Fluids and Barriers of the CNS

LETTER TO THE EDITOR Open Access

# Commentary on "Structural characterization of SLYM – a 4th meningeal membrane"

Julie Siegenthaler[1*] and Christer Betsholtz[2,3]

**Abstract**

For centuries, the meninges have been described as three membranes: the inner pia, middle arachnoid and outer dura. It was therefore sensational when in early 2023 *Science* magazine published a report of a previously unrecognized — 4th — meningeal membrane located between the pia and arachnoid. Multiple features were claimed for this new membrane: a single cell layer marked by the transcription factor Prox1 that formed a barrier to low molecular weight substances and separated the subarachnoid space (SAS) into two fluid-filled compartments, not one as previously described. These features were further claimed to facilitate unidirectional glymphatic cerebrospinal fluid transport. These claims were immediately questioned by several researchers as misinterpretations of the authors' own data. The critics argued that (i) the 4th meningeal membrane as claimed did not exist as a separate structure but was part of the arachnoid, (ii) the "outer SAS" compartment was likely an artifactual subdural space created by the experimental procedures, and (iii) the 4th membrane barrier property was confused with the arachnoid barrier. Subsequent publications in late 2023 indeed showed that Prox1 + cells are embedded within the arachnoid and located immediately inside of and firmly attached to the arachnoid barrier cells by adherens junctions and gap junctions. In a follow-up study, published in this journal, the lead authors of the *Science* paper Kjeld Møllgård and Maiken Nedergaard reported additional observations they claim support the existence of a 4th meningeal membrane and the compartmentalization of the SAS into two non-communicating spaces. Their minor modification to the original paper was the 4th meningeal membrane was better observable at the ventral side of the brain than at the dorsal side where it was originally reported. The authors also claimed support for the existence of a 4th meningeal membrane in classical literature. Here, we outline multiple concerns over the new data and interpretation and argue against the claim there is prior support in the literature for a 4th meningeal membrane.

**Keywords** Cerebrospinal fluid, Meninges, Inner arachnoid, Arachnoid barrier, Prox1, Dpp4, E-cadherin, Claudin-11

*Correspondence:
Julie Siegenthaler
Julie.Siegenthaler@cuanschutz.edu
[1]Department of Pediatrics, Section of Developmental Biology, University of Colorado, Anschutz Medical Campus, 12800 East 19th Ave, MS-8313, Aurora, CO 80045, USA
[2]Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden
[3]Department of Medicine-Huddinge, Karolinska Institutet, Huddinge, Sweden

In a recent paper in *FBCNS* [1], Kjeld Møllgård, Maiken Nedergaard and colleagues report new data that they claim provides additional support for a 4th meningeal membrane, originally reported by the same corresponding authors in *Science* in January 2023 [2] as a membrane distinct from the pia, arachnoid and dura maters. The 4th membrane, called SLYM (for ***s***ubarachnoid ***ly***mphatic-like ***m***embrane), was claimed to be a Prox1+mesothelium that subdivides the SAS into inner and outer compartments by holding a molecular barrier allowing unidirectional glymphatic CSF transport [2]. All of these claims were called into question by post- publication comments by several scientists that study the meninges (https://www.science.org/doi/10.1126/science.adc8810#elettersSection), including the authors of this commentary. We and others argued that meningeal Prox1+cells were instead constituents of the arachnoid, and the reported barrier properties unrelated to Prox1+cells but instead mediated by the adjacent arachnoid barrier cell layer (ABCL). Two papers [3, 4] published later in 2023 provided evidence that Prox1+cells are located immediately inside the arachnoid barrier cell layer(s), part of what has classically been referred to as the inner arachnoid amongst other names [5–8]. In their *FBCNS* paper [1], the authors acknowledge that SLYM is likely identical to the previously described inner arachnoid. Nevertheless, the authors insist their data show inner arachnoid/SLYM regionally detached from the arachnoid and forming a membrane of its own causing subdivision of the SAS into two compartments. We scrutinized the new data and identified multiple problems in experimental design and interpretations summarized below. We provide alternative interpretations of the data and suggest further experiments to test the alternatives. The authors' figures are referred to as Fig. X [1] and ours are Figure/Table Y.

