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Prevalence and Clinical Relevance of Exon 2 Deletion of *COMMD1* in Bedlington Terriers in Korea

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Background: Deletion of exon 2 of *copper metabolism domain containing 1* (*COMMD1*) results in copper toxicosis in Bedlington terriers (CT-BT).

Objectives: This study was conducted to identify the prevalence and clinical relevance of the *COMMD1* mutation in Bedlington terriers in Korea.

Animals: A total of 105 purebred Bedlington terriers (50 males, 55 females) from the kennels and pet dog clubs in Korea were examined during the period 2008–2013.

Methods: A multiplex PCR was carried out to detect exon 2 deletion of *COMMD1*. Clinical analysis was performed on each genetic group, and clinical status of the dogs was followed up to estimate survival probability.

Results: Of the 105 samples, 52 (49%) were wild-type homozygote, 47 (45%) were heterozygote, and 6 (6%) were mutant-type homozygote. Plasma alanine aminotransferase (ALT) activity was increased in the mutant-type homozygous group >2 years of age ($P < .0001$). The survival probability of 6 mutant-type homozygotes surviving 2.5 years was 0.67, and 4 years was 0.5.

Conclusions and Clinical Importance: Results show the prevalence and clinical relevance of exon 2 deletion of *COMMD1* and could help establish a structured selective breeding program to prevent CT-BT in Korea.

Key words: Copper metabolism; Copper toxicosis; Delete mutation; Survival probability.

The *copper metabolism domain containing 1* (*COMMD1*) gene (formerly *MURR1*) on dog chromosome 10 encodes a protein that functions as a regulator of copper metabolism and homeostasis.[1,2] Deletion of exon 2 of *COMMD1* results in an autosomal recessive disorder characterized by high concentrations of copper in the liver, because it leads to the complete absence of the protein.[3–5] Copper toxicosis in Bedlington terriers (CT-BT) was first reported in the United States.[6] Copper accumulates predominantly in hepatocellular lysosomes of affected terriers due to a genetic disorder in the biliary copper excretion pathway.[7–9] Clinical signs of CT-BT vary and can be classified depending on disease progression and age of onset.[10,11] In the acute form of the disease, nonspecific clinical signs including an enlarged liver, jaundice, and hemolytic anemia develop usually between 2 and 6 years of age. The chronic form produces progressive liver damage in middle-aged and older dogs, but the clinical signs are less severe.

Abbreviations:

COMMD1	*copper metabolism domain containing 1*
CT-BT	copper toxicosis in Bedlington terriers

Affected terriers usually are presented with normal CBC results, but anemia, hemolysis, and coagulopathy can be observed.[6,11] Although the severity of abnormalities varies depending on the progression of the disease, the results of plasma biochemistry typically indicate abnormal liver function. In early studies, serum ALT activity was reported to be a more reliable indicator of diagnosing CT-BT than other biochemical tests.[10] Liver copper concentrations in affected terriers can be measured either quantitatively or semiquantitatively by various methods. A liver biopsy specimen traditionally has been used for definitive diagnosis, but this approach has practical and clinical problems. Liver copper concentrations do not always correlate with the extent of liver damage and disease progression.[12,13] In addition, liver biopsy will not necessarily establish a definitive diagnosis at an early age before a substantial amount of copper accumulates in the hepatocytes.

Molecular diagnostic methods are used for genotyping of patients. In 2005, an exon 2 deletion of 39.7 kb of *COMMD1* was reported.[14] Molecular diagnosis by a multiplex PCR is a reliable way to identify the genetic type of the terriers. Genetic tests are widely used for the diagnosis of CT-BT in many countries including the United States, Europe, and Australia.[14–16] Although the Bedlington terrier population in Korea originated from Europe and Australia, it is important to verify the

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existence of the same genetic defect and frequency of the mutant gene. However, little information on CT-BT in Asian countries, including Korea, is available, and there is no report on the molecular diagnosis in the Asian Bedlington terrier population. Therefore, we conducted a molecular diagnostic test to screen for the genetic defect and evaluate clinical information including survival probability.

