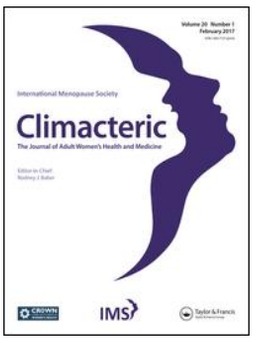

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ORIGINAL ARTICLE

Microsatellite variation of *ESR1*, *ESR2*, and *AR* in Serbian women with primary ovarian insufficiency

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ABSTRACT

Objective: This study aimed to investigate the potential role of microsatellite polymorphisms of the estrogen receptor alpha gene (*ESR1*) TA repeat, estrogen receptor beta gene (*ESR2*) CA repeat, and androgen receptor gene (*AR*) CAG and GGN repeats among Serbian women with primary ovarian insufficiency (POI). These microsatellites have been reported to be associated with POI in different racial/ethnic populations.

Methods: A cohort of 196 POI cases matched with 544 fertile controls was recruited by the Institute for Endocrinology, Diabetes and Metabolic Disorders of Serbia between 2007 and 2010. DNA was extracted from saliva. The four microsatellites were genotyped using a PCR-based assay to determine the repeat lengths.

Results: POI patients carried shorter repeat lengths of *ESR2* $(CA)_n$ than controls ($P = 0.034$), but the difference was small. *ESR1* $(TA)_n$ was on the borderline of statistical differences between groups ($P = 0.059$). *AR* $(CAG)_n$ and $(GGN)_n$ showed no association with POI.

Conclusions: We cautiously conclude that microsatellite polymorphisms of gonadal steroid receptor genes might contribute to the genetic basis of POI in Serbian women, but a larger-scale study and family-based studies are warranted to validate our findings even though the sample size in this study is larger than any previously published in this field.

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Primary ovarian insufficiency; premature ovarian failure; estrogen receptor; androgen receptor; microsatellite polymorphism

Introduction

The average age for menopause in Western populations is approximately 51 years. Primary ovarian insufficiency (POI) (previous called premature ovarian failure) is characterized by amenorrhea, hypoestrogenism, and elevated gonadotropins, and affects 1% of women under the age of 40[1,2]. The causes of POI are heterogeneous, including chromosome X defects, infections, iatrogenic effects (surgery, chemotherapy, radiation), and autoimmune diseases[3]. Approximately 20–30% of women with POI will have other affected female family members[4], hence a genetic basis for the disorder is a likely cause for this clinical scenario. It is known that normal ovarian development and function require the expression and proper coordination of many genes[5]. The underlying mechanisms are largely unknown and, despite the genetic defects identified in several candidate genes[6–9], in a large proportion of POI cases no cause has been found; hence they are classified as idiopathic POI.

Genotype-to-phenotype association mapping commonly utilizes two major classes of variants: single nucleotide polymorphisms and copy number variation. Microsatellites or short tandem repeats are informative genetic markers in the human genome and some might have biological functions depending upon their locations (coding regions or regulatory regions), so they are well established as a third major class of genotypic variation[10]. Considering that the initial follicular pool size and the rate of follicular depletion are associated with the age of menopause and given the fact that these germ cells express gonadal steroid receptors at various stages of development, it is plausible that genetic variants in sex hormone receptor genes involved in maintaining ovarian function could affect the risk of POI[11].

Ligand-bound gonadal steroid receptors act as DNA transactivation factors and are thus responsible for mediating the effects of steroids on development, reproduction, proliferation, cellular homeostasis, and gene expression[12,13]. Estrogen contributes to the regulation of cyclic gonadotropin release via its action on estrogen receptor alpha, encoded by *ESR1*, in the hypothalamic–hypophyseal axis and to enhancing folliculogenesis through its actions via estrogen receptor beta, encoded by *ESR2*, in the ovary[14]. A dinucleotide TA tandem repeat polymorphism is located in the promoter region of *ESR1*, while a dinucleotide CA tandem repeat is located in intron 5 of *ESR2*. The functional significance of *ESR1* $(TA)_n$ and *ESR2* $(CA)_n$ in POI remains unknown, although previous

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studies have shown associations between these two microsatellites and POI[15,16].