Based on data in Fig. 1 [1], the authors argue that tissue fixation and dehydration cause shrinkage of the skull-enclosed mouse brain with resulting displacement of the meningeal membranes. They argue that this could explain why others (including us) consistently find the ABCL and inner arachnoid layers adherent to each other, not separated by an outer SAS. While tissue fixation may indeed artificially cross-link tissue structures, the presence of adherens junctions and gap junctions between arachnoid barrier and inner arachnoid cells as demonstrated by transmission electron microscopy (TEM) in multiple species [4, 7, 9–11] provide evidence that these layers are naturally linked, without an intervening space, in a live animal. Junctions of these types do not form between non-contacted cells. The relevant transcripts such as *Gja1* (encoding the gap junction protein connexins 43) and *Cdh5* (VE-Cadherin) are highly expressed in arachnoid cells [4, 12–14]. Two papers cited by the authors in support of their model [7, 11] both describe cell- cell junctions between arachnoid barrier cells and inner arachnoid cells in detail.

In Fig. 1D [1], the authors claim to show ABCL adherent to the skull bone, while the inner arachnoid/SLYM is adherent to the brain surface, the two separated by an outer SAS. The authors' use of claudin-11 (referred to as Cld-11 in Fig. 1D [1]) as a marker for ABCL has significant limitations, since its cognate mRNA, *Cldn11*, is expressed also in dural fibroblasts [4]. The claudin- 11 immunoreactive structure indicated by arrowheads in Fig. 1D [1] is interpreted by the authors as ABCL, but we believe its morphology, thickness and location is consistent with the dura. The authors' own results indicate that the dura is indeed claudin-11 IHC-positive in Fig. 4A [1]. The thin, weak claudin-11 staining also observed coinciding with the Prox1-GFP signal at the brain surface could well represent the ABCL, which is substantially thinner than the dura (Figure 1). Our concerns with the data in Fig. 1 [1] and their interpretations, including additional experiments that we believe would resolve the issues are summarized in Table 1.

Fig. 2 [1] shows whole mount preparations of brain and skull doubly labeled by the transgenic reporter *Prox1-GFP* and dipeptidyl peptidase-4 (Dpp4). The authors conclude that *Prox1-GFP*+inner arachnoid/SLYM and Dpp4+ABCL separate when the brain is removed from the skull, leaving inner arachnoid/SLYM at the brain surface and ABCL at the inside of the calvarium (Fig. 2D [1]). This contrasts with observations by us and others that Dpp4+and *Dpp4*- CreERT-labeled cells remain

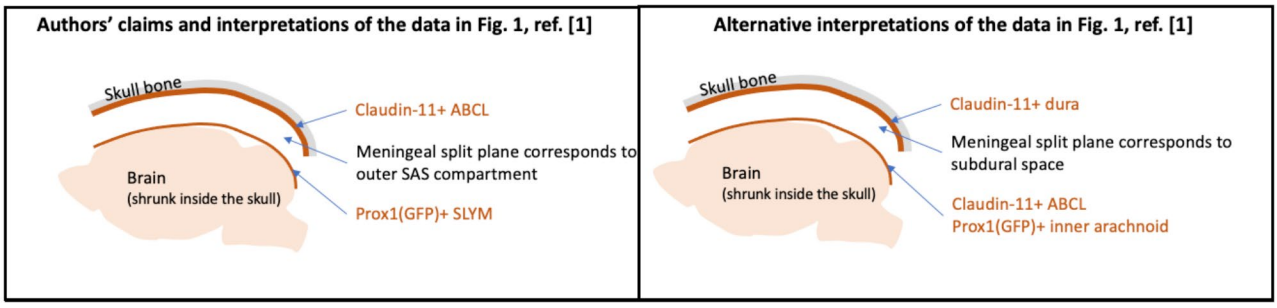


**Figure 1** Illustration of the discrepant interpretations of Cldn11 and Prox1-GFP immunohistochemistry analyses presented in Fig. 1D ref. [1]

