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Deletions in the *COL10A1* gene are not associated with skeletal changes in dogs

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Running head: *COL10A1* deletions do not cause canine skeletal changes

Abstract:

Type 10 collagen alpha 1 (*COL10A1*) is a short-chain collagen of cartilage synthesized by chondrocytes during the growth of long bones. *COL10A1* mutations, which frequently result in COL10A1 haploinsufficiency, have been identified in patients with Schmid metaphyseal chondrodysplasia (SMCD); a cartilage disorder characterized by short-limbed short stature and bowed legs. Similarities between SMCD and short stature in various dog breeds suggested *COL10A1* as a candidate for canine skeletal dysplasia. We report the sequencing of the exons and promoter region of the *COL10A1* gene in dog breeds fixed for a specific type of skeletal dysplasia known as chondrodysplasia, breeds that segregate the skeletal dysplasia phenotype and control dogs of normal stature. Thirteen single nucleotide polymorphisms (SNPs), one insertion and two deletions, one of which introduces a premature stop codon and likely results in nonsense mediated decay and the degradation of the mutant allele product, were identified in the coding region. There appear to be no causal relationships between the polymorphisms identified in this study and short stature in dogs. Although COL10A1 haploinsufficiency is an important cause of SMCD in humans, it does not seem to be responsible for the skeletal dysplasia phenotype in these dog breeds. In addition, homozygosity for the nonsense allele does not result in the observed canine skeletal dysplasia phenotype.

Introduction:

Type 10 collagen alpha 1 (*COL10A1*) is a short-chain collagen of cartilage synthesized by chondrocytes during the growth of long bones (Schmid *et al.* 1985). It is a homotrimer with non-triple-helical regions flanking the central short triple-helical domain (Yamaguchi *et al.* 1991). The coding region of this gene in humans is represented by the

last two of three exons. Exon 2 encodes the amino-terminal non-collagenous domain (NC2) and exon 3 encodes part of NC2, the collagenous domain, and the carboxy-terminal non-collagenous domain (NC1) (Thomas *et al.* 1991).

The *COL10A1* gene has been associated with specific forms of skeletal dysplasia in humans. Skeletal dysplasias are a heterogeneous group of inherited defects of endochondral ossification, the developmental process by which cartilage is converted to bone (Mortier 2001). Approximately 200 different skeletal dysplasias have been identified in humans, ranging in clinical severity from mildly affected short stature to lethal forms (Tuysuz, 2004). One such type of skeletal dysplasia, chondrodysplasia, which affects cartilage and the growth of long bones, results in various forms of disproportionate short stature, or dwarfism. Diagnoses of specific forms of chondrodysplasia are based mainly on radiographs, but the identification of some of the underlying genetic defects have recently enabled biochemical and DNA analyses to be used to confirm radiographic evaluations (Mortier 2001).

Schmid metaphyseal chondrodysplasia (SMCD), a specific type of chondrodysplasia, is known to be caused by mutations in the *COL10A1* gene (Warman et al. 1993). SMCD is an autosomal dominant cartilage disorder (Reichenberger *et al.* 1992) characterized by short-limbed short stature, bowed legs, and a waddling gait (Sawai *et al.* 1998). The majority of reported mutations associated with SMCD are in the region of the gene that encodes the NC1 domain (Bateman *et al.* 2005). It has been shown that COL10A1 haploinsufficiency is a significant molecular cause of SMCD, specifically in patients with nonsense or frameshift mutations (Bateman *et al.* 2003, Chan *et al.* 1998).

Similarities between SMCD and skeletal dysplasia in various dog breeds suggested *COL10A1* as a candidate gene for the canine phenotype. In both species, asynchronous

growth of the radius and ulna result in curvature of the radius and thickening of the bones of the limbs, resulting in the characteristic short-limbed, bow-legged phenotype. Splaying of the metaphyses is also seen in both humans and dogs. SMCD, like canine skeletal dysplasia, is a comparatively mild short stature phenotype with no other associated health problems.

Canine skeletal dysplasias, including chondrodysplasia, are primarily diagnosed by radiograph. Breeds such as Basset Hounds, in which there is no segregation of the phenotype, are considered to be fixed for chondrodysplasia (Stockard 1941). These breeds often experience complications that require treatment or surgery, such as intervertebral disk disease (IVDD), that are directly linked to the chondrodysplastic phenotype (Verheijen *et al.* 1982). Crosses between breeds fixed for chondrodysplasia and dogs of normal stature have demonstrated an autosomal dominant mode of inheritance in these breeds (Stockard 1941).

