**RESEARCH NOTE**

# Isolation, characterization and molecular screening of canine *SLC26A2* (sulphate transporter) in German Shepherd dogs with hip dysplasia

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## Introduction

The *SLC26A2* gene codes for a sulphate transporter (the gene is also known as diastrophic dysplasia sulphate transporter gene; *DTDST*) and is a causative gene for human diastophic dysplasia due to undersulphation of proteoglycans in the cartilage matrix (Hastbacka *et al*. 1994). Several mutations in the *SLC26A2* gene have been found in human diastrophic dysplasia (Hastbacka *et al*. 1994, 1996), achondrogenesis type IB (Superti-Furga 1994), and multiple epiphyseal dysplasia (Superti-Furga *et al*. 1999; Czarny-Ratajczak *et al*. 2001). Over 30 mutations in the *SLC26A2* gene have been identified in human to date, including heterozygous single base deletions and splicing site mutations (Hastbacka *et al*. 1994, 1996; Superti-Furga *et al*. 1996, 1999; Czarny-Ratajczak *et al*. 2001; Rossi and Superti-Furga 2001).

Canine hip dysplasia is a developmental orthopedic disease in which an abnormal formation of the hip leads to looseness in the hip joints, causing cartilage damage (LaFond *et al*. 2002). Progressive arthritis can result, and when it does, it can be crippling. Hip dysplasia is not the same thing as arthritis in the hips, rather it is the most common cause of arthritis in the hips. Hip dysplasia is most common among larger breeds of dogs, especially German Shepherds, Rottweilers, Labrador Retrievers, Golden Retrievers, Mastiffs and Saint Bernards (Guilliard 2003). It is also seen in smaller breeds such as Cocker Spaniels and Springer Spaniels, as well as in mixed breeds (Guilliard 2003)

Hip dysplasia is known to be transmitted genetically (Todhunter and Lust 2003), and recent genetic studies have found major genes for hip dysplasia in four Finnish dog populations (Maki *et al*. 2004), as well as quantitative trait loci (QTL) contributing to hip dysplasia in Portuguese Water Dogs (Chase *et al*. 2004). Further, the recent QTL mapping study using traits affecting hip dysplasia in dogs found several putative QTLs, one of which is located at chromosome 4, where the *SLC26A2* gene resides (Todhunter *et al*. 2005).

In this study, we report the first isolation and characterization of the canine *SLC26A2* gene. This canine homologue has high homology in genomic structure and functional domains to other *SLC26A2* across a number of different species. Given the critical role of *SLC26A2* in sulphation of proteoglycans in cartilage matrix, as seen in human dystrophic bone diseases, the availability of the canine *SLC26A2* provides a good starting point for identifying mutations that may be responsible for certain forms of dystrophic bone diseases in dogs. However, in this screen, we could not find any mutations and polymorphisms associated with phenotypic consequences. The expression levels of the *SLC26A2* between affected and unaffected dogs were not significantly different.

## Materials and methods

### *Isolation and characterization of canine SLC26A2 gene*

To identify genomic DNA and mRNA sequences of the *SLC26A2* gene, DNA and RNA samples were obtained from articular cartilage collected from an 11-month-old male Beagle dog, using commercial DNA and RNA isolation kits (Qiagen, Germany). The coding region of canine *SLC26A2* mRNA was amplified using primers designed from the sequence obtained from the GenBank (predicted *SLC26A2* sequence, LOC612273; table 1 of electronic supplement). Based on the sequence of canine *SLC26A2* mRNA, the genomic sequence (NC-No.006586) was obtained from GenBank using mRNA sequence alignment. To screen genomic DNA obtained from peripheral blood, three pairs of primers were designed from 5′ and 3′-flanking regions of the introns of each exon. Because the exon 2 was too big to amplify using single primer pair, it was amplified by two primer pairs (table 1 of electronic supplement).

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**Keywords.** hip dysplasia; *DTDST*; *SLC26A2*; dystrophic bone disease; dog.

