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Sulfation pattern of chondroitin sulfate in human osteoarthritis cartilages reveals a lower level of chondroitin-4-sulfate

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ABSTRACT

Chondroitin sulfates (CS) account for more than 80% of the glycosaminoglycans of articular cartilage, which impart its physiological functions. We quantified the absolute concentration of the CS components of the full thickness cartilages from the knees of patients with terminal-phase osteoarthritis. Osteochondrol biopsies were removed from the medial femoral condyle and lateral femoral condyle of sixty female patients received total knee arthroplasty, aged from 58 to 83 years old. We found the total CS concentrations and chondroitin-4-sulfate disaccharide were significantly lowered in osteoarthritic samples. Microstructure analysis indicated while chondroitin-0-sulfate was equally distributed across different zones of the osteoarthritic cartilages, chondroitin-4-sulfate is significantly less in the deep zones. Down-regulation of sulfotransferases, the enzymes responsible for CS sulfation, in the lesion site of cartilage were observed. Our study suggested chondroitin-4-sulfate down-regulation can be a diagnostic marker for degraded osteoarthritis cartilage, with potential implications in cartilage regeneration.

1. Introduction

Osteoarthritis (OA) is a leading cause of chronic disability and effective general treatment is lacking (Van Manen, Nace, & Mont, 2012). Non-steroidal anti-inflammatory drugs (NSAIDs) may mitigate pain and slow down disease progression, but their effects are limited in preventing the terminal OA. The current treatments are total knee transplantation or spinal fusion (Bachmeier et al., 2001). Cell-based therapy is believed to have potential for the treatment of extensive joint damage and OA (Wyles, Houdek, Behfar, & Sierra, 2015). We have developed a cylindrical biphasic osteochondral scaffold loaded with collagenase-treated cartilage for autologues chondrocyte implantation (ACI) to treat cartilage defects of the knee (Chiang et al., 2013). Patients with cartilage defects received ACI therapy showed completely filled grated sites and regenerated cartilage that appeared to be hyaline microscopically. Although the quality of the degenerative cartilage has been evaluated by MRI (Chiang & Jiang, 2009), analysis of extracellular matrix (ECM) components of the articular cartilage is needed.

Chondrocyte secretes ECM containing 20 ~ 35% solid matrix and 65 ~ 80% water to maintain cartilage function and to support tissue regeneration (Cucchiarini et al., 2016). ECM is organized into three zones: superficial, middle, and deep zones. In addition, the solid matrices of ECM are categorized into two subgroups: type-II collagen and proteoglycans. Collagen is embedded deeply into the bony matrix and extended upward to the top or tangential zone of cartilage, forming a layer of “protective armor” which contributes to the mechanical strength of the structure against abrasive forces during joint motion (Hunziker, 2002). Proteoglycans which form the “space fillers” between fibers, consist of a heavily glycosylated core protein bearing attached glycosaminoglycan (GAG) chains. GAGs’ function lies in their enormous water-binding capacity and organizing properties. Resistance of articular cartilage to deformation was principally correlated with GAGs (Kempson, Muir, Swanson, & Freeman, 1970), (Poole, Ionescu, Swan, & Dieppe, 1994).

GAGs are high molecular weight linear polysaccharides composed of repetitive disaccharide units. The disaccharide units are made of an

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amino sugar (GlcNAc or GalNAc) and uronic acid (glucuronic acid (GlcA) or iduronic acid). There are four major classes of GAGs in cartilage, including hyaluronan (HA), keratan sulfate (KS), dematan sulfate (DS), and chondroitin sulfate (CS). CS accounts for more than 80% of GAGs in articular cartilage and CS imparts gel-like properties, such as lubrication, water retention, and resistance (Watanabe, Yamada, & Kimata, 1998). In the structure of CS, each GlcA can be 2-*O*-sulfated and GalNAc can be sulfated at C(O4) or C(O6) position. Chondroitin-4-sulfate (C4S; GlcAβ(1,3)-4-SO_3-GalNAc) and chondroitin-6-sulfate (C6S; GlcAβ(1,3)-6-SO_3-GalNAc) were two of the most common disaccharide unit among the internal linkage of CS. The non-reducing terminal unit of CS is either GalNAc4S (S stands for sulfate), GalNAc6S, or Gal4,6S (Plaas, Wong-Palms, Roughley, Midura, & Hascall, 1997). There is evidence suggesting that CS in aging cartilage samples has increased level of sulfation at the C(O6) relative to the C(O4) of GalNAc (Bayliss, Osborne, Woodhouse, & Davidson, 1999). Changes in the distribution pattern of CS in cartilage may also be important because the levels of C4S and C6S in joint fluids after treating with chondroitinase have been used as markers of cartilage metabolism (Shinmei, Miyauchi, Machida, & Miyazaki, 1992).

