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The *inv* mouse as an experimental model of biliary atresia☆

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Abstract

Background/Purpose: The causation of biliary atresia (BA) remains unclear. However, ductal plate malformation (DPM), maldevelopment of the intrahepatic bile ducts, is 1 of the preferred theories. The *inv* homozygous mouse (*inv* mouse), created by insertional mutagenesis, shows situs inversus and jaundice. This study investigated whether the *inv* mouse could be an experimental model of human BA.

Methods: In the *inv* mice (n = 12) and wild-type littermates (n = 12), we examined the liver function and morphologic changes in the biliary tract through serum biochemical study and morphological study.

Results: The level of serum total and conjugated bilirubin in the *inv* mouse was 8.1 ± 3.8 and 4.4 ± 2.4 mg/dL, respectively, significantly higher than in the wild type. Macroscopically, 11 (92%) of 12 *inv* mice had situs inversus, and 3 (25%) of 12 mice had preduodenal portal vein. Histologically, the continuity of the extrahepatic bile duct was preserved. However, DPM, showing proliferative biliary epithelium around the intrahepatic portal vein, was found in the liver of the *inv* mouse.

Conclusion: In the *inv* mouse, the pathologic changes in DPM were found in the intrahepatic biliary system, which were observed in some clinical cases of BA. Therefore, the intrahepatic biliary system of the *inv* mouse could be an experimental model of human BA with DPM.

Biliary atresia (BA), characterized by a progressive obliterative cholangiopathy, is the most common cause of obstructive jaundice in infants [1-4]. Despite improvements in the surgical management of patients with BA since the condition was first described, little is known about the pathogenesis of the disease. Some cases have been reported to be caused by defects in the morphogenesis of bile ducts, viral infections, or immune-mediated injury to the biliary tree, all of which are now regarded as common pathogenic factors in the development of BA [1,2,4,5]. Ductal plate malformation (DPM), maldevelopment of the intrahepatic bile ducts, is 1 of the preferred theories of the defects in morphogenesis of the bile ducts.

The *inv* mutant mouse, created by insertional mutagenesis, was first reported in 1993 by Yokoyama et al [6], who described situs inversus and severe jaundice in homozygous mutants (*inv* mice). Later, Mazziotti et al [7] examined 6 *inv* mice and revealed that the strain had cholestasis with conjugated hyperbilirubinemia and a functional obstruction to bile flow, reporting no excretion of radiolabeled mibrofenin and no filling of duct structures with trypan blue, despite their showing the presence of extrahepatic bile duct structures microscopically. They described similarities and differences in the features of the *inv* mouse compared to

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Table 1 Serum biochemical study (mean ± SD)

Mice	Total bil (mg/dL)	Direct bil (mg/dL)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)
inv (n = 12)	8.1 ± 3.8*	4.4 ± 2.4*	692 ± 418*	46 ± 11	269 ± 185*
Wild type (n = 12)	0.3 ± 0.3	0	131 ± 37	39 ± 13	3.7 ± 2.8

bil indicates bilirubin.
* $P < .05$ compared with Wild type.

human infants with BA. However, they were ambivalent on whether the strain represents an animal model of BA associated with situs inversus. Since then, there have been no reports focusing on the pathophysiologic features of the hepatobiliary system of the *inv* mice.

In this study, we have investigated the further morphology of the intra- and extrahepatic biliary systems of the *inv* mice. We also elucidate a mechanism related to obstructive jaundice. Finally, we discuss whether the *inv* mouse could be an experimental model of human BA with associated anomalies focusing on the intrahepatic biliary system and the extrahepatic biliary system, separately.

1. Materials and methods

1.1. Animals

Male and female OVE210 heterozygous *inv* mutant mice were mated. Twelve homozygous *inv* mice and 12 wild-type littermates were obtained. Once born, affected mice were easily identified by a visible stomach on the right side and jaundiced skin color, as compared with nonaffected siblings. All mice were cared for and handled according to the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The experimental protocol was approved by the Kyoto Prefectural University of Medicine Animal Studies Committee.

