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ORIGINAL RESEARCH

Copper toxicosis in Bedlington terriers is associated with multiple independent genetic variants

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Abstract

Background: Bedlington terrier copper toxicosis (CT) is due to a homozygous exon deletion in *COMMD1*. CT also occurs in Bedlingtons lacking this deletion. An association with two *ABCA12* single nuceotide polymorphism (SNP) splice variants was reported. Labrador retriever CT is associated with a missense mutation in *ATP7B*, and with a protective mutation in *ATP7A*.

Methods: Liver and DNA samples from 24 affected and 10 unaffected Bedlingtons were assessed for copper and genetic variants. Allelic frequencies were compared. The *ATP7B* mutation frequency was investigated in 144 dogs of other breeds.

Results: The *ABCA12* SNPs showed no differences between groups. The *COMMD1* deletion was less frequent in unaffected than in affected dogs and in affected dogs post-2001 than pre-2001. The *ATP7B* mutation was more frequent in affected than unaffected Bedlingtons. Thirty-five of 144 dogs of other breeds were homo- or heterozygous for the *ATP7B* mutation. The *ATP7A* mutation was absent from Bedlingtons.

Limitations: Clinical information and qualitative copper measurements were unavailable for most dogs.

Conclusion: The *COMMD1* deletion remains present in Bedlington terriers but is no longer the primary cause of CT. *ABCA12* SNPs were not associated with CT. The *ATP7B:c.4358G>A* mutation was significantly associated with Bedlington CT and was more common in dogs of this breed than in the 144 dogs of other breeds.

INTRODUCTION

Copper toxicosis (CT), in which excess copper builds up in the liver resulting in hepatitis and cirrhosis in severely affected cases, was first diagnosed in Bedlington terriers in the USA in 1975[1] and subsequently in Australia,[2] the Netherlands,[3] Finland[4] and the UK.[5] The disease presents as an excess accumulation of copper in the liver with a centrilobular distribution.[6] The prevalence in this breed was very high in the 1970s and 1980s, ranging from 26% to 46% in different Bedlington terrier populations.[5] CT was found to be inherited as an autosomal recessive trait causing a defect in the biliary excretion of copper into the bile.[7,8] It was hoped that the Bedlington terrier disease would be an analogue of Wilson's disease, a genetic disorder in humans mapped to a copper-transporting P-type adenosine triphosphatase, ATP7B. However, clinically, Wilson's disease causes periportal hepatic accumulation of copper and affects other organs, including the eye (causing Kayser–Fleischer rings) and the CNS,[9] whereas the disease in Bedlington terriers is confined to the liver, where copper accumulates centrilobularly. In addition, humans with Wilson's disease have low circulating serum ceruloplasmin, whereas Bedlingtons with CT have normal levels.[8]

In 1998, in the first whole genome linkage analysis study with microsatellite markers in dogs, CT was found to be linked tomicrosatellite marker C04107.[10,11] A DNA test based on this marker was soon commercially available to help breeders reduce CT incidence in the breed. The allele associated with CT was denoted as allele 2, so affected dogs were expected to be homozygous for allele 2 (C04107[2-2]). In 2002, further studies mapped the disease to *COMMD1* on *Canis familiaris* autosome (CFA, i.e., dog chromosome) 10 (previously called *MURR1*) and

associated it with a large exon deletion (*COMMD1*$^{del/del}$), which leads to a truncated protein of 94 amino acids rather than the usual 188.[12,13] With widespread testing of breeding dogs, first for the microsatellite marker and then for the *COMMD1* deletion, selective breeding resulted in a dramatic fall in Bedlington terrier CT. However, the disease association with *COMMD1*$^{del/del}$ had never been 100% concordant, and this became more obvious as the *COMMD1* mutation was reduced in the population. CT was identified in dogs that were not C04107[2-2] in the UK[14] or *COMMD1*$^{del/del}$ in the USA[15] and Australia,[16] suggesting the existence of more CT-causing genetic mutations in this breed.