Today only a few studies on CS in osteoarthritis patients' "CS and OA" have been reported. Mankin et al. extracted proteoglycans from arthritic cartilage and reported that about 90% of the GAGs were comprised of an equal mixture CS and KS (Mankin, Dorfman, Lippiello, & Zarins, 1971). Both Bollet et al. (Bollet & Nance, 1966) and Ishmaru et al. (Ishimaru, Sugiura, Akiyama, Watanabe, & Matsumoto, 2014) reported CS polysaccharide chain lengths of proteoglycans were shorter (5.36 ± 1.46 kDa in lesion cartilage v.s. 6.19 ± 1.84 kDa in remote cartilage)and the concentration of CS was reduced in OA samples. It was further reported that the enzymes responsible for the biosynthesis of CS were down-regulated in the osteoarthritic samples (Ishimaru et al., 2014).

CS bearing sulfate residues at the GalNAc C(O4) or C(O6) positions were regulated by specific sulfatase and sulfotransferase, which are key enzymes for modifying CS chain composition. Three types of sulfotransferase are involved in CS sulfation. Type-1 functions as sulfotransferring to 4-*O*-GalNAc, including carbohydrate (GalNAc 4-*O*) sulfotransferase 8 (CHST8) (Morina et al., 2010), carbohydrate (GalNAc 4-*O*) sulfotransferase 9 (CHST9) (Kang, Evers, Xia, Baenziger, & Schachner, 2001), carbohydrate (chondroitin 4) sulfotransferase 11 (CHST11) (Okuda et al., 2000), carbohydrate (chondroitin 4) sulfotransferase 12 (CHST12) (Hiraoka et al., 2000), chondroitin-4-*O*-sulfotransferase-3 (CHST13) (Kang, Evers, Xia, Baenziger, & Schachner, 2002), and carbohydrate sulfotransferase 14 (CHST14)). The type-2 enzymes transfer sulfate to 6-*O*-GalNAc, they are the carbohydrate (GalNAc-6-*O*) sulfotransferase 2 (CHST2) (Fukuta, Kobayashi, Uchimura, Kimata, & Habuchi, 1998), carbohydrate (chondroitin 6) sulfotransferase 3 (CHST3) (Fukuta et al., 1998), carbohydrate (*N*-acetylglucosamine 6-*O*) sulfotransferase 7 (CHST7) (Kitagawa, Fujita, Ito, & Sugahara, 2000). The type-3 of sulfo-transferase is GalNAc 4-sulfate 6-*O* sulfotransferase (CHST15) (Ohtake, Kimata, & Habuchi, 2003). Galactosamine (*N*-acetyl)-6-sulfatase (GALNS) (Sukegawa et al., 2000) and arylsulfatase (ARSB) (Pungor et al., 2009) have been identified to perform de-sulfation at GalNAc of C(O6) and C(O4) positions, respectively. 3'-Phosphoadenosine 5'-phosphosulfate synthase (PAPSS) synthesizes the sulphate donor 3'-phosphoadenosine 5'-phosphosulfate sulfate (PAPS) for PAPSS (Harjes, Bayer, & Scheidig, 2005).

Some of these enzymes invoke the pathology of genetic diseases, for examples the Ehlers-Danlos syndrome (Miyake, Kosho, & Matsumoto, 2014), Kashin-Beck disease (Han et al., 2017), mucoploysaccharidosis IVA (MPS IVA) (Hendriksz et al., 2013), and type VI (MPSVI) (Petry, Dieter, Burin, Giugliani, & Leistner, 2003). It was also reported that modification of GALNS and ARSB expression might regulate the content of CS (Bhattacharyya, Kotlo, Shukla, Danziger, & Tobacman, 2008). These clinical and laboratory observations suggested the de-regulation of sulfate group on the CS might cause abnormal CS structures and impaired cartilages.

As mentioned above, most existing "CS and OA" studies are limited to a small population of patients and did not take potential variations into account in the microanatomy of cartilages. Here, we designed a robust method to quantify and compare the absolute concentrations of CS components and their distributions in the articular cartilages of the knees of patients with terminal-phase osteoarthritis treated with total knee arthroplasty. Even terminal phase osteoarthritic patients with severely damaged cartilage still had some healthy cartilage, so healthy (remote) and osteoarthritic (lesion) samples from the same patients were simultaneously obtained. Our study will provide valuable insights into OA progression and cartilage regeneration.

2. Methods and materials

2.1. Cartilage samples

This study was approved by the Human Ethics Committee of National Taiwan University Hospital (NTUH). All patients provided informed consent. Human cartilage samples were acquired from the knees of 60 end-stage OA patients (58–83 years old females, age 71.1 ± 5.6, Han Chinese) from total knee arthroplasty. None of patients had chemotherapy or trauma to the knee. OA was diagnosed according to the criteria of the American College of Rheumatology (Altman et al., 1986). Two full-thickness cartilage samples were harvested from medial femoral condyle (MFC) and lateral femoral condyle (LFC). Even osteoarthritic patients in the terminal phase of the disease were still had some healthy cartilage, probably at the less weight-bearing area of LFC. The cartilage samples included the area from the surface of the articular cartilage to the subchondral bone. Cartilage samples were divided into two groups: lesion cartilage, harvested from the area adjacent to the most severely degraded cartilage (osteoarthritic area) in which the subchondral bone was exposed, and remote cartilage, harvested from a non-arthritic area or early osteoarthritic area. The full-depth cartilage was removed from the cylindrical sample. Cartilage samples for CS analysis were direct lyophilized and then stored at -80 °C before use. Cartilage for RNA extraction were frozen and ground in liquid nitrogen for immediately RNA extraction.

