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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

LETTERS TO THE EDITOR

Response to Spontaneous Cholemia in C57BL/6 Mice Predisposes to Liver Cancer in NASH

Dear Editors:

With great interest we have read the article by Gallage et al[1] titled "Spontaneous cholemia in C57BL/6 mice predisposes to liver cancer in NASH." The authors report a spontaneous heterogeneous phenotype in the *C57BL/6J* mouse strain that is widely used in biomedical research. Mice with spontaneous high serum total bile acids (TBA) seemed to remain lean and metabolically healthy when challenged by a Western-type diet, but did develop hepatocellular carcinoma.

Recently, we did describe spontaneous phenotypic heterogeneity related to serum TBA in the C57BL/6JOlaHsd strain fed the AIN-93G semisynthetic diet.[2] This semisynthetic diet contains all known nutritional requirements for mice, and 7% (weight/weight) fat. In approximately 25% of mice, we observed substantially elevated serum TBA, which coincided with increased serum liver damage markers, lower serum cholesterol and triglyceride levels (likely caused by decreased VLDL-secretion rates), and small livers (measured in weights). Mice with small livers had chronic liver inflammation with mixed inflammatory cell infiltrate, bile duct hyperplasia, karyocytomegaly, and elevated hepatic mitotic figures compared with mice with a "normal" liver weight. The difference in liver weight was not yet visible at weaning, but mice that later developed into "small livers" did already show lower food efficiency ratios (body weight gain per gram of food consumed) in the first week after weaning. The latter observation indicates that the phenotype was likely metabolically already present at weaning.

Earlier work by Cudalbu et al[3] described a phenotype in approximately 25% of C57BL/6J mice, characterized by high cerebral glutamine and low myoinositol, reminiscent of what has been described in chronic liver disease in humans. All assessed mice with high cerebral glutamine had a congenital portosystemic shunts (PSS). Portal vein anomalies were not seen in control mice.[3]

It is tempting to speculate that the various observations of a spontaneous phenotypic heterogeneity are related to PSS in a subset of the mice.[2-4] PSS leads to a small liver and elevated plasma bile acids in dogs.[5] In humans, unresolved congenital PSS can eventually result in liver adenomas and carcinomas. High-TBA was a common feature in the cited references.[1,2,4] It has not yet been tested whether high-TBA is caused by a congenital PSS. Another feature described by Gallage et al[1] that is compatible with a PSS is the high concentration of not only total but also unconjugated bile acids.[1] Under physiological conditions, intestine-derived bile acids, including unconjugated bile acids from passive colonic reabsorption, are effectively "first-pass" cleared by the liver. Under conditions of a PSS, however, the bile acids rather enter the systemic circulation and are only secondarily cleared.

We agree with the authors that high-TBA is an undesirable source of heterogeneity and potential bias. Accordingly, we support to determine TBA before experiments and to exclude mice with high-TBA. We postulate that the mechanism of the phenotypic heterogeneity relates to congenital, possibly genetic, PSS in *C57BL/6J* mice.

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References

1. Gallage S, et al. Cell Mol Gastroenterol Hepatol 2022;13:875–8.
2. Ronda OAHO, et al. PLoS One 2020;15:e0232069.
3. Cudalbu C, et al. PLoS One 2013;8:e69782.
4. Tarasco E, et al. Am J Physiol Gastrointest Liver Physiol 2018;315:G602–17.
5. D'Anjou M-A, et al. Vet Radiol Ultrasound 2004;45:424–37.

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Conflicts of interest
The authors disclose no conflicts.

Most current article
https://doi.org/10.1016/j.jcmgh.2022.01.012

Reply

We recently demonstrated the presence of spontaneous cholemia with high total bile acids (H-TBA) in a subset (~5%–25%) of all C57BL/6 mice obtained from various commercial breeders.[1] Cholemic/H-TBA mice are predisposed to develop accelerated liver cancer with elevated biliary damage (increased serum alkaline phosphatase and biliary expansion) and fibrosis on feeding of a high-caloric diet (eg, Western diet) but are resistant to obesity and metabolic dysfunction compared with littermate low-TBA control mice. Therefore, we proposed that investigators should exclude cholemic/H-TBA C57BL/6 mice in future studies to prevent potential bias and to avoid inconsistent or perplexing findings.

In line with our data, it was recently demonstrated that a subset (~25%) of C57BL/6JOlaHsd mice fed from birth a semisynthetic diet (AIN-93G) displayed elevated TBAs.[2] These mice with elevated TBAs displayed smaller livers but showed increased serum liver damage markers and bile duct hyperplasia and increased immune infiltration, liver fibrosis, and hepatocyte proliferation. Mice with smaller livers fed the semisynthetic diet also showed lower serum total cholesterol and triglyceride levels similar to Western diet feeding. Overall, this highlights that the occurrence of cholemic mice with H-TBA is a common phenomenon of C57BL/6 mice and that experimenters should be aware of this fact when conducting metabolic or cancer studies.

As postulated by Ronda and colleagues, we agree that a possible cause for the cholemic phenotype is the presence of congenital portosystemic shunts (PSS), which in humans is also termed the Abernethy malformation (AM) or congenital absence of the portal vein. Interestingly, it was shown that approximately 25% of C57BL/6 mice exhibit sporadic congenital PSS.[3] Importantly, patients with PSS/AM are also predisposed to develop liver masses including hepatocellular carcinoma.[4,5]

Patients with PSS/AM have altered hepatic vascular dynamics, which results in dysregulated hepatic metabolism of intestinal products including bile acids. It is very likely that this dysregulated metabolism contributes to the pronounced liver damage, fibrosis, and inflammation observed in cholemic/H-TBA mice on high-caloric diet (Western diet or choline-deficient high-fat diet) or semisynthetic diet feeding.[1,2]

Furthermore, we observed that the toxin carbon tetrachloride (CCl_4), which induces liver injury and fibrosis, did not exacerbate fibrosis in cholemic/H-TBA mice.[1] This suggests that cholemic/H-TBA mice may only be susceptible to dietary stress, which causes metabolic dysregulation and consequently liver damage, as opposed to all forms of liver injury.

Further studies should aim to unravel the mechanistic basis for spontaneous cholemia/H-TBA phenotype in C57BL/6 mice. It seems possible that genetic and epigenetic factors contribute to this disease phenotype. We observed spontaneous cholemia only in the C57BL/6 strain, but not in B6-FVB/N-129-mixed mice or in DBA/2 mice.[1] Whole-exome sequencing and selection for genetic alterations unique to C57BL/6 cholemic mice may therefore aid in the identification of genetic alterations driving this phenotype. Ultimately, it would be important to compare the genetic signature of cholemic/H-TBA mice with PSS/MA patients to determine whether similar genetic alterations are responsible for this abnormality in mice and humans.

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References

1. Gallage S, et al. Cell Mol Gastroenterol Hepatol 2022;13:875–8.
2. Ronda O, et al. PLoS One 2020;15:e0232069.
3. Cudalbu C, et al. PLoS One 2013;8:e69782.
4. Lemoine C, et al. J Pediatr Surg 2019;54:266–71.
5. Sharma R, et al. Hepatobiliary Pancreat Dis Int 2015;14:552–7.

Conflicts of interest
The authors disclose no conflicts.

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https://doi.org/10.1016/j.jcmgh.2022.01.013