**Table 1** Concerns with data in Fig. 1 [1] and their interpretations and suggestions for alternative explanations and experiments

| | Authors' claims and reported data | Data-Analysis- Interpretation Concerns | Alternative Explanations and Experiments |
|---|---|---|---|
| Fig. 1 | • Tissue shrinkage during fixation and post-fixation-processing collapses meningeal spaces, causes artifactual adhesion of Prox1 + SLYM to ABCL in some regions, giving the false appearance that the ABCL and SLYM are connected.<br>• Immunohistochemistry for claudin-11 or GFP (a proxy for Prox1) on adjacent paraffin sections from perfusion-fixed brains shows claudin-11 signal in meningeal tissue near bone (interpreted as ABCL) and GFP on meningeal tissue on brain. Authors conclude that "*the SLYM membrane is not always fused with the ABC layer*". | • Non-specific claudin-11 IHC signal in bone, a structure that does not express this protein.<br>• Claudin-11 marks a several cell layers thick membrane directly adherent to the bone. This morphology is consistent with the known anatomy of the dura and *Cldn11* mRNA is also expressed in dura.<br>• Low-magnification imaging and IHC for single marker on adjacent sections limits interpretation. | • Claudin-11 IHC specificity is ambiguous due to high background in bone.<br>• Weak claudin-11 IHC- positive signal in meninges near brain shows ABCL.<br>• Claudin-11 IHC-positive layers adhered to bone are dura.<br>• **Alternative Experiments**: E-cadherin labeling would unambiguously identify ABCL, and TEM immunogold-GFP would distinguish ABCL and Prox1-GFP from each other and from dura. |

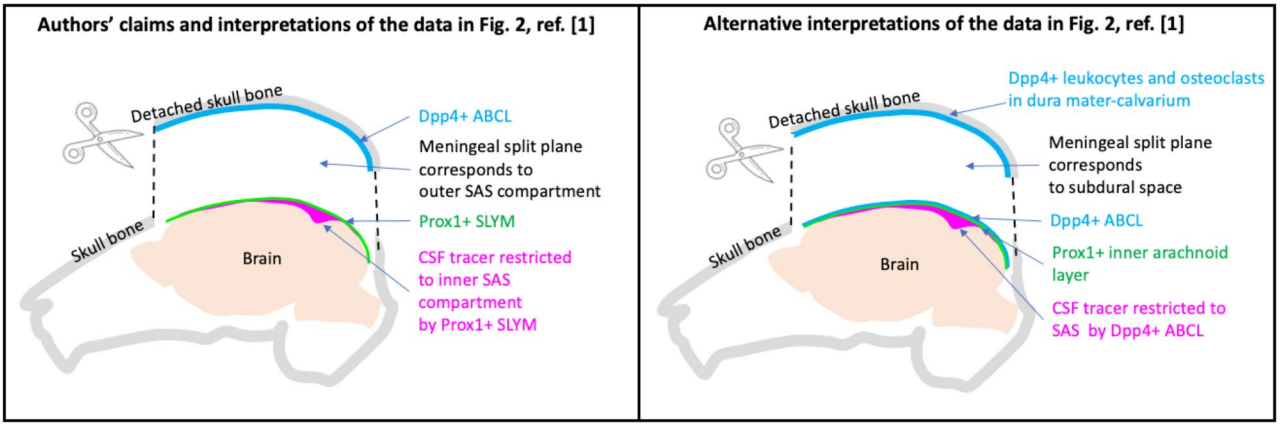


**Figure 2** Illustration of the discrepant interpretations of Dpp4 immunofluorescence and Prox1-GFP reporter expression in tissue whole mounts shown in Fig. 2, ref [1]

at the brain surface after removal of the skull [4, 19]. The observation of Dpp4 signal in the dura-calvarium may correspond to Dpp4+dural leukocytes [4] and Dpp4+osteoclasts in the calvarium [20, 21]. On closer examination of Fig. 2D [1], Dpp4 is observable also on the brain surface albeit weaker than in the dura-calvarium whole mount, consistent with ABCL being thinner than the dura. The divergent interpretations of the authors' data are schematically illustrated in Figure 2.

Unlike Claudin-11 and Dpp4, E-cadherin is a specific marker of ABCL not expressed by any other meningeal cells [4]. E-cadherin-positive cells consistently remain attached to the brain as part of the leptomeninges regardless of whether fresh or fixed specimen are analyzed (see [2, 4, 12, 15–18]). Adherence of ABCL to the brain surface is also evident in publicly available in situ hybridization data from the Allan Brain Atlas, which show continuous *Cdh1* (mRNA encoding E-cadherin) signal in the leptomeninges in all regions of the brain (http://developing-mouse.brain-map.org/experiment/show/100042112). Our concerns with authors' interpretations of their data in Fig. 2 [1] are summarized in Table 2.

