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Research Paper

Association of the *(TAAAA)n* repeat polymorphism of *SHBG* gene with the age at menopause in Greek postmenopausal women

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ABSTRACT

Objective: To assess the potential association of the pentanucleotide *(TAAAA)n* repeat polymorphism in the promoter of *SHBG* gene with the age at menopause in a Greek female population.
Study design: Cross-sectional study. Two hundred and ten postmenopausal women aged 46–63 years were enrolled. The age at the last menstrual period and anthropometric parameters were recorded in all participants. Blood sampling for genotyping of the *(TAAAA)n* polymorphism of *SHBG* gene was performed.
Main outcome measure(s): Frequency and association of the $(TAAAA)_n$ alleles with age at menopause.
Results: The alleles with seven and eight TAAAA repeats were associated with the age at menopause. The age at menopause was higher in carriers than in non-carriers of the $(TAAAA)_7$ allele (50.2 ± 3.1 years *vs.* 48.0 ± 4.8 years, $p = 0.026$). Furthermore, the age at menopause was lower in women carrying the $(TAAAA)_8$ allele (47.5 ± 4.8 years) than in women not carrying this allele (48.8 ± 4.4 years, $p = 0.048$).
Conclusions: The $(TAAAA)_7$ and $(TAAAA)_8$ alleles of the *SHBG* $(TAAAA)_n$ polymorphism may contribute to variation in the timing of natural menopause in postmenopausal women of Northwestern Greece.

1. Introduction

Menopause is characterized by cessation of reproductive function due to depletion of the follicle pool in the ovaries. The average age at natural menopause in Greek women is 48.7 years [1]. Multiple genetic and environmental factors as well as their interactions influence the age at menopause [2]. Linkage analysis in families and candidate gene association studies in populations indicate that heritability accounts for 49–87% of the variation in the age at menopause [3–5]. Although several genetic studies have been conducted to elucidate the genes involved in the determination of the age at menopause, only a few genes have been identified [2,6].

Sex steroids appear to play an important role in the regulation of reproductive function in women. In this regard, sex hormone-binding globulin (SHBG), a homodimeric glycoprotein produced mainly in the liver, binds sex steroids and regulates their access to target tissues [7,8]. Recent evidence suggests that SHBG is also produced in other tissues including the ovaries, endometrium, placenta and testis and, apart from being a plasma transport protein, it also exerts direct effects on sex steroid cellular uptake and cell proliferation through activation of specific plasma membrane receptors in hormone-responsive tissues [9–13]. As a consequence, variation in the levels of SHBG may influence sex steroid bioavailability and cellular effects with resultant clinical implications for reproductive function.

SHBG is under multifactorial regulation with hormonal, metabolic and nutritional factors influencing hepatic SHBG production [14–17]. Genetic factors may also contribute to variation in circulating SHBG [18,19]. In this context, recent studies have demonstrated that polymorphisms in the *SHBG* gene may influence SHBG levels [20–22].

A common *(TAAAA)n* pentanucleotide repeat polymorphism (PNRP) at the 5′ boundary of the human *SHBG* promoter has been described. It has been previously reported that this polymorphism influences the transcriptional activity of the promoter *in vitro* [23]. This functional polymorphism has been found to contribute to variation in SHBG levels, and to be associated with polycystic ovary syndrome, the age at menarche and hormone-sensitive breast cancer [24–26].

The aim of the present study was to assess the potential association of the pentanucleotide *(TAAAA)n* repeat polymorphism in the

Abbreviations: BMI, body mass index; LD, linkage disequilibrium; PCOS, polycystic ovary syndrome; PNRP, pentanucleotide repeat polymorphism; SHBG, sex hormone-binding globulin.

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promoter of the *SHBG* gene with the age at menopause in a cohort of postmenopausal Greek women.

2. Subjects and methods

2.1. Study subjects

All postmenopausal Greek women aged 46–63 years, who were referred for clinical evaluation and/or bone densitometry to our outpatient endocrine/osteoporosis clinic from May 2010 to December 2011, were recruited in the study. Women with a history of amenorrhea of more than or equal to 12 consecutive months and increased serum concentration of FSH were classified as having menopause. Women were excluded from the study if they had: (1) surgical menopause (history of oophorectomy) or other ovarian surgery, (2) early menopause at age < 40 years, (3) underlying diseases known to affect age at menopause such as polycystic ovary syndrome (PCOS), endometriosis, heart disease, *etc.* and (4) previous or current use of estrogens.

All participants were evaluated by means of a detailed medical history and a full physical examination. The current age, current medication and smoking habits were recorded. Information on menopause was obtained through interviews with the women. To attenuate recall bias, the exact year and month were recorded. Body mass index (BMI) was calculated.

The study was approved by the Institutional Ethics Committee and written informed consent was obtained from each participant.

