CASE REPORT

Hepatic copper accumulation in a young cat with familial variations in the ATP7B gene

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A 9-month-old intact crossbred female cat was presented with jaundice, intermittent anorexia and lethargy, increased hepatic enzyme activities, and hyperammonemia. Abdominal ultrasound and computed tomographic examinations determined that the liver had a rounded and irregular margin, and histopathological examination identified excessive accumulation of copper hepatocytes in the liver. Concentrations of both blood and urine copper were higher than in healthy cats. The patient responded well to treatment with penicillamine. Clinicopathological abnormalities and clinical signs improved within 2 months, and the patient was alive for >9 months after starting treatment. Genetic examination determined that the patient and its littermate had a single-nucleotide variation (SNV, p. T1297R) that impaired the function of the ATP7B gene product; the gene that is mutated in patients with Wilson's disease (WD). Hepatic copper accumulation was believed to be associated with the SNV of the ATP7B gene, and the patient had a genetic disorder of copper metabolism equivalent to WD in humans.

KEYWORDS
gene mutation, penicillamine, primary copper-associated hepatopathy, Wilson's disease

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A 9-month-old intact crossbred female cat was referred to the Veterinary Medical Center, the University of Tokyo (VMC-UT), Japan, with an 8-month history of intermittent anorexia and lethargy, increased hepatic enzyme activities, and hyperammonemia. An assessment performed at the referring veterinarian's clinic identified increased total bilirubin concentration (1.8 mg/dL; reference range [RR], 0.1-0.4 mg/dL). The anti-feline infectious peritonitis virus antibody titer was <1:100. The patient was treated with lactulose (0.5 g PO q8h) and ursodeoxycholic acid (25 mg PO q24h). The owner fed the cat a general commercial diet without any supplements containing additional copper. One month previously, a 1-year-old intact crossbred male littermate from the same queen also was referred to VMC-UT with jaundice. The littermate was diagnosed with liver failure because of jaundice without bile duct obstruction, increased total bilirubin concentration (11.6 mg/dL; RR, 0.1-0.4 mg/dL), and hyperammonemia (139 μg/dL; RR, 23-78 μg/dL), and died 4 days after presentation.

On initial evaluation at VMC-UT, the cat weighed 2.3 kg and had a body condition score of 2/5; physical examination disclosed jaundice on oral mucous membrane examination. No abnormality was identified in the CBC, whereas results of blood biochemical tests identified increased activities of alanine aminotransferase (ALT, 153 U/L; RR, 22-84 U/L) and alkaline phosphatase (ALP, 497 U/L; RR, 77-358 U/L). Total bilirubin concentration was increased at 1.2 mg/dL, and fasting hyperammonemia was noted (117 μg/dL). Results of feline leukemia virus antigen and feline immunodeficiency virus antibody tests were negative.

Thoracic and abdominal radiographs were unremarkable. Abdominal ultrasound imaging (HI VISION Preirus, Hitachi, Ltd., Chiba, Japan)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; CT, computed tomography; HE, hematoxylin and eosin; LOLA, L-ornithine-L-ascorbate; PCH, primary copper-associated hepatopathy; RR, reference range; SNVs, single-nucleotide variations; VMC-UT, Veterinary Medical Center, the University of Tokyo; WD, Wilson's disease.
determined that the liver had a rounded and irregular margin (Figure 1A). Computed tomographic (CT) examination (Aquilion PRIME, Toshiba Medical Systems Co., Ltd., Tokyo, Japan) also was performed under general anesthesia. The CT images identified multiple round nodules involving the entire liver (Figure 1B). Neither abnormal blood vessels indicating the presence of portosystemic shunts nor evident ascites was observed in abdominal ultrasound and CT examinations.