Figs. 3–7 [1] show paraffin sections of brain enclosed in the demineralized skull. Demineralization allows for visualization of all meningeal layers (pia, arachnoid, dura) without brain extraction from the skull, but disproportional shrinkage of the brain creates artificial splitting of the meninges resulting in widening of the SAS. Using specific markers and immunostaining techniques is therefore essential to correctly identify the meningeal layers. The authors used several markers to visualize the meninges in a few different areas; however, as summarized in Table 3, the lack of complete cell type-specificity for any of the markers limits interpretations. The authors claim that a Prox1+membrane separates from ABCL at the basal part of the brain. We question this conclusion because the reagents used are not specific and because double labeling for cell-type specific markers on the same section was not performed. The authors rely almost exclusively on claudin-11 antibody for identification of ABCL in Figs. 3–7 [1], but *Cldn11* is not ABCL-specific, as discussed above. E-cadherin staining (ABCL-specific) is shown in a single panel (Fig. 7C, [1]) but it is negative and lacks a positive control, hence a reliable landmark for ABCL is missing. Our concerns over the authors

**Table 2** Concerns with data in Fig. 2 [1] and their interpretations and suggestions for alternative explanations and experiments

| | Authors' claims and reported data | Data-Analysis- Interpretation Concerns | Alternative Explanations and Experiments |
|---|---|---|---|
| Fig. 2 | • Light and fluorescence microscopy (confocal and 2- photon) imaging of Prox1- GFP + brains infused with tracers into the CSF via the cisterna magna. Using Dpp4 as a marker for the ABCL, the authors report Dpp4 + cells attached to the calvarium but not to the brain. Prox1 + GFP signal is detected on brain above tracer.<br>• Authors argue that ABCL and the Prox1 + cells (SLYM) are separable, implying that the Prox1 + cell layer forms an outer barrier for tracers placed in the CSF. | • The origin of the weak Dpp4 staining on the brain surface is not addressed by the authors.<br>• Low magnification imaging of whole brain and calvarium with dura attached is insufficient to identify which cells express Dpp4. | • The strong Dpp4 signal in calvarium reflects other cells besides ABCL known to express Dpp4 (osteoclasts and immune cells) or could be autofluorescence from bone.<br>• The weak Dpp4 signal on brain surface likely represents ABCL as no other cell type at the brain surface is known to express Dpp4.<br>• **Alternative Experiments**: Vibratome sections of brain and calvarium with higher magnification imaging. Co-staining of brain and calvarium wholemounts with E-cadherin to mark ABCL and Dpp4 to label multiple cell types in the arachnoid and dura. |

**Table 3** Concerns with data in Figs. 3–7 [1] and their interpretations and suggestions for alternative explanations and experiments

| | Authors' claims and reported data | Data-Analysis- Interpretation Concerns | Alternative Experiments |
|---|---|---|---|
| Fig. 3 | • GFP + cells by IHC staining of paraffin sections from whole decalcified adult mouse heads. GFP + layer is variably stuck to the calvarium, brain surface or neither (i.e. free floating) within the artifactually enlarged space between calvarium and brain caused by brain shrinkage.<br>• The presence of a non-adhered Prox1 + layer supports that it is a separate meningeal membrane. | • Given the shrinkage of the brain after fixation, and the lack of co-staining of GFP with other definitive markers of other meningeal cell types, it is difficult to know which, if any, of the described attachments and thicknesses are real or artifacts.<br>• Many markers used such as Crabp2, plectin and ER-TR7 are not specific for a single meningeal cell type or layer.<br>• Lack of demonstrated specificity of the claudin-11 antibody.<br>• No identification of E-cadherin and GFP on the same section, prevents assessment of the proximity of Prox1- GFP + cells and ABCL.<br>• The "negative" E- cadherin staining in Fig. 7 is not validated by a positive control. | • Need for unambiguous identification of ABCL by E-cadherin staining or TEM.<br>• Co-labeling of E-cadherin with other markers on the same section is needed and can be achieved by antibody multiplexing or fluorescent in situ hybridization. |
| Fig. 4 | • **A**, **B** shows IHC staining for claudin-11 and Crabp2 (a meningeal marker not expressed in the pia) in paraffin sections from ventral brain. Here, Crabp2 + membrane appears as a separate membrane interpreted as SLYM without ABCL surrounded by inner and outer SAS. **C**, **D** shows a region where SLYM and ABCL are unattached to each other and other structures. | | |
| Fig. 5 | • GFP, Prox1 and collagen type 6 (ER-TR7) IHC of membranes at the basilar artery (**A-C**) and cisterna ambiens (**D**) suggest that SLYM and ABCL are separate layers at the basilar artery but adhered in the wall of cisterna ambience (**D**). | | |
| Fig. 6 | • Sagittal sections show a continuous GFP + membrane variably attached to the brain surface, the calvarium, or "free floating" in an artifactually widened space between the brain and the bone. A second, GFP-weak, membrane close to the bone is marked as ABCL. | | |
| Fig. 7 | • Coronal adjacent sections stained individually for claudin-11, E-cadherin, podoplanin, collagen type 6 (ER-TR7), LYVE1, Raldh2, plectin and Prox1.<br>• The authors conclude that the membrane surrounding the internal carotid artery contains Prox1 + cells (SLYM) as a middle layer in a triple-layered membrane. Based on negative E-cadherin staining, they conclude that ABCL is absent in this structure. | | |

