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Association of vitamin D receptor gene *Cdx2* polymorphism with bone markers in Turner syndrome patients

Abstract

Background: Turner syndrome (TS) patients usually have low bone mineral density (BMD) and increased risk of osteoporotic fractures. We have previously demonstrated an association of bb (*Bsm*I polymorphic site) and ff (*Fok*I polymorphic site) vitamin D receptor (VDR) genotypes with reduced BMD in TS patients.
Aim: To analyze the relationship between VDR-*Cdx2* polymorphism and BMD as well as bone metabolic variables in TS patients.
Methods: Fifty-five TS patients and 59 control women were studied. VDR-*Cdx2* genotypes were determined using TaqMan probes in a real time thermocycler. Lumbar and femoral BMD were determined by dual-energy X-ray absorptiometry (DEXA) and serum intact parathyroid hormone, osteocalcin and β-CrossLaps were determined by electrochemiluminescence.
Results: Patients with genotype GG had higher levels of both osteocalcin and β-CrossLaps as compared to patients with genotype GA ($p<0.01$ and $p<0.05$, respectively).
Conclusion: Patients carrying genotype GG have higher levels of bone formation and resorption markers. This indicates a more active bone turnover that could impact on their future bone mineral density.

Keywords: β-CrossLaps; bone mineral density; osteocalcin; Turner syndrome; vitamin D receptor- (VDR-)*Cdx2* genotypes.

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Introduction

Turner syndrome (TS) patients frequently have reduced bone mineral density (BMD) that appears in prepubertal stages and partially persists after estrogen replacement. This suggests a genetic predisposition to skeletal demineralization combined with the chronic estrogen deficiency due to gonadal disgenesis. The association of some polymorphisms in VDR gene with BMD, bone markers and osteoporotic fractures has been studied in different populations with varying results. Previous data from our laboratory have shown an association of bb (*Bsm*I polymorphic site) and ff (*Fok*I polymorphic site) VDR genotypes with reduced BMD in TS patients (1). *Cdx2*, a G-A polymorphic variant in the promoter of the VDR gene, has been found to be associated with low bone mineral density and susceptibility to fractures (2, 3). The aim of the present study was to analyze the relationship between *Cdx2* polymorphism and BMD, bone formation and resorption markers and other biochemical parameters of bone metabolism in TS patients.

Subjects and methods

Fifty-five TS patients from the Endocrinology Service of the Hospital de Niños de la Santísima Trinidad, Córdoba, Argentina (CA

15.83±0.96 years of age) and 59 healthy women from the same region (CA 18.11±0.68 years of age) were studied. Clinical diagnosis of TS was confirmed with the karyotype of 50 metaphases peripheral lymphocytes performed by standard cytogenetic techniques and GTG banding. Fluorescence in situ hybridization (FISH) was practiced to identify low-level mosaicisms. The phenotype of the patients was considered severe if they had organ malformations and mild if not. Thirty-three patients had been treated with recombinant human growth hormone (rhGH) (0.33 mg/weight/week) for an average period of 3 years and 24 patients with hormone replacement therapy (ethinyl estradiol, 0.07 μg/kg/day; range: 0.03–0.37 μg/kg/day) for an average period of 5 years. Subjects with: hepatic or renal disease; malabsorption syndrome; hyperparathyroidism; malignancy in the last 5 years or treated with estrogens for the last 3 months before the study; glucocorticoids or some other drug capable of altering calcium metabolism; were not included in the control group. Controls were studied only for genetic analysis. Written informed consent was signed by all participants or their tutors. The study project has been previously approved by the Comité de Etica de la Facultad de Ciencias Médicas, Universidad Nacional de Córdoba and the Comité Institucional de Etica en la Investigación en Salud del Niño y el Adulto.

Lumbar and femoral BMD were measured by dual-energy X-ray absorptiometry DEXA (Norland XR36 Quick Scan; Fort Madison, Wisconsin, USA) and were expressed in Z-score. Daily scans of an anthropomorphic phantom were performed in order to check the stability of the equipment. The coefficient of variance was lower than 0.48% during the period of this study. Serum calcium and inorganic phosphorus were assayed with standard methods. Serum intact-PTH, N-MID osteocalcin and β-CrossLaps were determined using electrochemiluminescence immunoassay kits from Roche Diagnostic Corporation, Indianapolis, IN, USA.

