Known and proposed role of peripheral myelin protein 2 in Schwann cells during myelination

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Abstract

Many peripheral myelination proteins, such as myelin protein zero and myelin basic protein, have defined roles within peripheral nervous system myelination. However, there are several proposed roles for peripheral myelin protein 2 (Pmp2) expression in peripheral nervous system myelination. Factors contributing to myelination are not well researched in relation to Pmp2. The function of Pmp2 in aging was assessed by using WT and Pmp2OE, as age has previously been examined with Pmp2 -/- mice at 56 days of age. The skew between g ratios of slides was from the sciatic nerve. An analysis of the g ratios for WT and Pmp2OE slides was completed using NIH ImageJ software. The statistical tests utilized were Shapiro-Wilks test for normality, Mann-Whitney U test, and linear regression analyses. The average g ratio for WT mice was 0.671 and 0.668 for Pmp2OE. G ratios were not normally distributed ($p = 0.4556$). No significant effects of Pmp2 overexpression on Schwann cells in mice at 8 months of age were observed ($p = 0.9314$). There was no skew in g ratio of each axon seen by comparing slide 1 and 2 of each genotype ($p = 0.4004$, and $p = 0.7136$). The results of this study provide information regarding the function of Pmp2 expression as age increases. They can also be used in medicine, regarding the starting age of various therapies for demyelinating diseases.

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Introduction

Schwann cells, a glial cell type that are responsible for the myelination of the peripheral nervous system (PNS), have properties that allow the unique function as a system insulator. The composition of myelin is between 70 and 85% lipids, and 15 to 30% proteins, whereas most other biological membranes have lipid and protein compositions at about ~50% lipids and ~50% proteins (Poitelon et al., 2020). This unique composition of myelin allows for structural properties that include wrapping and compression. While there are many different proteins that contribute to the composition of myelin, three that are involved in myelination include the following: myelin protein zero (Mpz), myelin basic protein (Mbp), and peripheral myelin protein (Pmp2). Mpz has several functions, among which include extracellular adhesion (Salzer 2015), whereas Mbp acts as an intracellular adhesion molecular within Schwann cells (Boggs 2006). However, there are several proposed and overlapping roles for Pmp2. While its role as a fatty acid binding protein (FABP), FABP8 specifically, is well established, consensus for the function of Pmp2 is not completely clear.

Due to its location within the cell membrane of Schwann cells, Pmp2 was thought to have a similar function to the other two proteins in stabilizing the membrane and compaction of myelin. However, it has been shown that in Pmp2 null mice, there is little disruption to the structural integrity of the myelin (Zenker et al., 2014). Additionally, Pmp2 can function as a transport protein, using collision transfer to transport fatty acids within the cell, maintaining lipid homeostasis within the myelin. The transfer occurred at higher rates if the receiving membranes or vesicles had a greater negative charge (Zenker et al., 2014). This further supports the role of Pmp2 as a FABP within Schwann cells.

Another function for Pmp2 revolves around neuregulin 1 type 3 (ngr1t3). As signaling for ngr1t3 increases, the result is an activation of the MAPK/ERK pathway. Downstream of the MAPK/ERK pathway, it was found that Pmp2 was produced. As Pmp2 production continued under sustained signaling of ngr1t3, myelination also saw an increase, in some cases becoming hypermyelination, showing how Pmp2 can aid in determining myelin thickness, but also illustrating its function as a FABP (Belin et al., 2019).

There is a third proposed function for Pmp2 besides the two listed previously. Since Pmp2 is also a FABP and functions in the transport of fatty acids, there is potential for it to play a role in the metabolism of fatty acids within the myelin (Poitelon et al., 2020). Alterations in this function may play a role in demyelinating diseases, such as autosomal dominant CMT1, where mutations to the Pmp2 gene may be an underlying cause of neuropathy. As Pmp2 typically holds fatty acids such as cholesterol, if the protein is no longer able to hold as much, lipid homeostasis is not maintained, and demyelination along with a neuropathic phenotype can come as a result (Hong et al., 2016)

The ability of Pmp2 to function as a transport protein, FABP, and lipid metabolizer, all factors which contribute to myelin thickness, suggests that function may be impacted by additional factors as well. These include sex, age, and exons affected by mutations. For example, Pmp2 was determined to have a statistically significant effect on myelin thickness at 56 days of age (Zenker 2014). However, studies are lacking regarding the role of Pmp2 on myelin thickness after 56 days of age. To determine the ability of Pmp2 to contribute to myelin thickness as age increases, g ratios of 8-month-old wild type (WT) and peripheral myelin protein 2 overexpressing (Pmp2OE) mice were analyzed.

