Proteolytic processing of cell adhesion molecule L1 (L1CAM) in nervous system development and regeneration

David Lutz
Department of Neurobiology and Molecular Brain Research, Ruhr University Bochum, Germany
Universitätsstr. 150, 44801 Bochum, tel.: +49 (0) 23432 24450, mailto: david.lutz@rub.de

Mutations in the L1CAM gene entail severe congenital neurodevelopmental disorders, collectively termed as the „L1-syndrome“. This syndrome is characterized by hydrocephalus, mental retardation, corpus callosum agenesis, and fiber tract malformations. No therapy is available to treat the L1-syndrome, as its etiology and pathomechanism have remained poorly understood. Mice engineered to carry a mutation in the murine L1CAM-gene, which prevents L1CAM cleavage by several extracellular matrix proteases, develop severe congenital ventriculomegaly with improperly anchored ependymal cell cilia and deficits in fiber tracts of the central nervous system, reminiscent of the abnormalities displayed by L1-syndrome patients. The clinical phenotype of these mutants expressing non-cleavable L1CAM suggests an essential function of L1CAM-proteolysis in proper development of the white matter as well as in the etiology of neurodegenerative diseases. Together with own preliminary findings on this novel L1-syndrome model mouse line, this study aims at treatment of the L1-syndrome in mutant mice with state-of-the-art techniques for *in utero* rescue of the aberrant L1CAM-proteolysis at critical neurodevelopmental stages.

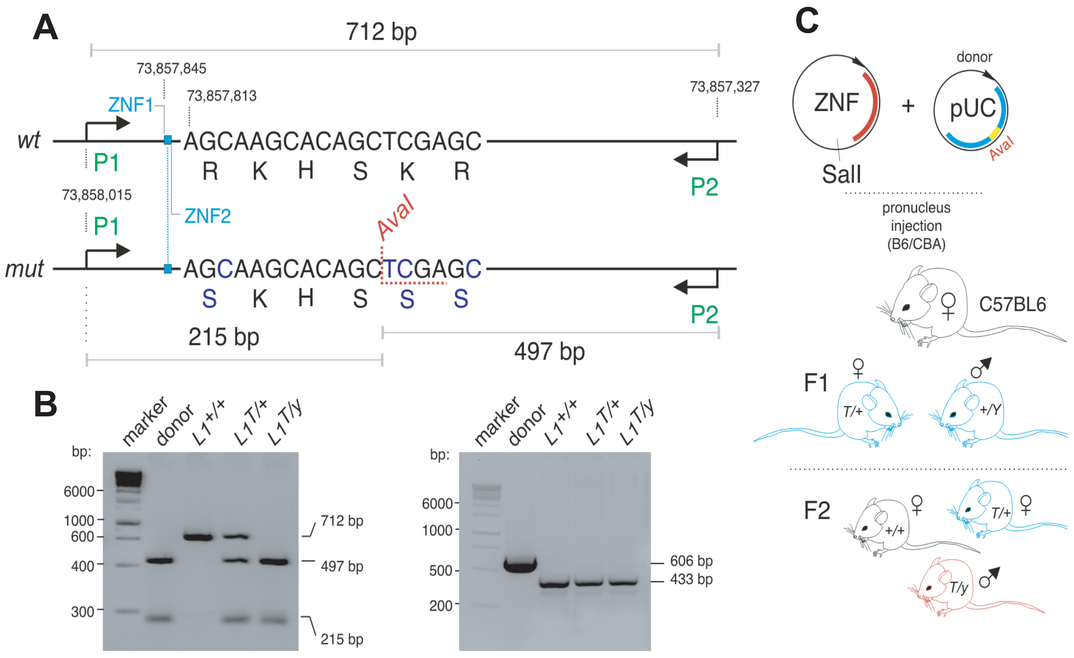


Fig. 1 Generation of mutant mice expressing the 840SKHSSS845 mutation in L1. (**A**) Schematic representation of the targeted mutation and genotyping strategy. Wild-type L1CAM sequence (wt) on the X-chromosome was used as a landmark for the design and screening of suitable zinc finger nucleases 1 and 2 (ZNF1 and ZNF2), which were found to bind close to the codon of interest (AGCAAGCACAGCTCGAGC). Genomic positions are indicated. In the mutated sequence (mut), this motif is replaced by the codons for SKHSSS (AGCAAGCACAGCTCGAGC), thus generating a restriction site for AvaI. Primers P1 and P2 were used for amplification of a 712 bp region. (**B**) After digestion with AvaI, the 712 bp amplicon was cleaved into 215 bp and 497 bp products. The cDNA expression for the mutated L1 was confirmed by reverse PCR and electrophoresis. (**C**) Zinc finger nuclease vector map and pUC donor with injection and breeding scheme; restriction, mutation sites and genotypes of mice are indicated.