Human cartilages for CS analysis of three zones were acquired from the knees of 15 end-stage OA patients (61–83 years old females, age 72.5 ± 6.1, Han Chinese) from total knee arthroplasty and were prepared as follows. Full-depth cartilages samples were directly sectioned at 10 μm with a conventional ultramicrotome and glass knife. By visualization under a dissection microscope, the thickness of hyaline articular was measured and defined into three zones: superficial (~200 μm), middle (~400 μm), and deep (~1200 μm) (Fig. 1). Three zones were carefully separated by a scalpel under dissection microscope. Each piece of tissue was direct lyophilized and then stored at −80 °C before use.

2.2. Chondroitin sulfate disaccharide assay

Lyophilized cartilage was obtained by excision with a scalpel and then shredded with scissors. Cartilage sample powder (ranging 0.8–6.2 mg; dry weight in each zone) was dissolved in a solution of 10 μL proteinase K (5 mg/mL in PBS buffer, Sigma-Aldrich) at 37 °C over 12 h. Proteinase K was then inactivated by incubation at 80 °C for 10 min and then centrifuged at 12000 g for 5 min at room temperature to remove the pellets. The post proteinase K treated samples were added into 100 μL of tris-acetate buffer and incubated with 2 μL (20 mU) of chondroitinase ABC (AMSBIO, Abingdon, UK) for 1 day at 37 °C. After, chondroitinase ABC was then inactivated by incubation at 80 °C for 5 min. The samples were filtered through on 0.22 μm filter prior to HPLC analysis. HPLC analysis was performed on the Hitachi system with L-2130 pump, L-2200 autosampler and L-2200 detector. The CS disaccharide standards and the 5 μL of digested samples were applied to

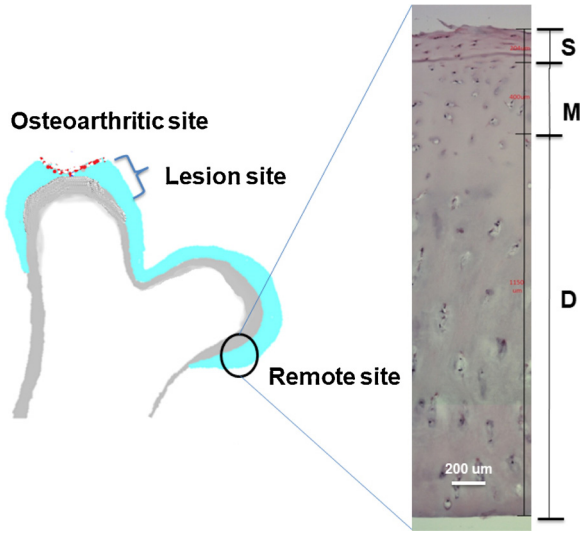


Fig. 1. Human cartilage harvest. A) Each knee provided two cartilage samples which were harvested from the area adjacent to osteoarthritis area, defined as lesion samples, and which were from the area of non-osteoarthritis or early osteoarthritis, defined as remote samples. B) A represented full thickness cartilage from remote site which was stained by safranin O-fast green (original magnification 10X). Three zones, superficial (S, ~200 μm), middle (M, ~400 μm), and deep (D, ~1200 μm) are shown.

a Hypersil SAX column (4.6 mm × 250, 5 μm; Thermo). Eluent A was water and eluent B was 2 M NaCl solution. Both solution A and B were acidified to pH 3.5 with 1.0 N HCl. In the first 4 min, the column was eluted with 100% of eluent A, and the eluent B was increased from 0% to 50% by a linear gradient in the following 40 min. The flow rate was maintained under 1.0 mL/min and the absorbance was measured at 232 nm. Generation of standard curve determining the concentration of CS disaccharide from HPLC and individual data can be seen in Supplemental (Fig. S1, and Table S2).

2.3. mRNA expression levels assay

Fresh cartilage samples, frozen in liquid nitrogen immediately after the surgery, were ground in liquid nitrogen using a mortar and pestle within 6 h of harvested. Ground tissues were kept frozen and immediately transferred to a tune, where they were mixed with 1 mL TRIzol reagent (Thermo Fisher Scientific, Carlsbad, CA, USA). Total RNA was extracted and purified by using RNeasy kit (Qiagen Inc., Valencia, CA, USA), and a reverse transcription reaction and quantitative PCR was carried out by SensiFAST™ SYBR® & Fluorescein One-Step Kit. The expression levels of GalNAc 6-*O*-sulfate sulfotransferase (*CHST2*、*CHST3*、*CHST7*), GalNAc 4-*O*-sulfate sulfotransferase (*CHST8*, *CHST9*, *CHST11*, *CHST12*, *CHST13*, *CHST14*) and *CHST15* mRNA, sulfate donor for sulfotransferase (*PAPSS1*, *PAPSS2*), GalNAc 6-*O*-sulfate sulfatase (*GALNS*), GalNAc 4-*O*-sulfate sulfatase (*ARSB*) mRNA expression level were determined by quantitative PCR. Expression levels of mRNA were normalized by GAPDH expression. The sequences of primers, Ct values of PCR and data can be seen in the supplemental (Tables S1 and S4–S5).