A genome-wide association study (GWAS) utilising 30 *COMMD1*$^{+/+}$ Bedlington terriers from the UK, comprising equal numbers of histologically confirmed CT-affected dogs and controls, identified a region on CFA 37 that was significantly associated with disease. Deep sequencing of the candidate region revealed two SNP splice variants in *ABCA12* that were both significantly associated with *COMMD1*$^{+/+}$ Bedlington terrier CT.[17] *ABCA12* codes for an ATP-dependent pump involved in divalent metal ion transport. This study only investigated Bedlington terriers that were *COMMD1*$^{+/+}$. Therefore, the relationship between the CFA 10 *COMMD1* deletion and the CFA 37 *ABCA12* splice sites as putative causes of CT in the breed remains unclear.

Copper storage disease is also recognised in other dog breeds, but the *COMMD1* deletion is currently confined to Bedlington terriers. There is a high prevalence of copper storage disease in Labrador retrievers in the Netherlands[18] and the USA.[19,20] A recent GWAS in 235 Labradors identified two SNPs on CFA 22 significantly associated with disease in the region of *ATP7B*. Subsequent Sanger sequencing identified a missense mutation in *ATP7B* (*ATP7B:c.4358G>A*, which results in ATP7B:p.Arg1453Gln), which was correlated with increased hepatic copper levels and showed functional significance in a hepatic cell line. Conversely, a canine *ATP7A* mutation (*ATP7A:c.980C>T*) on the X chromosome was also identified, which was negatively correlated with copper accumulation and showed functional significance in canine fibroblasts.[21] The association with clinical copper accumulation was not inherited in a simple autosomal manner (unlike the *COMMD1* deletion in Bedlington terriers). *ATP7B:c.4358G>A* appeared to have an additive effect, such that homozygotes accumulated more copper than heterozygotes. These effects were reduced by the concurrent presence of *ATP7A:c.980T* but with a more marked protective effect in male dogs, likely because it is X-linked and apparently explains the female predisposition to disease in Labradors. One small study of *ATP7B* polymorphisms in 10 closely related Bedlington terriers that were *COMMD1*$^{+/+}$ or *COMMD1*$^{+/del}$, published in 2008, found no apparent association between CT and *ATP7B* polymorphisms.[22] However, the authors recommended further studies, which have not been undertaken to date.

Copper storage disease in dogs represents an interaction between their genetic make-up and environment—in particular, the copper content of their diet. There is evidence that the amount of available copper in dog food has increased from 1980 to the present, with the amount of copper that is considered 'normal' in the livers of dogs both with and without hepatitis also increasing over this time period.[23,24] Changes in dietary copper concentrations as well as genetic make-up may also have impacted on the disease prevalence in *COMMD1*$^{+/+}$ Bedlington terriers over this time period.

One of the authors (S. H.) has maintained an ongoing relationship with Bedlington terrier breeders in the UK and Canada, which has enabled a unique collection of DNA samples and liver biopsies to be generated over a prolonged period of time. We aimed to use these samples to try to increase our understanding of the frequency and clinical significance of additional mutations. The aims of the current study were thus as follows:

1. To investigate the frequency of the two previously published *ABCA12* SNPs in Bedlington terriers with histologically confirmed CT, both with and without the *COMMD1* deletion, and compare with Bedlington terriers with histologically normal livers.
2. To investigate the frequency of *ATP7B:c.4358G>A* and *ATP7A:c.980C>T* in Bedlington terriers with histologically confirmed CT, both with and without the *COMMD1* deletion, and compare with Bedlington terriers with histologically normal livers.
3. To investigate whether there has been a change in the frequency of these three mutations over the last 25 years.

In addition, whole-genome sequencing (WGS) data provided by the Kennel Club Genetics Centre (KCGC) from 144 different dogs, representing 66 breeds (including four crossbreeds) were interrogated for the *ATP7B:c.4358G>A* allele.

