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ORIGINAL ARTICLE

Serum level of cartilage oligomeric matrix protein is lower in children with idiopathic scoliosis than in non-scoliotic controls

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Abstract
Purpose The etiology of idiopathic scoliosis remains unknown, but growth is a risk factor for progression. Growth pattern differs in children with and without scoliosis. Cartilage oligomeric matrix protein (COMP) may be associated with scoliosis and growth. We, therefore, studied COMP in children with and without idiopathic scoliosis.
Methods We included 105 children, with mean age 14.4 years (range 10–16), under observation or treatment for idiopathic scoliosis, and 103 children from an age-matched population-based cohort. COMP was measured in serum at the time of inclusion. Growth velocity was estimated from repeated height measurements. *T* tests, analysis of covariance or linear regression were used for statistical comparisons.
Results COMP was mean (SD) 11 (5) units/liter (U/L) in children with scoliosis and 13 (5) U/L in the control cohort ($p = 0.005$, adjusted for sex and sampling time of the day). When patients and controls were analyzed together, high COMP was correlated with high growth velocity ($\beta = 0.19$, $p = 0.003$). When patients and controls were analyzed separately, COMP was correlated with growth velocity in children with scoliosis ($\beta = 0.27$, $p = 0.007$), but not in children without scoliosis ($\beta = 0.02$, $p = 0.83$) (all analyses adjusted for age, sex and sampling time). Low COMP was significantly correlated with large curve size in children with scoliosis ($\beta = -0.29$, $p = 0.003$), but not after adjustment for age, sex and sampling time ($\beta = -0.16$; $p = 0.14$).
Conclusion COMP was lower in children with idiopathic scoliosis than in a control cohort. In children with scoliosis, high COMP was modestly correlated with high growth velocity, but not with curve severity.

Keywords Cartilage oligomeric matrix protein · Idiopathic scoliosis · Children · Growth velocity

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Introduction

Idiopathic scoliosis is characterized by an abnormal three-dimensional curvature of the spine, involving both the skeleton and the intervertebral discs. Idiopathic scoliosis is seen in about 3 % of otherwise healthy children and adolescents and is often detected at the time of the prepubertal growth spurt [1]. Growth in that period is faster in children with idiopathic scoliosis than in non-scoliotic children [2, 3]. Around one-tenth of the affected individuals will progress to a more severe form and may be subjected to treatment with bracing or surgery. However, it is difficult

to tell in which children the scoliosis will progress from a mild to a severe form. Frequent follow-ups with clinical inspection of the trunk, estimation of remaining growth and spinal radiographs are, therefore, needed to detect progression.

Cartilage oligomeric matrix protein (COMP) in serum has been associated with growth velocity in children with juvenile idiopathic arthritis [4]. A recent report describes that the COMP gene is down-regulated in osteoblasts from patients with idiopathic scoliosis, as compared to controls [5]. Mutations in the COMP gene cause pseudoachondroplasia or multiple epiphyseal dysplasia, conditions which may include spinal deformity [6, 7].

The functional role of COMP remains largely unknown. COMP exists in the extracellular matrix and has been found in cartilage, tendon, bone, synovium [7], and intervertebral discs [8], and is expressed in chondrocytes, osteoblasts [9] and in the growth plate [10]. COMP may contribute to stabilize cartilage extracellular matrix by interactions with other extracellular components, and interaction of COMP and extracellular matrix protein 1 (ECM1) in the growth plate has been suggested to regulate endochondral bone formation [11].

In summary, COMP may be associated with growth and spinal deformity. We, therefore, hypothesized that COMP may be different in patients with idiopathic scoliosis than in controls. The main aim of this study was to investigate if serum COMP differs between children with and without idiopathic scoliosis.

Materials and methods

This is an age and sex-matched case–control study in children with and without scoliosis, all 10–16 years of age.