The androgen receptor (*AR*), the only sex hormone receptor gene encoded on the X chromosome, is essential for the male reproductive process. *AR* expression in the development of follicles in rat ovary has been described[17], and excess androgen production causes infertility in polycystic ovary syndrome[18], so it is plausible that AR-mediated androgen signaling plays an important physiological role in the female reproductive system. Furthermore, *AR*-knockout mice have low follicle counts and eventually develop POI phenotypes[19]. Within the first exon of the *AR* gene lie two microsatellite polymorphisms, one with a CAG repeat [$(CAG)_n$] and one with a GGN repeat [$(GGN)_n$]. A limited number of studies have found an association of CAG repeats of the *AR* gene in POI[20,21] while others have not[16,22], and for the GGN repeats a significant association has been demonstrated with POI in Indian women[22].

Based on this evidence, we hypothesize that these key receptors of reproductive hormones might have roles in folliculogenesis and ovarian function; therefore, the present study was undertaken to investigate whether the *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, *AR* $(CAG)_n$, and *AR* $(GGN)_n$ repeat polymorphisms are associated with POI in Serbian women. This is the first study, with a larger cohort size than any previously published, to investigate these four microsatellites in gonadal steroid receptor genes in relation to POI in a well-defined ethnic group of patients and matched controls.

Methods

Patients and controls

The study population comprised native Serbian women of the same socioeconomic status and eating habits. The study population was selected from a larger group of young women with amenorrhea referred to the Institute for Endocrinology, Diabetes and Metabolic Disorders of Serbia, University of Belgrade. A cohort of 196 women diagnosed with idiopathic POI who fulfilled the inclusion criteria of the study was enrolled into the study. All patients completed a questionnaire on menstrual history, parity, family history of POI, education, and the experience of stresses prior to amenorrhea.

The diagnosis was made in otherwise healthy women who fulfilled the criteria of amenorrhea of $\geq$12 months duration before the age of 40 years and both follicle stimulating hormone (FSH) $\geq$40 IU/L and estradiol $<$50 pmol/L. Women who had undergone gynecological operations (hysterectomy, ovarian cystectomy, or oophorectomy), those who had a history of drug abuse, and those with conditions known to cause ovarian failure such as metabolic disorders (e.g. galactosemia), autoimmune disorders, irradiation, or chemotherapy were excluded. In addition, all women diagnosed with POI included in this study had normal results for the following: full blood count, fasting glucose and lipoprotein profile, prolactin, testosterone, androstenedione, dehydroepiandrosterone (DHEA) sulfate, 17-OH progesterone, thyroxine, thyroid stimulating hormone, parathyroid hormone, adrenocorticotropic hormone, and cortisol. All patients had low inhibin B and anti-Müllerian hormone levels. Normal results of a 2-h 75-g oral glucose tolerance test with glucose and insulin measured at 30-min intervals were part of the inclusion criteria. These patients also tested negative for antiovarian, anticardiolipin, anti-thyroglobulin, and anti-microsomal antibodies. In this study, all women who developed POI under the age of 30 years had a normal karyotype. Pelvic ultrasound scan on all patients showed no ovarian pathology or follicular activity.

As controls, we recruited 544 healthy regularly menstruating women not using any hormonal treatment which could interfere with menstruation. All women included in the present study have signed a written consent form approved by the local ethics committee in Belgrade.

The mean age at diagnosis of POI was 34.2 ± 3.9 years in the patient group and the control group had a mean age of 36.0 ± 3.6 years with 46% parity.

Microsatellite genotyping

Genomic DNA was extracted from saliva using Oragene® OG-300 DNA sample collection kits (DNA Genotek Inc., Canada) following the manufacturer's protocol. Microsatellite markers were amplified by conventional polymerase chain reaction (PCR), using fluorescently labeled primers from Sigma, UK.

Genotyping for the *ESR1* $(TA)_n$ and *ESR2* $(CA)_n$ polymorphisms was performed by PCR amplification in a total volume of 10 µl containing 10 ng DNA for *ESR1* $(TA)_n$ or 2 ng DNA for *ESR2* $(CA)_n$ after optimization, 0.25 mM each dNTP, 1.5 mM $MgCl_2$, 0.5 U Taq DNA Polymerase (KAPA Biosystems, USA), and 0.4 µM of each of the primers. We designed forward primer 5′-6FAM-TTAGGCTGCAGCAAAGGAAG-3′ and reverse primer 5′-TGTTACATTGTCGGTCTGGTC-3′ for the *ESR1* $(TA)_n$ analysis, and forward primer 5′-HEX-GGTCT GTACCCAGGTGTGTG-3′ and reverse primer 5′-TCAGGC TTGTCTCGAACTCC-3′ for the *ESR2* $(CA)_n$ analysis. The temperature profile was 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 20 s, and a final incubation at 72 °C for 2 min.