MATERIALS AND METHODS

Bedlington terriers were included in this study if they had both histological sections of liver and DNA samples available. All sections were taken as surgical liver biopsies as part of routine clinical work-up of suspected liver disease, which was clinically justified. DNA was extracted from EDTA blood either submitted for genetic screening for breeding dogs or left over from diagnostic samples taken during clinical investigations. Fully informed owner consent was obtained for all biopsies and blood samples. Clinical details, including serum liver enzymes and dietary details, were not available for most dogs because samples were collected by a number of veterinary surgeons over a prolonged period. Liver biopsies and blood samples collected from dogs prior to 2001 were donated to one of the authors (S. H.) for the study. Pedigree information was not available for most dogs. The date of blood sampling was available for most Bedlington terriers. Where not available, date of DNA analysis or date of birth (obtained from the Kennel Club Health Tests

Results webpage: www.thekennelclub.org.uk/search/health-test-results-finder/) was recorded.

Liver samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin and rhodanine stain for copper. Copper was quantified in only one case at Colorado State University using atomic absorption spectroscopy. In all other cases, histological sections were assessed by a veterinary histopathologist (S. H.) with 37 years of experience of CT in Bedlington terriers who assigned each dog to either affected or unaffected groups, blinded to the genetic results. Dogs were considered to be CT-affected if their livers contained histochemically demonstrable copper in hepatocytes in zone 3 with concurrent chronic hepatitis, consistent with a liver copper content of more than 400 µg/g Cu dry weight, as previously described.[6] Fibrosis was present in some dogs but not others. In progressive disease, fibrosis became bridging, leading to cirrhosis. Dogs were considered unaffected if livers lacked histochemically demonstrable copper apart from occasional scattered cells.[6]

Blood samples from affected and control dogs were sent to Animal DNA Diagnostics and genotyped by one of the authors (J. S.). DNA was extracted from blood samples using a commercially available kit (E.Z.N.A. Blood DNA Mini Kit from Omega Bio-Tek) according to their instructions. Extracted DNA was then assessed for the presence of previously CT-associated variants in *COMMD1*[12] using fragment length polymorphism and *ATP7B*, *ATP7A*[21] and *ABCA12*[17] using direct sequencing. Allelic frequencies in cases and controls were compared using Fisher's exact test using an online calculator www.socscistatistics.com/tests/fisher/default2.aspx.

WGS data from 144 different dogs, representing 66 breeds (including four crossbreeds) were aligned against the CanFam4 reference genome,[25] and variants were called per sample and then consolidated to joint-call across all samples according to GATK Best Practices recommendations.[26,27] The variant call format file was analysed for the *ATP7B:c.4358G>A* variant (rs851958524).

RESULTS

Histological liver sections and paired DNA were available from 24 affected dogs (six taken pre-2001 and 18 post-2001) and 10 control dogs without CT (all post-2001). Numbers were limited by the requirement to have paired samples. All the cases sampled pre-2001 were from the UK. Dogs sampled post-2001 and control dogs were all from the UK apart from one control dog from Canada, one affected dog from the USA, one affected dog from Finland and two unaffected dogs from Canada (Table 1). The most recently affected and unaffected dogs were sampled in May 2021. One affected dog post-2001 had copper measured quantitatively at 1385–1520 ppm. This dog was homozygous affected for the *ATP7B* mutation but homozygous wild type for the other alleles.

Table 1 shows the results for *COMMD1* deletions, *ABCA12* SNPs and *ATP7B* mutations in all dogs in three groups (affected pre-2001; affected post-2001 and unaffected with CT). All affected dogs were homozygous for either the *COMMD1* deletion or the *ATP7B* variant. Two of the 10 normal dogs were wild type for all genes investigated, none was homozygous for the *COMMD1* deletion and only three were homozygous for the *ATP7B* variant. *COMMD1* deletions became less frequent with time but remain present in the UK Bedlington terrier population. In CT-affected dogs, all six Bedlingtons tested pre-2001 were $COMMD1^{del/del}$, whereas in the 18 post-2001 dogs, seven were $COMMD1^{del/del}$, three dogs were $COMMD1^{+/del}$ and the other eight dogs were $COMMD1^{+/+}$. The three most recent $COMMD1^{del/del}$ dogs came from the UK and were diagnosed in May 2017, May 2018 and August 2019. In the control population, three dogs were $COMMD1^{+/del}$ and the other seven were $COMMD1^{+/+}$.