A condition similar to chondrodysplasia has been observed to segregate in Chesapeake Bay Retrievers, Newfoundlands, and Nova Scotia Duck Tolling Retrievers. The disorder is also characterized by short stature and bowed, thickened front legs but appears to be less severe than chondrodysplasia (Figure 1). The mode of inheritance has not been determined in these breeds. Since this condition is not known to be allelic to canine chondrodysplasia, we refer to it as skeletal dysplasia. Variation in phenotypic expression complicates the diagnosis in the aforementioned breeds and mildly affected individuals are often mistakenly classified as normal. This is important since these individuals appear capable of producing severely affected offspring. In severe cases, the condition can be so debilitating that surgery or euthanasia is required. The current methods of diagnosis by clinical and radiological analysis are effective for diagnosing severe cases, but they are often inconsistent in cases with milder presentations. Understanding the genetic basis of canine

skeletal dysplasias would enable more accurate diagnosis and facilitate genetic counseling to help breeders avoid producing affected dogs.

We report the sequencing of the three *COL10A1* exons as well as 350 base pairs of the promoter region in four dog breeds that are fixed for chondrodysplasia, three breeds that segregate the skeletal dysplasia phenotype and three control dogs of normal stature. Due to the structure of dog breeds, diseases seen within breeds are identical by descent, making it possible to identify the molecular cause for a particular canine disease by evaluating affected individuals within the breed (Ostrander & Kruglyak 2000). Thirteen single nucleotide polymorphisms (SNPs), one insertion and two deletions were identified in the coding region of the *COL10A1* gene. There appear to be no causal relationships between these polymorphisms and skeletal dysplasia in these breeds. One of the identified deletions introduces a premature stop codon, which likely results in nonsense mediated decay and the degradation of the mutant allele product. This deletion was seen in dogs of several different breeds in the heterozygous as well as the homozygous state and does not correlate with the skeletal dysplasia phenotype under investigation.

Materials and Methods:

DNA isolation:

Blood samples were acquired from patients of the University of California at Davis Veterinary Medical Teaching Hospital. Genomic DNA was isolated using the QiaAmp DNA Blood Mini Kit (Qiagen Inc). Additional DNA samples, extracted primarily from buccal swabs, were acquired from the Veterinary Genetics Laboratory at the University of California at Davis. Buccal swab samples from Nova Scotia Duck Tolling Retrievers and Chesapeake Bay Retrievers were obtained from breeders and owners. DNA was extracted

from buccal swabs by adding 500ul of 50 mM NaOH to each swab, heating the sample at 96°C for five minutes and adding 75ul of 1M Tris.

For breeds that segregate the skeletal dysplasia phenotype, samples were obtained from two affected individuals. The affected Newfoundlands that were tested are full siblings, as are the Chesapeake Bay Retrievers. The two Nova Scotia Duck Tolling Retrievers are a severely affected individual and its affected dam.

Primer development:

Canine sequence for exons 2 and 3 was obtained by a cross-species comparison using a discontiguous MegaBLAST of human *COL10A1* to the canine trace archives (http://www.ncbi.nih.gov/genome/guide/dog/). Primers were designed across exon/intron boundaries and are listed in Table 1.

Promoter and exon 1 sequences were obtained from the UCSC genome browser (http://www.genome.ucsc.edu/) by performing a BLAT search with human *COL10A1* promoter and exon 1 sequence. Boundaries for canine exon 1 were determined based on homology with human exon 1 sequence. Primers pF1 and pR1 were used for PCR and sequencing of the promoter region. Primers pF2 and pR2 were used to provide additional sequence in areas that did not provide adequate sequence with the PCR primers (Table 1). Overlapping sequence fragments from these primers were assembled using the ContigExpress function of Vector NTI (Informax).

PCR:

PCR was performed on a GeneAmp PCR system 9700 (Applied Biosystems) with 1µL DNA, 2.5mM dNTP, 0.5U AmpliTaq Gold Polymerase (Applied Biosystems), 1µL 10X Buffer II with 1.5mM MgCl2, and water to 20µl. Amplification parameters were: 95°C

for 12min, 35 cycles of 94^{o}C for 30s, 60^{o}C for 60s, and 72^{o}C for 2min, with a final extension of 72^{o}C for 20min. Products were purified using the QIAquick PCR Purification Kit (Qiagen Inc).

Sequencing:

Purified PCR products were sequenced with the ABI Prism BIGDye Terminator Cycle Sequencing Kit (version 3.1) on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and visualized using Chromas2 (Technelysium). Sequences were obtained from breeds fixed for chondrodysplasia: Basset Hound, Dachshund, Pekinese, Pembroke Welsh Corgi, three individuals affected with skeletal dysplasia: Chesapeake Bay Retriever, Newfoundland, and Nova Scotia Duck Tolling Retriever and two controls of normal stature: a Dalmatian and a wolf (*Canis lupus*). The available canine genome sequence (Boxer) was used as an additional normal control. Dalmatian sequences were submitted to GenBank (accession numbers: DQ286968, AY903955, and AY903956). Sequence alignment was performed using VectorNTI (Informax).

