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The Exon 2 Deletion of the *COMMD1* Causing Copper Toxicosis in Bedlington Terriers in Korea

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Abstract : This study was performed to survey prevalence of *Copper metabolism domain containing 1* (*COMMD1*) mutation using molecular diagnostic method in a population of Bedlington terriers in Korea. *COMMD1* gene (formerly *MURR1*) functions as a regulator of sodium transport and copper metabolism. The deletion of exon 2 of the *COMMD1* gene causes copper toxicosis in Bedlington terriers. Bedlington terriers with this autosomal recessive disorder were shown to have the elevated liver copper levels due to genetic derangement in the biliary copper excretion pathway. DNA samples were extracted from whole blood collected from 257 Bedlington terriers (109 males, 148 females) of pet dog clubs in Korea. A multiplex PCR was carried out to detect of exon 2 deletion of *COMMD1* gene. In this study, it was possible to know the existence and prevalence of exon 2 deletion of *COMMD1* in Bedlington terriers in Korea. Of the 257 samples, 131 (51%) were wild type homozygous for the normal *COMMD1* gene, 108 (42%) were heterozygous, having both normal and mutated copy of the *COMMD1* gene. The eighteen (7%) were mutant type homozygous. The results of genetic analysis could help establish proper management strategy and selective breeding program to prevent *COMMD1* mutation in Bedlington terriers in Korea.

Key words **:** Copper toxicosis, Bedlington terriers, *COMMD1* mutation.

Introduction

The deletion of exon 2 of the *Copper metabolism domain containing* (*COMMD1*) gene (formerly *MURR1*) causes copper toxicosis in Bedlington terriers (CT-BT). *COMMD1* encodes a protein that functions as a regulator of copper metabolism (3,14). CT-BT is characterized by a progressive accumulation of copper in the hepatocellular lysosomes (11,17). Although copper is an essential micronutrient, excessive copper can be highly toxic. Excessive free copper ions can make free radicals by Haber Weiss reaction (1). Affected terriers suffer from hepatitis, progressive cirrhosis of the liver and even death in severe cases (7,16).

Traditional diagnostic method of copper toxicosis is liver biopsy. Liver copper levels can be measured either quantitatively or semi-quantitatively using various methods. But clinical signs of affected terriers have not been obvious until liver copper levels exceed 2000 μg/g dry weight (16). Although liver biopsy has traditionally been the definitive diagnostic tool, it has many practical and clinical problems (5). Alternatives to liver biopsy are the molecular diagnostic methods. In 1997, a microsatellite marker linked to the CT-BT, C04107, has been reported in the USA (18). However, the marker alone cannot make a reliable diagnosis since recombination between the marker and the disease gene has been existed (2,5,6,8). Another molecular diagnostic approach is a detection of the exon 2 deletion using a multiplex PCR (4). It is not only simple, rapid and robust method, but also reliable way to detect *COMMD1* mutation.

Genetic tests are widely used for diagnosis of CT-BT worldwide, including the United States of America, Europe, and Australia (4,12,13). It is important and necessary to verify the existence of same genetic defect or frequency since the Bedlington terrier population in Korea originates from Europe and Australia. However there is no public report on the molecular diagnosis in the Asian Bedlington terrier population, including Korea. In the present study, we conducted molecular diagnosis of the exon 2 deletion of the *COMMD1* in Korean Bedlington terriers and evaluated prevalence of the mutation.

Materials and Methods

Animal blood sample collection & DNA extraction

Blood samples for PCR were obtained from 257 Bedington terriers (109 males and 148 females), aged between 2 months and 11 years, of pet dog clubs in Korea. Signalment and pedigree information of animal samples were obtained from the owners. Three milliliters of blood was sampled by veinpuncture and stored in EDTA-coated tubes. DNA was extracted from blood samples using the G-DEXIIb genomic DNA extraction kit (iNtRON Biotechnology, Seongnam, Korea) as per the manufacturer's protocol. The concentrations of all DNA samples were normalized using NanoVue spectrophotometer (GE Healthcare Bio-Science, Piscataway, USA).