Comparing allelic frequency for *COMMD1* deletion between all 24 affected and 10 control dogs, there was a very significant difference with many fewer deletions in the controls ($p = 0.0011$). Significantly fewer CT-diagnosed dogs carried the *COMMD1* deletion post-2001 ($n = 17$) compared to pre-2001 ($n = 6$) ($p = 0.0005$).

There was a significantly higher allelic frequency of *ATP7B* mutations in affected dogs (0.77) compared with unaffected dogs (0.45) ($p = 0.021$), but there was no significant difference in *ATP7B* between dogs seen pre- and post-2001 ($p = 0.705$), which is also clearly visible in Table 1.The affected dog from Finland was homozygous *ATP7B:c.4358A*. All the dogs were tested for the *ATP7A* mutation except for one control case, which did not have a suitable sample. All dogs only carried the *ATP7A:c.980C* allele and were therefore negative for the protective mutation.

The allelic frequency of the *ATP7B* mutation in the KCGC crossbreed dataset of 144 samples from 66 different breeds was 0.135. Four samples were homozygous for the *ATP7B:c.4358G>A* variant, and a further 31 samples, from 25 different breeds, carried a single copy of the mutant allele. Table 2 shows the breeds in the dataset along with counts of samples for each *ATP7B:c.4358G>A* status. The homozygous alternative (non reference) allele samples had been submitted for other research studies and none of those dogs were reported to be affected with CT.

The two *ABCA12* SNPs were seen to be widespread in Bedlington terriers and there was no obvious difference between affected and control dogs or pre- and post-2001 dogs. Only two dogs in the study (both affected, post-2001) were also included in the previously published study (see Table 1). Both dogs were homozygous for the *ABCA12A* SNP and one was homozygous and one heterozygous for the *ABCA12B* SNP. Seven out of the 24 affected dogs were negative for both SNPs (one pre-2001 and six post-2001), and four out of 10 unaffected dogs were negative for both SNPs. There appeared to be an even distribution of affected and unaffected SNPs across both groups. This lack of

TABLE 1 Genotype analysis of study Bedlington terrier dogs separated into groups: pre-2001 copper toxicosis (CT) affected ($n = 6$), post-2001 CT affected ($n = 18$) and CT unaffected (controls) ($n = 10$).

	Date of blood taken or date of tested or DOB	COMMD1		ABCA12				ATP7B	
				ABCA12B		ABCA12A			
Affected Pre-2001									
UK	DOB 1993	del	del	A	A	C	C	G	G
UK	DOB Oct 1996	del	del	A	A	C	C	A	A
UK	DOB Oct 1996	del	del	A	A	C	C	A	A
UK	DOB Jan 1998	del	del	A	A	C	C	A	A
UK	DOB Sept 2000	del	del	G	A	A	C	A	A
UK	DOB Sept 2000	del	del	G	G	A	A	A	A
Affected Post-2001									
Canada	Unknown but post-2001	del	del	G	A	A	C	G	G
UK	Unknown but post-2001	wt	wt	G	G	A	A	A	A
UK	Blood taken Feb 2008	wt	wt	A	A	C	C	A	A
UK*	Blood taken 2008	wt	del	G	A	C	C	A	A
UK*	Blood taken 2008	wt	wt	A	A	C	C	A	A
UK	Blood taken April 2010	wt	wt	G	G	A	A	A	A
UK	Blood taken 2014	wt	wt	G	G	A	A	A	A
UK	Tested Aug 2016	del	del	G	A	A	C	G	A
UK	Tested Aug 2016	del	del	A	A	C	C	G	G
USA	Blood taken May 2017	del	del	G	A	A	C	G	A
UK	Blood taken Aug 2017	del	del	G	G	A	A	A	A
UK	Blood taken Dec 2017	wt	del	G	A	A	C	A	A
UK	Blood taken Dec 2017	wt	wt	G	A	C	C	A	A
UK	Blood taken May 2018	del	del	G	A	C	C	G	G
UK	Blood taken 2019	wt	wt	G	G	A	A	A	A
UK	Blood taken April 2019	wt	wt	G	A	A	C	A	A
UK	Blood taken Aug 2019	del	del	G	A	A	C	G	A
Finland	Blood taken May 2021	wt	del	G	G	A	A	A	A
Controls									
UK	Blood taken 2018	wt	del	G	A	A	C	A	A
UK	Blood taken Feb 2018	wt	wt	G	G	A	A	G	G
UK	Blood taken Oct 2018	wt	wt	G	A	A	C	G	A
UK	Blood taken Jan 2019	wt	wt	G	A	A	C	G	G
UK	Tested Nov 2019	wt	wt	G	G	A	A	G	G
UK	Blood taken Jan 2020	wt	del	A	A	C	C	G	G
UK	Blood taken Jan 2020	wt	wt	G	A	A	C	G	A
UK	Blood taken Nov 2020	wt	del	G	A	A	C	G	A
Canada	Blood taken May 2021	wt	wt	G	G	A	A	A	A
Canada	Blood taken May 2021	wt	wt	G	G	A	A	A	A

