Lukasova *et al. Metab Target Organ Damage* 2024;4:48
DOI: 10.20517/mtod.2024.127

Metabolism and Target Organ Damage

Reply

Open Access

Check for updates

Reply to "comment on 'onion-skin type of periductular sclerosis in mice with genetic deletion of biliary kindlin-2 as tight junction stabilizer: a pilot experiment indicating a primary sclerosing cholangitis (PSC) phenotype'"

Martina Lukasova[1], Katharina Weinberger[2], Ralf Weiskirchen[3], Wolfgang Stremmel[4]

[1]Pharmacy of University Clinics of Heidelberg, Ruprecht Karls University Heidelberg, Heidelberg D-69047, Germany.
[2]Institute of Pharmacy and Molecular Biotechnology (IPMB), Ruprecht Karls University Heidelberg, Heidelberg D-69047, Germany.
[3]Institute of Molecular Pathobiochemistry, Experimental Gene Therapy and Clinical Chemistry (IFMPEGKC), RWTH University, Hospital Aachen, Aachen D-52074, Germany.
[4]Medical Center Baden-Baden, Baden-Baden D-76530, Germany.

Correspondence to: Dr. Wolfgang Stremmel, Medical Center Baden-Baden, Beethovenstraße 2, Baden-Baden D-76530, Germany. E-mail: wolfgangstremmel@aol.com

How to cite this article: Lukasova M, Weinberger K, Weiskirchen R, Stremmel W. Reply to "comment on 'onion-skin type of periductular sclerosis in mice with genetic deletion of biliary kindlin-2 as tight junction stabilizer: a pilot experiment indicating a primary sclerosing cholangitis (PSC) phenotype'". *Metab Target Organ Damage* 2024;4:48. https://dx.doi.org/10.20517/mtod.2024.127

Received: 4 Dec 2024 **Accepted:** 6 Dec 2024 **Published:** 10 Dec 2024

Academic Editor: Amedeo Lonardo **Copy Editor:** Ting-Ting Hu **Production Editor:** Ting-Ting Hu

Reply to *Metab Target Organ Damage* 2024;4:47.

We appreciate the thoughtful feedback provided by Chenghao Zhanghuang, MD, Na Long, and Bing Yan, MS, regarding our recent pilot study focused on the role of *kindlin-2* in bile duct cells and its implications for a phenotype analogous to primary sclerosing cholangitis (PSC). We welcome the critical evaluation of our work as it contributes to the scientific debate surrounding this important topic. We would like to address several points raised in their letter.

Sample size and experimental design: The rationale for generating a mouse model of PSC stemmed from previous kinetic experiments with the human cholangiocellular carcinoma-derived cell line Mz-ChA-1. These experiments demonstrated that phosphatidylcholine (PC) was transported from the basal side of the polarized cell layer to the apical side through tight junctions (TJs) in transwell culture dishes[1]. Similar findings in the intestine, where mucus PC derived from systemic sources through TJ was shown to protect against microbiota invasion[2], further inspired these studies. It was noted that deficient mucus PC, resulting from interference with this translocation process, led to an ulcerative colitis (UC) phenotype[3]. Given the frequent association of PSC and UC in humans, we hypothesized that both diseases share the same pathogenesis. The lack of protective biliary mucus PC may make the epithelial cell layer vulnerable to bile acids in the biliary lumen.

To test this hypothesis, we utilized a knock-out mouse model with a deletion of *kindlin-2* as the TJ adapter protein in biliary epithelial cells. This deletion was meant to confirm the findings from the kinetic experiments with polarized biliary epithelial cells. The consequence of biliary *kindlin-2* deletion and disrupted TJ function was the impairment of PC transport to the mucus, compromising its protective qualities. The expected histologic features of PSC, such as onion-skin type periductular fibrosis, were observed in the *kindlin-2* deleted mice, supporting our hypothesis.

In contrast to PSC mouse models induced by various inflammatory stimuli, our model focused on the early stages of PSC with minimal systemic inflammation and the absence of overt cholestasis. The use of young mice with the *Hnf1β* promoter allowed us to mimic these early stages. However, due to technical limitations, further studies on biochemical features typical of PSC pathophysiology could not be conducted in our laboratory. This study serves as a proof-of-concept pilot experiment, providing a novel perspective on the cause of PSC.

Moving forward, increasing the sample size in future studies is crucial for enhancing reliability. This includes examining the time course of PSC-related histologic alterations, inflammation, and cholestasis, as well as conducting ultrastructural analysis[4]. A comprehensive analysis of cell biology in living mice, bile flow, bile composition, metabolic alterations, biochemical changes, mechanisms of inflammation and cholestasis, and cell proliferation is essential. These approaches will require a large number of mice to ensure the reliability of the data. Therefore, the present study is considered a pilot experiment, as stated in our title, disclosing a novel aspect of the cause of PSC.