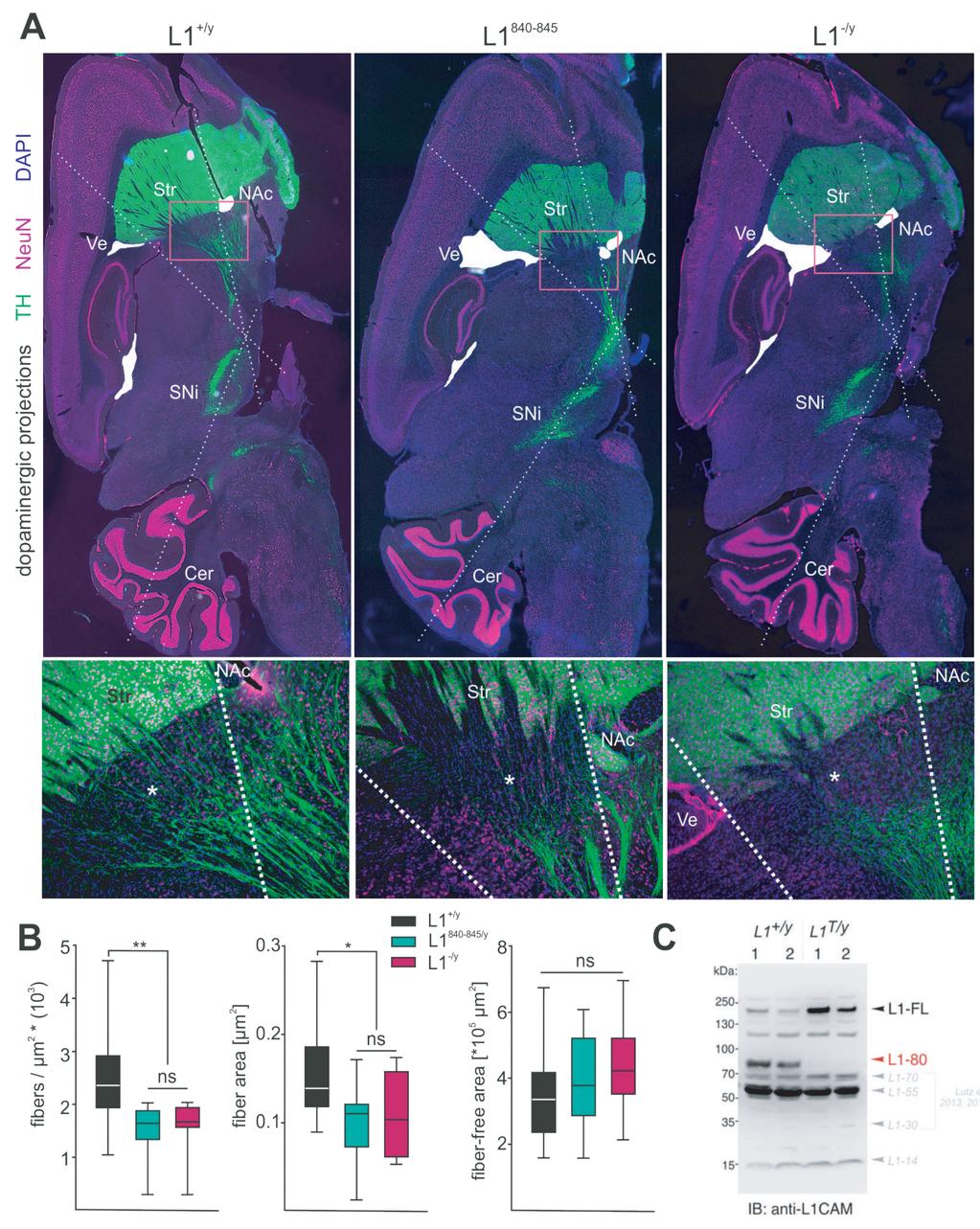
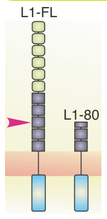


Fig. 3 Mutant mice expressing the 840SKHSSS845 mutation in L1 display fiber tract projection deficits. (**A,B**) Tyrosine hydroxylase immunostaining (green) reveals reduced fiber density and fiber area along the nigro-striatal axis which enters the striato-accumbenal gate (*) in L1 mutant ($L1^{840-845}$) and L1-deficient ($L1^{-/y}$) mice; Str – striatum; NAc – nucleus accumbens; Ve – ventricles (in white); SNi – substantia nigra; Cer – cerebellum; neurons are immunostained for NeuN (violet); nuclei are stained in blue (DAPI); *p<0.05, **p<0.005, ns – not significant (one-way ANOVA with Multiple Comparison Test). (**C**) Immunoblot analysis for L1CAM confirms abolished cleavage of mutated L1 due to lack of the L1-80 generated by proteases that target the 840RKHSKR845 motif.



Stimulation of proteolysis of L1 via injection of L1-fragments or proteases active on L1 or L1 mimetics is beneficial for development and regeneration of the diseased nervous system.