Genomic DNA was isolated from peripheral leukocytes in EDTA blood samples by a standard phenol-chloroform extraction procedure. Genotyping was performed by the 5′ nuclease TaqMan assay, using a Brilliant QPCR Core Reagent Kit (Agilent Technologies, Stratagene, La Jolla, CA, USA). PCR reactions were carried out in a quantitative PCR thermocycler (StratageneMx 3000P, Agilent Technologies, Santa Clara, USA) under standard conditions recommended by the manufacturer. The primers were: forward 5′-CATTGTAGAACATCTTTTGTATCAGGAACT-3′ and reverse 5′-GGTCTTCCCAGGACAGTATTTTTCA-3′. The probe used to identify allele A was VIC-5′-AGGTCACA**A**TAAAAAC-3′ (5 μM) and the probe for allele G was FAM-5′-AGGTCACA**G**TAAAAAC-3′ (5 μM) (4). Statistical analysis was carried out with SPSS software (Version 17.0) for Windows XP (SPSS Inc., Chicago, IL, USA). A χ^2-test was used to compare observed genotype frequencies with those expected under the Hardy-Weinberg equilibrium. The association between BMD Z-scores and bone markers with karyotypes, phenotypes and VDR-*Cdx*2 genotypes was analyzed by a general linear model (GLM)-ANOVA procedure followed by a Bonferroni post hoc test and adjusted by age and current estrogen therapy. Differences were considered statistically significant when $p<0.05$.

Results and discussion

Thirty-eight (69.09%) of the TS patients had 45,X karyotype, fourteen (25.46%) had different kind of mosaicisms and three (5.45%) carried a structural abnormality. Nineteen patients (34.54% of the studied TS population) presented a severe phenotype, having at least one visceral anomaly, while the rest had mild phenotypes. The frequency of severe phenotypes was higher among patients with 45,X karyotype (42.11%) than in patients with mosaicisms (14.29%). This tendency to major severity in 45,X carriers agrees with data reported in the literature (5).

Biochemical variables and bone formation and resorption markers were within the normal range (calcium, 2.51±0.02 mmol/L; phosphorus, 1.5±0.04 mmol/L; PTH, 3.03±0.22 pmol/L; osteocalcin, 13.85±0.8 nmol/L and β-CrossLaps, 12.55±0.86 nmol/L), indicating an accurate control of extracellular calcium and phosphorus homeostasis. However, most patients had osteopenia, with an average BMD Z-score at the femoral neck of –1.42±0.15 and at the lumbar spine of –1.38±0.16. Almost 30% of the patients had BMD more than 2 SD below the reference value.

The frequency of alleles G and A was similar in both groups (relative frequency of allele G was 0.80 in TS patients vs. 0.77 in controls; relative frequency of allele A was 0.20 in TS vs. 0.23 in controls) and the

Genotypes	TS patients	Control women	p-Value
Cdx-2			
GG	60.0% (33)	54.2% (32)	
GA	40.0% (22)	45.8% (27)	0.12
AA	0.0% (0)	0.0% (0)	

Table 1 Genotype distribution of VDR-*Cdx*2 polymorphic site in TS patients and control women.
The frequency of genotypes is expressed as percentage of the total population in each group. *Cdx2,* polymorphic site in the promoter of VDR gene; ( ), number of cases. χ^2-test was used for frequency analysis.

	VDR-*Cdx*2 genotype	
	GG	GA
Calcium, mmol/L	2.52±0.02 (29)	2.49±0.04 (22)
Phosphorus, mmol/L	1.57±0.05 (29)	1.47±0.05 (22)
Osteocalcin, nmol/L	15.98±1.20 (29)[a]	11.04±1.42 (22)
β-CrossLaps, nmol/L	14.18±1.19 (28)[b]	10.38±1.10 (21)
Femoral neck BMD (Z-score)	–1.29±0.20 (32)	–1.50±0.26 (22)
Lumbar spine BMD (Z-score)	–1.31±0.19 (33)	–1.58±0.23 (22)

Table 2 Relationship between VDR-*Cdx*2 genotypes and serum calcium, phosphorus, bone markers and femoral neck and lumbar spine BMD in TS patients.
Calcium, phosphorus, bone markers and BMD are expressed as means±standard errors. ( ), number of cases. Statistical analysis: one way ANOVA and Bonferroni post-hoc test. [a]$p<0.01$ vs. GA; [b]$p<0.05$ vs. GA.

polymorphism was in Hardy-Weinberg equilibrium within each group. Genotypes at *Cdx*2 polymorphic sites were similarly distributed in subjects with TS and in control women as shown in Table 1. Genotype AA was not found among the study participants, presumably due to the low frequency of this genotype among the Caucasian population (6).

The relationships between the VDR-*Cdx*2 genotypes and bone variables are presented in Table 2. Serum calcium and phosphorus were independent from the genotypes and so were lumbar and femoral BMD. However, patients with genotype GG had significantly higher levels of osteocalcin and β-CrossLaps than heterozygote patients, indicating a more active bone turnover in TS patients carrying the genotype GG. The higher bone formation and resorption rate could eventually impact on the future BMD of TS patients carrying genotype GG. Longitudinal studies could probably shed light concerning the impact of higher skeletal turnover associated with VDR genotypes on BMD and bone health throughout time.

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