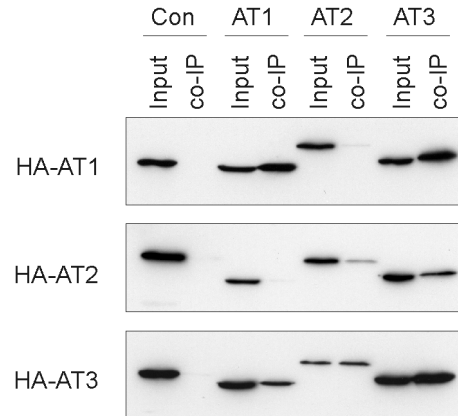


AT1	---MAKN-----RRDRNS-----WGGFSEKT-----YEWS--SEEEEPVKK	31
AT2	---MAEGDEAARGQQPHQGLWRRRRTSDPSAAVNHVSSSTSLGENYEDDDLVSDEVMKK	57
AT3	-mrtagrdrpvsagllqfp----kdgstqrpanrarfvpartprpsplhpccfcfegggsMLS	3
AT1	AGFVQVLIV-KDDHSFELDETALNRILLSEAVRDKEVAVSVAGAFRKGKSF [◆] FLMDFMLRY	90
AT2	PCPVQIVLAHEDDHNFELEEALEQILLQEHIRDLNIVVSVAGAFRKGKSFLLDFMLRY	117
AT3	PQRVAAAAS-----RGADDAMESSILLQDHIRDLVIVVSVAGAFRKGKSFILDFMLRY	57
AT1	MYNQESV---DWVGDYNEPLTGF [*] SWRGGSERETTGIQI [◆] WSEIFLINKPDGKKVAVLLMDT	147
AT2	MYNKDSQ---SWIGGNNEPLTGF [*] TWRGGCERETTGIQVWNEVFVIDRPNGTKVAVLLMDT	174
AT3	LYSQKESGHSNWLGDPEEPLTGF [*] SWRGGSDPETTGIQI [◆] WSEVFTVEKPGGKKVAVLLMDT	117
AT1	QGT [◆] FDSQSTLRDSATVFALSTMISSIQVYNLSQNVQEDDLQHLQLFTEYGR [◆] LAMEETFLK	207
AT2	QGAFDSQSTIKDCATVFALSTMTSSVQVYNLSQNIQEDDLQHLQLFTEYGR [◆] LAMEEITYQK	234
AT3	QGAFDSQSTVKDCATIFALSTMTSSVQIYLSQNIQEDDLQQLFTEYGR [◆] LAMDEIFQK	177
AT1	PFQSLIFLVRD [*] WSFPYEFSYGADGGAKFLEKRLK [◆] VSGNQHEELQNV [◆] RKHIHSCFTNISC [◆] F	267
AT2	PFQTLMFLIRD [*] WSYPYEHSYGLEGGKQFLEKRLQV [◆] KQ [◆] NQHEELQNV [◆] RKHIHNCFSNLGCF	294
AT3	PFQTLMFLVRD [*] WSFPYEYSYGLQGGMAFLDKRLQV [◆] KEHQHEEI [◆] QNV [◆] RNHIHSCFSDVTCF	237
AT1	LLPHPGLKVATNP [◆] NFDGKLKEIDDEFIKNLKILIPWLLSPESLDIKEINGNKITCRGLVE	327
AT2	LLPHPGLKVATNP [◆] SFDGRLKIDIDEDFKRELRLNLPVLLLAPENLVEKEISGSKVTCRDLVE	354
AT3	LLPHPGIQVATSPDFDGLKLDIAGEFKEQLQALIPYVLNPSKLMEKEINGSKVTCRGLLE	297
AT1	YFKAYIKIYQGEELPHPKSMLQATAEANNLAAVATAKDTYNKKMEEICGGDKPFLAPNDL	387
AT2	YFKAYIKIYQGEELPHPKSMLQATAEANNLAAVAGARTYCKSMEQVCGGDKPYIAPSDL	414
AT3	YFKAYIKIYQGEDLPHPKSMLQATAEANNLAAAASAKDIYNNMEEVCGGKEKPYLSPDILL	357
AT1	QTKHLQLKEESVKLFRGVKKMGGE [◆] EF [◆] SRRYLQQL [◆] ESEIDELYIQYIKH [◆] NSKNI [◆] FHAART	447
AT2	ERKHLDLKEVAIKQFRSVKKMGGE [◆] DFCRRYQDQLEAEIEETYANFIKH [◆] NDGKNI [◆] FYAART	474
AT3	EEKHCEFKQALALDHF [◆] KTKKMGKDF [◆] SFRYQQL [◆] E [◆] EEIKELYEN [◆] FCKHNGSKN [◆] VESTFRT	417
AT1	PATLFVVI [◆] FITYVIAGVTGFIGLDIIASLCNMIMGLT [◆] LI [◆] TLCTWAYIRYSGEYRELGA [◆] VI	507
AT2	PATLFAVMFAMYIISGLTGFIGNLSIAVLCNLVME [◆] LALI [◆] FLCTWAYVKYSGEFREIGT [◆] VI	534
AT3	PAVLE [◆] TGIVALYIASGLTGFIGNLE [◆] VVAQL [◆] FNCMVGLLLIALLTWGYIRYSGQYRELGA [◆] VI	477
AT1	DQVAAALWDQGSTNEALYKLYSAAATHRHLYHQAFPTPKSESTEQSEK [◆] KKM	558
AT2a	DQIAETLWEQVFS-----KLFEVTRRRMVHRALSSAQRQRLSSNNNKK [◆] KN	579
AT2b	DQIAETLWEQVLKPLGD---NLMEENIRQSVTNSIKAGLTDQVSHHARL [◆] KTD	583
AT3	DFGAAVLEQASSHIG---NSTQATVRDAVGRPSMDKKAQ	515