2.4. Statistical analysis

Results are the mean ± S.D. of at least three biological determinations and two technical replicates of each. SAS Version 9.2 (SAS Institute, Cary, NC) was used for the statistical analysis. The data of GAG concentration, each CS concentration of lesion and remote cartilage passed the normality tests, and these differences between lesion and remote cartilage were determined by using two-tailed paired *t*-test. The data of relative gene expressions (*CHST2*、*CHST3*、*CHST7, CHST8*, *CHST9*, *CHST11*, *CHST12*, *CHST13*, *CHST14*, *CHST15*, *PAPSS1*, *PAPSS2, ARSB*, and *GALNS*) differences between both cartilages were determined by using Wilcoxon signed rank sum test. A p value of < 0.05 was considered statistically significant.

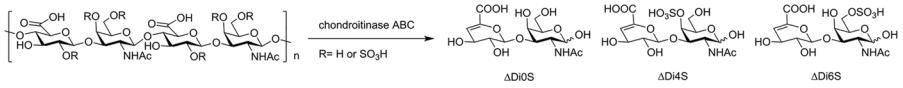
3. Results

3.1. CS composition in the remote and lesion cartilage samples

Three disaccharides ΔDi4S, ΔDi6S and ΔDi0S were identified in HPLC analysis of the cartilage samples (Table 1, Supporting Information, Fig. S3), confirmed by spiking with commercial unsaturated CS disaccharide standards (C-kit, Seikagaku Corporation). Disulfated or trisulfated CS disaccharides were not detected, which might be below the detection limit. The linear regression analyses of three subtypes were created using the commercial standard unsaturated CS disaccharides (Figs. S1 & S2).

The concentration of CS disaccharides in the samples could be determined from the peak area obtained from HPLC analysis by reference to its corresponding standard regression line. The concentrations of ΔDi0S, ΔDi4S and ΔDi6S were measured and pair comparison between the lesion and remote cartilage samples were calculated (Table 1). Pair comparison is the difference of mean value obtained from individual concentration difference between two sites (remote concentration subtracts lesion concentration of each individual). ΔDi6S was the major constituent (~80%) of CS in the cartilage. Both the concentration of ΔDi4S and total CS (sum of ΔDi0S, ΔDi4S, and ΔDi6S) showed significantly higher concentration in the remote samples, as seen in Table 1, they were are 0.008 μg/ mg and 0.015 μg/ mg more in the remote than lesion samples, respectively. On the other hand, no

Table 1
Treating of cartilage with chondroitinase ABC to afford calculated data for the CS disaccharides, as detected by strong anion HPLC.[a]



	Remote cartilage	Lesion cartilage	Pair comparison[b]	P value[c]
ΔDi0S	1.99 ± 1.10	1.85 ± 1.2	0.0008	0.13
ΔDi4S	7.02 ± 2.18	6.37 ± 2.00	0.008	0.01
ΔDi6S	40.1 ± 8.73	39.0 ± 8.81	0.002	0.06
Total CS[d]	49.1 ± 16.0	47.1 ± 17.9	0.015	0.04

[a] Data presented mean ± SD, the unit of the value is μg/mg of dried weight, n = 60.
[b] Mean value of remote concentration subtracted lesion concentration of each individual.
[c] Statistically analysis of pair comparisons.
[d] Total CS: sum of ΔDi0S, ΔDi4S and ΔDi6S.