1.2. Serum biochemical study

Blood specimens were obtained from the *inv* mice and wild-type littermates at the age of 0, 2, 4, and 6 days. Then, serum concentrations of bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), and gamma glutamyl transpeptidase (GGT) were examined. The results were

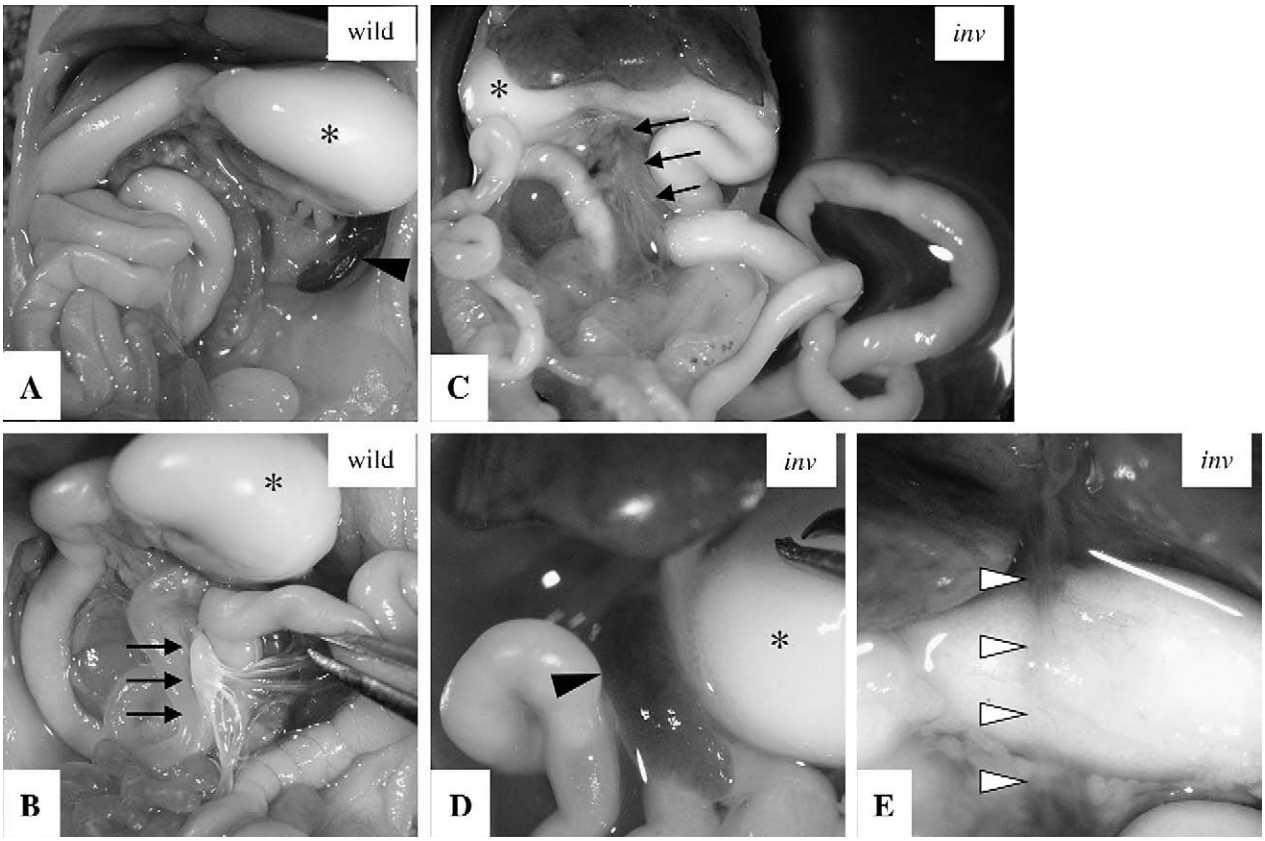

Fig. 1 Macroscopic observation of intra-abdominal anomalies. Wild-type mouse (A, B); *inv* mouse (C, D); another *inv* mouse (E). In the wild type, the stomach (*) was found on the left side, the duodenum on the right side, and a single spleen (arrowhead) on the left. The supra mesenteric artery (arrow) and Treiz ligament were found in all wild type mice. In most *inv* mice, the stomach (*) was found on the right side, the duodenum on the left side, and a single spleen (arrowhead) on the right. In some *inv* mice, intestinal malrotation (C) and/or preduodenal portal vein (open arrowhead; E) were found.