Cloning:

PCR products that gave unclear results by direct sequencing were cloned using the TOPO TA Cloning Kit (pCR Topo 2.1-TOPO vector) (Invitrogen). Sequencing proceeded as described.

Genotyping:

Genotyping of polymorphisms in samples from additional breeds was performed on an ABI Prism 3100 Genetic Analyzer using GeneScan 400 HD Rox size standard (Applied Biosystems). Product sizes were analyzed using STRand software (Toonen 2001).

Results:

The three exons, as well as 350 base pairs of the promoter region, of the *COL10A1* gene were sequenced in nine individuals from seven breeds with short stature and two normal legged controls. No differences were identified in exon 1 or the promoter region between breeds. A three base pair difference in Exon 2 was characterized as an insertion based on its absence in the wolf sequence, which was defined as wildtype. The insertion results in the addition of a proline in the NC2 domain of the protein. This polymorphism was heterozygous in the Dalmatian control and the insertion was homozygous in the Boxer. Results for the affected breeds are presented in Table 2. Fluorescently-labeled primers were designed (Table 1: Ex2gF and Ex2gR) to assay this polymorphism in additional samples. A PCR product size of 144 base pairs was associated with "wildtype" sequence and 147 base pairs signified sequence that contained the insertion.

A total of 13 SNPs were identified in exon 3. Six of these SNPs do not cause an amino acid change. The remaining SNPs were sequenced in a second Basset Hound to determine if they warranted further testing. It was assumed that, since Basset Hounds are fixed for the skeletal dysplasia phenotype, all individuals would be homozygous for a causative mutation. SNPs 229, 241, 430, 1103 and 1285 were found to be heterozygous in the second Basset Hound and were not tested further. The SNP at base pair 440 is homozygous in the Basset Hound and Dachshund that were sequenced (Table 2) and appears to cause a significant amino acid change (arginine to histidine). This SNP was further assayed in a panel composed of individuals from 24 different breeds by amplifying the product with primers Ex3F2 and Ex3R2 and then digesting the product with the restriction enzyme *Bsa*HI. The G allele has one *Bsa*HI site, giving bands of 228 and 739 base pairs. The A allele has no *Bsa*HI restriction sites (product size: 967 base pairs). The SNP at base

pair 838 was homozygous in the second Basset Hound that was sequenced and appears to cause a significant amino acid change (proline to serine). The affected amino acid is also highly conserved between other mammalian COL10A1 protein sequences. This SNP was amplified with the same primers and assayed in the 24 breed panel with a *Sma*I restriction digestion. The T allele is cut at two sites, yielding bands of 570, 280 and 122 base pairs. The C allele is digested at three sites, giving bands of 340, 280, 230 and 122 base pairs. These SNPs were assayed in an additional affected individual from each of the breeds that segregate skeletal dysplasia (Table 3). As shown in Table 2, SNP 1285 was homozygous in the Chesapeake Bay Retriever. A second severely affected Chesapeake Bay Retriever was sequenced and was homozygous for the G allele present in the control dogs. These results indicated that this SNP does not cause skeletal dysplasia and it was not tested further.

A nine base pair deletion at the end of the triple helix region was also identified in Exon 3 (Table 2). This results in the deletion of a glycine and two prolines, amino acids 513 through 515 of the protein. Fluorescently-labeled primers were designed to assay this polymorphism in samples from additional breeds (Table 1: Ex3gF and Ex3gR). The PCR product size was 200 base pairs; a PCR product size of 191 base pairs signified sequence that contained the nine base pair deletion. Upon analysis, it was discovered that some individuals have a four base pair deletion in this region (PCR product size of 196 base pairs) which causes a frameshift at codon 514 resulting in 13 novel amino acids and the subsequent introduction of a premature stop codon at amino acid 526 of the protein. The premature stop codon would result in the predicted deletion of 150 of the 158 amino acids in the NC1 domain (Figure 2).

In order to determine how frequently these deletions were found in other breeds, a total of 647 dogs, representing 149 different breeds, were genotyped. The full length allele was seen most often, with an allele frequency of 0.708. The nine base pair deletion had an allele frequency of 0.243 and the four base pair deletion had an allele frequency of 0.049. The four base pair deletion was found as a heterozygote with either the full length allele or the 9 base pair deletion in the following breeds: Bichon Frise, Catahoula Leopard Dog, Cavalier King Charles Spaniel, Chesapeake Bay Retriever, Chihuahua, Dachshund, English Cocker Spaniel, Labrador Retriever, Newfoundland, Nova Scotia Duck Tolling Retriever, Shih Tzu, Spinone Italiano, Toy Fox Terrier, and Vizsla. Homozygotes for the four base pair deletion were identified in Labrador Retrievers and Shih Tzus. The four base pair deletion allele was sequenced in Labrador Retrievers, Shih Tzus, Chesapeake Bay Retrievers, Nova Scotia Duck Tolling Retrievers, and Newfoundlands and was determined to be the same allele in these breeds. Primary sequence data for the deletions is presented in Figure S1.