Statement of Purpose

The purpose of this study is to evaluate the role of overexpression of Pmp2 in Schwann cells, seen through myelin thickness, as age increases. Myelin thickness was measured via g ratio.

Hypotheses

At eight months of age, overexpression of Pmp2 yields an increase in myelin thickness when compared to 56 days. Within each genotype, there will be skew present in axon diameter, which is consistent with healthy mice.

Methodology

Slide collection

Slides were prepared by lab members at Albany Medical College's Myelin Laboratory. Slides included the sciatic nerve of 8-month-old mice, WT (n=10) and Pmp2OE (n=12).

Slide analysis

ImageJ version 1.5.4, an image analysis software developed by the NIH, was used for the analysis of each slide. The procedures for installation and use were previously described in Ferreira et al., 2011.

The files to be analyzed were categorized according to genotype. The two genotypes are C57BL/6 and C57BL/6 *Pmp2*-OE, *Mpz*-Cre, which will be subsequently referred to as WT and Pmp2OE. Each slide was analyzed separately, with results being combined for statistical analysis.

A scale of 10 micrometers per 220 pixels was established and continued for each slide.

Once a scale was established, inner circumference was traced on the axon, followed by outer circumference. This process was repeated for 100 axons per slide. Data was extracted and exported to excel for analysis of g ratios.

G ratio calculation

Procedures for g ratio determination were previously described in Rushton 1951. Briefly, axon diameter divided by fiber diameter yields a g ratio. This is a metric by which myelin thickness can be measured, with a high g ratio indicating decreased myelin thickness.

Circumferences gathered via ImageJ were divided by 3.14 in order to determine the diameter. Once all diameters were found for an individual animal and slide, g ratios were calculated by dividing inner diameter by outer diameter and repeated for the other axons. The average g ratio for each slide was calculated, and upon completion, average g ratio for each genotype was calculated, along with medians, and first quantiles and third quantiles.

Statistical analysis

Statistical analysis was conducted using RStudio software version 2023.09.0 +463 with R version 4.3.1. The Shapiro Wilks test was used to determine normal distribution. Data that was

determined to be nonparametric was further evaluated using the Wilcoxon rank sum test. Further analysis of g ratio relationship between each genotype was done via a linear regression analysis with axon diameter and g ratios as the x and y variables respectively. To assess any skew within calculated g ratios, linear regression analyses were performed. Statistical significance was set at $p < 0.05$.

Results

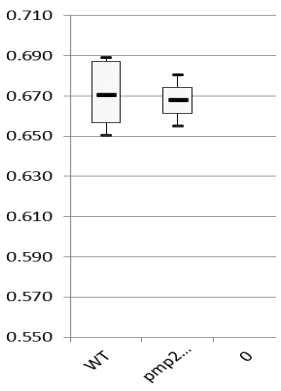
Initial analysis of g ratios

In order to conduct in-depth analysis of myelin thickness via g ratios, an initial analysis of g ratios was performed. Minimum and maximum g ratios were found for each genotype. There was a range of 0.458 – 0.865 and 0.461 – 0.823 for WT and Pmp2OE mice respectively. Average g ratios for each genotype were then calculated. WT mice had an average g ratio of 0.671, while the average g ratio in PMP2OE mice was 0.668 (Figures 1a and b). Data was then analyzed to determine parametric or nonparametric distribution. In using Shapiro Wilks test of normality, average g ratios for each slide were found to be nonparametrically distributed ($p = 0.2477$) (Figure 1c).

a

	WT	Pmp2OE
Mean	0.671	0.681
Min	0.650	0.655
Max	0.689	0.681
1st Quartile	0.657	0.661
Median	0.673	0.672
3rd Quartile	0.687	0.674

b



c

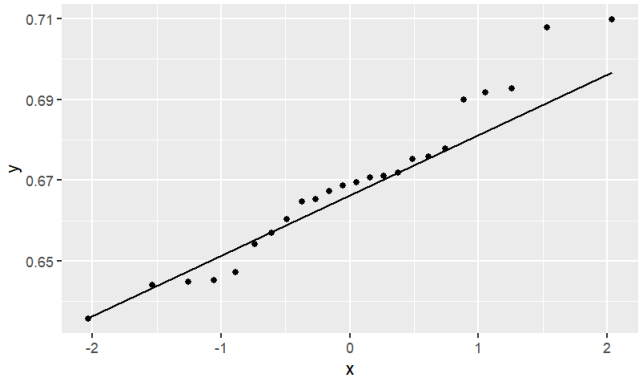


Figure 1: Initial analysis of g ratios (a) Display of values. A table of values in relation to g ratios of each genotype is provided. (b) Graphical representation of data from (a) within the form of a box-and-whisker plot. Graph was created with the use of Excel. (c) Quantile-quantile plot. The average g ratio for each animal was used to determine distribution. Data did not meet theoretical line of best fit ($p = 0.4456$). Tests that assumed nonparametric were used for the rest of the analysis.