Genotyping for the *AR* $(CAG)_n$ and $(GGN)_n$ polymorphisms was performed by PCR in a total volume of 10 µl containing 10 ng DNA for *AR* $(CAG)_n$ or 2 ng DNA for *AR*$(GGN)_n$ after optimization, 0.3 mM each dNTP, 2.0 mM $MgCl_2$, 0.2 U High-Fidelity HotStart DNA Polymerase (KAPA Biosystems, USA), and 0.3 µM of each of the primers. We designed forward primer 5′-HEX-AGATTCAGCCAAGCTCAAGG-3′ and reverse primer 5′-CTCATCCAGGACCAGGTAGC-3′ for the CAG repeats analysis, and forward primer 5′-6FAM-CTCTTCA CAGCCGAAGAAGG-3′ and reverse primer 5′-GGATAG GGCACTCTGCTCAC-3′ for the GGN repeats analysis. The temperature profile was 95 °C for 5 min, followed by 30 cycles of 98 °C for 20 s and 60 °C for 15 s, and a final incubation at 72 °C for 5 min.

For fragment-length determination, 1.0 µl of PCR product was mixed with 0.2 µl of MapMarker® 1000-ROX (BioVentures, Inc., USA) and 9.8 µl of Ultra-pure formamide (AGTC Bioproducts Ltd, UK). Upon denaturation for 5 min at 96 °C and cooling for 5 min on ice, samples were run on an ABI

3130xl Genetic Analyzer (Applied Biosystems, USA) and the repeat lengths were subsequently checked and assigned in Genemapper® software (version 4.0; Applied Biosystems).

Quality control

One anonymous genomic DNA sample and a reaction without a DNA template were included in each set of PCR amplifications performed and run for every microsatellite length-determination experiment, as positive and negative controls, respectively. This ensured consistency of allele calling between PCR amplification batches. In addition, 10% of samples were randomly selected and the length determinations repeated to test the assay reproducibility. Furthermore, to confirm the precise repeat number, 16 PCR amplification products of different sizes as determined by Genemapper® analyses were subjected to direct sequencing on an Applied Biosystems 3730 sequencer (Applied Biosystems, USA). Repeat lengths measured by sequencing were successfully assigned to corresponding peak positions determined by fluorescence-based length-determination genotyping.

Statistical analysis

The repeat lengths for *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, *AR* $(CAG)_n$, and *AR* $(GGN)_n$ were classified as both continuous and categorical variables, separately. For the continuous variable analysis, allele frequencies are summarized by the mean and standard deviation. As no hypothesis existed about which alleles would be associated with POI, a two-tailed Student's *t* test was used to compare allele frequencies between POI patients and controls.

In addition, the genotype was also classified using the median repeat length in the control group as the cutoff value[16,23], where microsatellite repeat alleles were coded as long repeats (denoted by L) and short repeats (denoted by S). As a result, three genotype classes can be defined for each of the four short tandem repeats: LL, LS, and SS. The cutoff limits were 17 ($S < 17$; $L \geq 17$) for *ESR1* $(TA)_n$, 23 ($S < 23$; $L \geq 23$) for *ESR2* $(CA)_n$, 22 ($S < 22$; $L \geq 22$) for *AR* $(CAG)_n$, and 23 ($S < 23$; $L \geq 23$) for *AR* $(GGN)_n$. Comparisons of genotype distributions between the case and control groups were then performed using logistic regression models. Odds ratios were calculated by logistic regression analysis with 95% confidence intervals (CIs). All statistical results with $P < 0.05$ were considered statistically significant.

Results

The allele distributions of each individual microsatellite *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, *AR* $(CAG)_n$, and $(GGN)_n$ between the 196 POI cases and the 544 controls are similar (Figure 1).

ESR1

The TA repeat lengths in *ESR1* ranged from 7 to 27 with a median length of 16 in POI patients and from 10 to 29 with a median length of 17 in controls. This polymorphism displayed a bimodal distribution, with peaks at 15 repeats and 24 repeats (Figure 1). The difference in the frequency distribution of the TA dinucleotide repeat polymorphism of the *ESR1* gene between 196 POI cases (392 chromosomes) and 544 controls (1088 chromosomes) was not significant ($P = 0.059$) (Table 1). POI patients tended to have shorter repeat lengths, but no significant difference in the $(TA)_n$ repeat genotype distribution (SS, SL, and LL) was observed between the two groups ($P = 0.121$) (Table 2).

