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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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Conflicts of interest
The authors disclose no conflicts.

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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.