QUANTIFICATION OF RIB COL1A2 GENE EXPRESSION IN HEALTHY AND FLUOROSED INNER MONGOLIA CASHMERE GOATS

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Summary: Damage to collagen protein and its gene expression caused by excessive fluoride (F) ingestion plays an important role in the etiology of skeletal fluorosis. Recently we found that industrial F pollution significantly increased the expression level of type II collagen gene (COL2A1) in rib cartilage of Inner Mongolia cashmere goats. With the same goats and methods, we have now quantified another important collagen gene, the rib COL1A2 gene, which encodes an α2(I) polypeptide chain assembled into collagen molecules. The results showed that the expression level of COL1A2 and COL1A2/β-actin increased by 88% and 81%, respectively.

Keywords: Cashmere goats; COL1A2 gene; Collagen; Fluoride pollution; Gene expression; Real-time RT-PCR; Rib collagen.

INTRODUCTION

Skeletal fluorosis is a well-known chronic metabolic bone disease caused by ingesting excessive amounts of fluoride (F), especially from water, and usually or less often from food and air. The unique mechanical properties of bone depend on the composition and organization of its extracellular matrix, and collagens are major structural components of the extracellular matrix.[1] As suggested by Susheela in the 1970s, collagen protein is a target damaged by excessive F ingestion.[2] Thus, investigation of the effect of F on collagen protein by quantification of some related collagen gene expressions should help us understand the molecular mechanism of F toxicity.

A number of reports show that F affects the collagen metabolism of cartilage and bone.[3-6] In our previous work, we also found that the collagen of fluorosed tooth matrix had undergone obvious morphological changes and the total amino acid content of the fluorosed matrix was significantly greater than that of nonfluorosed control tooth matrix.[7-11] Furthermore, we recently reported that industrial fluoride pollution increases the expression level of type II collagen gene (COL2A1) that encodes the cartilage-specific collagen in rib cartilage.[12]

In ribs the predominant form of collagen is collagen type I encoded by COL1A2 gene. The question then arises: Can F also increase the COL1A2 gene expression in ribs? Using the same goats studied in our recent report,[12] we developed and validated real-time RT-PCR (reverse transcription polymerase chain reaction) assays[13] to quantify the expression level of COL1A2 gene in the ribs of Inner Mongolia cashmere goats.

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MATERIALS AND METHODS

The Inner Mongolia cashmere goats and their treatment were the same as described previously.[12] The rib tissue preparation and the total RNA extraction were performed following the methods described in that report.[12]

Two pairs of specific primers (Table 1) were designed according to the alignments of the published cDNA sequences of COL1A2 and ß-actin genes in humans and sheep.

Table 1. Primer sequences with their corresponding PCR product size and position

Gene	Primers (5'→3')	Primer locations	Product (base pairs)	Genbank accession No.
COL1A2	GCCTAGCAACATGCCAATC AGCAAAGTTCCCACCGAGA	524-705	182	NM_000089
β-actin	ATCGGCAATGAGCGGTT CGTGTTGGCGTAGAGGT	834-977	144	U39357

The conventional RT-PCR amplification and identification of the above two gene fragments were also the same as those described previously.[12] Real-time RT-PCR conditions and analysis of the COL1A2 gene expression level were the same as described previously as well as the statistical analysis.[12]

RESULTS

Conventional RT-PCR for COL1A2 and β-actin: Amplification products of the expected size were obtained from each pair of primers (Figure 1). After digestion of COL1A2 and β-actin gene recombinant T-easy vectors with Eco RI, size-specific bands were confirmed. Sequencing of the purified and cloned products showed that the COL1A2 gene fragment was 92% homologous with the human COL1A2 gene, and the β-actin gene fragment was 98% homologous with the sheep β-actin gene.

Quantification of COL1A2 gene expression: The standard curve obtained by correlation of the Ct values (threshold cycles) with the dilution series of the COL1A2 gene exhibited a relatively low intra-assay variation (Figures 2 and 3). Specificity of RT-PCR amplification was identified by melting curve profile analysis (Figure 4).

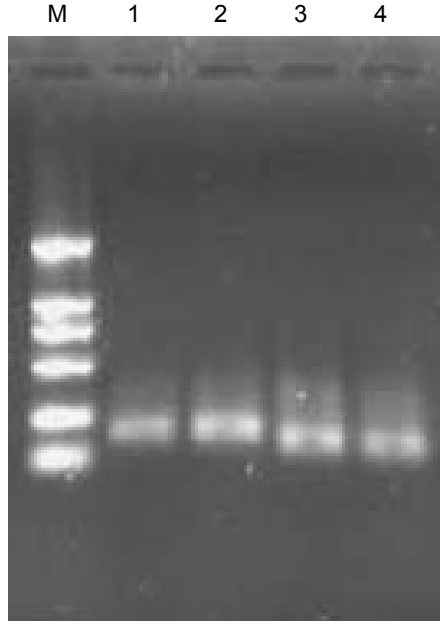

Figure 1. Electrophoresis of conventional RT-PCR products of COL1A2 and β-actin genes from Mongolian cashmere goat rib. M: molecular weight markers (DL2000); Lane 1 and 2 respectively show COL1A2 (182 base pairs) of control and fluorosed group; Lane 3 and 4 respectively show β-actin (144 base pairs) of control and fluorosed group.

