Late Onset Neuropathy with Spontaneous Clinical Remission in Mice Lacking the POZ Domain of the Transcription Factor Myc-interacting Zinc Finger Protein 1 (Miz1) in Schwann Cells*  

Adrián Sanz-Moreno†, David Fuhrmann†, Armin Zankel§, Herbert Reingruber‡, Lara Kern‡, Dies Meijer§, Axel Niemann∥, and Hans-Peter Elsaßser∥  

From the †Department of Cytobiology and Cytopathobiology, Philipps University of Marburg, Robert-Koch-Strasse 6, 35033 Marburg, Germany, §Graz University of Technology, 8010 Graz, Austria, ¶Erasmus Medical Center, 3015GE Rotterdam, Netherlands, and ∥ETH Zurich, CH-8093 Zurich, Switzerland

Background: The Myc-binding transcription factor Miz1 has multiple functions in different cells and tissues.  

Results: Ablation of the Miz1 POZ domain in Schwann cells causes a late onset peripheral neuropathy with spontaneous remission.  

Conclusion: Miz1 plays an essential role in myelin homeostasis of peripheral nerves.  

Significance: Ablation of Miz1 function in Schwann cells leads to a new model for remitting peripheral neuropathies.

The transcription factor Miz1 (Myc-interacting zinc finger 1) is a known regulator of the cell cycle but also has cell cycle-independent functions. Here we analyzed the role of Miz1 in the peripheral nervous system, using an early embryonic conditional knock-out model in which the Miz1 POZ domain is ablated in Schwann cells. Although the development of myelinated nerve fibers was not impaired, Miz1ΔPOZ mice acquired behavioral signs of a peripheral neuropathy at the age of 3 months. At this time, ultrastructural analysis of the sciatic nerve showed de- and dysmyelination of fibers, with massive outfoldings and a focal infiltration of macrophages. Although the expression of genes encoding structural myelin proteins, such as periaxin, myelin basic protein, and myelin protein zero, was decreased, genes associated with a negative regulation of myelination, including c-Jun, Sox2, and Id2, were up-regulated in Miz1ΔPOZ mice compared with controls. In animals older than 4 months, the motor disabilities vanished, and the ultrastructure of the sciatic nerve exhibited numerous tomacula and remyelinated fibers, as indicated by thinner myelin. No second acute attack was observed up to the age of 1 year. Thus, the deletion of the Miz1 POZ domain in Schwann cells induces an acute neuropathy with a subsequent regeneration in which there is ongoing balancing between de- and remyelination. Miz1ΔPOZ mice are impaired in the maintenance of myelinated fibers and are a promising model for studying remyelination in adult peripheral nerves.

The transcription factor Miz1 (Myc-interacting zinc finger protein 1; Zbtb17) contains 13 zinc finger motifs in the middle and C-terminal parts of the protein and a BTB/POZ domain at the N terminus (1). DNA binding of a Miz1 tetramer (2, 3) occurs at the initiation region of target genes, and tetramerization depends on the POZ domain (4, 5). Miz1 can either activate or repress transcription, depending on its binding partners. Coactivators include nucleophosmin and p300, and known corepressors are Myc, Bcl-6, Gfi1, and Zbtb4 (6). Miz1 has a broad expression profile and has also been detected in neural precursor cells (7, 8) and in different parts of the adult brain (9, 10). The constitutive knockout of Miz1 is lethal on embryonic day 7.5 (7). Miz1 function in skin, the hematopoietic system, mammary gland, and neurons of the central nervous system has been investigated in a conditional knock-out mouse model by deletion of the POZ domain encoded by exons 3 and 4 of the Miz1 gene (also known as Zbtb17) (2, 3, 10–13).

In the peripheral nervous system, Schwann cells are the myelin-forming cells and remain tightly connected to the axon (14–16). This connection is lost following a nerve injury because the axon degenerates distally from the lesion. Schwann cells engaging this degenerating segment dedifferentiate and finally remyelinate the regrowing fiber. Molecular pathways driving these de- and redifferentiation processes have been elucidated (17, 18). Thereby, p21cip1, encoded by the Miz1 target gene Cdkn1a, is central in the regulation of Schwann cell proliferation following a nerve injury as well as in development (19). An important signaling pathway driving demyelination and dedifferentiation of Schwann cells is the Ras/Raf/MEK/ERK pathway (20), which is accompanied by an inflammatory response to support the removal of tissue debris (21). Interestingly, activated Ras can also cause oncogene-induced senescence (22) by activating p53/p21cip1 and p16INK4a (23).

Here we ask whether a Schwann cell-specific POZ domain deletion of Miz1 has an impact on peripheral nerve myelination. We show that Miz1ΔPOZ mice develop a late onset peripheral neuropathy characterized by de- and dysmyelination, increased levels of p21cip1, and elevated senescence mark-
Miz1 POZ Domain Deletion in Schwann Cells

ers. Finally, the neuropathy progresses to a spontaneous clinical remission.

EXPERIMENTAL PROCEDURES

Mice—Miz1^flox/lox^ mice (2, 11) were crossed with the desert hedgehog (Dhh)-Cre driver line (C57Bl6-Tg(Dhh-cre)1Mejr) (24) to achieve conditional ablation in Schwann cells of Miz1 exons 3 and 4, which encode the POZ domain. Mice had a mixed C57Bl6 and 129S2/SvHsd genetic background. Here, animals that were Dhh-Cre^+/Miz1^flox/lox^ are designated Miz1^ΔPOZ^, whereas Dhh-Cre^-/Miz1^flox/lox^ mice were used as control animals and named Ctr. Genotyping was performed by standard PCR, using the REDEXtract-N-Amp tissue PCR kit (XNATR; Sigma). Primers used for genotyping are listed in Table 1.

Research with mice was conducted according to the German Animal Protection Law (Tierschutzgesetz). The application for the experiments was reviewed and approved by the responsible local authorities (Regierungspräsidium Giessen, reference numbers V54-19c20 15-MR20/10 Nr. A18/2010 and V54-19c20 15h01-MR20/10 Nr. 14/2014).