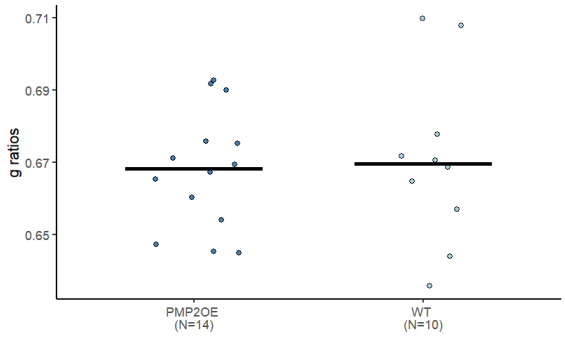
Effect of Pmp2 on myelin thickness

To assess the effect of PMP2 on myelin thickness as a factor of age, g ratios from WT mice and Pmp2OE mice were compared. The relationship between g ratios of WT and PMP2OE was found to be not statistically significant ($p = 0.9314$). This suggests that there is not a significant increase in myelin thickness produced by Schwann cells as there is an increase in age (Figure 2a). Axon diameter as compared to g ratios was also analyzed. There was a significant relationship observed between axon diameter and g ratios ($p < 2.2 e -16$). These results indicate that there is not a significant relationship between Pmp2 expression and myelin thickness.

Analysis of skew between slides of each genotype

Skew appeared to be present between axon diameters within each genotype (Figure 3a). To assess this, a linear regression between axon size on slide one of each animal and slide two. There was not any skew seen between slides of either group with p values of p = 0.8114 and p = 0.5441 respectfully (Figure 3b). This reveals that axon diameters are not evenly distributed between slide 1 and slide 2 of each genotype. G ratios were then assessed for skew between slides. The skew was seen again, with p values of p = 0.4004 and p = 0.7136 for WT and Pmp2OE (Figure 3c).

