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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"

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Comment of *Metab Target Organ Damage* 2024;4:36.

We have taken a keen interest in the recent follow-up study conducted by Lukasova *et al.*[1]. The study aimed to investigate whether the genetic deletion of the tight junction protein kindlin-2 in bile duct cells would induce a phenotype reminiscent of primary sclerosing cholangitis (PSC) in mice. By knocking out the *kindlin-2* gene in bile duct cells within a murine model, the researchers explored the impact of the absence of this tight junction protein on the functionality of bile duct cells and observed that such deletion led to a periductal onion-skin fibrosis phenotype, which is analogous to that observed in human PSC. However, we have several queries regarding this study.

Firstly, we noted that the study utilized six control mice and six *kindlin-2* knockout mice, all induced at seven weeks of age, with observations extending over periods of four and eight weeks. Within each group, three mice were subjected to a four-week induction, while the other three underwent an eight-week induction. The study did not account for the failure rate of the modeling process or the reduction in the number of subjects due to unexpected mortality, which are significant considerations that should have been addressed in the experimental design. Additionally, in the context of animal model research, a larger sample size is instrumental in mitigating the impact of random variation, thereby enhancing the reliability of the study outcomes[2]. Consequently, we recommend increasing the sample size based on the success rate of the modeling and other objective factors to enable a more precise assessment of disease progression.

Secondly, the study conducted a limited number of immunohistochemical assays on liver sections, revealing some intriguing findings, as noted by the authors, who found the molecular changes occurring in primary bile duct cells lacking *kindlin-2* to be of interest. However, why were no further sequencing and bioinformatics analyses performed on these fascinating alterations, followed by molecular-level immunofluorescence assays based on these findings? We believe that such an approach could provide a deeper exploration of the specific molecular mechanisms underlying these changes[3]. These techniques have the potential to uncover the molecular dynamics during disease progression, offering additional insights into the pathogenesis of the disease[4].

Thirdly, in Table 1, we observe that the authors have grouped and compared mice observed for four weeks with those observed for eight weeks without presenting the *P*-values. This approach raises concerns, as there may be inherent differences between mice naturally growing for four versus eight weeks, which could introduce bias when these groups are combined for statistical analysis. Additionally, in the "Kindlin-2-deleted mice" group, we notice a standard deviation of only three for the Alanine aminotransferase (ALT) values, which is significantly different from other groups. We would appreciate it if the authors could further elucidate the potential reasons for this discrepancy in the discussion section. It is crucial to address whether the duration of observation influences the physiological and biochemical parameters being measured, as this could have implications for the interpretation of the study's findings. The variation in standard deviation for ALT levels in the "Kindlin-2-deleted mice" group might suggest a unique response or a technical issue in sample processing that warrants further investigation and clarification. A detailed discussion on these points would enhance the transparency and robustness of the study's conclusions[5].

Lastly, in Figure 3. We found that the differences in the images were most likely due to the intensity of the light rather than the liver itself. It is recommended to use a picture with a consistent background to make the article more convincing.

In summary, this study provides important tools and information for the understanding of pathological mechanisms, early diagnosis, and development of novel therapies for PSC. We acknowledge this study by Lukasova *et al.*[1]. First, the present study found that genetic deletion of *kindlin-2* leads to disruption of tight junctions, revealing a PSC-like phenotype, supporting the hypothesis that reduced phosphatidylcholine (PC) content in bile duct mucus may contribute to bile acid attack on bile duct epithelial cells. More importantly, this study provides a new perspective on understanding the molecular pathological mechanisms of PSC and provides clues to potential therapeutic targets. We kindly ask the authors to address our concerns. With further research, it is likely that more innovative animal models and therapies will be developed in the future to better mimic human PSC and improve therapeutic efficacy.

DECLARATIONS

Authors' contributions

Performed data analysis and interpretation: Zhanghuang C, Long N
Approved the version to be published and agreed to be accountable for all aspects of the work: Zhanghuang C, Yan B
Performed the draft: Zhanghuang C
Critical revision of the intellectual content: Zhanghuang C, Yan B

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication.

Not applicable.

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