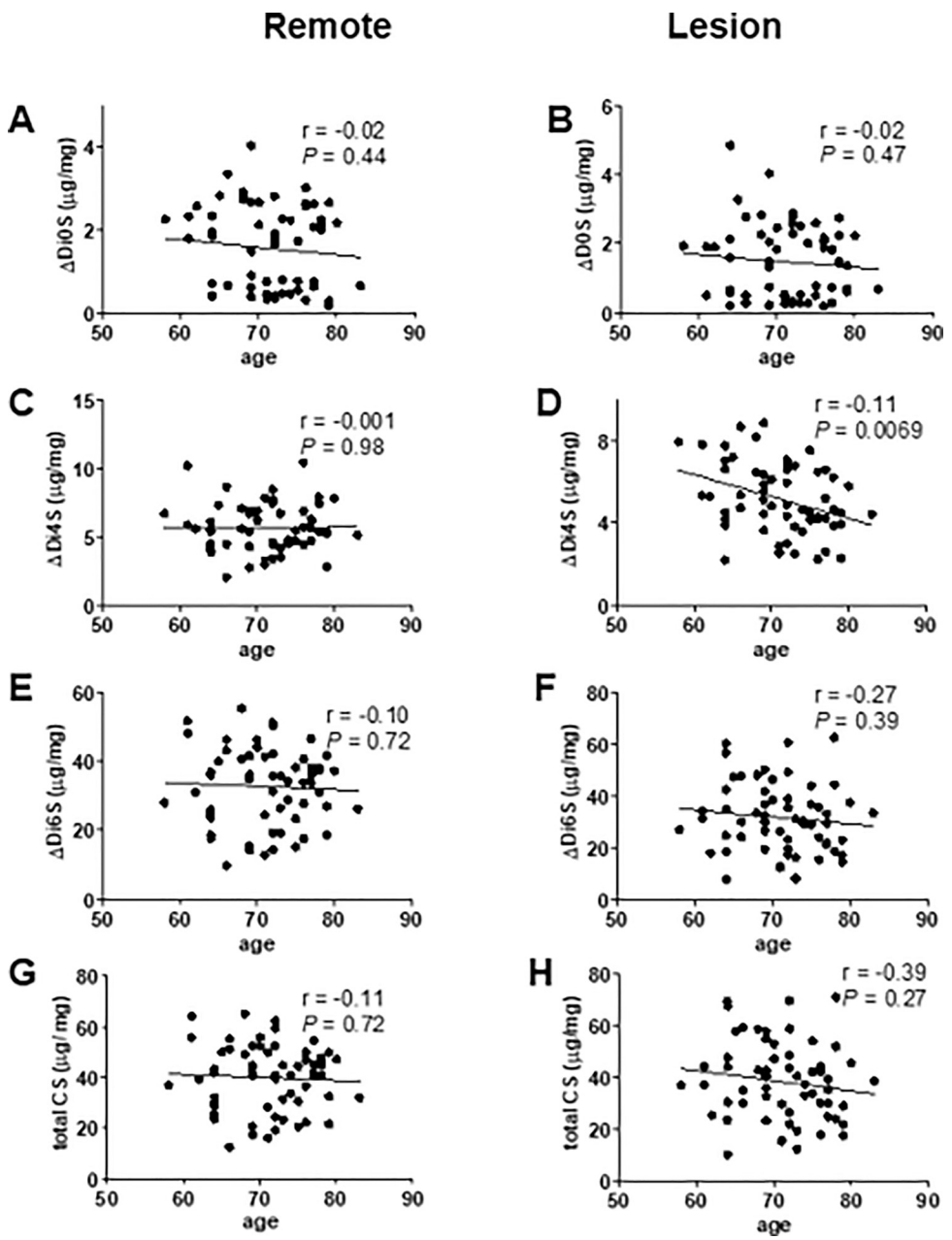


Fig. 2. Correlation of the age and the concentration CS disaccharides. The concentration of Di0S (A), Di4S (C), Di6S (E), total CS (G) are from the remote samples and Di0S (B), Di4S (D), Di6S (F), total CS (H) are from the remote samples. Each scattergram includes the data of 60 patients harvested from remote or lesion cartilages. Concentration of each CS disaccharide is μg of per mg of dried weight.

significant difference was noted in the concentration of ΔDi6S and ΔDi0S in each cartilage.

3.2. Correlation between the structure of CS and age

The concentrations of each CS disaccharide (ΔDi0S, ΔDi4S, ΔDi6S) were found to correlate with the age of the patients (Fig. 2). In lesion cartilage, age had a significant inverse correlation with the ΔDi4S concentration (lesion cartilage: $r = -0.11$, $P = 0.0069$) (Fig. 2D). This phenomenon was not seen in remote cartilages (ΔDi4S in remote cartilage, $r = -0.001$, $P = 0.98$, Fig. 2C). In remote cartilages, concentrations of each CS disaccharide had no significant correlation with the age the patients.

3.3. CS composition in each zone from the remote and lesion cartilages

Additional 15 cartilage samples were collected and CS disaccharides in each zones were quantified (Kuiper & Sharma, 2015) (Table 2). Although the lesion samples were considered to be full-thickness cartilages, their superficial zones were not completed (some of the samples were in fact damaged in the superficial zones, or it was too thin which could be contaminated with tissues of middle zone). Therefore, only middle and deep zones from lesion cartilages were collected. There was significantly more ΔDi4S, ΔDi6S and total CS in deep zone than the middle and superficial zones (Table 2). ΔDi0S was distributed evenly in each zone. Pair comparisons of CS disaccharides in each zone between the remote and lesion area found that only ΔDi4S was significantly less in the deep zone (Fig. 3).

Table 2
The calculated data for the CS saccharide, as detected by strong anionic HPLC. The values represent the mean concentration (μg/mg of dried weight) and their 95% confidence intervals (CI; lower limit, upper limit) of 15 patients.