Clinical Assessment—To assess the degree of motility impairment, the score devised by King et al. (25) was used. Mice were judged in five categories: leg cross, grid walk, hind and front leg grasp, and tail bending. Scores from 0 = normal to 2 = abnormal were given in each category, resulting in overall scores between 0 and 10 (normal to highly impaired). Scoring was performed independently by two observers blinded to the animals’ age and genotype. Walking pattern (26) was documented for control and Miz1^ΔPOZ^ mice by dipping foot pads of the hind paws into India ink and allowing the animal to walk on 50 × 10-cm paper strips placed in a dark runway of 10-cm height. Mice were habituated to the runway for 5 min, and then four runs were performed. To measure the grip strength of the hind paws into India ink and allowing the animal to walk on succinate dehydrogenase staining—Calf muscles were prepared, covered with Tissue-Tek O.C.T. Compound (Sakura, Netherlands) and quick frozen in isopentane cooled by liquid nitrogen. Cryostat sections were air-dried and subsequently incubated in a 37 °C prewarmed solution containing 0.1 M phosphate buffer, pH 7.6, 0.1 M sodium succinate, and 0.1% nitro blue tetrazolium chloride (AppliChem, Darmstadt, Germany) for 1 h. Slides were rinsed in water, dehydrated with ethanol, incubated in xylene, and embedded in Entelan (Merck, Darmstadt, Germany). Sections were stained with X-Gal (Roth) at 37 °C overnight.

Succinate Dehydrogenase Staining—Calf muscles were prepared, covered with Tissue-Tek O.C.T. Compound (Sakura, Netherlands) and quick frozen in isopentane cooled by liquid nitrogen. Cryostat sections were air-dried and subsequently incubated in a 37 °C prewarmed solution containing 0.1 M phosphate buffer, pH 7.6, 0.1 M sodium succinate, and 0.1% nitro blue tetrazolium chloride (AppliChem, Darmstadt, Germany) for 1 h. Slides were rinsed in water, dehydrated with ethanol, incubated in xylene, and embedded in Entelan (Merck Millipore).

Ultrastructural Analysis—Sciatic nerve fragments were fixed in a mixture of 2.5% glutaraldehyde, 2.5% paraformaldehyde, and 0.05% picric acid in 67 mM cacodylate buffer (pH 7.4) according to Itó and Karnovsky (29). Postfixation was performed in 1% osmium tetroxide followed by an overnight incubation with 0.3% uranyl acetate dissolved in 50 mM sodium cacodylate, and 150 mM NaCl at room temperature. Then tissue was incubated in LacZ rinse buffer containing 5 mM potassium hexacyanoferrate (III), 5 mM potassium hexacyanoferrate (II), and 1 mg/ml X-Gal (Roth) at 37 °C overnight.

Three-dimensional Reconstructions—For the generation of three-dimensional reconstructions, serial block-faced scanning electron microscopy (30) was performed using a prototype in situ ultramicrotome from Gatan (Pleasanton, CA) and an environmental scanning electron microscope (FEI ESEM Quanta 600 FEG, FEI, Eindhoven, Netherlands). For imaging, backscattered electrons were used, resulting from primary electrons with an energy of 4 keV. Serial sectioning and imaging was done in the low vacuum mode of the environmental scanning electron microscope with water vapor as imaging gas at a pressure of about 107 pascals. The chemically pretreated sample blocks were trimmed to small cuboids of about 300 × 400 × 500 μm and using a Diatome® diamond knife “Trim 90” and then glued on

---

The abbreviations used are: H3K9me3, histone 3 trimethylated on lysine 9; SA, senescence-associated; Pn, postnatal day n; P0, myelin protein zero.
### QUANTITATIVE PCR PRIMERS

| Primers | fw | reverse | Primers | fw | reverse |
|---------|-----|---------|---------|-----|---------|
| Ambra1  | GAGCACCCCAATTTACCCAGA | GATATGTCCTGGGGAATTAGTA | Mtmr2 | GGAAACTGTATGACCCCTTC | CACAGAGTTACATGCTGTTATATC |
| Ccl2    | CATCCACGTGTTGGGCTCA | GATCCTCCTGGTGAATGAGT | Mtmr6 | GCCAAATACGCCTTCTTCAA | AATATGCTGACGACAGCTCC |
| Ccl3    | TGCCCTTGTGCTTCTCTCT | GGAATTCGGCATGCTGTTACG | Mtmr7 | ACCTCATGTGCTGGAAGGA | CAGAATGCTTTGCAATTTTT |
| Ccnd1   | TTTCCTATCTCAGATCGGT | TGACTTCCAGAGCGCTTCCCA | Mtmr9 | GCAGACACACGAGGAGA | ACCAGAGCCACACACATC |
| Cxcl10  | GTGCCGCTATTTTCTGC | TCACTCAGGACGCTCTCCTC | Mtmr11 | GTCAGCTCAGCCATCTGTGG | CAGAATGCTTTGCAATTTTT |
| Cxcl14  | GACACAGCAGCCAGGACAC | TTCCAGAAGCCGCTCTCCTC | Mtmr13 | CAACAGCATCTGCAAAACA | CGAAGAATGCTTTGCAATTTTT |
| Dctn6   | CTCCGGAGCGAGTAGTGTG | AGGGTTGATCCGCTCTCC | Ngfr | ACTGAGCCCTGATGTCAG | CAGAATGCTTTGCAATTTTT |
| Dlg1    | TTTCCGGAGAATTTCCCCTTCT | TGAGCACTTACGATGCTTCTCC | Nrg1 (I) | GGGAGGCGAGAGGAAGG | TTCCACAGGAGGCCAGAGC |
| Egr1    | CCTATGAGACCTCACCGACCA | AGGTTGATCCGCTCTCC | Pikfyve | GGGAGGCGAGAGGAAGG | CGAAGAATGCTTTGCAATTTTT |
| Exoc2   | GAGGAGAAGCTGGGACTGTT | CCGAGGAGCTGCTGCTGTT | Pten | AGGGAGAGGCTGCTGCTGTT | CAGAATGCTTTGCAATTTTT |
| Exoc4   | TGCCACACATCAGATGCTCC | GCGGACGTCGATACTC | Prx | AGGGAGGCTGCTGCTGCTGTT | CAGAATGCTTTGCAATTTTT |
| Emr1    | GGAGACCTCTTCAGCAAGCC | AGGTTGATCCGCTCTCC | Rorc | AATCTTTATTGGAAGACATTA | CAGAATGCTTTGCAATTTTT |
| Fgd4    | TACATGATGCGGCTCCTCA | CCGAGGAGCTGCTGCTGTT | Rufy | TGAGGCGGATGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Gapdh   | GAGGCAATGCGGAGTTGATG | GAGGCAATGCGGAGTTGATG | Saa1 | CAGAGATGAGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Id2     | GCCGAGGAGCTGCTGCTG | GAGGCAATGCGGAGTTGATG | Saa2 | GAGGAGGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| c-Jun   | CAGAGGGATGCTGCTGCTG | GAGGCAATGCGGAGTTGATG | Sh3tc2 | GGGCGACTCTCCTTCAC | CAGAATGCTTTGCAATTTTT |
| Lrp12   | CAGGCTGCTGCTGCTGCTG | GAGGCAATGCGGAGTTGATG | Sox2 | GGAGGAGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Mbp     | GAGGCAATGCGGAGTTGATG | GAGGCAATGCGGAGTTGATG | Spast | GAGGAGGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Miz1    | GAGGCAATGCGGAGTTGATG | GAGGCAATGCGGAGTTGATG | Vamp4 | GAGGAGGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Mpz     | CAGGCTGCTGCTGCTGCTG | GAGGCAATGCGGAGTTGATG | Vps13d | GAGGAGGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |
| Mtmr1   | GAGGCAATGCGGAGTTGATG | GAGGCAATGCGGAGTTGATG | Vps28 | GAGGAGGCTGCTGCTGCTG | CAGAATGCTTTGCAATTTTT |