Discussion:

More than thirty *COL10A1* mutations, including missense mutations and mutations that introduce premature stop codons (nonsense and frameshift mutations), have been linked to SMCD in humans (Bateman et al. 2005). Almost all of the known *COL10A1* mutations in humans have been identified in the critical NC1 trimerization domain. The actual mechanism by which these mutations cause SMCD has been a point of some debate. One possibility is that missense mutations could exert a dominant negative effect (Gregory et al. 2000, Marks et al. 1999, McLaughlin et al. 1999). Mutant proteins may be unable to trimerize properly, resulting in their interference with normal chain assembly and secretion

(McIntosh et al. 1995, Chan et al. 1996, Chan et al. 1999, Marks et al. 1999, Wilson et al. 2002, Bateman et al. 2004). Association of mutant and normal chains has been shown *in vitro*, but an interaction *in vivo* has yet to be proven (Chan et al. 1999, Bateman et al. 2005). Transgenic mice with a dominant negative *COL10A1* mutation exhibit dwarfism characteristics such as short stature and bowed legs. Perinatal lethality at three weeks after birth was revealed in some lines of transgenic mice and variable dwarfism, ranging from severe to very mild, was seen in other lines (Jacenko et al. 1993).

Research in humans and mice has shown that mutant transcripts containing premature stop codons can be degraded by way of nonsense-mediated decay in cartilage cells, leading to COL10A1 haploinsufficiency (Chan *et al.* 1998, Bateman *et al.* 2003). Most recent studies support the theory that COL10A1 haploinsufficiency, specifically in patients with nonsense or frameshift mutations, is the most common cause of SMCD (Bateman et al. 2005).

Despite the identification of SNPs and insertion/deletions, the results of sequencing the exons and promoter region of the *COL10A1* gene in dogs did not identify any mutations that appear to cause canine skeletal dysplasia in the breeds studied. The four and nine base pair deletions in the NC1 domain, as well as the two SNPs identified in exon 3 that appear to cause significant amino acid changes, were seen in the homozygous and heterozygous states in affected dogs and in additional dogs of normal stature. The three base pair insertion polymorphism seen in exon 2 is present in the homozygous and heterozygous states in the controls (Table 2). Based on the fact that almost all human *COL10A1* mutations are in the NC1 domain, it was assumed that mutations in this region would be significant in dogs as well. Only one significant sequence change, the nine/four base pair deletion, was seen in the

canine NC1 domain. In addition, most of the known NC1 mutations in humans are missense mutations which result in misfolding of the protein. None of the observed canine missense mutations are in the NC1 domain. These findings indicate that the observed polymorphisms are not associated with canine skeletal dysplasia in these breeds.

It is interesting to note that, while COL10A1 haploinsufficiency is extremely significant in human chondrodysplasia, this study shows that it does not appear to be responsible for canine skeletal dysplasia. The four base pair deletion identified in Exon 3 of the canine *COL10A1* gene would result in a protein that is missing 150 residues of the NC1 domain. In humans, the mutant allele product would be degraded and the truncated protein would not be made. It is reasonable to assume that the same result would be seen in dogs. In humans, this type of deletion would result in COL10A1 haploinsufficiency and manifest itself in the form of SMCD. This deletion was observed at a low frequency in the heterozygous state in affected and unaffected dogs, suggesting that COL10A1 haploinsufficiency does not cause canine skeletal dysplasia.

Initially, no dogs homozygous for the four base pair deletion were identified, leading to the hypothesis that this deletion was homozygous lethal. Upon further testing, seven homozygotes were found, two Labrador Retrievers and five Shih Tzus. Although it is not possible to definitively rule out that these dogs may have a very mild skeletal phenotype, they do not have the striking skeletal dysplasia phenotype that was under investigation. The promoter region was sequenced to verify that there were no mutations that might alter transcription and none were identified. Unfortunately, growth plates from affected dogs are not available to investigate potential expression differences between the full length allele and the four base pair deletion allele. Therefore, it cannot be conclusively determined that

the mutant protein is not functional. Research in humans and mice has concluded that the NC1 domain is critical to COL10A1 function so it is reasonable to assume that it is important in dogs as well. Perhaps the deletion confers a phenotype that is not associated with skeletal changes.

The breeds in which the four base pair deletion were identified are not necessarily closely related breeds. This indicates that the deletion may be an older mutation or that it has arisen more than once. Four wolves and one wolf hybrid were genotyped in the course of this study and all had the full length *COL10A1* allele. Due to the low allele frequency of this deletion in dogs and the number of wolves tested, it cannot be concluded that it does not exist in wolves or other canids.