	Zone[a]	Mean concentration (lower limit, upper limit)		*P* value[e]
		Remote	Lesion	Remote-Lesion
ΔDi0S	S	0.46 (0.10, 0.85)	–	–
	M	0.49 (0.13, 1.28)	0.11 (0.05, 0.25)	0.20
	D	0.47 (0.09, 1.16)	0.32 (0.06, 1.65)	0.13
ΔDi4S	S	1.74 (0.68, 5.62)	–	–
	M	3.33 (1.48, 5.27)**[b]	1.38 (0.41, 2.77)	0.13
	D	4.62 (2.70, 6.23)**[c]	3.19 (0.35-5.64)*[d]	0.0015*
ΔDi6S	S	15.7 (0.09, 41.8)	–	–
	M	34.0 (17.4, 51.8)**[b]	17.6 (10.9, 32.9)	0.11
	D	46.5 (20.8, 67.3)**[c]	39.9 (9.69, 60.3)**[d]	0.15
Total CS	S	17.6 (0.09, 45.1)	–	–
	M	37.8 (19.7, 58.0)**[b]	19.6 (11.4, 34.9)	0.11
	D	51.6 (26.7, 73.0)**[c]	43.4 (10.0, 64.5)**[d]	0.10

*P value < 0.05; ** P value < 0.001
[a] Cartilage samples obtained from remote area of OA cartilages were separated into superficial (S), middle (M), and deep (D) zones. P values obtained from pair-comparison from .
[b] superficial and middle (M-S).
[c] superficial and deep (D-S).
[d] middle and deep (D-M). [e] statistically analysis of pair comparison.

3.4. Gene expression in the remote and osteoarthritic cartilage mRNA expression levels assay

Fresh cartilage samples, frozen in liquid nitrogen immediately after the surgery, were ground in liquid N_2 using a mortar and pestle within 6 h of harvested. Ground tissues were kept frozen and immediately transferred to a tune, where they were mixed with 1 mL TRlozol reagent (Ambion). Total RNA was extracted and purified by using RNeasy kit (Qiagen), and a reverse transcription reaction and quantitative PCR was carried out by SensiFAST™ SYBR® & Fluorescein One-Step Kit. The expression levels of GalNAc 6-*O*-sulfate sulfotransferase (*CHST2, CHST3, CHST7*) were downregulated to have ≤ 10% of mRNA expression in lesion area compared to remote cartilage (Fig. 4A). However, GalNAc 4-*O*-sulfate sulfotransferase (*CHST8*, *CHST9*, *CHST11*, *CHST12*, *CHST13*, *CHST14*) had distinct results, *CHST8*, *CHST12*, *CHST13* and *CHST14* had ≤ 5% of less mRNA expression in lesion cartilage (Fig. 4B). It was found gene expression of 4,6-sulfotransferase (*CHST15)* completely lost in OA cartilage. mRNA expression of *CHST9* had no difference between two groups. *CHST11* was 14-fold mRNA upregulated in lesion cartilage than remote cartilage in this study.

4. Discussion

Articular cartilage is a soft hyaline cartilage that covers the surface of bones. It acts as boundary lubricant. In addition to its poor regenerative capacity, articular cartilage is further broken down by various proteolytic enzymes, e.g. matrix metalloproteases (MMPs) and aggrecanase-1 (ADAMST-4), where the core polypeptide backbones of aggrecan are hydrolyzed, leading to the subsequent loss of GAG and collagen and permanent damage of hyaline cartilage (Lark et al., 1997; Reboul, Pelletier, Tardif, Cloutier, & Martel-Pelletier, 1996). Some reports suggest that the fine structures of GAG becoming more heterogeneous in aging and OA patients, possibly as a results of up/down regulation of specific GAG biosynthetic enzymes, but evidence supporting the claim is lacking. More specifically, large sample size study on the structures and biosynthesis of CS in cartilages of OA patients has not been reported.

In this study, we first quantified CS using anion HPLC analysis. We have demonstrated that the overall CS concentration was lower in severely damaged cartilages, consistent to previous findings in Bollet et al. (Bollet & Nance, 1966) and Ishmaru et al. (Ishimaru et al., 2014) Next we reported the disaccharide composition of CS in the lesion and remote cartilages of 58–83 years old females (n = 60) and we have found that both cartilage tissues have similar components, ΔDi0S, ΔDi4S, ΔDi6S at approximately 5%, 15%, and 80%, respectively (Table 1). Although the sulfation patterns of CS have been reported to vary in both youth (below 20) and mature (20–85 years) patients, the mature cartilages were found to show similar composition of CS (Bayliss et al., 1999). In pairwise comparison of remote and lesion sites, we found that only ΔDi4S was statistically lower in the lesion cartilages. It is also noted that the ΔDi4S pattern is inversely correlated with advancing age in the lesion cartilage, as shown in Fig. 2D. Indeed, we found the concentration of ΔDi4S was lower in the more degraded cartilages. Although we did not investigated the mechanisms that could be responsible for the decreasing ΔDi4S in the aging lesion cartilage, we proposed an imbalance of CS metabolism and altered ΔDi4S can be a potential diagnostic benchmark for the degraded cartilages. One Japanese study including 5 male and 19 female patients with knee OA revealed that C6S/C4S in synovial fluid decreased after arthroscopic surgery, indicating that the content of C4S was an important factor in both male and female patients (Nakajima et al., 2013).