### GENOTYPING PRIMERS

| Primers | Primers | Primers | Primers |
|---------|---------|---------|---------|
| Cre     | GAACGCGATGATTTCGACCCAC | AACCAGGCTTTTTGGCCTTCGC | Miz1 | 
| Miz1    | Primer 1: GATATCCTCGTGGGGAATTAGTA | Primer 2: GCCAAATACGCCTTCTTCAA | Primer 3: GAGGAGGCTGCTGCTGCTG |
special aluminum rivets with superglue (31). A voxel size of 74 × 74 × 100 nm³ is determined by a slice thickness of 100 nm and a pixel resolution of 1024 × 1024 at an image width of 76 μm. Three-dimensional models were created with the Avizo® Fire software (Visualization Sciences Group, Mérignac, France).

**Morphometric Analysis**—Morphometric analyses of the sciatic nerve were performed blinded on electron microscopic images taken at ×1100 magnification. The g-ratios of 100–120 randomly selected myelinated fibers were determined in each of three or four animals per genotype and time point. Morphological alterations (myelin outfoldings and aberrant axonal features) were determined for all myelinated axons on 10–17 randomly chosen EM pictures per animal. Between three and eight animals were analyzed per genotype and time point, resulting in the blinded counting of 1200–5000 fibers/data point.

Nerve sections were double-stained for neurofilament-M (secondary antibody labeled with Alexa-488) and unphosphorylated neurofilament-H (secondary antibody labeled with Alexa-546), and three randomly chosen pictures were taken from each individual sample with a fixed exposure time for each fluorochrome (10 ms for Alexa-488; 100 ms for Alexa-546). Intensities were evaluated with Adobe Photoshop, and the ratio of the fluorescence intensities was calculated. Nerve tissue from four individual animals was analyzed for each genotype.

**RNA Isolation and Quantitative PCR**—Sciatic nerve pieces were stored in RNAlater (Sigma, R9091) until homogenization in TRI Reagent (Sigma, T9424). RNA was extracted, DNase-treated (Macherey-Nagel, catalog no. 740963), and further purified using a NucleoSpin RNA column-based kit (Macherey-Nagel, catalog no. 740948) according to the manufacturer’s instructions. 0.1 μg of total RNA was reverse transcribed with the RevertAid first strand cDNA synthesis kit (Thermo Scientific, catalog no. K1622) using random hexamer primers. SYBR Green-based real-time polymerase chain reactions (Thermo Scientific, AB1167) were run in triplicate using an Mx3005P quantitative PCR system (Stratagene, Heidelberg, Germany) and undiluted cDNA. Gene expression was normalized to GAPDH and analyzed by the comparative cycle threshold method (ΔΔCt). “No template controls” were included in each run, and product specificity was verified by dissociation curve analysis. The primers used are summarized in Table 1.

**Statistics**—Comparisons between Ctrl and Miz1ΔPOZ animals were analyzed by two-tailed Student’s t tests. All statistical tests were performed with Prism version 5.0 software (GraphPad) (not significant, p > 0.05; *, p = 0.01–0.05; **, p = 0.001–0.01; ***, p < 0.001. Data are shown as mean ± S.D.

**RESULTS**

**Deletion of the Miz1 POZ Domain in Schwann Cells**—Because Miz1 expression in the peripheral nervous system had not been elucidated in detail, we analyzed it in the sciatic nerve at different time points after birth by immunohistochemistry. Miz1 expression was detected in sciatic nerve samples on days 10, 30, 60, and 90 postpartum (Fig. 1A), and the Miz1 signal colocalized to the SOX-10-positive Schwann cell nuclei (Fig. 1B representative for all time points). Consistently, expression of Miz1 mRNA levels was similar at these four postnatal time points in sciatic nerve lysates, as determined by quantitative RT-PCR (Fig. 1C).

To study the functional relevance of Miz1 expression in Schwann cells, we used a mouse model in which Miz1 exons 3 and 4 are flanked by loxp sites, leading to a truncated form of Miz1 lacking the POZ domain upon Cre-mediated recombination (2, 11) (Fig. 1D). We crossed Miz1lox/lox mice with a transgenic mouse line expressing the Cre recombinase under the control of desert hedgehog gene (Dhh) regulatory elements (32). This construct drives Cre expression in the Schwann cell lineage from embryonic day 12 onward (33). Miz1lox/lox animals either expressing Cre (Miz1ΔPOZ) or not expressing it (Ctrl; control) were employed. Deletion of exons 3 and 4 was evaluated by PCR using DNA isolated from skin and from sciatic nerve samples (Fig. 1D).

The embryonic deletion of the Miz1 POZ domain driven by the Dhh-Cre is not embryonic lethal, unlike in the case of the constitutive knockout of Miz1 (7), and mutant mice were born in normal Mendelian ratios (data not shown). We could not identify major differences between 30-day-old Ctrl and Miz1ΔPOZ nerves on semithin sections (data not shown) or in electron microscopic pictures (Fig. 1E). The arrangement and density of nerve fibers, the g-ratio (Fig. 1F), and the axon and nerve diameters were similar in 30-day-old Ctrl and Miz1ΔPOZ mice, indicating that the deletion of the Miz1 POZ domain has no major impact on peripheral nerve development within the first 30 postnatal days.

