

Table of Contents

1	Introduction	1
1.1	The Vertebrate Peripheral Nervous System and its Myelin	1
1.2	The Origin and Development of Schwann Cells	2
1.3	The Process of De- and Remyelination in the PNS	3
1.3.1	Wallerian Degeneration	6
1.3.2	Charcot-Marie-Tooth Neuropathy.....	7
1.3.3	Inflammatory Neuropathies	9
1.4	The Dynamin Superfamily	10
1.5	Dynamin 2.....	11
1.6	Cellular Functions of Dynamin 2.....	12
1.7	Dynamin 2 in Disease	13
1.8	The Heterotetrameric Adaptor Complex Family	14
1.9	The Adaptor Protein Complex 2	15
1.10	Clathrin-Mediated Endocytosis	16
1.11	Iron	17
1.12	Objective of the Study	19
2	Results	21
2.1	SC-Specific Ablation of Dynamin 2 in Adult Mice Results in a Remitting Neuropathy.....	21
2.1.1	Acute De- and Remyelination upon Dynamin 2 Ablation.....	22
2.1.2	Ablation of Dynamin 2 Causes Schwann Cell Dedifferentiation.....	24
2.1.3	Schwann Cells Coordinate an Acute Inflammation upon Dynamin 2 Ablation	26
2.1.4	Dynamin 2-Depleted Cells are Replaced by Non-Recombined Schwann Cells	29
2.2	SC-Specific Ablation of AP2 μ 2 in Adult Mice Results in a Late Onset Neuropathy.....	31
2.2.1	Aged AP2 ^{iko} Mice Develop Myelin Aberrations	33
2.2.2	Ablation of AP2 μ 2 Leads to the Recruitment of Macrophages.....	36
2.2.3	The Number of Schwann Cells Increases in Aged AP2 ^{iko} mice.....	37
2.3	The Surfaceome of CME-Impaired Primary Rat Schwann Cells.....	38
2.4	Schwann Cells and Iron Deficiency	40
2.5	Iron Deficiency in Different Knockout Mice	41
2.6	The Contribution of Iron Deficiency to the Observed Phenotype in Dnm2 ^{iko} Mice	43
2.7	Transcriptome Analysis after Dynamin 2 or AP2 μ 2 Ablation	46
3	Discussion	49
3.1	SC-Specific Ablation of Dynamin 2 in Adult Mice Results in a Remitting Neuropathy.....	50
3.2	SC-Specific Ablation of AP2 μ 2 in Adult Mice Results in a Late Onset Neuropathy.....	54
3.3	Iron Deficiency is a Potential Contributor to the Observed Phenotypes	57
4	Material and Methods	63
4.1	Solutions and Buffers.....	63
4.2	Schwann Cell-Specific Dynamin 2 Ablation in Adult Mice	64
4.3	Schwann Cell-Specific AP2 μ 2 Ablation in Adult Mice.....	64
4.4	Genotyping	65
4.5	Gait Analysis	66

4.6	Electron Microscopy.....	66
4.6.1	Nerve Preparation for Electron Microscopy.....	66
4.6.2	Sample Preparation and Electron Microscopy.....	67
4.7	Immunological Methods	67
4.7.1	Nerve Preparation for Immunoblotting	67
4.7.2	Protein Gel Electrophoresis.....	68
4.7.3	Protein Transfer to PVDF Membrane.....	68
4.7.4	Immunoblotting.....	68
4.7.5	ELISA	69
4.8	Histological Methods.....	70
4.8.1	Nerve Preparation and Cryosectioning	70
4.8.2	Immunohistochemistry	70
4.8.3	Proliferation Assay.....	71
4.8.4	Evens Blue Injections.....	71
4.8.5	Iron Staining	71
4.8.6	Fluorescence In Situ Hybridization	72
4.8.7	Muscle Staining	72
4.9	Quantitative Real Time – Polymerase Chain Reaction (qRT-PCR).....	72
4.9.1	RNA Extraction.....	72
4.9.2	Reverse Transcription.....	73
4.9.3	qRT-PCR	73
4.10	Cell Culture	74
4.10.1	Commonly used Media and Solutions.....	74
4.10.2	Isolation of Primary Rat Schwann Cells	75
4.10.3	Culture of Primary Rat Schwann Cells	75
4.10.4	Deferoxamine Treatment of Cells	75
4.10.5	Dorsal Root Ganglia Explant Cultures.....	76
4.10.6	Production of Low-Titer Lentivirus and Infection of Rat Schwann Cells	76
4.10.7	Transferrin Uptake Assay	77
4.10.8	Production of Inducible shRNAs.....	77
4.10.9	Cell Surface Capturing	78
4.11	Quantification and Statistics	78
4.11.1	Morphometric Analysis and Quantification of EM Images	78
4.11.2	Morphometric Analysis and Quantification of Immunological Methods.....	78
4.11.3	Quantification of qRT-PCR.....	79
4.11.4	Gait Analysis Statistics	79
4.11.5	Statistical Analysis	79
5	Appendix	81
6	References.....	95
7	Curriculum Vitae	107
8	Acknowledgement	109