Cases

Between 2004 and 2010, patients diagnosed with juvenile or adolescent idiopathic scoliosis, were invited to take part in a multicenter study concerning heredity and genetics. Details of the study have been published elsewhere [12]. Inclusion criteria were an idiopathic scoliosis with a Cobb angle of 10° or more, and no signs or symptoms associated with a non-idiopathic scoliosis. For this specific study, only patients between 10 and 16 years ($n = 105$) and currently under treatment or observation at the Skåne University Hospital in Malmö were included.

The Cobb angle was registered by the same observer for the largest curve at the time of inclusion. If surgery had been performed, the Cobb angle was determined from the last radiograph prior to surgery. For boys, age at diagnosis was 12.0 (2.6) years and the largest curve 33 (14)° and in girls 11.1 (2.3) years and 38 (16)°, all values mean (SD).

The patients were treated according to the protocols at the clinic. In general, brace treatment was started in patients with curves between 25° and 45° with remaining growth, whereas surgery was indicated in patients with curves larger than 45° and remaining growth. At the time of inclusion, 67 patients were observed, 18 were brace treated and 20 had been surgically treated for idiopathic scoliosis.

Controls

Age-matched controls were selected from a longitudinal population-based project of school children that were included with beginning in 1999 [13], and still are followed prospectively. The main aims of this study are to compare bone health, physical activity and school performance in children in a school with more frequent physical education compared to children in schools with less frequent physical education. In total, 668 children were invited and 600 children took part in the study at least once. Serum samples were collected from the volunteers among these children. All controls had been imaged with a whole-body and spinal dual-energy X-ray absorptiometry (DXA) scan for the purpose of studying bone density. DXA has been reported to be a reliable method to evaluate scoliosis, and comparable to conventional radiographs [14].

Matching procedure

Among the 105 children with scoliosis, 88 were girls and 17 were boys. Cases and controls were intended to be age-matched as follows; girls 1:1 and boys 1:2. During matching, it was found that there was a relative shortage of girls with serum samples in the same age among the controls, and matching to a 1:1 ratio was not possible. Three female controls were found to have scoliosis and were excluded from further analysis. Among the 103 controls, 69 were girls and 34 were boys. At the time of serum sampling, 29 children went to schools with 60 min weekly physical education and 74 children to a school with 200 min weekly physical education.

Serum sampling procedure

Non-fasting venous blood sampling was performed at the same institution for both cases and controls. Mean sampling time was 12:00 h (range 8:15–15:51 h). Samples were allowed to clot for at least 30 min and were then centrifuged within 2 h from sampling. Serum was saved and stored in −80 °C until analysis.

Cartilage oligomeric matrix protein

Serum levels of COMP were measured with an enzyme-linked immunosorbent assay (ELISA), as described by the manufacturer (COMP ELISA, AnaMar, Uppsala, Sweden), and expressed as units/liter (U/L). One U/L is approximately 0.1 mg/ml of COMP. According to the manufacturer, the intra-assay precision is less than 3.0 % and the inter-assay precision is less than 4.2 %. All samples were analyzed at the same time at the Skåne University Hospital Clinical Chemistry Laboratory in Lund, Sweden in June 2012.

Anthropometry

Body weight and height were measured in a standardized way at the same facility for all subjects. Body mass index (BMI) was calculated as kg/m^2. In addition, body height for the patients with scoliosis was corrected according to Bjure et al. [15]. One additional body height measurement about a year from the inclusion was available in 94 patients and 83 controls. Growth velocity was calculated as the difference between two uncorrected body height measurements, divided by the time elapsed between the two measurements, and expressed as cm/year.

Skeletal maturity

In the patients with scoliosis, data on skeletal maturity were collected from the routine radiographical examination of the hand skeleton performed close to the inclusion in the study. In the controls radiographical examination of the hand was part of the research protocol. Skeletal age was assessed by an experienced radiologist according to the standard described by Greulich and Pyle. To compare skeletal age between patients and controls, the difference between chronological age and skeletal age was calculated.