a



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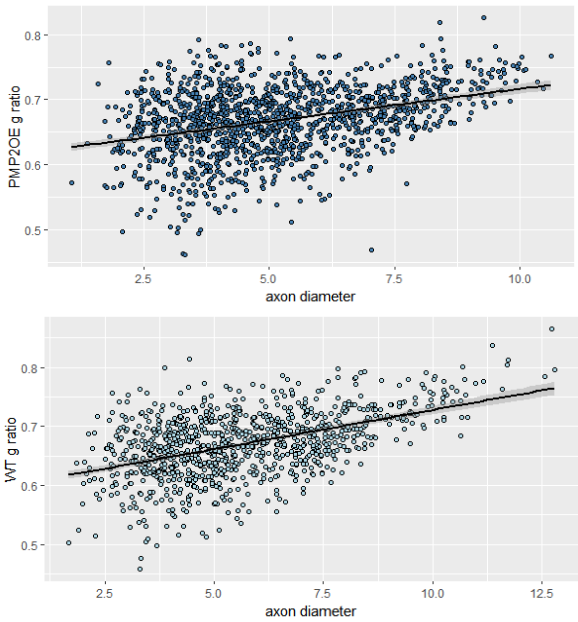
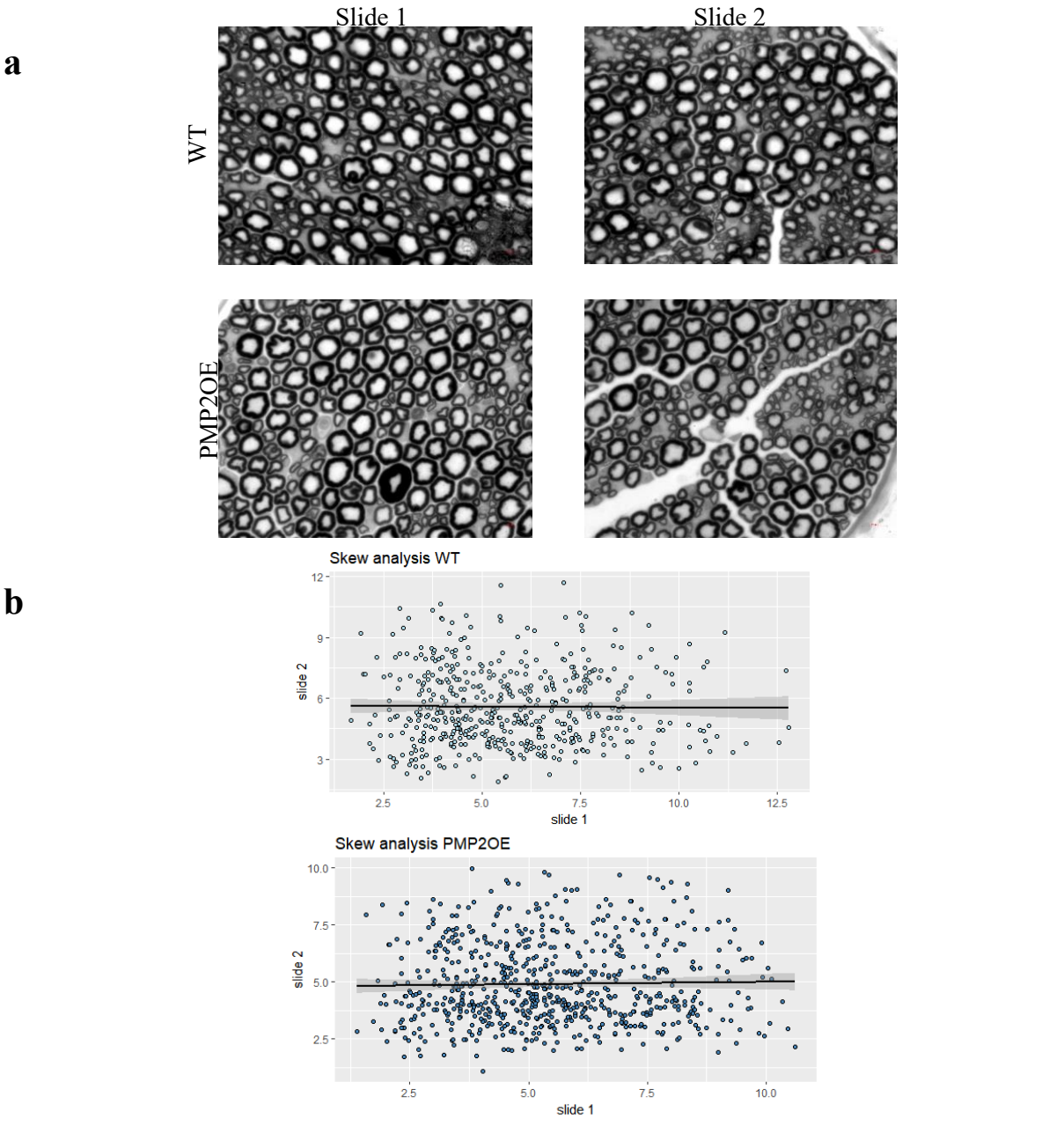


Figure 2: The effect of PMP2 expression on myelin thickness. (a) Jitter plot of all g ratios for both WT and PMP2OE genotypes, serves as a visual representation of Mann-Whitney U test. One value excluded due to inaccurate calculations. No significant relationship seen between g ratios of PMP2OE mice and those of WT mice. Black line indicates the median. (b) Linear regression analysis of axon diameter as compared to g ratio. Significant relationship seen between axon diameter and WT g ratio along with axon diameter and PMP2OE g ratio. Shaded area indicates 95% confidence interval.