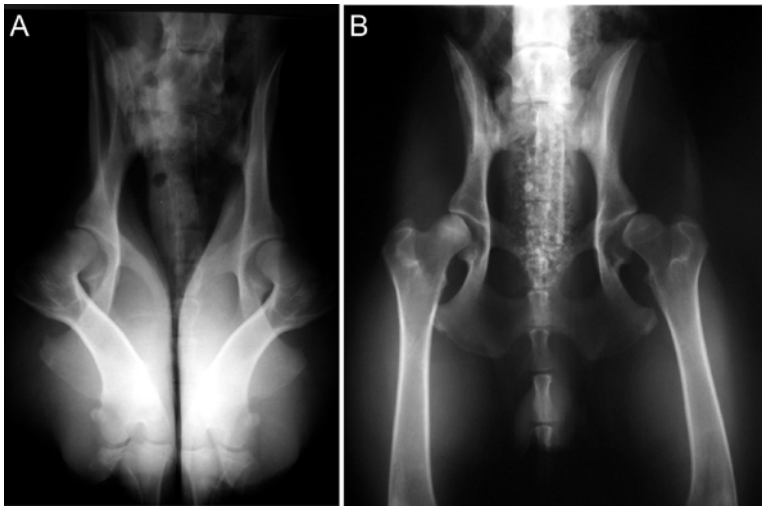


**Figure 1.** Radiographs taken from affected dog (II:3 in figure 1 of electronic supplement). (A) DLS (dorsolateral subluxation) method and (B) Radiograph taken following the OFA guidelines.

***Molecular screening of canine SLC26A2 gene in German Shepherds with hip dysplasia***

Fourteen affected German Shepherds (two dogs each in two familial cases; 10 sporadic cases), eight related German Shepherds (unaffected dogs from the affected families), and twenty unrelated normal German Shepherds were used in this screen (figure 1; table 2 of electronic supplement). Clinical diagnosis was made by the guidelines described by the Orthopedic Foundation for Animals (OFA) (Rendano and Ryan 1985; Henry 1992), and the dorsolateral subluxation method (Farese *et al*. 1999; figure 1),. The severity of clinical signs was based on the degree of lameness.

***The expression level of canine SLC26A2 gene in German Shepherds with hip dysplasia***

The expression level of the *SLC26A2* was evaluated in the affected (group 1), the related unaffected (group 2) and the unrelated dogs (group 3), using real-time PCR with RNA samples obtained from the blood and bone cartilage. The real-time PCR protocol for this study is described in table 2 of the electronic supplement. Three RNA pools were made from the three different groups of dogs (groups 1, 2 and 3). Each RNA pool was made from eight individuals (randomly selected). The relative expression levels were normalized to those of *GAPDH* in the same samples (Cao *et al*. 2006). To validate the result, this experiment was repeated three times with freshly pooled RNA samples.

## Results and discussion

The full length of coding exons of canine *SLC26A2* was isolated and identified (GenBank No. DQ220791). The canine *SLC26A2* was located in the chromosome 4 spanning from 62.27M to 62.28M (Cfa.43194). The amino acid sequence homology of canine *SLC26A2* to other *SLC26A2* sequences revealed that the sulphate transporter and STAS domains were highly conserved (figure 2). Further, the genomic structure consisting of two exons is similar to that seen in humans. Sequence analysis placed in the canine *SLC26A2* are closer to the human *SLC26A2* mRNA and amino acid sequences in comparison to other species (table 1). *SLC26A2* has two important domains, the sulphate transporter domain and the STAS domain. The highest sequence identity was observed in human and canine *SLC26A2* amino acid sequences in these domains. These findings suggest that these domains are involved in growth regulation of chondrocytes mediated by sulphated proteoglycans in dogs, as seen in human and mouse studies. In our screen, we found two base changes (c.316C > A, c.2229 + 23G > A) in affected individuals. Although the c.316C > A base change causes L106M amino acid change, there might be no association between these base changes and phenotypic consequences, since these polymorphisms were also found in unaffected individuals.