**Phenotype of Postnatal Day 90 (P90) to P120 Mice—Miz1ΔPOZ mice did not show an obvious behavioral phenotype during the first 2 months after birth. In contrast, P90 Miz1ΔPOZ animals displayed characteristic signs of a peripheral neuropathy, with cramping of hind limbs and gait abnormalities on plain and smooth surfaces (data not shown). When placed on a grid, Miz1ΔPOZ mice were hardly able to walk over the grid because they could not grab the bars properly, and their hind legs dropped between them (Fig. 2A). Thus, 3-month-old mice with a Miz1 POZ domain deletion in Schwann cells exhibited notable motor disorders.

In order to document the motor disorder in more detail, we analyzed animals of different ages using a score sheet as originally described by King et al. (25) and further used by others (34, 35). The mean score value was not different between P60 control and Miz1ΔPOZ animals. However, P90 and P120 Miz1ΔPOZ mice exhibited a significant increase of the mean score value (Fig. 2B). This was confirmed also in a footprint assay by brush marks of the hind limbs seen in P90 and P120 Miz1ΔPOZ but not in control animals, whereas P60 Miz1ΔPOZ animals showed normal footprints (Fig. 2C). Finally, a grip assay was performed to test the strength of the forelimbs, which was lower in P120 male (Fig. 2D) and female (data not shown) Miz1ΔPOZ mice, compared with control mice.

To characterize the underlying neuropathy, sciatic nerve samples were analyzed by light and transmission electron microscopy. Semithin sections of the sciatic nerves from P90 Miz1ΔPOZ mice showed fewer myelinated fibers per field of view, with corresponding larger areas of endoneurial space (Fig. 3A). In addition, myelin outfoldings and signs of demyelination were present in semithin sections of Miz1ΔPOZ animals, which...
were confirmed by ultrastructural analysis (Fig. 3B). In blinded countings, using low power electron microscopic sections, a significant proportion of myelinated axons exhibited aberrant myelin structures and axonal impairment in Miz1ΔPOZ animals compared with controls (Fig. 3C). However, myelinated fibers without outfoldings had a normal g-ratio, independent of the axonal caliber (Fig. 3D).

To determine whether myelin fragments present on ultrathin cross-sections are completely separated from the myelin sheet or represent cuts through longitudinal outfoldings, we performed serial block-faced scanning electron microscopy for a three-dimensional reconstruction of P90 nerves. Myelin of control animals formed a tube-like structure around the enwrapped axon without major extensions toward the axon or

FIGURE 1. Expression of Miz1 in peripheral nerves and conditional knockout of the Miz1 POZ domain in Schwann cells. A, immunohistochemical stainings of Miz1 in sciatic nerve tissue from control mice of different ages, as indicated. B, confocal double immunofluorescence pictures from stainings of nerve tissue from P30 control mice using antibodies against Miz1 and Sox10. C, expression of Miz1 mRNA measured by quantitative RT-PCR. n = 3 independent samples were used for each time point, whereas each sample contained sciatic nerve tissue from three mice at P10, from two mice at P30, and from one mouse at P90, respectively. D, exons 3 and 4 encoding the Miz1 POZ domain were flanked with loxP sites. The indicated primers (arrows) were chosen to discriminate between the floxed allele (311 bp) and the recombined allele (180 bp), respectively (see Table 1 for primer sequences). The gel shows a representative PCR analysis with genomic DNA isolated either from skin or from sciatic nerve and performed with primers for Cre recombinase and Miz1. *, faint bands most likely arising from nerve tissue in the skin. E, low power electron microscopic pictures from sciatic nerves of P30 control and Miz1ΔPOZ mice. F, average g-ratios obtained from electron microscopic pictures, as shown in E. Bar, 50 μm (A), 10 μm (B), and 5 μm (E). NS, not significant. Error bars, S.D.
the cytoplasmic space (Fig. 4, A and B). In contrast, myelin sheets from *Miz1ΔPOZ* nerve fibers exhibited outfoldings and cytoplasmic myelin fragments in ultrastructural sections and possessed multiple excrescences with variable morphology yet still connected to the original myelin sheet (Fig. 4, C and D). Conversely, outfoldings and myelin fragments observed in thin
FIGURE 3. Motor disabilities are accompanied by structural changes in the sciatic nerves of P90 Miz1 ΔPOZ mice. A, light microscopy of 1-μm thin sections from sciatic nerves of control (left) and Miz1 ΔPOZ mice (right). B, low power electron microscope pictures (left) from control and Miz1 ΔPOZ animals. The right-hand image shows a higher magnification of a Schwann cell with a myelinated axon (Ax) and outfoldings (arrowheads and asterisk). C, percentage of nerve fibers exhibiting outfoldings and other aberrantly myelinated fibers in sciatic nerves of control and Miz1 ΔPOZ mice. D, scatter plot correlating the diameter of the axon with the diameter of the entire nerve fiber in sciatic nerves from P90 control and Miz1 ΔPOZ mice. Inset, g-ratios of nerve fibers from the sciatic nerves of control and Miz1 ΔPOZ mice. Bar, 25 μm (A), 23 μm (B, left and middle), and 900 nm (B, right). Error bars, S.D. **, p = 0.001–0.01; ***, p < 0.001; NS, not significant (p > 0.05).

Miz1 POZ Domain Deletion in Schwann Cells

JANUARY 9, 2015 • VOLUME 290 • NUMBER 2

JOURNAL OF BIOLOGICAL CHEMISTRY

733
sections could be related to the myelin excrescences (Fig. 4, E–H). Thus, the nerve ultrastructure reflects a massive blebbing of the myelin sheaths.

Besides degenerative structures, signs of remyelination were detectable in P90 sciatic nerves of Miz1ΔPOZ mice. Larger caliber axons without myelin were engaged with Schwann cells in a 1:1 ratio (Fig. 5A). A subpopulation of these cells exhibited extended autophagic vacuoles containing degradative material reminiscent of myelin fragments (Fig. 5, B and C).
To elucidate whether the loss of Miz1 POZ expression allowed dedifferentiation and remyelination, we determined the expression of differentiation and dedifferentiation markers at P90 by quantitative RT-PCR (20). We found that the expression of genes encoding differentiation markers, such as myelin protein zero (gene \textit{Mpz}), periaxin (\textit{Prx}), and myelin basic protein (\textit{Mbp}), was significantly decreased in mutant sciatic nerves (Fig. 5D; \( p < 0.05 \)). The expression of genes associated with...
Miz1 POZ Domain Deletion in Schwann Cells