This study does not exclude *COL10A1* as the gene responsible for skeletal dysplasia as intronic sequences were not explored. However, the presence of multiple heterozygous SNPs in the affected breeds, as well as pairs of affected samples from breeds that segregate skeletal dysplasia, makes it unlikely that mutations in noncoding regions cause the phenotype. No mutations were found in the exons or promoter region of *COL10A1* that are linked to or cause skeletal dysplasia in these dog breeds.

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Table 1. **Sequences of primer pairs used to amplify the *COL10A1* promoter region and exons 1-3.** Genomic location is in reference to the July 2004 canine whole genome shotgun assembly v1.0, accessible on the UCSC genome browser (http://www.genome.ucsc.edu/index.html?org=Dog&db=canFam1&hgsid=61050165).

Exon	Primer sequence (5'-3')	Genomic Location	Product size (base pairs)
1 & promoter	**pF1** TGGGGTAAGCTACTTGCATAAAA **pR1** TGGAAACCACATTGCAAGTAA	74788219-74787340	879
	pF2 TCTGCCTGCCCAATAGAACT	74787840	Sequencing primer
	pR2 TGTGTCCAGGTGAGCAGAAG	74787706	Sequencing primer
2	**Ex2F** CTTGCAATGTGGTTTCCACT **Ex2R** TGTTTCTCCAGGCACATCTTT	74787318-74787906	588
	Ex2gF 6FAM-AGAACATGCTGCCACAAACA **Ex2gR** GGCATAGGGAATGAAGAACTG	74787117-74786970	147
3	**Ex3F1** TGGACTGGTTGATGGTGTGA **Ex3R2** GCACCTCTTGGACCAGTCTC	74783369-74782149	1220
	Ex3F2 CCATCTGGACCAAAAGGAGA **Ex3R2** GCACCTCTTGGACCAGTCTC	74783078-74782149	929
	Ex3F3 AGAGGGTCTCCTGGTCTTCC **Ex3R3** CAATTCCGCTGCGCCCCACAAAAT	74782199-74781387	812
	Ex3gF 6FAM-CCGGATTCCCTGGATCTAAA **Ex3gR** TGCCTTTATGAAGCCCTCAG	47482063-74781863	200

Table 2. **Polymorphisms found in exons 2 and 3 of the canine *COL10A1* gene.** Base pairs are in reference to the beginning of the coding sequence, designated as base pair 1, for each exon. * indicates polymorphisms that were tested in additional breeds. Δ designates a deletion. Bases in bold in the context sequence indicate the location of the polymorphism. Bases in bold in the table signify a base pair change. Heterozygous bases are separated by a /. Boxer sequence was obtained from GenBank. The Dalmatian, wolf and Boxer are unaffected controls, the Basset Hound, Dachshund, Pembroke Welsh Corgi, and Pekinese are affected individuals from breeds that are fixed for skeletal dysplasia, the Nova Scotia Duck Tolling Retriever, Newfoundland, and Chesapeake Bay Retriever are severely affected individuals from breeds that segregate skeletal dysplasia.