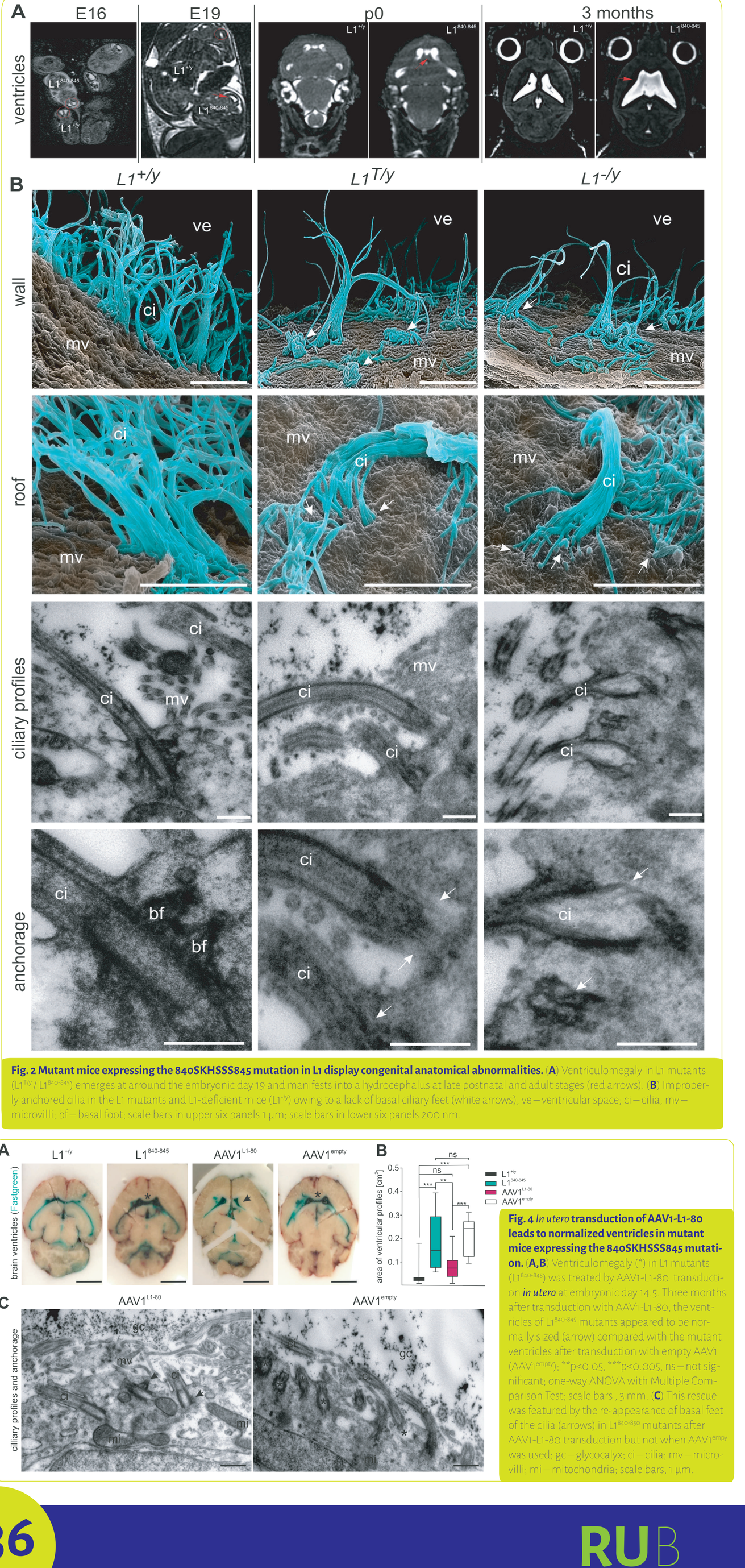


Fig. 2 Mutant mice expressing the 840SKHSSS845 mutation in L1 display congenital anatomical abnormalities. (**A**) Ventriculomegaly in L1 mutants ($L1^{T/y}$ / $L1^{840-845}$) emerges at arround the embryonic day 19 and manifests into a hydrocephalus at late postnatal and adult stages (red arrows). (**B**) Improperly anchored cilia in the L1 mutants and L1-deficient mice ($L1^{-/y}$) owing to a lack of basal ciliary feet (white arrows); ve – ventricular space; ci – cilia; mv – microvilli; bf – basal foot; scale bars in upper six panels 1 µm; scale bars in lower six panels 200 nm.

Fig. 4 *In utero* transduction of AAV1-L1-80 leads to normalized ventricles in mutant mice expressing the 840SKHSSS845 mutation. (**A,B**) Ventriculomegaly (*) in L1 mutants ($L1^{840-845}$) was treated by AAV1-L1-80 transduction *in utero* at embryonic day 14.5. Three months after transduction with AAV1-L1-80, the ventricles of $L1^{840-845}$ mutants appeared to be normally sized (arrow) compared with the mutant ventricles after transduction with empty AAV1 ($AAV1^{empty}$). **p<0.05, ***p<0.005, ns – not significant; one-way ANOVA with Multiple Comparison Test; scale bars , 3 mm. (**C**) This rescue was featured by the re-appearance of basal feet of the cilia (arrows) in $L1^{840-850}$ mutants after AAV1-L1-80 transduction but not when $AAV1^{empty}$ was used; gc – glycocalyx; ci – cilia; mv – microvilli; mi – mitochondria; scale bars, 1 µm.