Schwann cell dedifferentiation, including Ngfr, Ccnd1, Sox2, c-Jun, and Id2, was clearly up-regulated in Miz1ΔPOZ animals compared with age-matched controls (Fig. 5E). Because c-Jun is also highly expressed in macrophages (36) and because these invade the nerve (see below), we performed double immunofluorescence stainings with antibodies against c-Jun and the Schwann cell and neuronal marker S-100 (Fig. 5G). In control animals, only a few nuclei were positive for c-Jun, most of which were not correlated with the S-100 signal. In contrast, the number of c-Jun-positive nuclei increased in nerves from Miz1ΔPOZ mice. Some of these positive nuclei were clearly located in Schwann cells, indicating that the increase of c-Jun expression is at least partly related to Schwann cells. The dedifferentiation marker Krox24 (encoded by the gene Egr1) was slightly but not significantly up-regulated (Fig. 5E; p = 0.0670). Furthermore, the expression of Schwann cell-derived neuregulin 1 (Type I), normally induced after adult nerve injury (37), was increased around 350-fold in 90-day-old knock-out sciatic nerves (Fig. 5F). In summary, ablation of the Miz1 POZ domain in Schwann cells induces myelin outfoldings and a late onset demyelination/dedifferentiation in 3-month-old mice, which can explain the motor disabilities of mutant mice already described.

Occasionally, we observed myelin-like structures in the interstitial space of 90-day-old Miz1ΔPOZ nerves (Fig. 6A). Beside Schwann cells, we also found other cells with a pleomorphic shape in the interstitial space (Fig. 6B). These cells also contained strongly degraded myelin-like material similar to what was observed in Schwann cells (compare with Fig. 5, B and C). When examined at higher magnification, these cells clearly lacked a basal lamina, whereas adjacent Schwann cells exhibited a continuous one (Fig. 6C). We hypothesized that cells competent for phagocytosis entered the sciatic nerve to clear myelin debris. Consistent with this notion was the focal increase of cells expressing the macrophage marker F4/80 antigen (Fig. 6D) (38) and an increase of Emr1 expression, encoding F4/80 (Fig. 6E). Furthermore, quantitative PCR data (Fig. 6F) showed an increased expression of the cytokines Ccl2 (macrophage chemoattractant protein 1; MCP-1), Ccl3 (macrophage inflammatory protein 1a; Mip-1a), Cxcl10 and Cxcl14 (macrophage inflammatory protein 2γ; Mip-2γ), known as a potent monocyte chemoattractant substance, and of serum amyloid A1 and A2 (Saa1 and Saa2) with multiple functions in inflammatory processes and acute phase responses (39, 40). However, neither by light nor by electron microscopy could we observe infiltration of lymphocytes or granulocytes. Also, the number of mast cells present in the nerve tissue did not differ between control and Miz1ΔPOZ mice.

Phenotype in P200 to P300 Mice—Unexpectedly, the severe clinical symptoms visible in mutant mice of about 90–120 days of age disappeared in animals older than 120 days, although slight motor disabilities, such as a mild cramping of the hind legs, remained. This could be documented by motility score assessment. In P150 Miz1ΔPOZ mice, the mean disability score was significantly lower than in P120 mice of this genotype, but it was still elevated compared with control animals (Fig. 2B). Furthermore, in the footprint assay, brush marks were no longer seen in P150 mice (Fig. 2C), and the strength of the forelimbs recovered to normal values (Fig. 2D).

Consistently, electron microscopy revealed a persistent myelinization phenotype in knock-out mice but with a decreased number and extension of outfoldings (4.2 ± 0.7%). Instead, a subpopulation of nerve fibers appeared to be remyelinated, as indicated by the presence of thin myelin sheets in relation to the diameter of the enwrapped axons (Fig. 7A). This observation was confirmed by a significantly increased g-ratio in sciatic nerves from Miz1ΔPOZ mice (Fig. 7E) and a parallel shift of the regression lines in the scatter plot of axon to nerve fiber diameter (Fig. 7F). In addition, we identified earlier stages of remyelination in mutant nerves. We observed axons that were only touched and not enwrapped by Schwann cell processes (Fig.
Other axons of large diameter were surrounded by Schwann cells in a 1:1 ratio, but compact myelin had not yet been formed (Fig. 7C). Finally, we found basement membrane foldings not associated with cells, indicating that the corresponding Schwann cell had detached from this structure (Fig. 7, B and D). Taken together, these different structures suggest that demyelination and remyelination are processes ongoing simultaneously in Miz1ΔPOZ animals older than 120 days.

Analysis of serial block-faced microscopy stacks of images as well as conventional transmission electron microscopy revealed that some Schwann cells had lost their myelin completely (e.g. see Fig. 5A). The corresponding axon appeared darker and less granular, most likely due to changes of the neurofilament and microtubule cytoskeleton. Therefore, we co-stained sciatic nerves from P90 Miz1ΔPOZ and control mice with an antibody against unphosphorylated neurofilament-H from the SMI-32 clone and against neurofilament-M. SMI-32 positivity is a marker for degenerating axons, whereas the neurofilament-M antibody recognizes neurofilaments independently of the phosphorylation status (41, 42). Morpho-
metric quantifications uncovered no significant increase in SMI-32 signal intensity in Miz1ΔPOZ compared with control mice (Fig. 8A). In line with this observation was an unaltered pattern of succinate dehydrogenase staining in calf muscles of Miz1ΔPOZ mice at P90, P120, P150, P180, and P240, indicating that no sprouting had taken place (Fig. 8B).

Deletion of the Miz1 POZ Domain Affects Expression of Genes Involved in Myelin Homeostasis—A variety of Miz1 target genes, involved in vesicular transport, endocytosis, and autophagy, are down-regulated when the Miz1 POZ domain is deleted in Purkinje cells of the cerebellum and in mammary gland epithelial cells (10, 13). In line with this observation, the accumulation of polyubiquitinated proteins and p62 led to the hypothesis that Miz1 regulates the autophagic flux (10). Because myelin degradation also depends on autophagy (43, 44), we tested whether the expression of Miz1 target genes, encoding proteins relevant to vesicular transport and autophagy, is affected in Schwann cells of Miz1ΔPOZ mice. Among the 11 genes described previously (10, 13), only Vamp4 was down-regulated, whereas Pikfyve and Spast were even more highly expressed (Fig. 9A). All other genes tested were not regulated, indicating that in Schwann cells, the deletion of the Miz1 POZ domain does not have the same impact on the expression of genes involved in vesicular transport and autophagy as has been described for Purkinje and mammary gland epithelial cells.