Note: The two Bedlington terriers also included in Haywood et al.[17] are each indicated by an asterisk.
Abbreviations: del, deletion; DOB, date of birth; wt, wild type.

difference between affected and unaffected dogs for both SNPs was confirmed with Fisher's exact test (for A, $p = 0.1826$; for B, $p = 0.2957$).

DISCUSSION

This is the first study to investigate the genetic background of Bedlington terriers with CT over a long period of time, facilitated by one of the authors' life-long involvement in the disease and ongoing relationship with Bedlington terrier breeders in the UK and Canada. This study clearly demonstrates a reduction in the *COMMD1* deletion over time, facilitated by selective breeding. Selective breeding started in 1998 after identification of the microsatellite marker associated with CT in Bedlington terriers,[10,11] so the comparison of dogs pre- and post-2001 is very informative. One author has previously suggested that the *COMMD1* deletion has now been eradicated in

TABLE 2 Dog breeds available in the crossbreed dataset of 144 samples, showing counts of homozygous wild type, heterozygous and homozygous alternative genotypes for the *ATP7B:c.4358G>A* variant

Breed	Homozygous wild type (GG)	Heterozygous (GA)	Homozygous affected (AA)	Total number of dogs
One each of: Affenpinscher, Beagle, Bull Terrier, Cesky Terrier, Chow Chow; English Setter; French Bulldog; Glen of Imaal Terrier; Great Dane; Havanese; Irish Terrier; Japanese Akita Inu; Lakeland Terrier; Maltese; Norwich Terrier; Pyrenean Mountain dog; Retriever (Chesapeake Bay); Rottweiler; Shetland Sheepdog; Spaniel (American Cocker); Spaniel (Clumber); Spaniel (field); Tibetan Spaniel; West Highland White Terrier	24			24
One each of: Collie (Rough); Doberman; German Spitz (Mittel); German Wirehaired Pointer; Irish Setter; Lancashire Heeler; Mexican Hairless Dog (Xoloitzcuintli); Lhasa Apso; Picardy Sheepdog; Retriever (golden); Retriever (Nova Scotia Duck Tolling); Russian Black Terrier; Shih Tzu; St. Bernard		14		14
One each of: Cavalier King Charles Spaniel; Papillon			2	2
Airedale Terrier	1	1		2
Basset Hound	16			16
Bearded Collie	2			2
Border Collie	5	2		7
Border Terrier	12			12
Briard	1	1		2
Cairn Terrier	3			3
Chinese Crested	2	1		3
Crossbreed	2	2		4
Dachshund (Miniature Long Haired)	2			2
Dachshund (Miniature Wire Haired)	2			2
Dandie Dinmont Terrier	4			4
English Shepherd	2			2
Italian Spinone	3	1		4
Keeshond	1	3		4
Leonberger	1	1		2
Norwegian Buhund	1	2		3
Retriever (Labrador)	4	2		6
Scottish Terrier	5			5
Shar Pei	3			3
Siberian Husky	2		1	3
Skye Terrier	2			2
Soft Coated Wheaten Terrier	2			2
Spaniel (Cocker)	3	1		4
Tibetan Terrier	2		1	3
Whippet	2			2
Total number of dogs	109	31	4	144