Ethical consent

Written informed consent was obtained from all study participants. The ethical review board at Lund University approved all parts of the study: LU 363-02 (cases) and LU 453-98, LU 368-99, LU 369-99, LU 266-00 and 369/2004 (controls).

Statistics

Normal distribution of the variables was assessed by visual inspection. Serum COMP and growth velocity were not normally distributed. Visual inspection and plot of residuals revealed a better fit after logarithmic transformation of serum COMP and growth velocity. The logarithmically transformed variables were, therefore, used in all statistical analyses. Independent samples t test or analyses of covariance (ANCOVA) were used to compare groups. Linear regression was used to study the relationship between continuous variables. Some analyses were adjusted for differences in sex, age and sampling time (samples taken before or after noon), and in controls, information on whether they attended a school with 60 or 200 min weekly physical education. The statistical analyses were performed in IBM SPSS Statistics, version 22. A p value of <0.05 was considered significant.

Results

The mean (SD) S-COMP was 11 (5) U/L in the group of children with scoliosis and 13 (5) U/L in the control group ($p = 0.005$, adjusted for sex and sampling time differences). When looking separately at boys and girls, we found that girls with scoliosis had lower S-COMP than girls without scoliosis ($p = 0.006$) (Tables 1, 2). The difference between chronological age and skeletal age was similar in patients and controls (Tables 1, 2).

Children with scoliosis had similar body height, weight and BMI as children without scoliosis ($p = 0.23$, $p = 0.40$ and $p = 0.12$, respectively, all analyses adjusted for sex differences). When height was corrected according to Bjure et al. [15], children with scoliosis were taller and had lower BMI than children without scoliosis ($p = 0.001$ and $p < 0.001$, respectively, both analyses adjusted for sex differences). Separate data for boys and girls are shown in Tables 1 and 2. Growth velocity was lower in children with

Table 1 Descriptive data for boys with idiopathic scoliosis and boys without scoliosis, shown as mean and standard deviation (SD)

	Patients ($n = 17$)		Controls ($n = 34$)		p
	Mean	SD	Mean	SD	
Serum COMP (U/L)	12	5	14	5	0.18
Age at investigation (years)	15.0	1.0	14.9	0.9	0.87
Corrected body height (m)[a]	1.77	0.10	1.73	0.07	0.10
Body weight (kg)	57.6	7.4	63.4	13.9	0.12
BMI (kg/m^2)[a]	18.4	1.7	21.0	3.8	0.008
Difference in chronological age and skeletal age (years)[b]	−0.1	1.1	−0.4	1.2	0.40
Growth velocity (cm/year)	3.5	4.2	5.2	2.7	0.024

p value for t test is shown

[a] Body height for patients is corrected for curve severity according to Bjure et al. [15]

[b] Data on skeletal age were available in 13 boys with scoliosis and 29 boys without scoliosis

Table 2 Descriptive data for girls with idiopathic scoliosis and girls without scoliosis, shown as mean and standard deviation (SD)

	Patients ($n = 88$)		Controls ($n = 69$)		p
	Mean	SD	Mean	SD	
Serum COMP (U/L)	11	5	13	5	0.006
Age at investigation (years)	14.3	1.2	14.1	1.2	0.51
Corrected body height (m)[a]	1.68	0.07	1.63	0.08	<0.001
Body weight (kg)	54.0	11.2	54.0	10.2	0.98
BMI (kg/m^2)[a]	19.0	3.4	20.2	3.0	0.025
Difference in chronological age and skeletal age (years)[b]	−0.1	1.1	0.0	1.0	0.49
Growth velocity (cm/year)	2.0	2.4	3.1	2.6	0.016

p value for t test is shown

[a] Body height for patients is corrected for curve severity according to Bjure et al. [15]

[b] Data on skeletal age were available in 73 girls with scoliosis and 63 girls without scoliosis

scoliosis than in controls ($p = 0.001$, adjusted for sex differences). Also, when looking separately at boys and girls, children with scoliosis had a lower growth velocity than children without (Tables 1, 2).