Immunohistochemical assays and molecular analyses: We understand the desire for additional sequencing and bioinformatics analyses to accompany our immunohistochemical findings. While we did not include these analyses in the current pilot study, we felt it was prudent to first validate the phenotypic changes and ensure replicability of our results in this initial exploration. The principal mechanism of TJ disruption as a cause of insufficient PC incorporation in biliary mucus was detailed in the kinetic studies with the polarized biliary epithelial cell line[1]. The employed mouse model with biliary deletion of *kindlin-2* is just an example of the destruction of the TJ-mediated pathway of PC translocation to the mucus and served as a proof of concept. This requires sophisticated imaging techniques to document the defective PC translocation process. However, it does not imply that a mutation only in the *kindlin-2* gene is pathogenetic for PSC. Instead, it is more likely that the general impairment of the TJ barrier, involving a variety of proteins responsible for its function, underlies the dysfunction of the PC transport system. Even the transport of PC from lipoproteins across the endothelium, the distribution of PC within the interstitial space between cholangiocytes, the driving forces for translocation across TJ, and finally, the binding to mucin 3 and 2 - all contribute to the establishment of a hydrophobic mucus layer protective against the luminal bile acids.

Elucidating the varying partners of this transport machinery requests a bioinformatic approach to analyze genetics, proteomics, and metabolomics of patients with PSC, potentially those with concomitant UC, in comparison to controls to detect molecular candidates of pathogenetic significance. Biopsy analyses can also help detect pathomechanisms that are involved. The challenge will be to prove their pathogenic significance *in vivo*, as shown for *kindlin-2* in the presented study.

Statistical comparisons and data presentation: The data presented in Table 1 were surprising to us. In fact, no difference was observed when mice were exposed to tamoxifen for 4 or 8 weeks ($P > 0.05$). The small standard deviation for alanine aminotransferase (ALT) and alkaline phosphatase (AP), obtained in our routine clinical laboratory, may differ from values reported by other groups. The higher variability in aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) was also unexpected. The latter was interpreted as a hemolytic component when blood was taken by heart puncture. We could not validate this discrepancy, and a laboratory mistake is not excluded, although samples were taken at different times and the laboratory is certified. This issue can be resolved by enlarging the sample size in future studies and continuously monitoring serological parameters over the observation period. The conclusion drawn from the present data is simply that serum parameters remained in the normal range regardless of *kindlin-2* deletion during tamoxifen exposure. To exclude a technical issue in data analysis, exchanging information with other laboratories working with mouse models of PSC will be helpful.

Image consistency and presentation: The images displayed were captured using conventional microscopy, as suggested in the literature[4]. The intensity of light may have influenced the appearance of cellular structures, but the qualitative and quantitative assessment of the main feature of periductular fibrosis remained consistent across the various sections analyzed. For future studies, more advanced analyses should be conducted.

In conclusion, we would like to express our gratitude to the authors for their constructive critiques and insights, which will undoubtedly help us refine the focus of our ongoing research. We are dedicated to uncovering the complex mechanisms underlying PSC and improving the potential for therapeutic advancements in this field. As researchers, we value the ongoing dialogue within our community and welcome any suggestion to enhance the comprehensiveness of our mouse model with biliary deletion of *kindlin-2*. We are eager to collaborate with all groups interested in PSC to critically evaluate the quality of available mouse models, with the goal of advancing our understanding of PSC pathogenesis and developing treatment options.

We appreciate the constructive criticism, identification of shortcomings and unanswered questions, and recommendations for enhancing our work. Overall, Chenghao Zhanghuang, Na Long, and Bing Yan have recognized our mouse model as innovative, with the potential to serve as a valuable tool for understanding the pathophysiology of PSC and developing new therapies.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the study: Weiskirchen R, Stremmel W
Performed data analysis and interpretation: Weiskirchen R, Stremmel W
Performed the draft: Weiskirchen R, Stremmel W
All authors made critical revision of the intellectual content.
All authors approved the version to be published and agreed to be accountable for all aspects of the work.

Availability of data and materials
Not applicable.

Financial support and sponsorship
None.

Conflicts of interest
Weiskirchen R is the Associate Editor and Stremmel W is an Editorial Board member of *Metabolism and Target Organ Damage*, while the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

REFERENCES
1. Stremmel W, Staffer S, Weiskirchen R. Phosphatidylcholine passes by paracellular transport to the apical side of the polarized biliary tumor cell line Mz-ChA-1. *Int J Mol Sci* 2019;20:4034. DOI PubMed PMC
2. Stremmel W, Ehehalt R, Staffer S, et al. Mucosal protection by phosphatidylcholine. *Dig Dis* 2012;30 Suppl 3:85-91. DOI PubMed
3. Stremmel W, Staffer S, Schneider MJ, et al. Genetic mouse models with intestinal-specific tight junction deletion resemble an ulcerative colitis phenotype. *J Crohns Colitis* 2017;11:1247-57. DOI PubMed PMC
4. Fickert P, Pollheimer MJ, Beuers U, et al; International PSC Study Group (IPSCSG). Characterization of animal models for primary sclerosing cholangitis (PSC). *J Hepatol* 2014;60:1290-303. DOI PubMed PMC