ESR2

The CA repeat lengths in *ESR2* ranged from 10 to 28 with a median length of 22 in POI patients and from 15 to 29 with a median length of 23 in controls. The mean number of $(CA)_n$ repeats was lower in POI patients compared to the controls ($P = 0.034$), but this difference was small (Table 1). According to the categorical repeat length cutoff value of 23, comparisons of the *ESR2* $(CA)_n$ genotypes (SS, SL, and LL) between POI case and control groups showed no significance ($P = 0.075$), although POI patients are more likely to have the SS genotype (27.6% vs. 21.5%), and individuals with SS and SL genotypes (enrichment of the S allele) are more common in the POI patient group ($P = 0.045$) (Table 2).

AR

The CAG repeats in *AR* ranged from 12 to 31 with a median length of 22 in POI patients and from 8 to 35 with a median length of 22 in controls. The difference of the distribution of CAG repeats was not significant between POI cases and controls ($P = 0.071$). The GGN repeats in *AR* ranged from 7 to 29 with a median length of 23 in both POI patients and controls, and there was no statistical difference observed between POI cases and controls ($P = 0.620$) (Table 1).

Discussion

The results of the present study demonstrate that microsatellite *ESR2* $(CA)_n$ could play a potential role in the genetic mechanism for the etiology of POI, but *ESR1* $(TA)_n$, *AR* $(CAG)_n$, and *AR* $(GGN)_n$ are not associated with POI in Serbian women.

The *ESR1* gene has a very complex promoter organization. It contains multiple promoter regions with alternative splice sites, resulting in expression of alternative first exons and different protein isoforms[24]. It has been reported that the number of *ESR1* $(TA)_n$ repeats differs by ethnicity, with estimated major peaks at 14 repeats in European populations and 15 repeats in Asian populations[25]. In the present study, the major peak of *ESR1* $(TA)_n$ was at 15 repeats in our Serbian population, rather than 14 repeats in other European populations. We found that Serbian patients with POI had slightly shorter repeat lengths than controls, but the *P* value was at the borderline of significance. This finding is consistent with a study in Korean populations suggesting that *ESR1* $(TA)_n$ does not contribute to the development of POI in women[11]. Furthermore, it has been reported that short alleles in *ESR1* $(TA)_n$ were significantly less