Next, we stained the cartilages at full thickness to study their cell expression pattern and morphology (Sophia Fox, Bedi, & Rodeo, 2009). By analysing the composition of cartilages toward each zone, our study provided the first microstructure analysis of CS in the OA cartilages. In the deep and middle zones of the tissue, the ratio of ΔDi6S to ΔDi4S and also the total CS were significantly higher (Table 2). Since highly sulfated CS bears more anionic charges allowing higher water retention and improved elasticity, such physicochemical properties can counteract against swelling pressure and cope with variable compressive

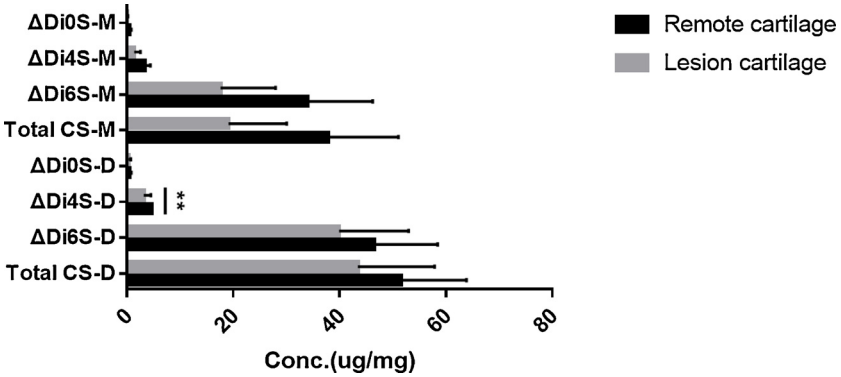


Fig. 3. Bar presentation of disaccharide concentrations (mean, μg/mg of dried weight) of middle and deep zones from remote and lesion cartilages. ΔDi4S was significantly less in the deep zone. ** P value < 0.001.

