Association of collagen Type I α1 gene polymorphism with bone density in survivors of childhood cancer - preliminary report

Muszyńska-Rosłan K[1], Galicka A[2], Sawicka M[1], Krawczuk-Rybak M[1], Wołczyński S[3]

[1]Department of Paediatric Oncology, [2]Department of Medical Chemistry,
[3]Department of Gynaecological Endocrinology, Medical University of Białystok, Poland

Abstract

A candidate gene, involved in the regulation of bone mass is the COLIA1 gene encoding type I collagen, the major protein of bone matrix. The disease per se, the age of its onset and treatment options might exert an impact on bone mineralization in survivors of childhood malignancy. We examined possible allelic influences of COLIA1 gene polymorphism on BMI, BMD spine and total body in 41 survivors (15 girls) of childhood cancer (the mean age 8.9 years). Genotype distribution was 33 (80.5%) SS and 8 (19.5%) Ss. There were no differences in SDS BMD and SDS BMI between patients with SS and Ss genotype. A tendency towards lower SDS values of BMD spine and BMI was observed (not significant). In conclusion, our preliminary observations suggest that COLIA1 genotype may affect bone accrual in a population treated for childhood cancer. Further investigations in a greater population are needed.

Key words: childhood cancer, collagen type I, polymorphism, bone, osteoporosis.

Introduction

Osteoporosis is a common disease, characterized by reduced bone mineral density (BMD), damage of the architecture of bone tissue and an increased risk of non-traumatic fractures. Variation in the attainment of peak bone mass plays an important role in the development of osteoporosis. Based on family and twin studies, peak bone mass has been estimated to be up to 85%, as genetically determined. Several polymorphisms in "candidate" genes have been investigated as genetic factors that could influence bone mineral density [1]. In many studies, polymorphism Sp1 binding site, in the first intron of the collagen type Iα1 (COLIA1) gene, has been proved to be associated with low bone density [2, 3]. Treatment for malignancy in childhood can result in diminished peak bone mass and osteoporosis development in adults [4]. We hypothesize that COLIA1 genotype might affect anthropometric or densitometric characteristics of bone in children and young adults exposed to negative environmental factors, connected with malignant disease and its treatment. Therefore, we tried to investigate the incidence and association between COLIA1 polymorphism and bone characteristics in survivors of childhood cancer.

ADDRESS FOR CORRESPONDENCE:
Katarzyna Muszyńska-Rosłan
Department of Paediatric Oncology
Medical University of Bialystok
Waszyngtona 17 15-274 Białystok, Poland
e-mail: kmroslan@amb.edu.pl

Material and methods

We examined 41 patients (14 girls) diagnosed for leukaemia and lymphoma (n= 34) and solid tumour (n= 7) at the mean of age of 8.9 years (range 1.66 - 19.83). The mean age of diagnosis was 3.7 years. All the subjects had received chemotherapy (CHT), 24 additionally steroidotherapy (ST) and 7 - radiotherapy (RTX). All the patients underwent a physical examination; their height was assessed, using a fixed stadiometer, their weight on a standard clinical balance, the pubertal development, according to Tanner's scale. Body mass index was calculated, according to the formula: weight/height^2 (kg/m^2) and expressed as standard deviation score (SDS). Bone mineral density (BMD g/cm^2) was determined by dual energy x-ray absorptiometry (Lunar DPX-L Madison WI version 1.35) of the lumbar spine and total body in children over 5 years of age (n= 26) - the acquired results were compared with the results of 473 healthy references and expressed as SDS. The G to T polymorphism ratio in the Sp1 binding site in the COLIA1 gene was detected by the polymerase chain reaction-based method with primers

and reaction conditions, as described previously, using DNA extracted from whole blood. The reaction products were digested with the restriction enzyme MscI and analysed by agarose gel electrophoresis. The genotype was defined as "S" or "s", according to either the absence or presence of restriction site. Statistical analysis: to correct for the age differences between the genotype, anthropometric parameters and bone characteristics were expressed in age and sex-matched standard deviation scores (SDS). The data were analyzed by using t-test for unpaired samples. Linear regression was made to assess the dependence of bone mineral density on the independent variables, such as clinical characteristics and genotype. P-values of less than 0.05 were considered significant. The study was approved by the Medical Ethics Committee of the Medical Academy of Białystok

Results

The characteristics of the studied subjects are shown in Table 1. The genotype distribution was 33 (80.5%) SS - homozygous for absence of MscI site; 8 (19.5%) Ss heterozygous; we did not find ss - homozygous for presence of MscI site. There were no differences in SDS values of BMI and BMD total and spine between SS and Ss genotypes. We observed a tendency towards lower SDS values of BMI (0.654 ±2.11 vs -0.138 ±2.44) and BMD spine (0.43 ±1.33 vs -0.75 ±1.48) in children with Ss genotype; after an adjustment for BMS SDS, the COLIA1 genotype effect on BMD spine disappeared. There was no significant association between the age of diagnosis, the kind of neoplastic disease, body mass index and genotype.