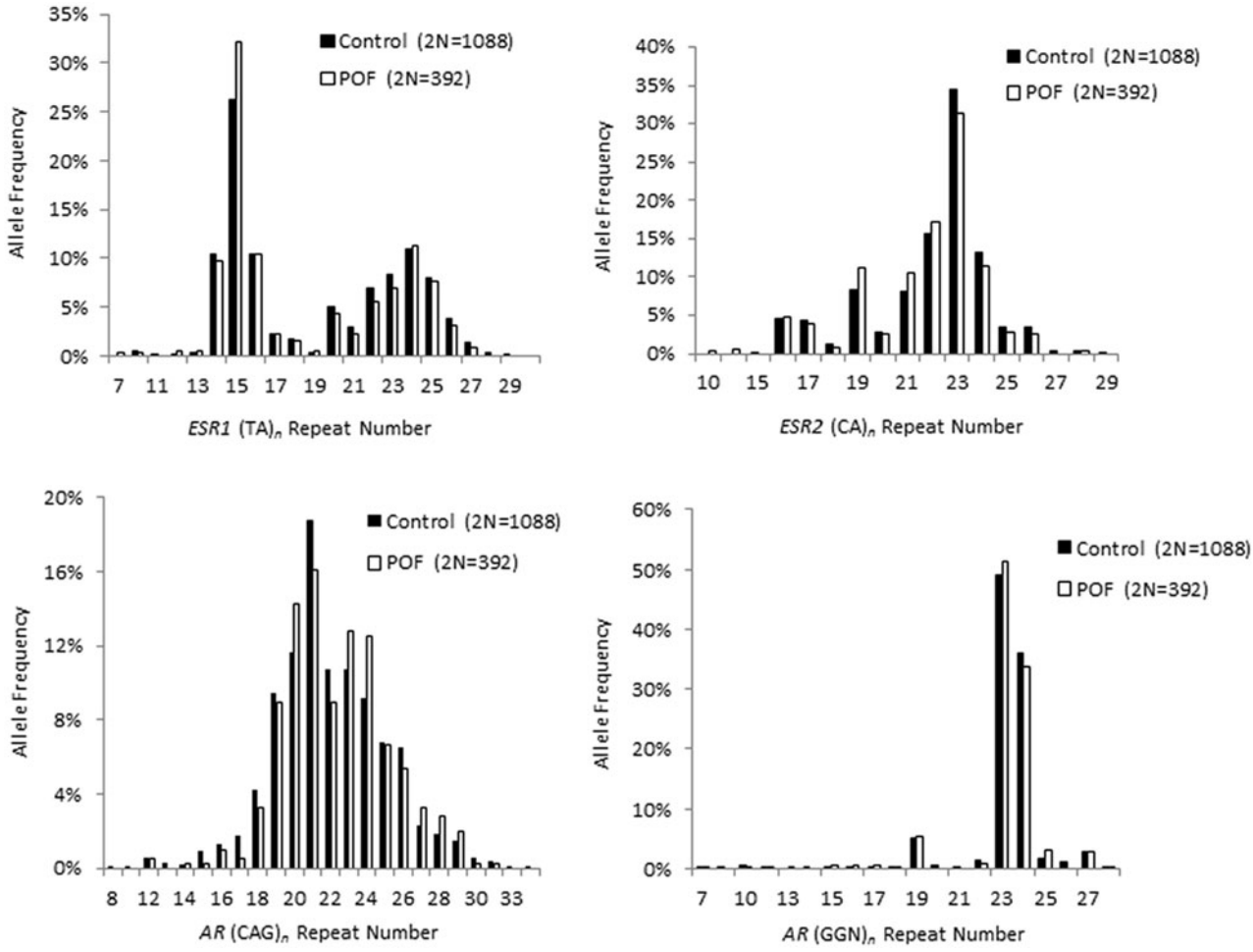

Figure 1. Allele distribution of *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, *AR* $(CAG)_n$, and $(GGN)_n$ repeats in POI cases and controls. *ESR1*, estrogen receptor alpha gene; *ESR2*, estrogen receptor beta gene; *AR*, androgen receptor gene; POF, premature ovarian failure; POI, primary ovarian insufficiency.

Table 1. Repeat polymorphism values for *ESR1*, *ESR2*, and *AR* genes for POI cases and controls.

	Repeat length		
Microsatellite	*Range*	*Mean ± SD*	*p*-Value
ESR1 $(TA)_n$			
POI ($n = 196$)	7–27	18.56 ± 4.33	
Controls ($n = 544$)	10–29	19.05 ± 4.39	0.059
ESR2 $(CA)_n$			
POI ($n = 196$)	14–32	21.67 ± 2.48	
Controls ($n = 544$)	19–33	21.97 ± 2.43	0.034*
AR $(CAG)_n$			
POI ($n = 196$)	12–31	22.29 ± 2.93	
Controls ($n = 544$)	8–35	21.96 ± 3.13	0.071
AR $(GGN)_n$			
POI ($n = 196$)	7–29	23.06 ± 2.08	
Controls ($n = 544$)	7–29	23.12 ± 2.03	0.620

*Significant at $P < 0.05$.
ESR1, estrogen receptor alpha gene; *ESR2*, estrogen receptor beta gene; *AR*, androgen receptor gene; *POI*, primary ovarian insufficiency; *SD*, standard deviation.

common in POI, and SS alleles were rare in patients with POI in a Caucasian with Asian admixture population[16], but we found that the SS genotype in Serbian patients with POI was more common than in controls (30.6% vs. 23.3%).

For *ESR2* $(CA)_n$, our analysis of continuous variables showed that more patients with POI had shorter repeat lengths. This means that the women who carry the S allele might have greater chance of developing POI. *ESR2* $(CA)_n$ was first characterized as a highly polymorphic (CA) dinucleotide repeat in a Japanese population in 1998[26]. Since then, the only published report investigating a possible link between *ESR2* $(CA)_n$ and POI failed to establish any significant association[16]. To the best of our knowledge, this is the first report demonstrating that the *ESR2* $(CA)_n$ repeat polymorphism is statistically associated with POI. The POI patients in this study also tended to have shorter TA repeat lengths in *ESR1* (although the association did quite reach statistical significance), hence S alleles in *ESR1* and *ESR2* might be considered as risk factors in POI in Serbian women.