Supplementary Material, Figure S1. Atlastin family of proteins in humans. Residues identical in all three atlastins are highlighted in orange, while those residues identical in at least two of the atlastins (exclusive of different splice variants of the same atlastin) are shaded green. Bold lines are above predicted transmembrane domains. A variant of atlastin-3 with a 26-residue insertion following Ser²² (GenBank accession number CAB56010) and a variant of atlastin-1 lacking exon 13 (b isoform; coding for residues 518-522), both generated by alternative splicing, are also known. Numbering for the atlastin-3 start site is based on results shown in Fig. 1C (Trans-S), but additional residues at the N-terminus of atlastin-3 predicted if an alternate start site (Trans-L) were utilized are shown in lower case. GTP-binding motifs are boxed. A filled diamond (◆) denotes the site of the dominant-negative, GTP-binding deficient mutation and an asterisk (*) identifies the site of a *SPG3A* mutation investigated in this study. Potential C-terminal ER retrieval motifs are shaded in blue.

A

pGAD \ pBHA		pBHA		
		AT 1	AT 2	AT 3
AT 1	+++	++	+++	
AT 2	++	++	+++	
AT 3	+++	++	+++	

B

Supplementary Material, Figure S2. Hetero-oligomerization of atlastins upon overexpression. (A) Matrix of yeast two-hybrid tests showing interactions of atlastin-1, -2, and -3 with one another. Strength of interaction was assayed by *HIS3* and β -galactosidase induction and semi-quantitated as described previously (35). In all cases, *HIS3* and β -galactosidase induction correlated. (B) Co-immunoprecipitation (co-IP) of overexpressed atlastin proteins with one another. HeLa cells were co-transfected with Myc-tagged atlastins (across top) and HA-tagged atlastins (left) in all nine possible combinations and immunoprecipitated with anti-Myc antibodies. Immune complexes were resolved by SDS-PAGE and immunoblotted with anti-HA antibodies. In control experiments (Con), immunoprecipitations were performed on extracts from cells expressing only the HA-tagged constructs. On longer exposures, co-precipitations of HA-AT1 and Myc-AT2 as well as HA-AT1 and Myc-AT2 are apparent.