Myelin outfoldings similar to the pathology observed in Miz1ΔPOZ mice are characteristic histopathological features in a limited number of inherited peripheral neuropathies (45–50). The underlying pathomechanism has been attributed to dysregulated phosphoinositide levels and impaired vesicular transport (45, 46, 48, 50–52). We determined the expression of these genes by quantitative RT-PCR in Miz1ΔPOZ and Ctr mice. In addition, we chose genes encoding proteins that interact with the proteins associated with these disease genes. We found an altered expression of Mtmr2 (myotubularin-related protein 2), Mtmr7, Mtmr9, Fdg4 (FYVE/RhoGEF/PH domain-containing 4), Dig1 (discs large homolog 1), Pten (phosphatase and tensin homolog), and Exoc4 (exocyst complex component 4). However, except for Mtmr7, all of these genes were expressed on a higher level when the Miz1 POZ domain was deleted (Fig. 9B), most likely secondarily due to the dysmyelination. Our data suggest that neither previously identified Miz targets involved in vesicular transport and autophagy nor genes related to peripheral neuropathies with similar histopathologic changes are likely to be causative for the phenotype observed in Miz1ΔPOZ mice.

Deletion of the Miz1 POZ Domain Induces a Senescence-like Phenotype in Schwann Cells—Ablation of the Miz1 POZ domain in keratinocytes leads to an increased expression of the cyclin- dependent kinase inhibitor p21Cip1 due to a relieved repression of Cdkn1a via functional Myc-Miz1 complexes (12). p21Cip1 is also up-regulated in Schwann cells following a nerve injury (19). Quantitative RT-PCR together with immunohistochemical stainings of sciatic nerves from P90 mice revealed a strong up-regulation of p21Cip1 in Schwann cells from Miz1ΔPOZ animals compared with age-matched controls (Fig. 10, A and B). Staining for the proliferation marker Ki67 revealed...
a 5-fold higher labeling index in nerve tissue from Miz1ΔPOZ mice compared with control animals, and this was confirmed by changes in the expression of Mki67 (Fig. 10C). Because p21Cip1 expression inhibits cell cycle progression (53), we performed immunohistochemical staining of Ki67 and the macrophage marker F4/80 to unravel the identity of the proliferating cells in the tissue. Most Ki67-positive cells also expressed F4/80 (Fig. 10D), indicating that the increased Ki67 labeling index is mainly related to the proliferation of macrophages.

Besides its role in cell cycle regulation, p21Cip1 has been identified as a crucial player in the establishment and maintenance of senescence (54, 55). To this end, we prepared sciatic nerve fragments and performed whole-mount SA/β-galactosidase staining to probe for this senescence marker (13). Nerve fragments from Miz1ΔPOZ mice were, in contrast to those from controls, positive for SA/β-galactosidase, albeit to a variable extent (Fig. 10E). In addition, we used an antibody against the senescence-associated H3K9me3 (56) and found a higher percentage of positive Schwann cell nuclei in Miz1ΔPOZ animals compared with control animals (Fig. 10, F and G).

**DISCUSSION**

Ablation of the Miz1 POZ domain in Schwann cells did not affect the establishment of myelination but was followed at around 3 months of age by demyelination, as observed in sciatic nerve fibers. This was associated with motor constraints of knock-out mice. These motor disabilities were first observed around P90 and were accompanied by features of dys- and demyelination, such as myelin outfoldings, tomacula, and degradation of myelin by Schwann cells and invading macrophages with a severity ranging to complete loss of myelin sheets around axons. Interestingly, careful ultrastructural analysis and determination of the g-ratio in sciatic nerves from P30 Miz1ΔPOZ mice revealed no abnormalities when compared with tissue from control animals, although Miz1 is expressed in Schwann cell nuclei already at P10. We conclude that Miz1 is not necessary for the development of myelin sheets and that the de- and dysmyelinating processes are initiated between P30 and P90. Late onset neuropathies in a similar time range have been described in other animal models. However, most of these
Miz1 POZ Domain Deletion in Schwann Cells

A

**Cdkn1a**

Relative Fold Change

CTR

ΔPOZ

B

**p21**

CTR

ΔPOZ

C

**Mki67**

Relative Fold Change

CTR

ΔPOZ

**Ki67+ nuclei (%)**

CTR

ΔPOZ

D

**Ki67**

**F4/80**

**Hoe**

CTR

ΔPOZ

E

**SA-β-galactosidase**

CTR

ΔPOZ

F

**Histone H3K9me3**

CTR

ΔPOZ

G

**H3K9me3+ nuclei (%)**

CTR

ΔPOZ
models mimicking analogous neuropathies are progressive in the development of their phenotype (57, 58). In Miz1ΔPOZ animals, the neuropathic symptoms observed were reduced starting from the age of 120 days. In P200 knock-out mice, motor disabilities were hardly observable, indicating that the peripheral nervous tissue spontaneously regenerated. Ultrastructural analysis of knock-out sciatic nerves at this time point revealed that the number of outfoldings decreased, although tomacula were regularly observed and most axons were hypomyelinated, which was also reflected by an increased g-ratio. Our observations suggest that after initiation of an acute neuropathy at 3 months of age, regenerative processes are activated to compensate for the damage to peripheral nerve tissue. The increased expression of genes, such as Mtmmr2, Mtmmr9, Exoc4, Dgl1, Ddg4, or Pten, all involved in myelin homeostasis (45, 46), also underlines the notion of a regenerative process. Although the myelination is not completely restored even at P200, ongoing damage and regeneration could be counterbalanced in such a way that the motor abilities of Miz1ΔPOZ mice are almost normal.

The development of a late onset phenotype followed by its remission was unexpected because the loss of function mutation in Miz1 was induced at embryonic day 12 in the Schwann cell lineage and thus is also present in the remyelinating Schwann cells. The results demonstrate that Miz1 function is neither needed for myelin formation in development, which is completed around P30 (15), nor for regeneration. Based on the presented data, we suggest that loss of a functional Miz1 could interfere with normally suppressed pathways that promote demyelination. Consistently, we found that the expression of negative regulators of myelination, including c-Jun, Sox2, and Id2, was significantly up-regulated in Miz1ΔPOZ mice (Fig. 5E). Following nerve injury, c-Jun, Sox2, and Id2 are crucial for adaptation of Schwann cells because they promote a transdifferentiation to an immature type of Schwann cells (36, 59). However, other pathways driving demyelination, such as the Ras/Raf/MEK/ERK pathway (20), were not up-regulated in Miz1ΔPOZ mice (data not shown). This demonstrates that the tissue damage observed in Miz1ΔPOZ sciatic nerves does not lead to a general up-regulation of dedifferentiation factors and supports the hypothesis that the up-regulation of c-Jun, Id2, and Sox2 could play a central role in the pathology of Miz1ΔPOZ mice. Interestingly, Miz1 has been shown to inhibit TNFα-dependent activation of c-Jun N-terminal kinase (JNK1) (60), which in turn activates c-Jun. The loss of a functional Miz1 could promote c-Jun activation by relieving Jnk1 inhibition; ongoing experiments test this hypothesis.