Bedlington terriers by selective breeding,[28] but this study demonstrates that it remains present in the breed, albeit at a reduced frequency, and Bedlington terriers *COMMD1*$^{del/del}$ were identified in the UK for this study as recently as 2 years ago. More than half of the Bedlington terriers diagnosed with CT after 2001 were *COMMD1*$^{+/+}$. A commercial test is available for this deletion, and Bedlington terrier breeders in the past have assumed that *COMMD1*$^{+/+}$ dogs would not develop CT. The current study confirms that this is not true, supporting previous studies in Finnish dogs.[15]

The finding that *ATP7B:c.4358A*, originally identified in Labrador retrievers,[21] was also common in affected Bedlington terriers was unexpected. A study in 1999 excluded the involvement of *ATP7B* in Bedlington terriers on the basis of gene mapping.[29] A subsequent small study of nine related Bedlington terriers from one extended Finnish pedigree failed to find any obvious association.[22] The current study is the first to investigate this polymorphism in a larger number of Bedlington terriers over different pedigrees and time periods. The results suggest that *ATP7B* mutations may play an important role in CT in *COMMD1*$^{+/+}$ Bedlington terriers because of the high frequency of the mutated allele in affected dogs compared with control normal Bedlington terrier dogs, including homozygous *ATP7B:c.4358A* in all but one affected *COMMD1*$^{+/+}$ dog. It is interesting to note that all but one of the dogs tested prior to 2001 were also homozygous for *ATP7B:c.4358A* as well as the *COMMD1* deletion, suggesting that this potential causative mutation was always present in the breed and became more dominant as the *COMMD1* deletion was selectively bred out of the Bedlington terrier population. This suggests that selective breeding to remove one genetic cause of copper storage disease has led to the discovery of other genes involved in the disease in the breed, which is a cautionary tale for selective breeding programmes. The single Finnish dog in the current study was also homozygous for *ATP7B:c.4358A*. The finding of three unaffected Bedlingtons that were homozygous for the mutation is not unexpected, as this has been reported previously in Dobermans and Labradors, where the mutation is associated with increased hepatic copper accumulation but only contributes to a proportion of the inheritance of copper storage disease in the breeds.[21,30] Its expression is likely affected by dietary copper concentrations and other modifier genes. *ATP7A* is one such modifier gene reported in Labradors. None of the Bedlington terriers in the current study carried the protective *ATP7A:c.980C>T* mutation.

This is the first study to report the frequency of the *ATP7B:c.4358G>A* allele in a large number of dogs from a variety of breeds in the UK. Analysis of WGS data from 144 dogs of 66 different breeds indicates that the *ATP7B:c.4358G>A* allele is a common variant and segregates in multiple dog breeds, including many that have not been reported to have an increased prevalence of CT. Of note, two out of four crossbreeds were heterozygotes, and one Cavalier King Charles Spaniel was homozygous, a breed very recently reported with copper storage disease in the UK and Israel.[31] This suggests that if the allele is in fact a risk factor for CT, additional risk factors (genetic or environmental) likely play a role in the development of disease, and these additional risk factors might vary between breeds. Until additional, breed-specific risk factors are better understood, it is unwise to use selection against *ATP7B:c.4358G>A* as a means to reduce the prevalence of CT in any breed for which association with disease has not been demonstrated.

The roles of ATP7B and ATP7A are well described. They are large transmembrane proteins that actively transport copper across cellular membranes.[32] COMMD1 is involved in many protein degradation pathways by its involvement in the ubiquitin–proteosome pathway and is reported to downregulate ATP7B by facilitating its degradation.[32]

This study failed to confirm a disease association between CT and the two *ABCA12* SNPs associated with Bedlington terrier CT in a previous study of 15 affected and 15 controls, none of which was homozygous *COMMD1*$^{del+/del+}$.[17] The current study showed that these SNPs were widespread in both affected and control dogs and over both time periods, with no obvious association with disease. In fact, it would be possible to explain disease association in all dogs in the current study either by *COMMD1*$^{del/del}$ or homozygosity for *ATP7B:c.4358A*. This does not rule out a contribution of *ABCA12* SNPs to CT, which is likely to be polygenic and could also involve contributions from this and other genes not investigated in this study. This finding was surprising given the high segregation of the *ABCA12* SNPs with disease in the previous study, although neither SNP segregated perfectly with disease. In the current study, about one-third of the affected dogs were negative for both SNPs, whereas about a half of unaffected dogs were negative. This might suggest that a difference could be found with increased numbers of cases, although numbers were similar to the previous study. However, two UK blood lines dominated in the previously published study, which may partly explain the difference.