interpretations of their data in Figs. 3–7, [1] are summarized in Table 3.

Concerns over the data and interpretations aside, we also question the claim that the inner arachnoid/SLYM has barrier properties independent of the ABCL [1, 2]. Even if the inner arachnoid were regionally separated from ABCL, the inner arachnoid's lack of canonical tight junction gene transcripts [4, 19] argues against it being able to form a barrier to low molecular weight compounds. Absence of a size-restrictive barrier at the inner arachnoid is also supported by older literature [8] and by a recent study showing passage of cisterna magna-injected horseradish peroxidase (40 kDa) through all leptomeningeal layers except the ABCL [19].

## Concerns with the authors' placement of the 'SLYM' in context of prior studies of the meninges

We do not agree with their interpretation of the graphics reproduced from Nabeshima et al., 1975, and Orlin et al., 1991 [5, 11] shown with pseudo-coloring of the original diagram and image (Fig. 8 [1]). Our expert opinion, and we also believe the original authors' interpretations of the green-colored layer in Fig. 8 [1] is that this represents the pia (see Figure 3 for a comparison between the authors' and our placement of Prox1 + cells within the classical Nabeshima cartoon).

The diagram in Nabeshima et al., 1975 [5] summarizes the meningeal ultrastructural features of several mammalian species, whereas the image in Orlin et al., 1991 [11] shows porcine meninges. As the subpial space and other features of the meninges in larger species are larger than in mice, images and summary diagrams of mouse meninges cannot be meaningfully aligned with those of