As shown in Fig. 5D, the expression of myelin protein zero (P0), periaxin, or myelin basic protein is decreased in P90 Miz1ΔPOZ sciatic nerves compared with controls. This is underscored by a simultaneous increase in the expression of genes encoding proteins such as Krox24, p75, or cyclin D1, which promote the transition of Schwann cells back to a pro-myelinating state (15, 61), and by a dramatic increase of neu-regulin 1 (type I) as an indicator of disturbed Schwann cell/axon interaction (37). It is also conceivable that a direct down-regulation of the expression of Pmp22 (as observed in our preliminary DNA array data) or one of the other structural myelin proteins mentioned earlier could be the initial trigger for the phenotype observed after Miz1 POZ domain ablation, similar to the dose-dependent onset in Pmp22-induced peripheral neuropathy models (62, 63). However, a direct binding of Miz1 to genes encoding myelin proteins remains to be elucidated.

Recently, a function of Miz1 in the regulation of autophagy has been described in Purkinje cells based on a correlation between genes to which Miz1 binds and their down-regulation in Nestin-Cre Miz1ΔPOZ mice. Many of these genes are important for vesicular transport in general or autophagy in particular (10). Similar results were recently obtained using primary mammary cells from control and Miz1ΔPOZ lactating glands (13). In Schwann cells, autophagic removal of myelin debris after nerve damage has been described (43). Additionally, myelin debris can be cleared by macrophages (44). However, among the known Miz1 target genes associated with autophagy (10, 13), only the expression of Vamp4 was reduced in nerve tissue from Miz1ΔPOZ mice; two genes were even more strongly expressed, and all others were not regulated. This indicates that a loss of expression of the tested genes associated with autophagy is not likely to be causative for the observed phenotype.

After the discovery of Miz1 as a Myc-binding protein, the favored model saw the Miz1-Myc complex as a repressor of gene expression (64, 65). This was originally shown in cell culture for the cyclin-dependent kinase inhibitor gene Cdkn2b, encoding p15INK4b (28, 66), and was extended to a variety of other genes (13, 54). The repressive function of the Miz1-Myc complex was confirmed, especially for Cdkn1a (encoding p21Cip1), in keratinocytes in vivo (11). In line with this model, skin tumorigenesis is attenuated in mice with a Miz1 POZ domain ablation in keratinocytes through an increased p21Cip1 expression, which inhibits proliferation and increases the expression of SA β-galactosidase (12). Schwann cells from 90-day-old Miz1ΔPOZ animals also have elevated levels of p21Cip1 when compared with those from control mice, where p21Cip1 was hardly detected. Interestingly, nerve injury also leads to a nuclear accumulation of p21Cip1 (19), indicating that an increase in p21Cip1 expression can be a common reaction of Schwann cells to diverse types of damage. Besides its role in the cell cycle, p21Cip1 is involved in other cellular processes, including regulation of the actin cytoskeleton, apoptosis, and senescence (67). The expression of SA β-galactosidase and the high amount of H3K9me3 in Miz1ΔPOZ sciatic nerves are reminiscent...
cent of cellular senescence. This can also be induced by p21<sup>Cip1</sup> (54), although the detailed molecular link between this protein and senescence is still obscure (67). According to these findings and our data, we propose that the deletion of the Mizi POZ domain in Schwann cells leads to a premature cellular senescence, which might be a direct cause of the up-regulation of the Mizi1 target gene Cdkn1a.

Acknowledgments—The expert technical assistance of Waltraud Ackermann, Claudia Mayrhofer, Ursula Lehr, and Brigitte Agricola is gratefully acknowledged. We thank Burkhard Schütz for the device to measure grip strength. We thank Klaus V. Toyka and Carsten Wessig for helpful discussions and Ned Mantei for carefully reading the manuscript.