2.2. Genetic analysis of (TAAAA)n repeat polymorphism

Genomic DNA was extracted from the peripheral blood leukocytes using NucleoSpin Blood QuickPure kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Amplification of the TAAAA repeat region within the *Alu* sequence in the *SHBG* promoter was accomplished using polymerase chain reaction (PCR) with a forward primer (5′-GCTTGAACTCGAGAGGCAG) and a reverse primer (5′-CAGGGCCTAAACAGTCTAGCAGT) corresponding to a sequence at −651/−673 nt within the upstream promoter sequence. Amplified products were separated by 10% non-denaturing polyacrylamide gel [acrylamide/bis-acrylamide (29:1) 40% in water (Fluka, Sigma–Aldrich)], followed by silver staining, and the number of individual alleles was determined, using as control previously sequenced PCR products.

2.3. Statistical methods

Women were classified in two groups (carriers and non-carriers) according to the presence of each allele of the *(TAAAA)n* repeat polymorphism. Carriers included women homozygous or heterozygous for each particular allele and non-carriers included women who did not carry the specific allele. For example, regarding the $(TAAAA)_6$ allele, carriers of the $(TAAAA)_6$ allele (6/6 and 6/*x* genotypes) were compared with non-carriers of this allele (*x*/*x* genotype, $x \neq 6$) and tested for differences in all characteristics (including age at menopause). The same procedure was applied for carriers *vs.* non-carriers of the $(TAAAA)_7$, $(TAAAA)_8$, $(TAAAA)_9$, $(TAAAA)_{10}$ and $(TAAAA)_{11}$ alleles.

In order to detect differences between the two groups (carriers and non-carriers of each allele of the *(TAAAA)n* polymorphism), Chi-squared test was applied for categorical variables. Comparisons between the two groups for continuous variables with normal distribution were made by Student's *t*-test, while Mann–Whitney *U* test was applied for continuous variables with non-normal distribution. The categorical variables included smoking habits. The continuous variables included age, age at menopause, years since menopause and BMI. The association of each allele of the *(TAAAA)n* repeat polymorphism with the age at menopause was examined separately by the Mann–Whitney *U* test or the *t*-test as appropriate. To adjust age at menopause for confounding factors such as BMI and smoking, linear regression analysis was performed. All tests were two-sided and the significance level was set at 0.05. Statistical analysis was performed using SPSS Statistics, version 17.0.

Table 1
Characteristics of the study population ($n = 210$).

Age (years)	56.9 ± 4.8
Age at menopause (years)	48.2 ± 4.6
Years since menopause	8.7 ± 6.1
BMI (kg/m^2)	27.9 ± 4.3
Smoking (%)	28 (13.3)

Results are expressed as mean ± SD for continuous variables and number of subjects (% percentage) for categorical variables.

3. Results

3.1. Characteristics of the study population

Two hundred and ten postmenopausal women were enrolled in the study. The characteristics of the subjects at enrollment are presented in Table 1. The mean age of the population studied was 56.9 ± 4.8 years (mean ± SD) (range 46–63) and the mean age at menopause was 48.2 ± 4.6 years.

3.2. Genotype distribution and age at menopause

Genotype analysis for the *(TAAAA)n* polymorphism revealed *SHBG* alleles containing 6–11 TAAAA repeats. Alleles with nine, six and eight TAAAA repeats were the most common, with respective frequencies of 30.5%, 29.1% and 24.1% (Table 2).

An association between the $(TAAAA)_7$ allele of the *(TAAAA)n* repeat polymorphism and the age at menopause was observed. Women carrying the $(TAAAA)_7$ allele had higher age at menopause than women not carrying this allele (50.2 ± 3.1 years *vs.* 48.0 ± 4.8 years, $p = 0.026$) (Table 3). The aforementioned association remained significant after statistical adjustment for potential confounding factors including BMI and smoking ($p = 0.019$) (Table 4). There were no significant differences in current age, years since menopause (YSM), BMI and in smoking habits between the two groups (Table 3).

Regarding the allele with eight TAAAA repeats, the age at menopause was lower in carriers of this allele than in non-carriers (47.5 ± 4.8 years *vs.* 48.8 ± 4.4 years, $p = 0.048$) (Table 3). The aforementioned difference remained significant after adjustment for BMI and smoking ($p = 0.043$) (Table 4). No significant differences in the other characteristics were noted between the two groups (Table 3).

The characteristics of the carriers and non-carriers of the alleles $(TAAAA)_7$ and $(TAAAA)_8$ are summarized in Table 3. No association of the other alleles with the age at menopause was detected (Table 5).

Table 2
The allele frequencies of the *(TAAAA)n* polymorphism.

Allele	Frequency (%)
$(TAAAA)_6$	*29.1*
$(TAAAA)_7$	*7.1*
$(TAAAA)_8$	*24.1*
$(TAAAA)_9$	*30.5*
$(TAAAA)_{10}$	*9.1*
$(TAAAA)_{11}$	*0.2*