When patients and controls were analyzed together, high S-COMP was correlated with high growth velocity ($\beta = 0.19$, $p = 0.003$, adjusted for age, sex and sampling time differences) (Fig. 1). When patients and controls were analyzed separately, high S-COMP was correlated with high growth velocity in children with scoliosis ($\beta = 0.27$, $p = 0.007$), but not in children without scoliosis ($\beta = 0.02$, $p = 0.83$) (both analyses adjusted for age, sex and sampling time differences). Additional adjustment in children without scoliosis for amount of physical education in school did not change the result ($\beta = 0.03$, $p = 0.77$).

In the group of children with scoliosis, low S-COMP was significantly correlated with large curve size ($\beta = -0.29$, $p = 0.003$), but not after adjusting for age, sex and sampling time differences ($\beta = -0.16$; $p = 0.14$) (Fig. 2).

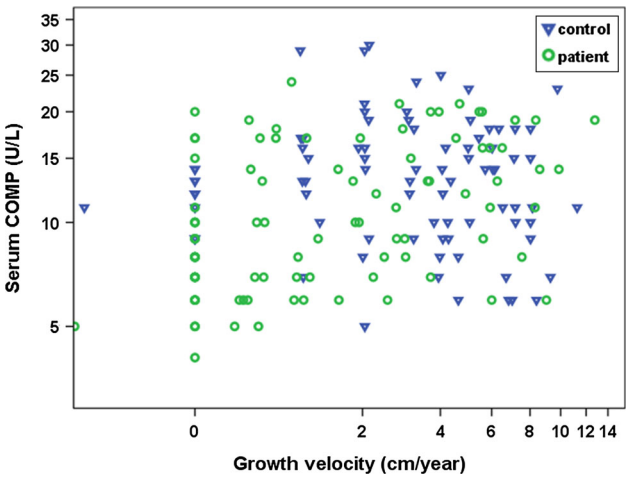

Fig. 1 Serum levels of cartilage oligomeric matrix protein (COMP) in relation to growth velocity in patients (*green circles*) and controls (*blue triangles*). The scales of both axes are logarithmic. The β-value from the linear regression between the logarithmically transformed serum level of COMP and growth velocity including both patients and controls was 0.19 ($p = 0.003$). When patients and controls were analyzed separately the β-value was 0.27 in children with scoliosis ($p = 0.007$) and 0.02 in children without scoliosis ($p = 0.83$) (all analyses were adjusted for age, sex and sampling time differences)

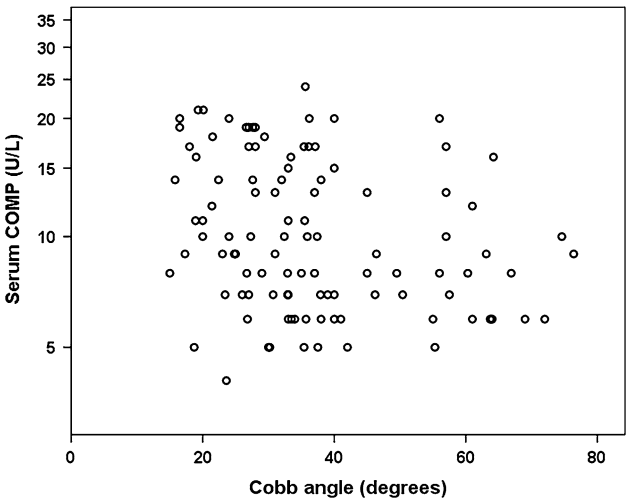

Fig. 2 Serum levels of cartilage oligomeric matrix protein (COMP) in relation to curve severity according to the largest Cobb angle in the 105 patients with idiopathic scoliosis. The scale of the *Y*-axis is logarithmic. The β-value from the linear regression between the logarithmically transformed serum COMP and the maximum Cobb angle was −0.29, $p = 0.003$. After adjustment for age, sex and sampling time differences, β was −0.16 ($p = 0.14$)

Discussion

In this study, we found that children with scoliosis had a lower level of serum COMP than children without scoliosis. High S-COMP was modestly associated with high growth velocity in children with scoliosis, but not in controls. S-COMP was not associated with curve severity.