c

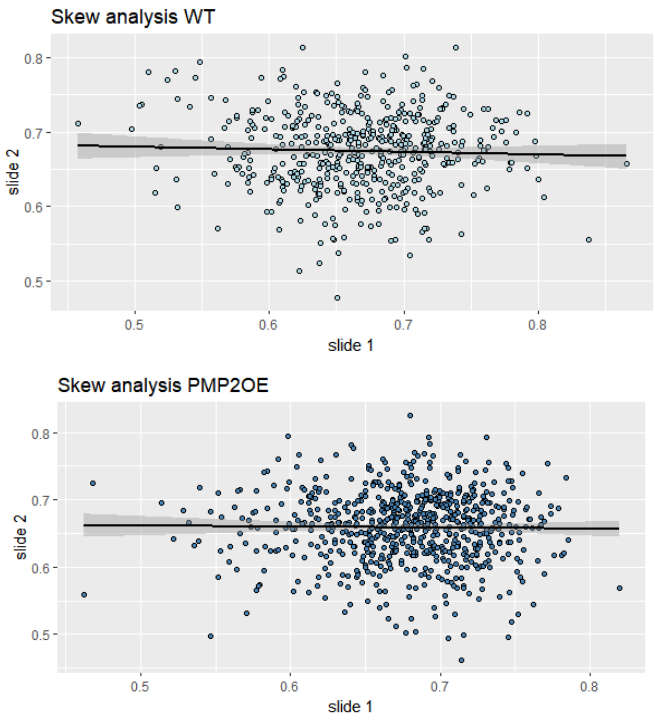


Figure 3: Analysis of skew between g ratios of each slide. (a) Slides of each genotype. Mouse 2694 was used as an example for WT mice, and mouse 2695 was used as an example for Pmp2OE mice. 2695 demonstrates how skew might appear: larger axons are seen on slide 1 as compared to axons seen on slide 2. (b) Linear regression comparing axon diameter of slide 1 to axon diameter of slide 2 for both WT and Pmp2OE. (c) Linear regression comparing g ratio of slide 1 to slide 2 for each genotype. Shaded area indicates 95% confidence interval.

Discussion

Proteins involved in myelination such as Mpz and Mbp have roles tied to membrane adhesion, but Pmp2 does not have a clearly defined role within peripheral nervous system myelination. Some proposed roles include regulating lipid transport and metabolism within

Schwann cells. However, consensus on whether or not Schwann cells are affected with increased expression of Pmp2 has not been achieved.

Interestingly, these data from Pmp2OE genotypes indicates that over expression of nonvariant Pmp2 does not increase myelin thickness created via Schwann cells in OE mice at eight months of age when compared to WT mice of the same age. There was no statistically significant increase in Schwann cell wrapping and compression seen via g ratios between mice with a genotype increasing Pmp2 expression and those without. Axon diameter as compared to g ratios also indicated that myelin thickness was not related to level of Pmp2 expression as no statistical differences were found between genotypes. Axon diameters and g ratios between slides of each animal ID demonstrated skew, consistent with histological findings from studies of axon distribution (Assaf et al., 2008).

In this study, values for g ratios between WT and Pmp2OE mice were seen to be similar. This suggests that myelin thickness is not significantly increased by the overexpression of Pmp2 as age increases. This is interesting, as Pmp2 is seen to play a role during peripheral nervous system myelination (Zenker et al., 2014). Similar results were found when analyzing both WT and p.I43N mutated Pmp2, indicating that overexpression of Pmp2 results in a CMT1 phenotype, regardless of whether or not the protein expressed was nonmutated or mutated (Hong et al., 2016). This may have an increased effect with age as demyelination occurs as age increases regardless of disease status (Verdú et al., 2000).

In contrast, myelin thickness has been seen to increase with age in some circumstances. One example of an increase with age is demonstrated in Pmp2 null mice. In this case, differences in g ratios were equilibrized between WT and Pmp2 null mice 14-days post crush injury,

illustrating an increase in myelin thickness over time in the absence of Pmp2 (Stettner et al., 2017). Another example of this was seen in an experiment observing changes in myelin morphology as age increases. The reason this study observed an increase in myelin thickness as the mice progressed during maturation and aging may be due to the fact that myelin proteins such as Mbp and Pmp22 were the focus of the study rather than Pmp2 (Shen et al., 2011)

There are limitations to this study. The sample size for the study was limited to a small number of mice. Additionally, only one age group was used. At least one other age group would provide additional data on how aging effects myelin thickness, and how protein expression levels also affect myelin thickness.

In conclusion, the aim of this study was to determine how expression of Pmp2 affected myelin thickness at eight months of age. Myelin thickness was investigated via the use of ImageJ to determine g ratios. Based on the findings of this study, an increased expression of Pmp2 at eight months of age does not increase myelin thickness. Also, it was shown that within Pmp2OE mice, there was no skew of axon diameter, consistent with studies on healthy WT populations. Additional research on the effect of the interaction and Pmp2 on myelin thickness would be valuable to understand how age effects the nervous system as individuals age. These results indicate that there is still much to be learned about how age affects myelin and the health of the PNS as a whole.

Conclusion

This study aimed to determine how expression of Pmp2 affected myelin thickness at eight months of age. Myelin thickness was investigated via the use of ImageJ to determine g ratios.

Based on the findings of this study, an increased expression of Pmp2 at eight months of age does not increase myelin thickness. Also, it was shown that within Pmp2OE mice, there was no skew of axon diameter, consistent with studies on healthy WT populations. Additional research on the effect of the interaction and Pmp2 on myelin thickness would be valuable to understand how age effects the nervous system as individuals age. These results indicate that there is still much to be learned about how age affects myelin and the health of the PNS as a whole.

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