Association of the *(CAG)n* repeat polymorphism of *AR* gene with bone mineral density in Greek peri- & postmenopausal women

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Background

Osteoporosis is a systemic skeletal disease with a strong genetic component. The androgen receptor (AR) is encoded by the *AR* gene and mediates the action of androgens, which play an important role in bone metabolism. Polymorphisms in the AR gene may be implicated in the pathogenesis of osteoporosis. A repeat polymorphism of the *AR* gene, *(CAG)n* has been described and is present in the exon 1 of the *AR* gene.

Objectives

The present study aimed to explore the influence of the repeat polymorphism *(CAG)n* in the promoter of the *AR* gene on BMD and serum levels of osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL) and bone metabolic markers in a Greek female population.

Methods

Two hundred and seventeen peri- and postmenopausal Greek women aged 42-63 years, who were referred for clinical evaluation and/or bone densitometry to our outpatient endocrine/osteoporosis clinic agreed to participate in the study. Women with any of the following criteria were not eligible for the study:
(1) surgical menopause,
(2) early menopause,
(3) diseases capable of affecting bone metabolism,
(4) previous or current treatment for osteoporosis or use of glucocorticoids.
All women were evaluated by means of a detailed medical history and a full physical examination. The age, age (year and month) at the last menstrual period, number of years since menopause (YSM), current medication, smoking habits and alcohol use were recorded.
Genomic DNA was extracted from the peripheral blood leukocytes using NucleoSpin Blood QuickPure kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Amplification of the *AR (CAG)n* polymorphism was accomplished using PCR with a forward primer (5'-TCCAGAATCTGTTCCAGAGCGTGC-3') and a reverse primer (5'-GCTGTGAAGGTTGCTGTTCCTCAT-3'). Amplified products were separated by 10% PAGE followed by silver staining and the number of individual alleles was determined. The number of CAG repeats in every particular allele was analyzed by sequencing the appropriate PCR products. A quality control assessment of our PCR method was carried out by random sampling and sequencing of the PCR products and duplication of PCR assays.
The study was approved by the Institutional Ethics Committee and written informed consent was obtained from each participant.

Results

The characteristics of the subjects at enrollment are presented in Table 1. The serum concentrations of bone metabolic markers, OPG, soluble RANKL (sRANKL) and sRANKL/OPG ratio are shown in Table 2. Genotype analysis for the *(CAG)n* polymorphism revealed *AR* alleles containing 15-30 CAG repeats. Alleles with twenty-five and twenty CAG repeats were the most common, with respective frequencies of 14.5% and 13.1%, respectively (Table 3).
A statistically significant association between the *(CAG)n* polymorphism of *AR* gene and lumbar spine BMD was observed. Women having biallelic mean CAG repeat number ≥21 had significantly lower spinal BMD (0.797±0.107 g/cm^2) than women having biallelic mean CAG repeat number <21 (0.841±0.129 g/cm^2), (p=0.007) (Table 4). The aforementioned association remained significant after statistical adjustment for potential confounding factors such as age, YSM and body mass index (BMI) (p=0.016). There were no significant differences in age, YSM, or BMI as well as in calcium supplement intake and smoking habits (Table 4).
Additionally, calcium concentration in serum was associated with the *(CAG)n* polymorphism. Calcium concentration in serum was higher in women with biallelic mean CAG repeat number ≥21 (9.9±0.5 mg/dl) than in women with biallelic mean CAG repeat number <21 (9.7±0.4 mg/dl) (p=0.017). No effect was observed on circulating levels of OPG and sRANKL (Table 5).
The characteristics and BMD of the two groups of the *(CAG)n* polymorphism are summarized in Table 4. Table 5 shows serum levels of bone metabolic markers, OPG and sRANKL in the two groups.