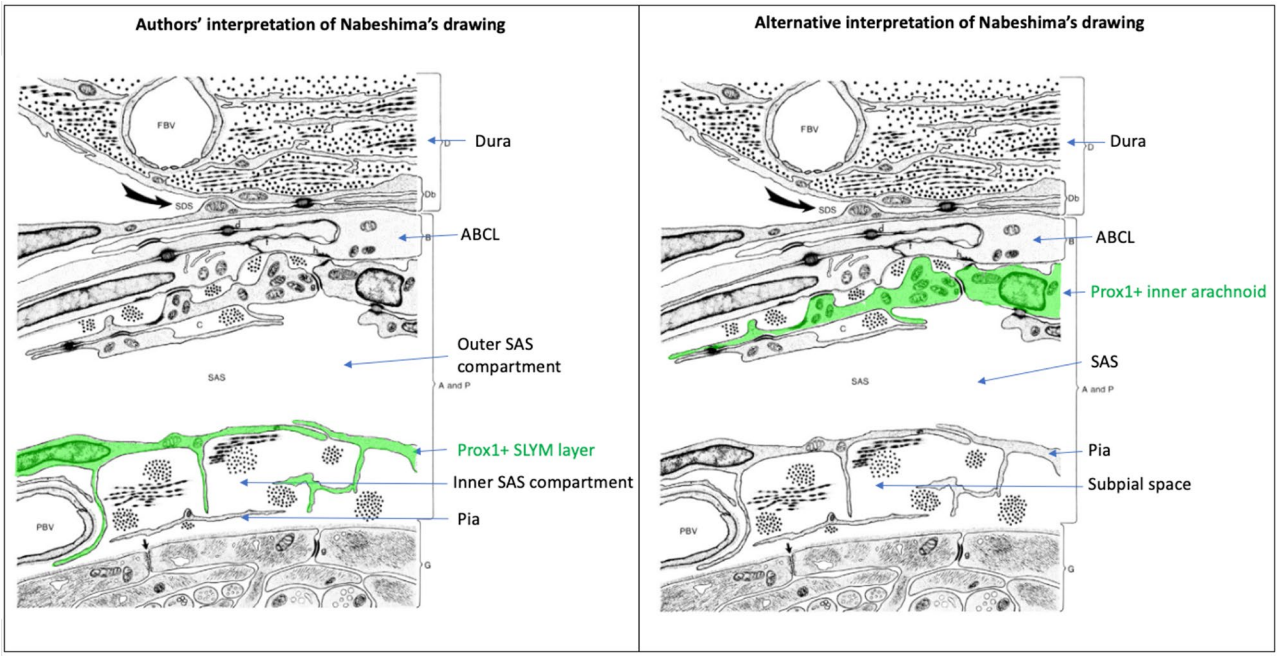


**Figure 3** Illustration of the discrepant interpretations of structure and location of pia and arachnoid in relation to Prox1 + cells

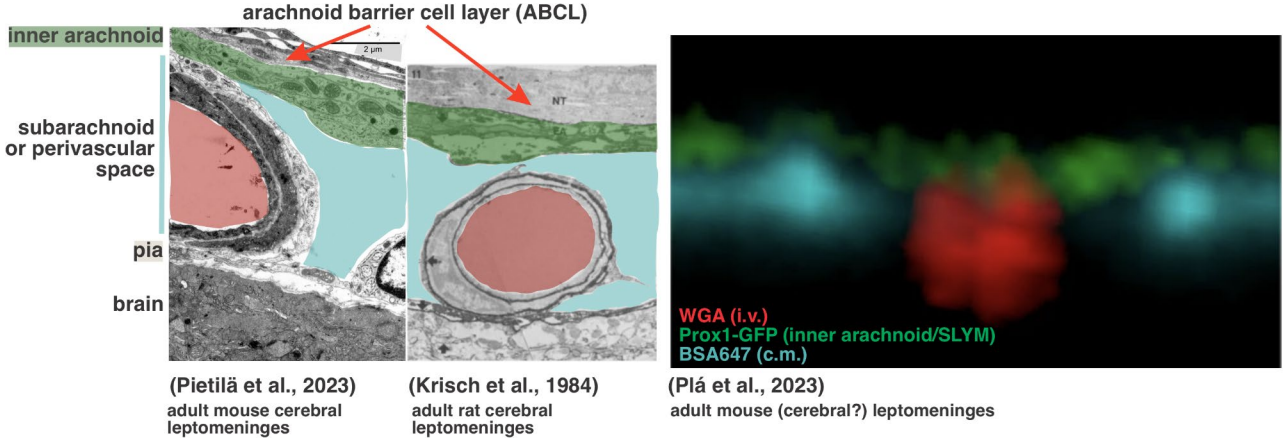


**Figure 4** *Where is SLYM in rodent meninges?* We aligned TEM images reproduced from [4] and [8] using the brain and blood vessels as reference landmarks: the inner arachnoid that contains Prox1 + cells is pseudo-colored green, the subarachnoid or perivascular space is blue and pial blood vessel lumen is red. To align this with an image containing the proposed SLYM, we used the lumen of the pial blood vessel and the *Prox1-GFP* (image reproduced from Fig. 8 [1]) to approximate the sizing and positioning

larger animals as the authors have done in Fig. 8 [1]. To make species appropriate-comparison of the location of SLYM in the context of prior literature, we aligned their image in Fig. 8 [1] of *Prox1-GFP* after injection of tracers into the blood vasculature (red) or cisterna magna (SAS, cyan) with TEM images of mouse [4] and rat [8] cerebral leptomeninges (Figure 4). In the TEM image, the inner arachnoid (containing Prox1+arachnoid cells), SAS and blood vessel lumen are pseudo-colored green, cyan and red, respectively, to match the authors' image. Figure 4 better enables evaluation of the authors' claim of the SLYM location in the context of neighboring structures by using a blood vessel as a reference in all three images. In comparing their image with aligned TEM images that show the cellular complexity of the leptomeninges, the green *Prox1-GFP* cells of the inner arachnoid form the roof of a single SAS and, based on the TEM, are connected to the arachnoid barrier layer without an intervening space. As we suggest above, use of TEM with *Prox1-GFP* mice in which GFP is detected with immunogold would permit unambiguous identification of the location of Prox1+arachnoid cells within the leptomeninges.