Multiplex PCR and sequence analysis

The multiplex PCR was performed in a TP600 cycler

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(Takara Bio Inc., Otsu, Japan) using 20 μl reactions containing 50 ng of genomic DNA as template, 1U Top-Taq DNA polymerase (CoreBio, Guro-dong, Korea), 200 μM dNTPs (CoreBio), 1X Top-Taq buffer (CoreBio) and 10 pmol of each oligonucleotide primer. A thermal program and sequence of primers were described by Forman *et al*. (2005); three pairs of primers were designedfor PCR for agarose gel visualization. Primers for characterization of the normal gene amplify DNA fragments from inside the deleted region. And the primers for characterization of the mutated gene amplify DNA fragments across the deletion break points. A positive, non-deletion control, amplify part of intron 2. Products were visualized by gel electrophoresis with ethidium bromide staining.

The PCR products of wild type homozygous, heterozygous and mutant type homozygous positive samples were purified from gel using MEGA-bead Agarose gel extraction kit (iNtRON Biotechnology) and these purified products were ligated with pCR2.1-TOPO vector (Invitrogen, Carlsbad, USA). *Escherichia coli* DH5α cells were transformed with the ligation products and isolation of the plasmid was performed with Plasmid Mini kit (Qiagen, Maryland, USA). The isolated plasmid DNAs were sequenced by ABI 3730XL capillary DNA sequencer (Applied Biosystems, Foster city, USA) at the SolGent inc, Hwaam-dong, Korea.

Results

Screening of the genomic DNA samples extracte from 257 Bedlington terriers for detecting exon 2 deletion of *COMMD1* was carried out by the multiplex PCR. Of the 257 samples, 131 terriers were wild type homozygote (51%) for the normal *COMMD1* gene, 18 samples were mutant type homozygote (7%) for the exon 2 deletion of *COMMD1*. And 108 samples were heterozygote (42%), having both a normal and mutated copy of the *COMMD1* gene (Table 1). On the basis of pedigree information, the pedigree trees of two family group were represented in Fig 1. In the pedigree A, male and female pairs of wild type homozygote and heterozygote were born to heterozygous male and wild type homozygous female. In the pedigree B, three males and female of wild type homozygote and heterozygous male were born to wild type homozygous male and heterozygous female.

Table 1. Prevalence and allele frequencies of the exon 2 deletion of *COMMD1* for Bedlington terriers in Korea

Genotypes	Heads (%)	Mutant allele frequencies
Wild type homozygote (*COMMD1* +/+)	131 (51%)	0.28
Heterozygote (*COMMD1* +/-)	108 (42%)	
Mutant type homozygote (*COMMD1* -/-)	18 (7%)	
Total	257 (100%)	

Disscussion

In this study, we investigate the existence and prevalence of the exon 2 deletion of *COMMD1* in Bedlington terriers in Korea using the molecular diagnostic method. Of the 257 Bedlington terrier samples in Korea, 18 were mutant type homozygote (7%) and the mutant allele frequencies were 0.26. In foreign cases, high prevalence rate of the CT-BT has been reported in many countries. Of the 51 Bedlington terrier samples in Belgium, 10 terriers were wild type homozygote (20%), 24 were heterozygote (47%), 17 were mutant type homozygote (33%) and mutant allele frequencies were 57% (13). And of the 147 samples in Australia, 60 terriers were wild type homozygote (40%), 47 were heterozygote (32%), 42 were mutant type homozygote (28%) and the mutant allele frequencies were 0.44 (12). Although the Bedlington terrier population in Korea originates from Europe and Australia, It is considered low that Prevalence and frequencies of *COMMD1* mutation in Korean population. The reasons are situations on initial Bedlington terriers introduction in Korea