Materials and Methods

Blood was collected from 105 Bedlington terriers (male 50, female 55), aged between 2 months and 11 years, of private pet dog clubs and the kennels in Korea. Among 105 terriers, 74 dogs were <2 years of age, 28 dogs were between 2 and 6 years, and 6 dogs were >6 years. Most dogs were private-owned and came from various parts of Korea, but 11 dogs were from 2 kennels. Pet owners and 2 kennels requested DNA testing to evaluate genetic conditions of dogs for health care and selective breeding. We obtained the signalment, pedigree information, history, and clinical signs of the dogs from the owners. Blood was collected into EDTA-coated and lithium heparin tubes. Genomic DNA was extracted from 0.5 mL of whole blood anticoagulated with EDTA with the genomic DNA extraction kit[a] according to the manufacturer's protocol. The concentrations of all DNA samples were adjusted to approximately 100 ng/μL with a spectrophotometer.[b]

The multiplex PCR was performed[c] to detect exon 2 deletion of the *COMMD1* gene as previously described.[14] The PCR products were visualized by 1.5% agarose gel electrophoresis with staining.[d] The products of wild-type homozygous, heterozygous, and mutant-type homozygous positive samples were purified from the gel with extraction kit[e] , and these were ligated.[f] *Escherichia coli* DH5α cells were transformed with the ligation products, and isolation of the plasmid was performed.[g] The isolated plasmid DNAs were sequenced[h] at SolGent Inc., Yuseong, Daejeon, Korea.

The CBC and plasma biochemistry tests were carried out using 46 blood samples. The CBC was measured[i] and plasma biochemistry was performed[j] to measure ALT, gamma-glutamyl transpeptidase (GGT), total bilirubin (Tbil) and total protein (TP). To perform comparative analysis, the results of CBC and biochemistry were divided into 3 groups based on the *COMMD1* gene type. The groups were further subdivided into 2 groups on the basis of age of onset (≤2 years or >2 years) in accordance with the classification described in previous studies.[10,11] Clinical signs associated with CT-BT and general health status of the mutant-type homozygous terriers were confirmed by continuous monitoring. When affected terriers died of CT-BT, clinical signs and primary diagnosis were obtained from the owners and local hospitals. The survival probability of 6 mutant-type homozygous dogs was estimated by the Kaplan-Meier method. All statistical evaluations for clinical analysis were performed by the ANOVA method using a statistics package, SPSS v19.0. A P value <.05 was considered statistically significant. All experimental procedures were performed in accordance with the guidelines for the Care and Use of laboratory animals of the Jeju National University.

Results

Identification of Exon 2 Deletion of COMMD1

To identify the exon 2 deletion of the *COMMD1* gene, DNA fragments from inside the deletion sequence and across the deletion break point were amplified utilizing genomic DNA extracted from 105 Bedlington terriers by multiplex PCR. The results of wild-type homozygote, heterozygote, and mutant-type homozygote are illustrated in Figure 1. Of the 105 samples, 52 terriers were wild-type homozygote (49%) for the normal *COMMD1* gene and 47 were heterozygote (45%) having both a normal and mutated copy of *COMMD1*. Six were mutant-type homozygotes (6%) for the exon 2 deletion of *COMMD1*, and the mutant allele frequency was 28% in the population. There were 52 dogs (32 males and 20 females) in the wild-type homozygous group with a mean (standard deviation [SD]) age of 1.5 (1.8) years and 47 dogs (15 males and 32 females) in the heterozygous group with a mean (SD) age of 1.3 (1.4) years. The mutant-type homozygous group consisted of 6 dogs (3 males and 3 females) with a mean (SD) age of 1.7 (1.2) years.

Clinical Relevance of CT-BT

There was no statistical difference in the results of the CBC in each genetic group. The WBC, RBC, and HCT results measured in each group were normal. The results of plasma biochemistry related to liver function showed a significant increase in ALT activity in the mutant-type homozygote group >2 years (P < .0001). There was no statistical difference in GGT activity, Tbil, and TP concentration in each group (Table 1). Through continuous monitoring of mutant-type homozygote group, deaths of 4 of the 6 were confirmed. They were examined at ages 2.1, 1.0, 2.2, and 3.7 years and died at ages 4.3, 2.3, 2.2, and 3.8 years. We obtained sire and dam information, including pedigree, of 6 mutant terriers and confirmed that they were not genetically related to each other within 3 generations. Four terriers (samples No. BT0901, BT0908, BT1108, and BT1110) in the mutant homozygote group showed clinical signs including vomiting, jaundice, and ascites related to copper toxicosis and were referred for genetic testing by pet owners. They were diagnosed as liver failure at local animal clinics. Two of these (samples No. BT1108 and BT1110) showed acute clinical signs of copper toxicosis, and 1 died during a test and another died approximately 1 month after it was diagnosed. The other 2