Investigations of the genetics of CT are not straightforward. Copper storage and transport in the liver is a complex process involving a number of enzymes, and the disease is likely to involve a variety of mutations in a variety of genes. In humans, the genetics of Wilson's disease is not simple, and to date, 600 different mutations in *ATP7B* have been identified, including missense or nonsense mutations.[9] Furthermore, disease phenotype also depends on dietary copper concentrations and availability.[23,24] Quantitative copper concentrations in the liver of Labradors both with and without chronic hepatitis increased significantly between 1980–1997 and 1998–2010.[23] This coincided with regulatory bodies in the USA recommending inclusion of more bioavailable copper in pet food, suggesting increased dietary intake as the cause. The current study investigated Bedlington terriers pre- and post-2001 because one of the authors had a collection of Bedlington DNA from pre-2001.

The concurrent potential increase in dietary copper concentrations after this date may have contributed to the unmasking of additional mutations in Bedlington terriers.

This study has a number of weaknesses inherent in the use of archived blood and tissue samples. There was no information about diet fed in the majority of dogs. The copper concentration was not quantified in the majority of dogs because of the small size of biopsy specimens and probable reluctance and difficulty for first opinion practitioners to access quantification. Nonetheless, CT was diagnosed on the basis of robust histological assessment by a very experienced pathologist. Pedigree information was only available for a small number of dogs, which precluded analysis of the inheritance pattern.

CONCLUSIONS

The *COMMD1* deletion is no longer the predominant cause of CT in Bedlington terriers. Testing for the deletion is still indicated prior to breeding, because it remains present in the breed, but copper storage disease cannot be ruled out by demonstrating that a dog is *COMMD1*$^{+/+}$. Liver biopsies are necessary to rule this out.

The mutation in *ATP7B* first identified in Labrador retrievers is widespread in Bedlington terriers and appears to play a role in CT in the breed. Its role may have become more important as the frequency of the *COMMD1* deletion was reduced. There was no evidence of the protective *ATP7A* mutation in the breed. However, the *ATP7B* mutation is surprisingly common on analysis of WGS data from a large number of dogs of a variety of breeds that were not reported to have liver disease.

The role of the *ABCA12* SNPs remains unclear. It is likely that CT in Bedlington terriers and other breeds is a polygenic disease involving an interaction between a number of genes involved in copper transport and dietary copper intake. Future investigations in Bedlington terriers and other dog breeds are indicated to confirm the involvement of *ATP7B*, identify additional genes and investigate the inheritance.

AUTHOR CONTRIBUTIONS

Susan Haywood provided the inspiration for this study and sourced the samples via connections in the breed society. Susan Haywood, June Swinburne, Fernando Constantino-Casas and Penny Watson were involved in the original study design. All five authors wrote and revised the manuscript. Susan Haywood, Fernando Constantino-Casas and Penny Watson collected, processed and examined all the liver pathology samples and co-ordinated DNA collection from cases. June Swinburne undertook the genetic analysis in the Bedlington terriers. Ellen Schofield undertook the analysis of whole-genome sequencing from the Kennel Club Genetics Centre.

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CONFLICT OF INTEREST STATEMENT

June Swinburne is Director of Animal DNA Diagnostics, which offers a commercial diagnostic test for the *COMMD1* deletion. None of the other authors has any conflicts of interest related to this study.

DATA AVAILABILITY STATEMENT

The Bedlington data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The data that support the findings for the other dog breeds in this study are openly available in the European Nucleotide Archive at www.ebi.ac.uk/ena/browser/ (reference number PRJEB36029).

ETHICS STATEMENT

Written consent was given by dog owners for use of liver biopsies and DNA samples in this study. The study was approved by the Department of Veterinary Medicine, University of Cambridge, Ethics and Welfare Committee (CR532).

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