Discussion

Type I collagen is the major protein of bone. Mutations in the coding regions of the COLIA1 genes are found in diseases associated with severe osteoporosis. Grant et al. detected polymorphism in the first intron of the COLIA1 gene, involving the binding site for the transcription factor Sp1, important for the regulation of gene expression and type I procollagen synthesis [5]. Most studies have shown a negative effect of COLIA1 "s" allele on BMD. However, they were performed in adults who had undergone substantial bone loss [2, 6]. Children had a shorter exposure to lifestyle and environmental factors which can influence the overall effect of genetic factors on bone development and mineralization. The studies in paediatric population have shown conflicting results because of different methods used to measure bone mass, the expression of data, the age and the gender. Berg et al. concluded that the polymorphism at the Sp1 binding site in the COLIA1 gene is not associated with BMD in healthy boys and girls [7]. No association was found between COLIA1 polymorphism and BMD or BMC (bone mineral content) in 428 prepubertal children examined by Willing et al. [8] On the other hand, Sainz et al. described lower bone density in prepubertal girls with the Ss and ss genotype, compared to girls with SS genotype, but no allele effect was found on bone size [9]. In childhood and especially during puberty, bone mineral density and body size change markedly. Van der Sluis et al.

Table 1. Clinical characteristics, anthropometric and densitometric parameters in relation to COLIA1 gene alleles.

Subjects	SS genotype	Ss genotype
N = 41	33	8
Age of study = 8,9 years	8.34 years	11.35 years
Sex: female N= 15 Male N= 16	F/ N=13 M/ N= 10	F/ N= 2 M/ N= 6

a) prepuberty N= 21
b) during puberty N= 10
c) post puberty N= 10
	a) N= 19;
	b) N= 8;
	c) N= 4
		a) N= 2;
		b) N= 2;
		c) N= 6

Treatment with CHT and RT N = 7	N= 5	N= 2
Treatment with CHT and ST N = 34	N= 28	N= 6
BMI (kg/m^2)	18.05 ±3.7	17.7 ±4.3
BMD total - (g/m^2)	0.883 ±0.15	0.947 ±0.22
BMD spine - (g/m^2)	0.719 ±0.22	0.820 ±0.35
BMD total SDS	-0.837 ±1.75	0.213 ±2.04
BMD spine SDS	0.436 ±1.33	-0.754 ±1.4
BMI SDS	0.655 ±2.12	-0.138 ±2.44

studied 148 healthy Caucasian children and showed that subjects with Ss and ss genotype had decreased SDS BMC and SDS BMD for both lumbar spine and total body and a shorter stature and smaller bones comparing to SS genotype. Similar associations were found at follow-up after 4 years [3]. The comparison various methods (DEXA, CT, and ultrasound) support the hypothesis that SP1 COLIA1 polymorphism is more strongly associated with bone morphology and quality than with mineralization [3, 7, 8, 9]. Children with malignant disease may be considered a subgroup at high-risk of developing osteoporosis. Unfavourable factors, e.g., cancer disease per se, the age of its onset, treatment options might influence bone mineralization in survivors of childhood malignancy [4]. In our preliminary study, we did not find any significant differences between genotype and SDS BMI and SDS BMD total and spine. The allele incidence rates of the COLIA1 variants were similar to those reported for other Caucasian population. We did not make any statistical analyses, dependending on Tanner's stage to compare children with SS and Ss genotype, due to a small population. We observed a tendency towards lower BMI SDS and BMD spine SDS in heterozygous patients, however, after a correction for BMI, the small differences between both groups decreased. The similar results, obtained in previous studies, indicate that the effect of the COLIA1 genotype on bone mineral density can partially be mediated by its genetic effect on body size. In summary, our preliminary observations suggest an influence of COLIA1 genotype on bone accrual in the population treated for childhood cancer. Further investigations on polymorphisms of other "candidate" genes in a greater cohort of children with neoplastic disease are needed.

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