## Conclusion

Use of single-cell RNA-seq, in situ identification of newly identified enriched genes, and transgenic mouse reporter lines for intravital imaging is enabling the definition and location of meningeal cell subtypes of the pia, arachnoid, and dura by combinations of molecular markers. These markers also make it possible to link new information on meningeal cell molecular identity to previous TEM-based anatomic studies of the meninges. Although Prox1 is a new marker of a subset of meningeal cells, we disagree with the interpretations of evidence for Prox1+cells as a 4th meningeal membrane with the properties claimed by the authors of the Plá and Møllgård papers [1, 2]. From our own data [4], we conclude that Prox1+cells are scattered amongst other cells in the inner arachnoid and do not form a separate meningeal membrane. The inner arachnoid also contains at least one additional cell type lacking *Prox1* and is distinct in other ways [4]. Currently, the function of the *Prox1* transcription factor in some of the inner arachnoid cells is not known. These cells have no resemblance to *lymphatics* beyond the expression of Prox1, which they share with numerous other non-lymphatic cells. Also, the properties ascribed to Prox1+cells in the inner arachnoid, including handling CSF in a way that is unique from other meningeal layers and forming a continuous connected layer [1, 2] are lacking convincing evidence. Therefore, the name SLYM is misleading. Other functional properties attributed to the inner arachnoid/SLYM are not supported by the evidence (ex: barrier to low molecular weight molecules) or are not specific to these meningeal cells. Other studies have identified interactions between leptomeningeal and immune cells (macrophages and T cells) [3, 22–27] but whether Prox1+cells contribute uniquely to these interactions is currently unknown. With new knowledge of meningeal cell molecular identity and location in hand, an important goal for the field now is to generate and use tools to target specific meningeal cell types to learn their functions, facilitate comparisons across species, and generate discoveries that will advance our understanding of the meninges in health and disease.

**Acknowledgements**
The authors thank Donald M. McDonald, MD/PhD and Britta Engelhardt, PhD for critical reading and feedback on this commentary.