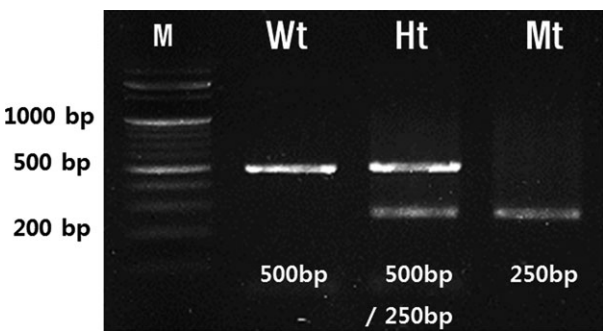

Fig 1. Results of multiplex PCR for wild-type homozygote (Wt), heterozygote (Ht), and mutant-type homozygote (Mt). The normal fragment is 500 bp, and affected fragment is 250 bp. Lane M: 100-bp ladder marker.

(samples No. BT0917 and BT1210) were tested to confirm their genetic type at an early age and did not have any clinical signs. They were alive during the follow-up time, and the ages at last assessment were 4.5 (BT0917) and 4.1 (BT1210) years. On the basis of this information, although the number of terriers of mutant-type homozygote group was small, the probability of survivors at the end of the time interval was estimated. The probability of affected terriers surviving 2.5 years was 0.67, and 4 years was 0.5.

Discussion

The diagnostic methods of CT-BT are liver biopsy and genetic testing. Liver biopsy presents practical and clinical problems, and in 1997, a microsatellite marker linked to CT-BT, *C04107*, was reported.[18] However, the marker alone cannot make a reliable diagnosis of mutant-type homozygote and heterozygote due to recombination between the marker and the disease gene.[12,19,20] Because indirect diagnostic methods with the microsatellite marker present a diagnostic dilemma, in the present study, we used a direct diagnostic method detecting exon 2 deletion in *COMMD1*.[14] Of 51 Bedlington terrier samples in Belgium, 10 terriers were wild-type homozygote (20%), 24 were heterozygote (47%), and 17 were mutant-type homozygote (33%), and mutant allele frequency was 57%.[16] Of the 147 samples in Australia, 60 terriers were wild-type homozygote (40%), 47 were heterozygote (32%), and 42 were mutant-type homozygote (28%), and the mutant allele frequency was 44%.[15] Mutant allele frequency of terriers in Korea is low in comparison with Belgium and Australia (Table 2). This likely is because introduction of Bedlington terriers is in its early stage in Korea and the population is much smaller than in other countries. In addition, selective introduction by a genetic test has been carried out when breeding imported dogs. Although the population is small, most of the dogs tested in the present study were privately owned from all parts of the country and did not share a common family tree. Therefore, we believe results represent current mutant allele frequency in the country.

The domestic incidence of heterozygotes is similar or higher than that of other countries, which suggests that some of the terriers introduced were not tested or were tested by inaccurate methods. Because breeding between heterozygotes can produce a mutant-type homozygote with a 1-in-4 chance, establishment of a selective breeding program by a reliable genetic test is necessary. In the case of other countries, mutant gene frequency has been maintained at a low level by excluding terriers with the genetic defect from breeding.[16] However, a senseless breeding strategy by widespread DNA testing could cause a reduction of genetic diversity in the Bedlington terrier population in Korea.[17] To ensure genetic health of the population, breeders should use quality carrier dogs for breeding with wild-type homozygous dogs. After the breeding, they can replace the carrier parents with quality normal offspring to safeguard the gene pool and exclude recessive genes.

In study, no terriers of mutant-type homozygote group had CBC abnormalities, and this finding is consistent with previous studies.[11,21] The CBCs of each group were normal, and intravascular hemolysis,

Table 1. Results of serum chemistry related to liver function in the each group.

			ALT (10–100 U/L)		GGT (2–10 U/L)		Tbil (0.1–0.7 mg/dL)		TP (5.5–7.8 g/dL)	
Group	Classification	Heads (n = 46)	Median	Range	Median	Range	Median	Range	Median	Range
Wild	≤2 years	10	42	16–108	6	5–10	0.3	0.2–0.8	6.9	5.4–8.0
	>2 years	10	59	39–166	7	5–10	0.3	0.1–0.6	6	5.2–8.0
Hetero	≤2 years	10	51.5	18–96	7	5–11	0.2	0.1–0.5	6.2	6.0–6.8
	>2 years	10	58.5	26–306	7.5	3–15	0.2	0.1–0.7	6.6	5.9–8.2
Mutant	≤2 years	3	79	71–141	4	1–6	0.2	0.1–0.4	6.5	6.1–7.0
	>2 years	3	348*	280–500	5	2–18	0.5	0.2–0.8	6.1	6.0–7.1

Wild, wild-type homozygote; Hetero, heterozygote; Mutant, mutant-type homozygote.
*$P < .0001$.