Exon	Base pair	Context (Dalmatian sequence)	Wolf	Boxer	Basset Hound	Dachshund	Pembroke Welsh Corgi	Pekinese	Nova Scotia Duck Tolling Retriever	Newfoundland	Chesapeake Bay Retriever	Amino Acid change	Position of amino acid change
* 2	111-113	CACCACC**ACC**/ΔCAACAC	Δ	**ACC**	Δ	ACC/Δ	Δ	ACC/Δ	ACC/Δ	Δ	ACC/Δ	P deletion	38
3	165	CCACTGG**G**AAGCC	G	G	**G/A**	**G/A**	G	G	G	**G/A**	G	G (no change)	108
	204	CAGGGTC**G/A**AAAG	G	**G**	**A**	**G**	**A**	**G**	**G**	**A**	**G**	S (no change)	121
	229	AAGGA**G**ATATTGG	G	G	**G/T**	**G/T**	G	**G/T**	G	**G/T**	G	D → Y	130
	241	GACCA**G**CTGGTTT	G	G	**G/T**	**G/T**	G	**G/T**	G	**G/T**	G	A → S	134
	360	CCAGGGG**A/C**TTTC	**C**	**A**	**C**	A/C	**C/T**	A/C	**A**	**C**	**A**	G (no change)	
	430	ATGGC**G**CTCCTGG	G	G	G	G	**G/T**	G	G	G	G	A → S	197
*	440	CTGGAC**G**CCCAGG	G	G	**A**	**A**	G	G	**G/A**	G	**G/A**	R → H	200
*	838	GTCAT**C**CCGGGGA	C	C	**C/T**	**C/T**	C	C	**C/T**	C	**C/T**	P → S	333
	1103	CTCCTG**G**TCTTCC	G	G	G	G	**G/T**	G	G	G	G	G → V	421
	1285	CTGGC**G**TGGCAAC	G	G	**G/A**	**G/A**	G	G	**G/A**	G	**A**	V → M	482
*	1377-1385	CC**AGGCCCCCC**GG	AGGCCCCCC	AGGCCCCCC	AGGCCCCCC	AGGCCCCCC	AGGCCCCCC	AGGCCCCCC	**AGGCCCCCC/Δ**	AGGCCCCCC	**AGGCCCCCC/Δ**	PPG deletion	513-515
	1377	GTCCCCC**A**GGCCC	A	A	**A/G**	**A/G**	A	A	**G**	A	**G**	P (no change)	512
*	1367-1384	CC**AGGTCCCCCCA GGCCCCCC**GGG	AGGTCCCCCCAGGCCCCCC	AGGTCCCCCCAGGCCCCCC	AGGTCCCCCCAGGCCCCC	**TGGGGGACCTGGGA**Δ/AGGTCCCCCCAGGCCCCCC	AGGTCCCCCCAGGCCCCCC	AGGTCCCCCCAGGCCCCC	**TGGGGGACCTGGGA**Δ/AGGTCCCCCCAGGCCCCCC	**TGGGGGACCTGGGA**Δ/AGGTCCCCCCAGGCCCCCC	**TGGGGGACCTGGGA**Δ/AGGTCCCCCCAGGCCCCCC	deletion of 150 amino acids	526
	1515	AGCTTA**T**CCGGC	**C**	T	**C**	T	**C**	T	T	**C**	T	Y (no change)	558
	1518	CTTATCC**G**GCCGT	G	G	**G/A**	G	G	G	G	G	G	P (no change)	559

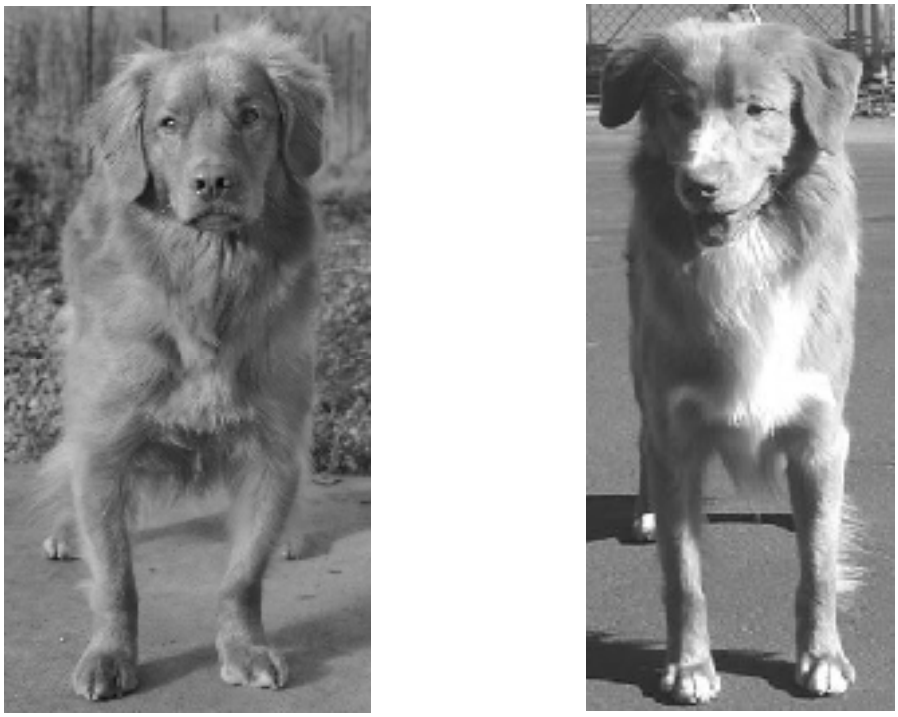
Table 3. Genotypes for assayed SNPs and deletions in pairs of affected dogs from three breeds that segregate the skeletal dysplasia phenotype. Base pairs are in reference to the beginning of the coding sequence, designated as base pair 1, for each exon. Δ designates a deletion. Heterozygous alleles are separated by a /.

Exon	Position of amino acid change	Base pair position	Nova Scotia Duck Tolling Retriever		Newfoundland		Chesapeake Bay Retriever	
			1	2	1	2	1	2
2	38	111-113	ACC/Δ	ACC/Δ	Δ	ACC/Δ	ACC/Δ	ACC
3	200	440	G/A	G	G	G	G/A	G
	333	838	C/T	C	C	C/T	C/T	C/T
	513-515, 526	1376-1384	Full length/9bp Δ	Full length	Full length	Full length/4bp Δ	Full length/9bp Δ	Full length

Figure captions:

Figure 1. Example of skeletal dysplasia phenotype and normal phenotype. a. Nova Scotia Duck Tolling Retriever affected with skeletal dysplasia. Note the comparatively short stature and bowed front legs. b. Nova Scotia Duck Tolling Retriever exhibiting a normal-legged phenotype.