**Author contributions**
JS and CB equally contributed to conceptulization, writing and editing of this article.

## Declarations

**Competing interests**
The authors declare no competing interests.

Published online: 09 September 2024

**References**

1. Plá V, Bitsika S, Giannetto MJ, Ladron-de-Guevara A, Gahn-Martinez D, Mori Y, et al. Structural characterization of SLYM—a 4th meningeal membrane. Fluids Barriers CNS. 2023;20:93.
2. Møllgård K, Beinlich FRM, Kusk P, Miyakoshi LM, Delle C, Plá V, et al. A mesothelium divides the subarachnoid space into functional compartments. Science. 2023;379:84–8.
3. Mapunda JA, Pareja J, Vladymyrov M, Bouillet E, Hélie P, Pleskač P, et al. VE-cadherin in arachnoid and pia mater cells serves as a suitable landmark for in vivo imaging of CNS immune surveillance and inflammation. Nat Commun. 2023;14:5837.
4. Pietilä R, Gaudio FD, He L, Vázquez-Liébanas E, Vanlandewijck M, Muhl L, et al. Molecular anatomy of adult mouse leptomeninges. Neuron. 2023;111:3745–64.
5. Nabeshima S, Reese TS, Landis DMD, Brightman MW. Junctions in the meninges and marginal glia. J Comp Neurol. 1975;164:127–69.
6. Alcolado R, Weller RO, Parrish EP, Garrod D. The cranial arachnoid and pia mater in man: anatomical and ultrastructural observations. Neuropathol Appl Neurobiol. 1988;14:1–17.
7. Vandenabeele F, Creemers J, Lambrichts I. Ultrastructure of the human spinal arachnoid mater and dura mater. J Anat. 1996;189(Pt 2):417–30.
8. Krisch B, Leonhardt H, Oksche A. Compartments and perivascular arrangement of the meninges covering the cerebral cortex of the rat. Cell Tissue Res. 1984;238:459–74.
9. Oda Y, Nakanishi I. Ultrastructure of the mouse leptomeninx. J Comp Neurol. 1984;225:448–57.
10. McLone DG, Bondareff W. Developmental morphology of the subarachnoid space and contiguous structures in the mouse. Am J Anat. 1975;142:273–93.
11. Orlin JR, Osen KK, Hovig T. Subdural compartment in pig: a morphologic study with blood and horseradish peroxidase infused subdurally. Anat Rec. 1991;230:22–37.
12. DeSisto J, O'Rourke R, Jones HE, Pawlikowski B, Malek AD, Bonney S, et al. Single-cell transcriptomic analyses of the developing meninges reveal meningeal fibroblast diversity and function. Dev Cell. 2020;54:43–e594.
13. DeSisto J, O'Rourke R, Bonney S, Jones HE, Guimiot F, Jones KL et al. A cellular atlas of the developing meninges reveals meningeal fibroblast diversity and function. bioRxiv. 2019;648642.
14. Vivatbutsiri P, Ichinose S, Hytönen M, Sainio K, Eto K, Iseki S. Impaired meningeal development in association with apical expansion of calvarial bone osteogenesis in the Foxc1 mutant. J Anat. 2008;212:603–11.
15. Hannocks M-J, Pizzo ME, Huppert J, Deshpande T, Abbott NJ, Thorne RG, et al. Molecular characterization of perivascular drainage pathways in the murine brain. J Cereb Blood Flow Metab. 2018;38:669–86.
16. Jones HE, Abrams KA, Siegenthaler JA. Techniques for visualizing fibroblast-vessel interactions in the developing and adult CNS. Neurophotonics. 2022;9:021911.
17. Cha J-H, Wee H-J, Seo JH, Ju Ahn B, Park J-H, Yang J-M, et al. Prompt meningeal reconstruction mediated by oxygen-sensitive AKAP12 scaffolding protein after central nervous system injury. Nat Commun. 2014;5:4952.
18. Derk J, Como CN, Jones HE, Joyce LR, Kim S, Spencer BL, et al. Formation and function of the meningeal arachnoid barrier around the developing mouse brain. Dev Cell. 2023;58:635–e6444.
19. Smyth LCD, Xu D, Okar SV, Dykstra T, Rustenhoven J, Papadopoulos Z, et al. Identification of direct connections between the dura and the brain. Nature. 2024;627:165–73.
20. Yang Q, Fu B, Luo D, Wang H, Cao H, Chen X, et al. The multiple Biological functions of Dipeptidyl Peptidase-4 in bone metabolism. Front Endocrinol (Lausanne). 2022;13:856954.
21. Huang J, Liu X, Wei Y, Li X, Gao S, Dong L et al. Emerging Role of Dipeptidyl Peptidase-4 in Autoimmune Disease. Frontiers in Immunology [Internet]. 2022 [cited 2024 Jan 29];13. https://www.frontiersin.org/articles/https://doi.org/10.3389/fimmu.2022.830863
22. Cloyd MW, Low FN. Scanning electron microscopy of the subarachnoid space in the dog. I. spinal cord levels. J Comp Neurol. 1974;153:325–68.
23. Bartholomäus I, Kawakami N, Odoardi F, Schläger C, Miljkovic D, Ellwart JW, et al. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. Nature. 2009;462:94–8.
24. Jones HE, Coelho-Santos V, Bonney SK, Abrams KA, Shih AY, Siegenthaler JA. Meningeal origins and dynamics of perivascular fibroblast development on the mouse cerebral vasculature. Development. 2023;50(19):dev201805.

25. Kierdorf K, Masuda T, Jordão MJC, Prinz M. Macrophages at CNS interfaces: ontogeny and function in health and disease. Nat Rev Neurosci. 2019;20:547–62.
26. Masuda T, Amann L, Monaco G, Sankowski R, Staszewski O, Krueger M, et al. Specification of CNS macrophage subsets occurs postnatally in defined niches. Nature. 2022;604:740–8.
27. Merlini A, Haberl M, Strauß J, Hildebrand L, Genc N, Franz J, et al. Distinct roles of the meningeal layers in CNS autoimmunity. Nat Neurosci. 2022;25:887–99.

## Publisher's note