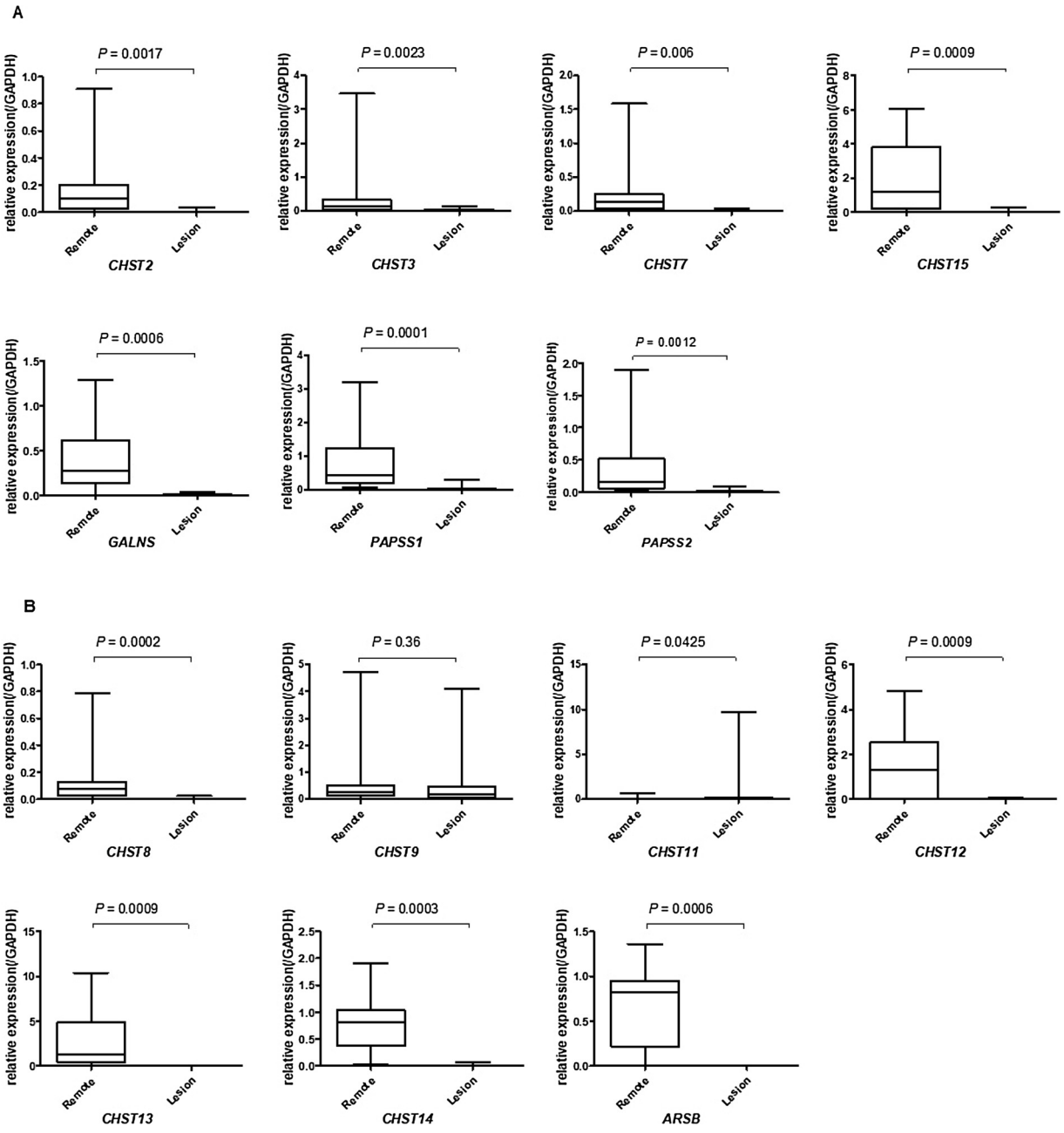


Fig. 4. Quantitative qPCR analysis of mRNA expressions level of sulfotransferases and sulfatases in both remote and lesion cartilages. A) GalNAc 6-*O*-sulfate anabolism (*CHST2*, *CHST3*, *CHST7*, and *CHST15*), PAPSS, and catabolism (*GALNS*) gene expression. B) GalNAc 4-*O*-sulfate anabolism (*CHST8*, *CHST9*, *CHST11*, *CHST12*, *CHST13*) and catabolism (*ARSB*) gene expression. The data are normalized to GADPH expression, which ate presented by whpser-box plots. Top and bottom whiskers represent the maximum and minimum observation, respectively.

loads swiftly. Our study was the first report of ΔDi0S detected in the superficial zone of elderly patient cartilage. Previous study did not detect ΔDi0S in the biopsies of 40 and 50 years old patients (Kuiper & Sharma, 2015), which could be a limitation of the detection method used (fluorphore-assisted carbohydrate gel electrophoresis, FACE). By using strong anion HPLC, we showed that the concentration of ΔDi4S in the deep zone was significantly lower (34% less) in the lesion cartilage.

We reasoned that the lowered ΔDi4S could be associated with the expression of mRNA encoding sulfotransferase (anabolic) and sulfatase (catabolic). We found that the transcript level of sulfotransferase and sulfatase were lower in lesion cartilage, suggesting homeostatic imbalance. We found GalNAc 6-*O*-sulfate sulfotransferases were down-regulated and have ≤10% of mRNA expression level in lesion area compared to remote cartilage (Fig. 4A). However, mRNA expression of GalNAc 4-*O*-sulfate sulfotransferases had distinct results, *CHST8*, *CHST12*, *CHST13*, and *CHST14* had ≤ 5%of less mRNA expression in lesion cartilage (Fig. 4B). The transcript level of *CHST9* had no obvious difference between two groups. 4, 6-sulfotransferase activity was completely abolished in the OA cartilage.

To our surprise, the transcript level of *CHST11* was 14-fold higher in the lesion cartilage compared to the remote cartilage. *CHST11* is involved in chondrocyte development and growth factor signalling during cartilage morphogenesis. Mice deficient in*CHST11* display a severe chondrodysplasia that was restricted to bones formed through endochondral ossification (Klüppel, Wight, Chan, Hinek, & Wrana, 2005). *CHST11* is a target gene of bone morphogenetic protein (BMP) signalling, which contributed to the intrinsic repair capacity of damaged cartilage after the inflammatory cytokines associated with OA

stimulation (Blaney Davidson et al., 2007; Fukui, Zhu, Maloney, Clohisy, & Sandell, 2003). Indeed, a high level of *CHST11* transcript in the lesion cartilage may have been the cartilage feedback mechanism to rehabilitate ΔDi4S, controlling the CS chain elongation (Little, Ballinger, Burch, & Osman, 2008). However, the transcriptome levels often do not correlate with the proteomic or glycomic levels. Whether high transcript level of *CHST11* gene would result in high level of *CHST11* expression or higher population of 4-sulfated CS, this remains to be explored. Herein, lesion area of osteoarthritic cartilage although remain visually normal, OA stimulating by inflammatory cytokines still took place, lowering level of ΔDi4S in the cartilage is an obvious signal for the loss of homeostasis in cartilage.

5. Conclusion

In summary, CS components in articular cartilages of 60 OA patients with total knee arthroplasty are reported. Although the CS composition varied among individuals, the concentration of ΔDi4S and total CS were significantly less in degraded cartilage. It was noted that ΔDi4S concentration was significantly decreased in lesion cartilage with increasing age and its content in deep zone became less compared to remote cartilage. These structure alterations to CS were associated with CS catabolic and anabolic enzymes. In particular, sulfotransferase and sulfatase gene expressions manipulated CS sulfate patterns, which seemed to be important variations in OA cartilages. We found that *CHST11* was upregulated in the lesion cartilage for rehabilitating ΔDi4S. Overall, this study provided valuable insights into the composition of OA cartilages. Moreover, detailed knowledge of cartilage microstructure would give insights into the development of cartilage cell therapies which is in turn a potential therapy for OA.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2019.115496.

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