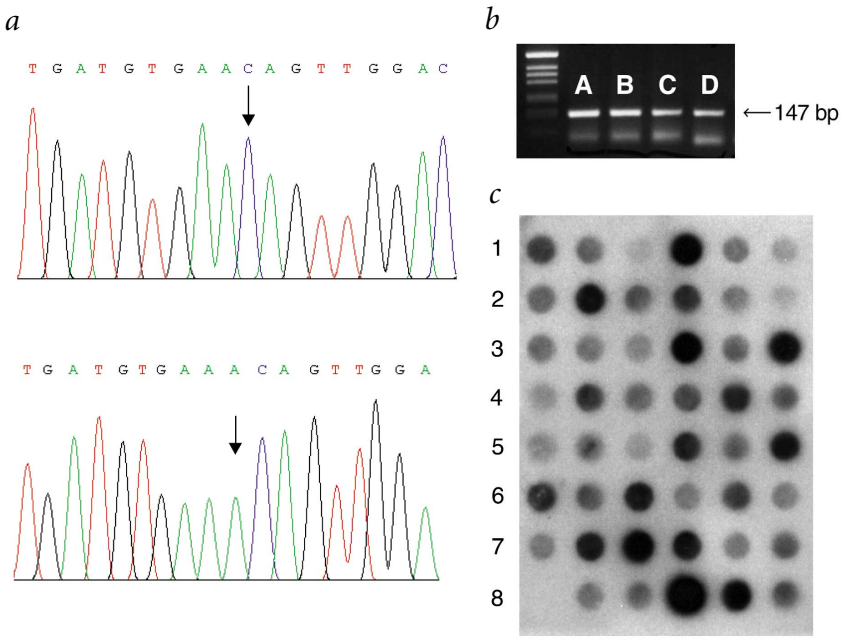

Fig. 1 Sequence of the 1110delA mutation and northern-blot analysis. ***a***, Sequence of *SPG20* exon 4 PCR products amplified from genomic DNA from an affected individual (top) and an unaffected sibling (bottom). ***b***, RT–PCR of *SPG20* exon 4 message (yielding a cDNA of 147 bp), using 0.55 μg RNA and F1/R1 primers, in a father (A), a mother (B), an affected (C) and a normal control (D). ***c***, Northern-blot analysis of *SPG20* mRNA. [^{32}P]dCTP-labeled exonic PCR products were hybridized to a multiple-tissue blot containing 5 μg of total RNA extracted from the indicated tissues, 50 μg human genomic DNA and 250 pg of pBg18 plasmid DNA. The same pattern and levels of expression were obtained when exon 4 alone was used to probe the blot (data not shown). Left to right: row 1, left atrium, frontal lobe, parotid, throat, kidney, thyroid; row 2, right atrium, temporal lobe, esophagus, broncheal trachea, bladder, pancreas; row 3, left ventricle, occipital lobe, stomach, left lung, prostate, adrenal gland; row 4, right ventricle, parietal lobe, small intestine, right lung, testis, tonsil; row 5, interventricle septum, thalamus, colon, diaphragm, uterus, thymus; row 6, pericardium, pons, rectum, skeletal muscle, breast, spleen; row 7, human DNA, cerebellum, liver, tongue, ovary, lymph node; row 8, plasmid DNA, spinal cord, gallbladder, adipose tissue, placenta, appendix. This work was approved by the St. George's Hospital Medical School ethics committee, and informed consent was obtained from the study participants.

molecule is located in its N-terminal region[9], which shares similarity with spartin. Thus, although the functions of spastin and spartin remain to be confirmed experimentally, the available data implicate spartin in endosomal trafficking, microtubule dynamics, or both, and suggest that there is a functional link between two genes responsible for HSP.

The large size, extreme polarity and diversified functions and shapes of neuronal cells place tremendous demands on intracellular transport systems. The microtubules provide the framework for the transportation of a large proportion of neuronal cargoes[10,11]. Evidence suggests that cellular transportation systems may constitute 'weak links' that may be susceptible to various insults, resulting in other neurodegenerative diseases. Molecules mutated in chorea-acanthocytosis (CHAC)[12], amyotrophic lateral sclerosis 2 (alsin)[13] and Niemann–Pick type C1 (NPC1)[14] are all implicated in trafficking dynamics. When considered with these studies, our findings highlight trafficking dynamics as a cellular mechanism worthy of further investigation in the pathogenesis of HSP and suggest that other molecules involved in this process may be candidates for this group of diseases.

GenBank accession numbers. AY123329, *SPG20* mRNA; AY123330–AY123337, *SPG20* exon organization.

Note: Supplementary information is available on the Nature Genetics website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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