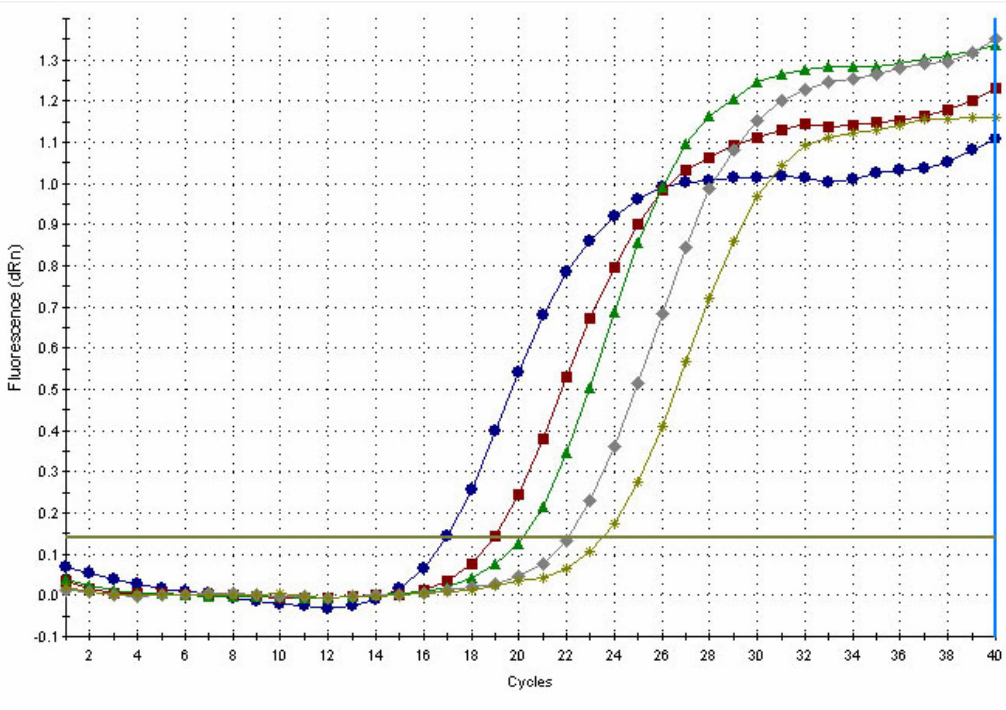

Figure 2. Amplification plots for COL1A2 gene after serial dilutions of total RNA, starting from 1 (undiluted, leftmost curve) to 0.03125 (1:32 dilution, rightmost curve).

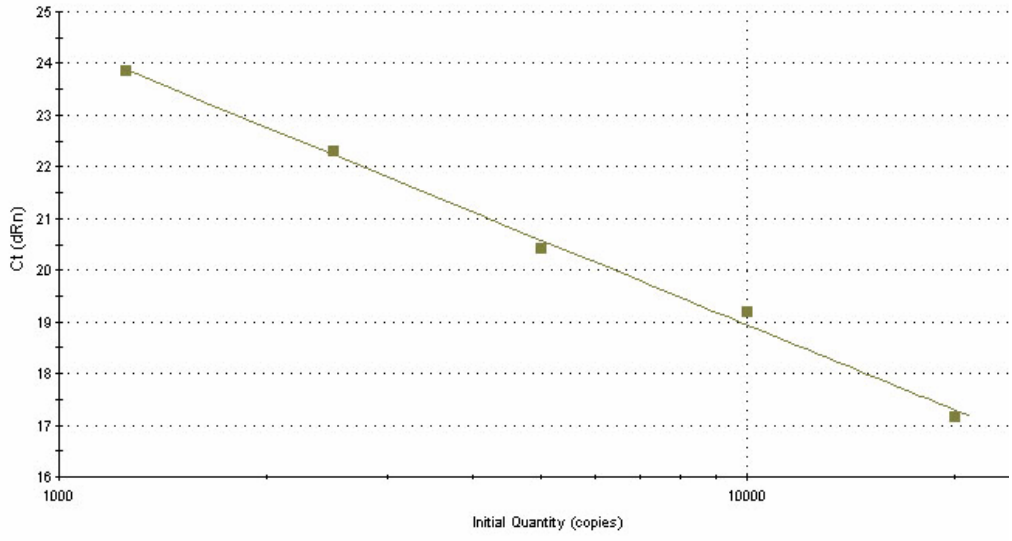

Figure 3. Standard curve for COL1A2 gene obtained by the correlation of the Ct values with the dilution series of the COL1A2 gene.

