Effect of spastic paraplegia mutations in KIF5A kinesin on transport activity

Bettina Ebbing[1,3], Klaudiusz Mann[1], Agata Starosta[1], Johann Jaud[3], Ludger Schöls[2], Rebecca Schüle[2], Günther Woehlke[3]

[1]Institute for Cell Biology, University of Munich, Schillerstr. 42, D-80336 Munich, Germany
[2] Hertie-Institute for Clinical Brain Research, Otfried-Müller-Str. 3, 72076 Tübingen, Germany
[3] Physics Department E22, Technical University Munich, James-Franck-Str., 85747 Garching, Germany

Hereditary spastic paraplegia (HSP) is a neurodegenerative disease caused by motoneuron degeneration. It is linked to at least 30 loci, among them SPG10, which causes dominant forms and originates in point mutations in the neuronal Kinesin-1 gene (KIF5A). Here, we investigate the motility of KIF5A and four HSP mutants. All mutations are single amino-acid exchanges and located in kinesin's motor or neck domain. The mutation in the neck (A361V) did not change the gliding properties *in vitro*, the others either reduced microtubule affinity, or gliding velocity, or both. In laser-trapping assays, none of the mutants moved more than a few steps along microtubules. Motility assays with mixtures of homodimeric wildtype, homodimeric mutant, and heterodimeric wildtype/mutant motors revealed that only one mutant (N256S) reduces the gliding velocity at ratios present in heterozygous patients, whereas the others (K253N, R280C) do not. Attached to quantum dots as artificial cargo, mixtures involving N256S mutants produced slower cargo populations lagging behind in transport, whereas mixtures with the other mutants led to populations of quantum dots that rarely bound to microtubules. These differences indicate that the dominant inheritance of SPG10 is caused by two different mechanisms that both reduce the gross cargo flux, leading to deficient supply of the synapse.