Liver biopsy was performed using laparotomy for definitive diagnosis by histopathologic examination. Before liver biopsy, prothrombin time and activated partial thromboplastin time were confirmed to be within RRs, and the cat was treated with vitamin K2 (menatetrenone, 0.9 mg/kg PO q48h) (Sannova Co., Gunma, Japan). Biopsy specimens were fixed in 10% phosphate-buffered formalin, routinely processed, and embedded in paraffin. The liver had multiple regenerative nodules (Figure 2A and B), fibrosis, and inflammatory infiltration with neutrophils, macrophages, and plasma cells around the nodules (Figure 2C). The hepatocytes were swollen, and eosinophilic cytoplasmic granules were identified diffusely in hepatocytes.
throughout the liver. These granules were red to brown on rhodanine staining (Figure 3), indicating severe hepatic copper accumulation. Based on these results, a histological diagnosis of hepatic cirrhosis with severe copper accumulation was made.

Blood and urine samples were collected before treatment for the measurement of copper concentrations to evaluate copper metabolism in the patient. Blood and urine samples also were collected from 10 healthy cats for comparison of the copper concentrations. A colorimetric assay and atomic absorption spectrometry were used to measure blood and urine copper concentrations, respectively. The Veterinary Medicine Institutional Animal Care and Use Committee of the University of Tokyo approved the study (approval number, P16-172), and informed consent was obtained from the owner of the cat before study enrollment. The results showed that blood and urine copper concentrations in the patient were 153 and 94 μg/dL, respectively. The median values of blood and urine copper concentrations in healthy cats were 80 μg/dL (range, 55-93 μg/dL) and 22.5 μg/dL (range, 16-64 μg/dL), respectively. In addition, the patient did not have a history of excessive intake of copper. Therefore, the copper metabolism was found to be impaired in the patient.

Based on the diagnosis, the cat was treated with penicillamine (20 m/kg PO q12h) (Taisho Pharmaceutical, Tokyo, Japan), prednisolone (1 mg/kg PO q24h) (Shionogi, Osaka, Japan), lactulose (650 mg/kg PO q8h) (Chugai Pharmaceutical, Tokyo, Japan), metronidazole (5 mg/kg PO q12h) (Shionogi), L-ornithine-L-aspartate (LOLA) (0.1 g/kg PO q12h) (Sigma-Aldrich, St. Louis, Missouri), and ursodeoxycholic acid (10 mg/kg PO q24h) (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan). A copper-restricted diet was not prescribed. After the initiation of treatment, hepatic enzyme activities, total bilirubin concentration, and ammonia concentration gradually decreased, and clinical signs (anorexia and lethargy) improved 55 days after starting initial treatment. Metronidazole and ursodeoxycholic acid were discontinued, and the dosages of prednisolone and lactulose were decreased (prednisolone 0.8 mg/kg PO q72h and lactulose 325 mg/kg PO q12h). Penicillamine and LOLA were continued. The general condition of the patient was good, with no abnormal clinical signs, 293 days after starting treatment. Results of blood biochemical tests showed that the activities of ALT and ALP had decreased to 95 U/L and 123 U/L, respectively. Total bilirubin concentration also had decreased to 0.1 mg/dL, and fasting ammonia concentration to 105 μg/dL. In addition, blood copper concentration decreased to 15 μg/dL, and urine copper concentration increased to 882 μg/dL.

Based on clinical data and age of onset, the cat was suspected to have primary copper-associated hepatopathy (PCH), which is similar to WD. Therefore, sequence analysis of the ATP7B and COMMD1 genes was conducted to investigate the cause of the hepatic copper accumulation. Mutations in the ATP7B gene have been known to cause WD, and the deletion of exon 2 of the COMMD1 gene has been reported in Bedlington Terriers, a well-characterized canine model of copper toxicosis. Genomic DNA samples were extracted from the peripheral blood of the patient and the 10 healthy cats that were used for the measurement of blood and urine copper concentrations. Twenty-four primer pairs and 3 primer pairs were synthesized to amplify the overlapping genomic DNA fragments spanning the coding region of the ATP7B (exon 1-21) and COMMD1 (exon 1-3) genes, respectively (see Supporting Information). The DNA samples were
Primary copper-associated hepatopathy in young cats has been reported in previous studies. Cats with PCH present with nonspecific clinical signs, such as vomiting, anorexia, lethargy, and weight loss, and copper accumulation are detected mainly in the centrilobular regions of the liver. However, the incidence of PCH is quite low, and there have been no investigations of genetic mutations in cats with PCH. The patient described here was 1 year old and diagnosed with hepatic cirrhosis with excessive copper accumulation in hepatocytes throughout the liver by histopathological examination. Based on the results of histological examination and the lack of history of excessive copper intake, the patient was suspected to have PCH. The difference in the distribution of copper accumulation between this patient and those of previous reports may be a consequence of the histological progression of PCH in this patient. One limitation of our report was that dry weight copper in the liver was not measured.