**Table 1.** Sequence comparison of *SLC26A2* across species. Main entries are the identities of the mRNA sequences in percentages. The identities of the amino acid sequences in percentage are given in parentheses.

| | Percentage in mRNA sequence | | |
|---|---|---|---|
| | *H. sapiens* | *M. musculus* | *R. norvegicus* |
| *C. familaris* | 89.2 (87.3) | 82.6 (81.7) | 82.1 (82.3) |
| *H. sapiens* | | 65.0 (80.9) | 65.3 (81.1) |
| *M. musculus* | | | 82.1 (92.4) |

GenBank accession number for sequences used, *H. sapiens* (NM000112); *M. musculus* (NM007885); *R. norvegicus* (NM057127).

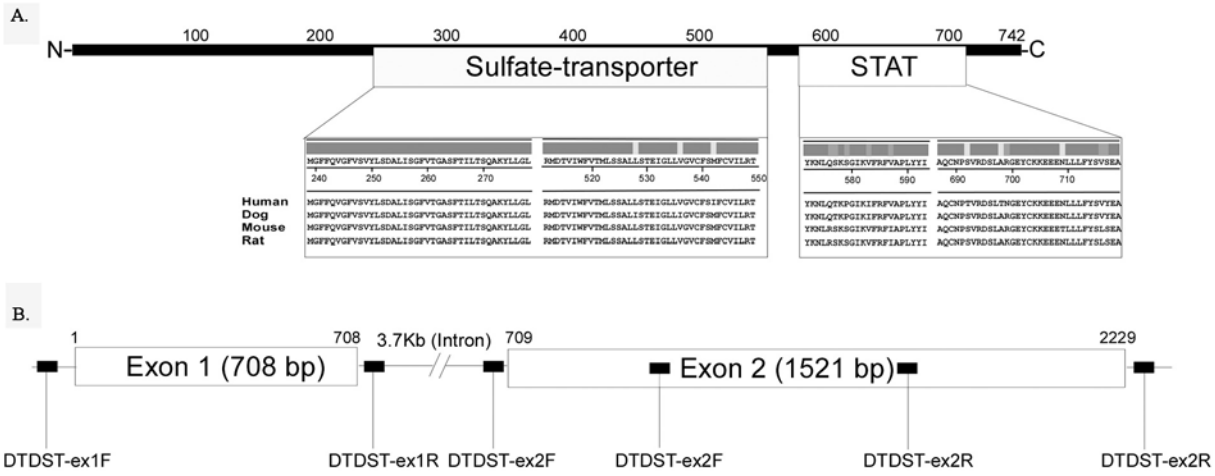


**Figure 2.** Genomic and mRNA structure of canine *SLC26A2*. Note that amino acid sequence in three main domains of *SLC26A2* is highly conserved across species. GenBank accession number for sequences used, *H. sapiens* (NP_004378); *M. musculus* (NP_032726); *R. norvegicus* (NP_446103); *G. gallus* (NP_990495).

We also investigated the different expressions of *SLC26A2* between the affected and unaffected dogs. However, the expression levels were not significantly different among the three groups of dogs compared (figure 3). Our finding suggested that the *SLC26A2* gene might not be involved in canine hip dysplasia in German Shepherd dogs. However, since there is a possibility that the *SLC26A2* gene is implicated in canine hip dysplasia in other dog breeds, further studies are warranted before we can rule out the association of the *SLC26A2* gene with hip dysplasia in other dog breeds.

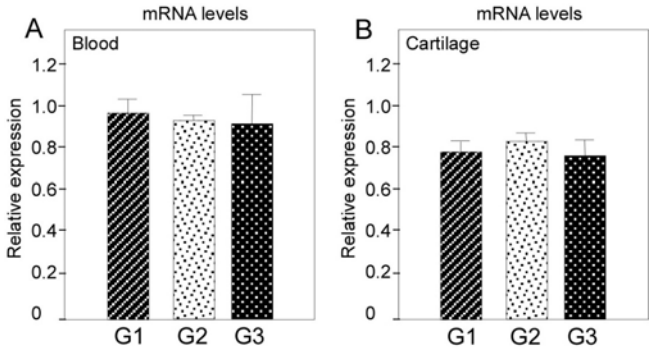


**Figure 3.** The canine *SLC26A2* mRNA levels in blood and cartilage in an affected dog (G1), a related but unaffected dog (G2), and an unrelated, unaffected dog (G3).

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