Table 1. Characteristics of the study population (n=217)

Age (years)	57.0 ± 4.7
Age at menopause (years)	48.5 ± 4.1
Years since menopause	8.5 ± 5.7
Weight (kg)	70.3 ± 11.0
Height (cm)	159 ± 5
BMI (kg/m^2)	27.8 ± 4.2
Calcium supplement intake (%)	87 (40.1)
Smoking (%)	28 (12.9)

Results are expressed as mean ± SD or number (% percentage)

Table 2. BMD and serum concentrations of bone metabolic markers, OPG, sRANKL and sRANKL/OPG ratio in the study population (n=217)

BMD lumbar spine (g/cm^2)	0.810 ± 0.120
Calcium (mg/dl)	9.80 ± 0.46
Alkaline phosphatase (IU/L)	74.31 ± 25.10
Urinary calcium to creatinine ratio (mmol/mmol)	0.41 (0.07-1.92)
OPG (pmol/L)	0.89 (0.09-5.64)
sRANKL (pmol/L)	0.034 (0.020-1.810)
sRANKL/OPG ratio	0.044 (0.004-4.410)

Results are expressed as mean ± SD or median (range)

Table 3. Allele frequencies of *(CAG)n* polymorphism

Allele	Frequency (%)
$(CAG)_{15}$	11.3
$(CAG)_{16}$	1.6
$(CAG)_{17}$	8.5
$(CAG)_{18}$	10.6
$(CAG)_{19}$	8.1
$(CAG)_{20}$	13.1
$(CAG)_{21}$	4.4
$(CAG)_{22}$	6.7
$(CAG)_{23}$	4.2
$(CAG)_{24}$	6.9
$(CAG)_{25}$	14.5
$(CAG)_{26}$	5.5
$(CAG)_{27}$	2.5
$(CAG)_{28}$	0.7
$(CAG)_{29}$	1.2
$(CAG)_{30}$	0.2

Conclusions

These findings demonstrate that the *(CAG)n* polymorphism of the *AR* gene may contribute to the determination of BMD at the lumbar spine in peri- and postmenopausal Greek women.

Table 4. Characteristics and BMD of the two groups of the *(CAG)n* polymorphism

	Women with biallelic mean (CAG) ≥21 (n=107)	**Women with biallelic mean (CAG) <21 (n=110)**	***p* value**
Age (years)	57.2 ± 4.3	56.8 ± 5.1	0.566
Age at menopause (years)	48.7 ± 3.9	48.2 ± 4.3	0.430
Years since menopause	8.5 ± 5.4	8.6 ± 5.9	0.925
BMI (kg/m$^{2)}$	27.5 ± 3.5	28.1 ± 4.8	0.269
Calcium supplement intake (%)	45 (42.1)	42 (38.2)	0.657
Smoking (%)	13 (12.2)	15 (13.6)	0.901
BMD lumbar spine g/cm^2	0.797 ± 0.107	0.841 ± 0.129	0.007

Results are expressed as mean ± SD or as number of subjects (% percentage)

Table 5. Serum levels of bone metabolic markers, OPG and sRANKL in the two groups of the *(CAG)n* polymorphism

	Women with biallelic mean (CAG) ≥21 (n=107)	**Women with biallelic mean (CAG) <21 (n=110)**	***p* value**
Calcium (mg/dl)	9.9 ± 0.5	9.7 ± 0.4	0.017
Alkaline phosphatase (IU/L)	72.6 ± 24.1	76.0 ± 26.1	0.317
Urinary calcium to creatinine ratio (mmol/mmol)	0.42 (0.27-0.65)	0.41 (0.25-0.59)	0.669
OPG (pmol/L)	0.92 (0.69-1.33)	0.86 (0.65-1.18)	0.212
sRANKL (pmol/L)	0.02 (0.02-0.15)	0.04 (0.02-0.15)	0.449
sRANKL/OPG ratio	0.04 (0.02-0.16)	0.05 (0.02-0.20)	0.282

Results are expressed as mean ± SD or median (range)

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