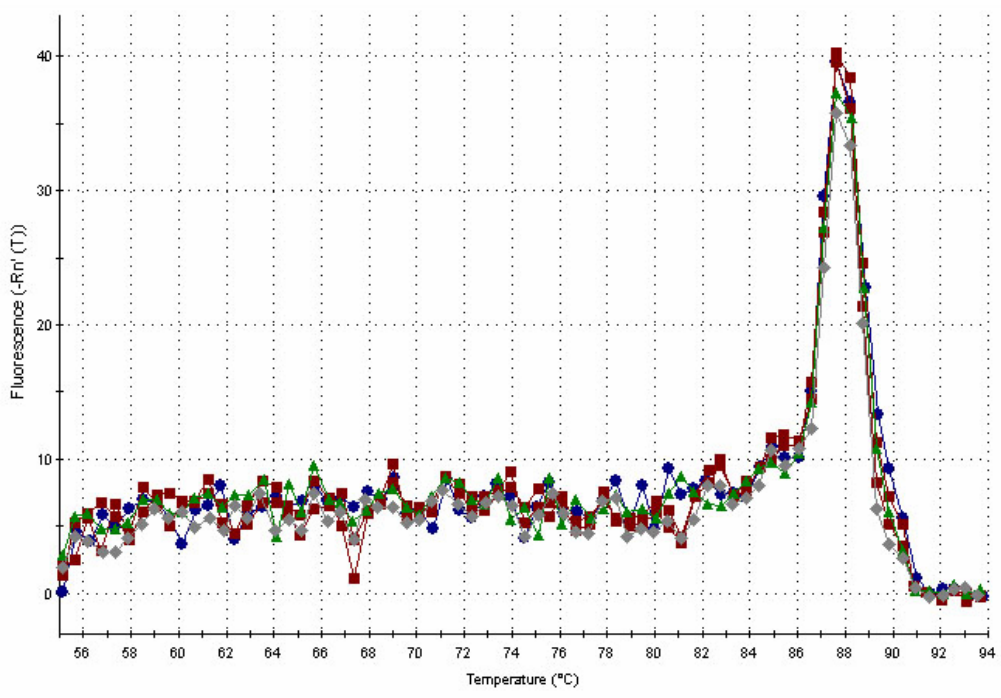

Figure 4. Melting curves for COL1A2. The single peak at 87.5°C indicates that no other transcripts were amplified in the real-time RT-PCR.

The expression level of COL1A2 gene, normalized to β-actin, is reported in Table 2. The expression level of COL1A2 gene in the fluorosed group is significantly higher than that in the control group ($P<0.01$), whereas there is no significant difference between the control and fluorosed group in the expression level of β-actin ($P>0.05$). The results showed that the expression of COL1A2 and COL1A2/β-actin increased by 88% and 81%, respectively.

Table 2. Expression level of COL1A2 gene in the control and fluorosed group (Mean± SEM)

Group	No. of samples (goats)	COL1A2 ($\times10^4$copies)	β-actin ($\times10^4$copies)	COL1A2/β-actin
Control	3	1.54±0.19	1.48±0.23	1.06±0.05
Fluorosed	5	2.90±0.13*	1.52±0.05	1.92±0.07*

*p<0.01

DISCUSSION

Although many experimental studies have shown that F can cause structural changes in collagen fibers, little is known about the molecular mechanism of these effects.[15-17] It is generally accepted that type I collagen is the main type of collagen in the rib responsible for its stability and cell biological functions.[18] The type I collagen gene (COL1A2) encodes an α2(I) polypeptide chain that is assembled into a collagen molecule.

In the present study, we quantified the COL1A2 gene expression level in ribs of Inner Mongolia cashmere goats by real-time RT-PCR. The results showed an 88% increase in COL1A2 expression and an 81% increase in COL1A2/β-actin expression.

Our findings thus confirm that industrial fluoride pollution increases not only the expression level of COL2A1 gene but also that of the COL1A2 gene in Inner Mongolia cashmere goats. Accordingly, we suggest that enhanced expression levels of the COL2A1 and COL1A2 gene are involved in skeletal fluorosis. Thus, it appears that F interferes with the balance between the synthesis and degradation of normal collagen by inducing the synthesis of large quantities of imperfect collagen or noncollagenous protein.

Finally, we note that the regulation of gene expression is complex and involves both transcriptional and post-transcriptional mechanisms.[19] Our initial results with the COL2A1 and COL1A2 gene expression remain to be verified with further studies. For this purpose, our research group is carrying out experimental studies of collagen protein expression in rabbits and rats.

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