A recent study reports a higher prevalence of joint hypermobility in children with idiopathic scoliosis when compared to healthy controls [16]. Another study of individuals without scoliosis reports that hypermobility is associated with low serum levels of COMP [17]. Taken together, these studies indicate an association between low serum levels of COMP and idiopathic scoliosis. Data on hypermobility status are not available in our cohorts, so whether hypermobility is more common in the scoliosis

patients than in the controls in the present study cannot be determined.

COMP has been suggested to be involved in endochondral bone formation and has been discussed to overcome growth inhibiting effects by ECM1 [11]. At the same time, COMP may act as a catalyst in collagen fibrillogenesis [18] which makes it attractive to speculate, that a down regulation of the COMP gene [5] reflected by lower serum COMP levels in the scoliosis cohort, in some way disturbs growth coordination in the spine. The expression and synthesis of COMP is influenced by application of dynamic and static load in vitro [19]. In vitro experiments suggest that depending on concavity and convexity, the composition of extracellular matrix in intervertebral discs and vertebral endplates is likely to be influenced by the changes in mechanical load [20]. Local changes in mechanical loading of the scoliotic spine may lead to a more uncoordinated endochondral growth in the vertebral disc and vertebral endplates and may end up in the development of idiopathic scoliosis.

Treatment with growth hormone in prepubertal children with short stature leads to increased levels of COMP [21]. A relationship between serum COMP and growth velocity has been reported in children with juvenile idiopathic arthritis [4], but there are no data available for healthy children. Serum COMP levels were modestly associated with growth velocity, at least in the children with scoliosis. Some caution has to be applied when interpreting the growth velocity data in the present study. Data were missing for some individuals, and exact growth velocity at the time of sampling could not be assessed. We have no clear explanation for the lack of association in children without scoliosis, and we cannot exclude the possibility that different growth patterns in patients with scoliosis and controls may have an impact on the finding. However, there were no differences in chronological and skeletal ages, or the distribution of these, in patients and controls. In addition, data were adjusted for chronological age.

There are some limitations with the study. The mean difference in S-COMP between patients and controls was 2 U/L, but non-significant when boys were analyzed separately. We believe it is likely that the main reason for this is the lack of power to detect a difference in the smaller male groups, rather than a true sex difference.

Most of the subjects were included after the prepubertal growth spurt [3], and to further elucidate the relation between COMP, scoliosis and growth velocity, studies on younger children as well as longitudinal studies would be appropriate.

Controls were selected from schools with different levels of physical education. Some studies reported serum COMP to increase by physical activity [22, 23], but not all [24]. Furthermore, COMP levels in serum return to baseline levels within 30 min after the end of physical exercise [25] and 12 weeks of exercise did not increase mean levels of serum COMP in men, aged 18–25 years [22]. In the present study, serum COMP levels in the controls did not differ between children with more or less physical education. The amount of physical education among the children with idiopathic scoliosis was not known. Neither patients, nor controls were told to refrain from exercise before venous sampling. All samples were taken at the same facility. We, therefore, believe that the sampling conditions for the patients and controls were similar.

Samples were taken during different times of the day. Some biochemical analytes are sensitive to diurnal variation. The diurnal variation in COMP does not seem prominent [26], and in this study there was no significant difference between samples taken before and after noon (data not shown). In addition, analyses were adjusted for sampling time.

In conclusion, we have described an association between idiopathic scoliosis and a biomarker associated with cartilage, skeletal growth and joint laxity. The findings indicate that there exist factors affecting serum COMP in children with idiopathic scoliosis, which is not found in children without scoliosis. Future studies need to clarify the pathophysiologic role of COMP in idiopathic scoliosis.

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Conflict of interest The authors have no conflicts of interest.

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