Figure 2. Canine *COL10A1* deletions. A. *COL10A1* nucleotide alignment, base pairs 1339 to 1432. Human *COL10A1* shows the human sequence in this region. *COL10A1* dog is the full length allele, *COL10A1* 9bp del is the allele for the nine base pair deletion in dog and *COL10A1* 4bp del is the four base pair deletion allele in dog. **B.** Proteins are shown from amino acid 389 of the triple helix domain to the end of the protein. Human COL10A1 is the human COL10A1 protein. COL10A1 dog represents the full length translation in dog. COL10A1 9bp del shows the translation for the 9 base pair deletion, with the absence of a glycine and two prolines. COL10A1 4bp del represents the protein for the 4 base pair deletion that causes a frameshift and a premature stop signal.



a. **b.**

Figure 1. Example of skeletal dysplasia phenotype and normal phenotype.

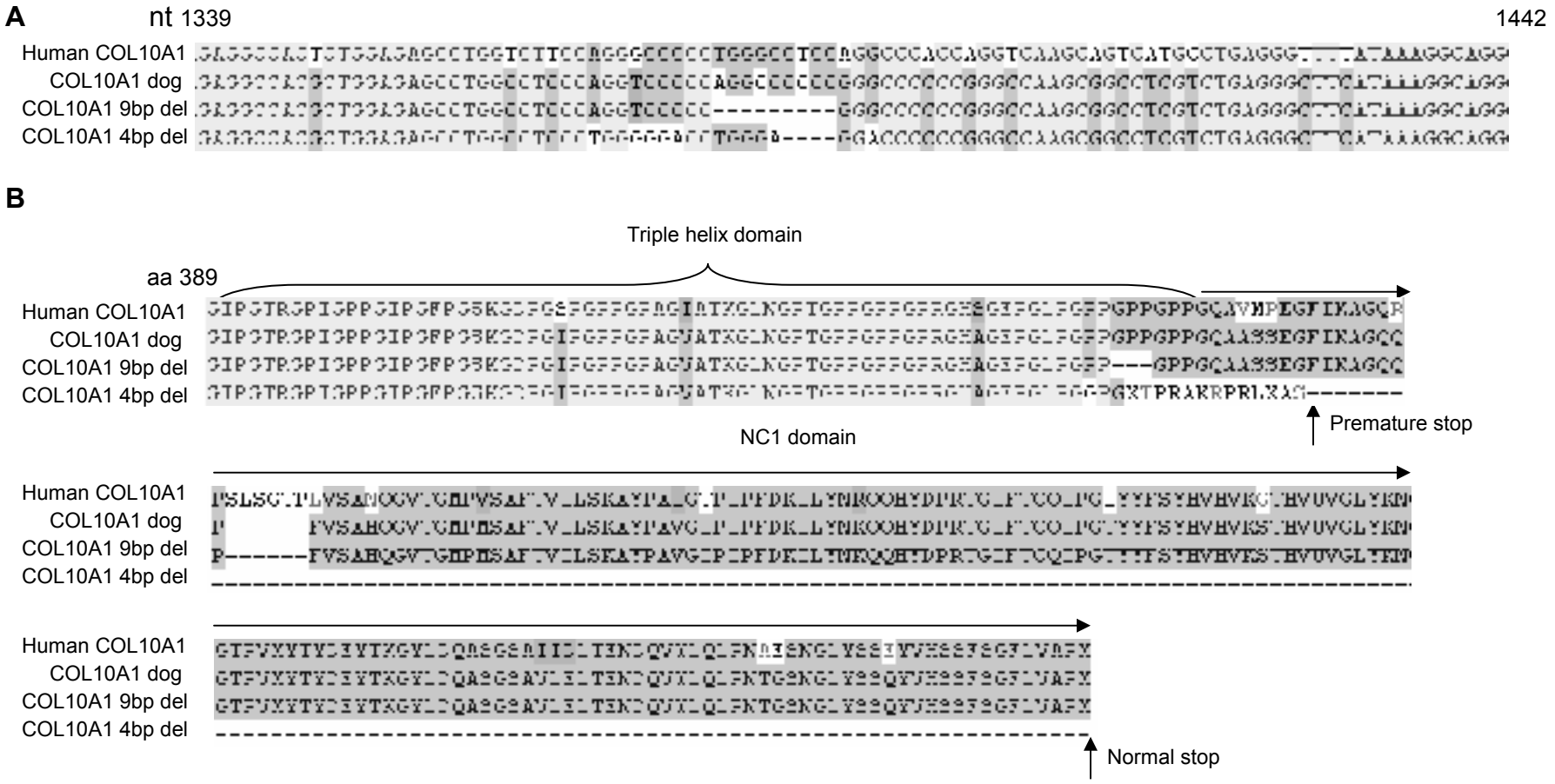


Figure 2. Canine COL10A1 deletions.