REFERENCES

1. Peukert, K., Staller, P., Schneider, A., Carmichael, G., Hänel, F., and Eilers, M. (1997) An alternative pathway for gene regulation by Myc. EMBO J. 16, 5672–5686
2. Kosan, C., Saba, I., Godmann, M., Herold, S., Herkert, B., Eilers, M., and Moröy, T. (2010) Transcription factor miz-1 is required to regulate interleukin-7 receptor signaling at early commitment stages of B cell differentiation. Immunity 33, 917–928
3. Moröy, T., Saba, I., and Kosan, C. (2011) The role of the transcription factor Miz-1 in lymphocyte development and lymphomagenesis: binding Myc makes the difference. Semin. Immunol. 23, 379–387
4. Stead, M. A., and Wright, S. C. (2014) Nac1 interacts with the POZ-factor Miz1. Biosci. Rep. 10.1042/BSR20140049
5. Stogios, P. J., Downs, G. S., Jauhal, J. J. S., Nandra, S. K., and Privé, G. G. (2005) Sequence and structural analysis of BTB domain proteins. Genome Biol. 6, R82
6. Herkert, B., and Eilers, M. (2010) Transcriptional repression: the dark side of myc. Genes Cancer 1, 580–586
7. Adhikary, S., Peukert, K., Karsunky, H., Beuger, V., Lutz, W., Elsässer, H.-P., Moröy, T., and Eilers, M. (2003) Miz1 is required for early embryonic development during gastrulation. Mol. Cell. Biol. 23, 7648–7657
8. Kerosuo, L., Piltti, K., Fox, H., Angers-Loustau, A., Häyry, V., Eilers, M., Renninger, C., Krohne, G., Asan, E., Roussel, M. F., and Eilers, M. (2013) Miz1 POZ Domain Deletion in Schwann Cells
9. Lein, E. S., Hawrylycz, M. J., Ao, N., Ayres, M., Bensinger, A., Bernard, A., Carter, R. J., Lione, L. A., Humby, T., Mangiarini, L., Mahal, A., Bates, G. P., Bremer, J., Baumann, F., Tiberi, C., Wessig, C., Fischer, H., Schwarz, P., Zankel, A., Kraus, B., Poelt, P., Schaffer, M., and Jessen, K. R. (2008) Novel signals controlling embryonic Schwann cell development, myelination and dedifferentiation. J. Peripher. Nerv. Syst. 13, 122–135
10. Sörensen, C. K., Wong, V. Y., Woodhoo, A., and Sommer, L. (2008) Development of the Schwann cell lineage from the neural crest to the myelinated nerve. Glia 56, 1481–1490
11. Bose, F. (2012) Extracellular and molecular mediators of peripheral axonal regeneration. Cell Tissue Res. 349, 5–14
12. Martini, R., Klein, D., and Grob, J. (2013) Similarities between inherited demyelinating neuropathies and Wallerian degeneration: an old repair program may cause myelin and axon perturbation under nonlesion conditions. Ann. I. Pathol. 183, 655–660
13. Atanasoski, S., Boller, D., De Ventura, L., Koegel, H., Boentert, M., Young, P., Werner, S., and Suter, U. (2006) Cell cycle inhibitors p21 and p16 are required for the regulation of Schwann cell proliferation. Glia 53, 147–157
14. Napoli, L., Noon, L. A., Ribeiro, S., Kerai, A. P., Parrinello, S., Rosenberg, L. H., Collins, M. J., Harrisingh, M. C., White, I. J., Woodhoo, A., and Lloyd, A. C. (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 73, 729–742
15. Harrisingh, M. C., Perez-Nadales, E., Parkinson, D. B., Malcolm, D. S., Mudge, A. W., and Lloyd, A. C. (2004) The Ras/Raf/ERK signaling pathway drives Schwann cell dedifferentiation. EMBO J. 23, 3061–3071
16. Gorgoulis, V. G., and Halazonetis, T. D. (2010) Oncogene-induced senescence: the bright and dark side of the response. Curr. Opin. Cell Biol. 22, 816–827
17. Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., and Lowe, S. W. (1997) Oncogenic ras provokes premature senescence in normal human diploid fibroblasts. Nature 383, 420–427
18. Jaegle, M., Ghazvini, M., Mandemakers, W., Pirsoo, M., Drüen, S., Levasseur, F., Raghoenath, S., Grosfeld, F., and Meijer, D. (2003) The POZ domain proteins Brn-2 and Oct-6 share important functions in Schwann cell development and Schwann cell lineage: from the neural crest to the myelinated nerve. Glia 44, 168–176
19. Wulf, E., Gebhardt, A., Kawauschi, D., Walz, S., von Eys, B., Wagner, N., Renningi, C., Krohne, G., Asan, E., Roussel, M. F., and Eilers, M. (2013) Mizi1 is required to maintain autophagic flux. Nat. Commun. 4, 2535
20. Gebhardt, A., Kosan, C., Herkert, B., Moröy, T., Lutz, W., Eilers, M., and Elsässer, H.-P. (2007) Miz1 is required for hair follicle structure and hair morphogenesis. J. Cell. Sci. 120, 2586–2593
21. Hönnemann, J., Sanz-Moreno, A., Wolf, E., Eilers, M., and Elsässer, H.-P. (2012) Miz1 is a critical repressor of cdkn1a during skin tumorigenesis. PLoS ONE 7, e43885
22. Sanz-Moreno, A., Fuhrmann, D., Wolf, E., von Eys, B., Eilers, M., and Elsässer, H.-P. (2014) Miz1 deficiency in the mammary gland causes a lactation defect by attenuated Stat5 expression and phosphorylation. PLoS ONE 9, e89187
23. Mirsky, R., Woodhoo, A., Parkinson, D. B., Arthur-Farraj, P., Bhaskaran, A., and Jessen, K. R. (2008) Novel signals controlling embryonic Schwann cell development, myelination and dedifferentiation. J. Peripher. Nerv. Syst. 13, 122–135
24. Hubert, E., and Eilers, M. (2014) Miz1 deficiency in the mammary gland causes a phenotype with reduced mammary gland size and ductal abnormalities. Breast Cancer Res. Treat. 1481–1490
25. Renninger, C., Krohne, G., Angers-Loustau, A., Häyry, V., Eilers, M., Renninger, C., Krohne, G., Angers-Loustau, A., Häyry, V., Eilers, M., and Eilers, M. (2013) Miz1 POZ Domain Deletion in Schwann Cells
47. Bolis, A., Coviello, S., Bussini, S., Dina, G., Pardini, C., Previtali, S. C., Niemann, A., Berger, P., and Suter, U. (2006) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 48, 1552–1565.

48. Bolis, A., Coviello, S., Visigalli, I., Taveggia, C., Chishti, A. H., Horn, M., Baumann, R., Pereira, J. A., Sidiropoulos, P. N. M., Somandin, C., Felser, D., Elsaesser, H.-P., and Eilers, M. (2005) Myc regulates keratinocyte differentiation by a monoclonal antibody to neurofilaments. Brain Res. 1021, 162–166.

49. Bouhy, D., and Timmerman, V. (2013) Animal models and therapeutic prospects for Charcot-Marie-Tooth disease. Annu. Neurol. 74, 391–396.

50. Boycott, K. M., and.SerializedName. The role of macrophages and eicosanoids in the pathogenesis of experimental allergic neuritis. J. Neurosci. 23, 10303–10312.

51. Boycott, K. M., and Suter, U. (2007) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 51, 355–367.

52. Boycott, K. M., and Suter, U. (2008) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 58, 563–576.

53. Boycott, K. M., and Suter, U. (2009) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 63, 513–526.

54. Boycott, K. M., and Suter, U. (2010) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 65, 525–538.

55. Boycott, K. M., and Suter, U. (2011) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 69, 585–598.

56. Boycott, K. M., and Suter, U. (2012) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 70, 9381–9390.

57. Boycott, K. M., and Suter, U. (2013) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 77, 355–367.

58. Boycott, K. M., and Suter, U. (2014) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 81, 355–367.

59. Boycott, K. M., and Suter, U. (2015) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 88, 355–367.

60. Boycott, K. M., and Suter, U. (2016) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 89, 355–367.

61. Boycott, K. M., and Suter, U. (2017) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 91, 355–367.

62. Boycott, K. M., and Suter, U. (2018) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 92, 355–367.

63. Boycott, K. M., and Suter, U. (2019) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 93, 355–367.

64. Boycott, K. M., and Suter, U. (2020) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 94, 355–367.

65. Boycott, K. M., and Suter, U. (2021) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 95, 355–367.

66. Boycott, K. M., and Suter, U. (2022) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuron 96, 355–367.