One of the best characterized PCH in dogs is autosomal recessive hepatic copper toxicosis in Bedlington Terriers, and deletion of exon 2 of the COMMD1 gene has been reported to cause PCH in some patients. However, a genome-wide association study recently identified SNVs in the ABCA12 gene (metal ion transporter) in Bedlington Terriers without deletion of COMMD1 gene. Therefore, it is suggested that PCH could result from mutations of various genes in cats. Although the patient in our report did not have mutations in the COMMD1 gene, other cases might have mutations in this gene or other genes.

Wilson's disease is an autosomal recessive disorder of copper metabolism in humans caused by mutations in the ATP7B gene. The ATP7B gene encodes a P-type ATPase that plays essential roles in ceruloplasmin synthesis and excretion of copper. Wilson's disease is diagnosed based on clinical signs, clinicopathological findings (including blood and urine copper concentrations), histopathological findings in the liver, and sequence analysis of the ATP7B gene. The patient and its littermate in our study had copper accumulation throughout the liver and the same 2 SNVs in the ATP7B gene, and 1 of the SNVs (p. T1297R) was predicted to have impaired the function of the ATP7B protein. Copper accumulation throughout the liver has been observed in human patients with WD, but the copper distribution often shows fluctuations in different stages of WD. Therefore, it was suspected that the 2 cats with the same mother had a familial disorder of copper metabolism equivalent to WD in humans. Although several animal models of WD have been identified, including the toxic milk mouse, Long-Evans Cinnamon rat, and Labrador Retrievers, no feline models of WD have been reported. Therefore, ours is the first case report of naturally occurring WD in cats. In Labrador Retrievers, concurrent mutations of the ATP7A gene, which cause copper deficiency disorder (Menkes disease), were indicated to modify the effect of ATP7B gene mutations on copper accumulation. It therefore is possible that severity and distribution of hepatic copper accumulation could vary among cats with WD. Further studies are needed to examine the association between gene mutations and hepatic copper accumulation.

The patient responded well to the treatment and was alive >300 days after first presentation. This outcome is consistent with an observation in a previous report, which showed long-term survival of human patients with WD and cats with PCH that are treated with penicillamine. In addition, blood copper concentration decreased and urine copper concentration increased after administration of penicillamine. These findings were consistent with those observed in human patients with WD. Thus, these findings suggest that penicillamine can be effective in the treatment of cats with PCH, and blood and urine copper concentrations can be used as the markers of the response to treatment. However, the RRs of blood and urine copper concentrations have not been determined previously, and the number of healthy cats used in our study was small. Thus, further investigations using a larger number of healthy cats are needed to determine the RR of blood and urine copper concentrations in cats.

In conclusion, we identified a young cat with PCH that had SNVs in the ATP7B gene that were damaging to the function of the ATP7B
protein, and our report indicates that cats may be new potential animal models of WD. Cats with mutations in the ATP7B gene may respond well to penicillamine-based treatment and survive for an extended period of time. Additional studies are needed to investigate the incidence of mutations in the ATP7B gene and describe the clinical characteristics of cats with WD.

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CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
The Veterinary Medicine IACUC of the University of Tokyo approved the study (approval number, P16-172).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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