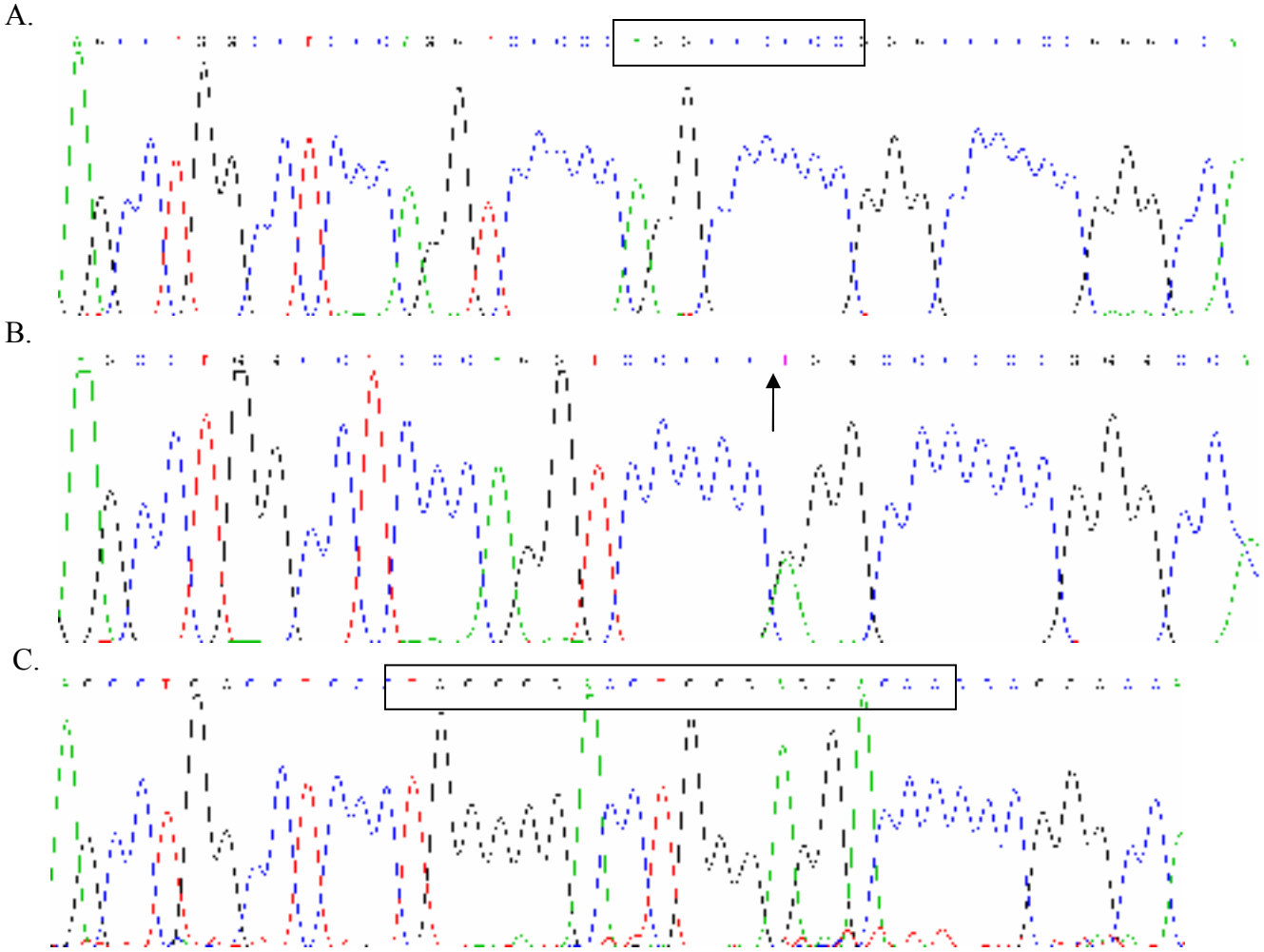


Figure S1. Primary sequence data for canine COL10A1 deletions. A. Full length sequence. B. Sequence with the nine base pair deletion. The bases that have been removed are boxed in A. The location of the missing bases is indicated by an arrow. This sequence also shows a heterozygous SNP (N in sequence) at base pair 1376 of exon 3 C. Sequence with the four base pair deletion. The unique sequence of this allele is boxed.