Table 2. Prevalence of the exon 2 deletion of *COMMD1* for Bedlington terriers in Korea and the other countries.

		Genotype No. (%)			
Country	Total Heads	Wild-Type Homozygote (*COMMD1* +/+)	Heterozygote (*COMMD1* +/-)	Mutant-Type Homozygote (*COMMD1* −/−)	Mutant Allele Frequency
Korea	105	52 (49%)	47 (45%)	6 (6%)	28
Belgium[16]	51	10 (20%)	24 (47%)	17 (33%)	57
Australia[15]	147	60 (40%)	47 (32%)	42 (28%)	44

hemoglobinemia, or hemoglobinuria was not detected. However, anemia and hemolysis may not be observed depending on the stage of the disease.[11] Although there were only 3 terriers in the mutant-type homozygote group >2 years of age and the results had a large range, a significant increase in ALT activity was observed. Because ALT is largely distributed in the cytoplasm of the hepatocyte, leak into the circulation could occur due to hepatocellular damage caused by the accumulation of copper in a few days. Despite considerable controversy,[22–24] increased ALT activity of affected terriers is known to have diagnostic value with a sensitivity of 34–45%.[10] There is no widely recognized diagnostic cutoff value for CT-BT, but affected terriers showed increases in ALT activity, and the mean, median value, and range were 234.2, 107, and 9-1378, respectively, in a previous study.[21] Although the increase in ALP activity was also observed in the mutant-type homozygote >2 years of age, there was no statistical significance and little correlation with disease status.

Although the number of affected terriers was small, the survival probability of the mutant-type homozygous group was 0.67 over 2.5 years and 0.5 over 4 years in the present study. Four terriers in the affected group died between ages 2 and 4 years and were diagnosed with liver failure at local animal hospitals. Because we could not examine or treat them directly, the omission of histopathological data is a limitation of this study. There have been no reports about the survival probability of CT-BT, and the prognosis is known to vary depending on the progression of the disease.[10,25] Because affected terriers showing mild or moderate clinical manifestations can respond to supportive care and have an improved prognosis, it is important to make an early diagnosis.[25] In the present study, 2 terriers (samples No. BT0917 and BT1210) in the mutant homozygote group were tested at an early age before showing clinical signs. They have been fed a commercial low copper diet after genetic diagnosis. Dog BT0917 was alive and 4.5 years old, but the current condition is unknown. Dog BT1210 is 4.1 years old and alive as of this writing. Although it could not be conducted in the present study because of the small number of affected terriers, an age-matched cohort study for disease progression and survival would provide important clinical information about CT-BT. Because CT-BT is a genetic disorder characterized by high mutant allele frequency and asymptomatic condition of the heterozygote, eradication is difficult and complicated. Therefore, a reliable genetic test-based breeding program is necessary and important to decrease the prevalence of CT-BT in Korea. Although the *COMMD1* gene mutation is not the single cause of CT-BT,[19] study demonstrates the prevalence and clinical relevance of CT-BT in Korea and helps us to understand the worldwide distribution and clinical importance of the *COMMD1* gene mutation.

Footnotes

[a] G-DEXIIb, iNtRON Biotechnology, Sungnam, Gyunggi, Korea
[b] NanoVue, GE Healthcare Bio-Science, Pittsburgh, Pennsylvania
[c] TP600 cycler, Takara Bio Inc., Kusatsu, Shiga, Japan
[d] RedSafe, iNtRON Biotechnology, Sungnam, Gyunggi, Korea
[e] MEGA-bead Agarose gel, iNtRON Biotechnology, Sungnam, Gyunggi, Korea
[f] pCR2.1-TOPO vector, Invitrogen, Grand Island, New York
[g] Plasmid Mini Kit, Qiagen, Valencia, California
[h] ABI 3730XL, Applied Biosystems, Grand Island, New York
[i] Hematology Analyzer, NIHON KOHDEN, Shinjuku-ku, Tokyo, Japan
[j] VETSCAN, ABAXIS, Union City, California

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Conflict of Interest Declaration: The authors declare no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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