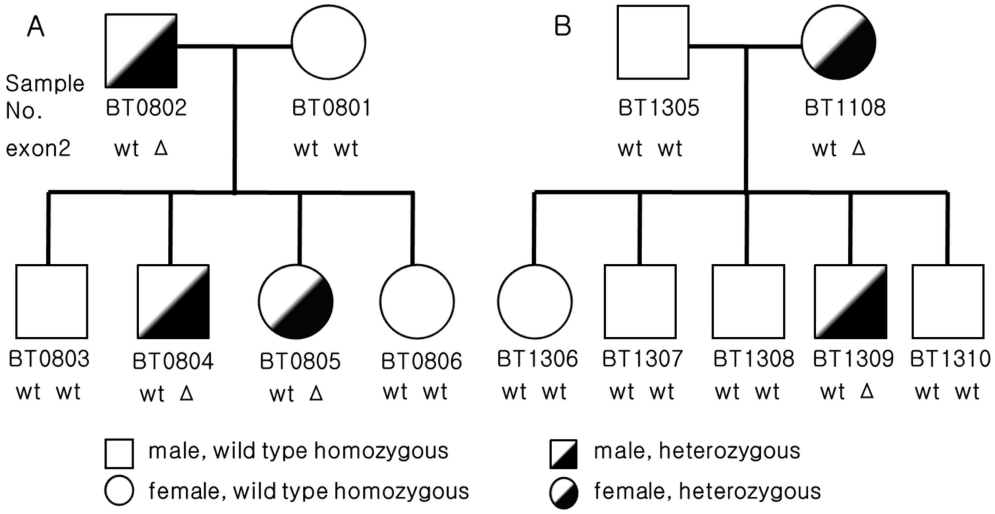

Fig 1. Bedlington terrier pedigrees status of *COMMD1* mutation in dogs used for current study. Legend upper left; sample number, exon2 (wild type [wt], deletion [Δ]).

and selective importation of terriers by high qualified breeders. But there is still the risk of outbreak of CT-BT in Korean population because of high domestic incidence of heterozygote. In addition, recent breeding situation suggests that some of the terriers introduced were not tested or tested by inaccurate methods, e.g. genetic test based on microsatellite marker and it have been reported that some affected terriers did not have the exon 2 deletion of *COMMD1* (9).

Because of asymptomatic clinical stage of heterozygote, the CT-BT has been a difficult problem for world wide breeders to work out. The only ideal long-term solution of the disease is a prevention of inheritance of affected gene by selective breeding program. Although exon 2 deletion of *COMMD1* gene is not the single cause of CT-BT (2), a reliable genetic test-based breeding program is important to decrease of prevalence rate of *COMMD1* mutaion in Korea by excluding terriers with genetic defect from breeding. Since copper toxicosis has been reported in Doberman Pinchers, West Highland White terrier and Skye terrier (5,10,15), genetic screening for these breeds including Korean domestic breeds is necessary to understand distribution of *COMMD1* mutation.

Conclusion

In the present study, we performed genetic screening to diagnosis exon 2 deletion of *COMMD1* in Bedlington terriers in Korea. Of the 257 samples, 131 (51%) were wild type homozygous for the normal *COMMD1* gene, 108 (42%) were heterozygous, having both normal and mutated copy of the *COMMD1* gene. The eighteen (7%) were mutant type homozygous. This result verifies the existence and prevalence of *COMMD1* mutation in Korea and could help establish structured selective breeding program.

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한국 베들링턴 테리어에서 구리중독증을 유발하는 *COMMD1* 유전자의 exon 2 결손변이

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요 약 : 개의 10번 염색체에 존재하는 *Copper metabolism domain containing 1* (*COMMD1*) 유전자는 체내 구리 대사를 조절하는 *COMMD1* 단백질을 합성한다. *COMMD1* 유전자의 exon 2 결손변이는 단백질의 결핍을 유발하여 베들링턴 테리어 견종에서 상염색체 열성 유전질환인 구리 중독증을 일으킨다. 본 증에 이환된 개체는 담즙을 통한 구리의 배설이 저해되어 간 내에 구리가 축적된다. 본 연구에서는 국내 베들링턴 테리어 257두(수컷 109두, 암컷 148두) 혈액 시료를 사용하여 genomic DNA를 추출하였다. 유전자 결손변이의 분자생물학적 진단을 위해 다중 중합효소연쇄반응법(multiplex PCR)을 이용하여 *COMMD1* 유전자의 exon 2 결손 발생 및 그 빈도를 조사하였다. 베들링턴 테리어 257두에서, 정상유전자 동협접합자가 131두(51%), 이형접합자가 108두(42%), 변이유전자 동형접합자가 18두(7%)로 확인되었다. 본 연구를 통해 한국 베들링턴 테리어 개체군의 유전변이 발생 및 그 빈도를 확인하였고, 이는 국내 베들링턴 테리어 개체군의 유전자 선택적 교배계획 설립 및 변이 유전자 확산을 예방하기 위한 기초 자료로서 의의가 있다.

주요어 : 구리중독증, 베들링턴 테리어, *COMMD1* 유전변이