We analyzed the coding-region tandem repeats of the *AR* gene: an 8–35 CAG repeat (encoding polyglutamine, polyQ) and a 7–29 GGN repeat (encoding polyglycine, polyG). Together, these polymorphisms result in approximately 90% of women being heterozygous for alleles of the *AR* gene. The polyglutamine and polyglycine tracts flank the activating function-1 domain of the AR protein and both have been reported to be modulators of AR transcription factor activity[27]. The CAG polyglutamine stretch in the amino-terminal domain of the AR gene appears to inversely influence the function of the receptor as a transcription factor, and is assumed to be involved in interactions between the AR

Table 2. Categorical variable analysis of *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, *AR* $(CAG)_n$, and *AR* $(GGN)_n$ alleles and POI risk.

Microsatellite	*Dichotomous cutoff value*	*Genotype*	*POI (n)*	*Controls (n)*	*OR (95% CI)*	*Logistic regression P value*
ESR1 $(TA)_n$	<17 vs ≥17	SS	60 (30.6%)	127 (23.3%)	1.0 (Reference)	
		SL	91 (46.4%)	270 (49.6%)	0.71 (0.48–1.05)	
		LL	45 (23.0%)	147 (27.0%)	0.65 (0.41–1.02)	
		Total	196	544		0.121
		LL vs SS + SL			0.80 (0.55–1.18)	0.266
		SL + LL vs SS			0.69 (0.48–0.99)	0.045
ESR2 $(CA)_n$	<23 vs ≥23	SS	54 (27.6%)	117 (21.5%)	1.0 (Reference)	
		SL	94 (48.0%)	252 (46.3%)	0.81 (0.54–1.21)	
		LL	48 (24.5%)	175 (32.2%)	0.59 (0.38–0.94)	
		Total	196	544		0.075
		LL vs SS + SL			0.68 (0.47–0.99)	0.045
		SL + LL vs SS			0.72 (0.50–1.05)	0.086
AR $(CAG)_n$	<22 vs ≥22	SS	39 (19.9%)	124 (22.8%)	1.0 (Reference)	
		SL	99 (50.5%)	288 (52.9%)	1.09 (0.71–1.67)	
		LL	58 (29.6%)	132 (24.3%)	1.40 (0.87–2.24)	
		Total	196	544		0.316
		LL vs SS + SL			1.31 (0.91–1.89)	0.144
		SL + LL vs SS			1.19 (0.79–1.78)	0.402
AR $(GGN)_n$	<23 vs ≥23	SS	3 (1.5%)	5 (0.9%)	1.0 (Reference)	
		SL	29 (14.8%)	90 (16.5%)	0.54 (0.12–2.39)	
		LL	164 (83.7)	449 (82.5%)	0.61 (0.14–2.58)	
		Total	196	544		0.672
		LL vs SS + SL			1.08 (0.70–1.68)	0.718
		SL + LL vs SS			0.60 (0.14–2.52)	0.483

ESR1, estrogen receptor alpha gene; *ESR2*, estrogen receptor beta gene; *AR*, androgen receptor gene; POI, primary ovarian insufficiency; OR, odds ratio; CI, confidence interval, L, long repeats; S, short repeats.

protein and different co-activators, with long repeats being inhibitory to these interactions which could explain the lower activity of the receptor[13]. Our analysis of continuous variables showed that variants in *AR* $(CAG)_n$ and *AR* $(GGN)_n$ did not appear to be associated with POI in our patient cohort. Among the four microsatellites examined, *AR* $(CAG)_n$ is the most commonly tested in POI in the literature[16,20–22], and the conflicting reports concerning allele lengths can possibly be explained by genetic differences in diverse populations and perhaps by different sample sizes. Based on our results, we can only cautiously draw a conservative conclusion that the variants of *AR* $(CAG)_n$ and $(GGN)_n$ do not play a major role in follicle dysfunction in POI.

Classifying the repeats as SS, SL, and LL genotypes revealed that the majority of the cases and controls had the SL genotype more frequently than the SS and LL genotypes for *ESR1* $(TA)_n$, *ESR2* $(CA)_n$, and *AR* $(CAG)_n$. However, for the *AR* $(GGN)_n$ repeat, the LL genotype was most frequent. To gain an insight into clinical significance, the CIs in the categorical variable genotype analysis of these four microsatellites (Table 2) were calculated. However, most CIs are quite wide, so the true difference between patients with POI and the controls cannot be ruled out, and a much larger cohort would be required to overcome this limitation.

We conclude that patients with POI harbor shorter repeats in *ESR2* $(CA)_n$, and S alleles in that gene might be considered a risk factor for POI in Serbian women. However, no convincing evidence was found in this study for an association between *ESR1* $(TA)_n$, *AR* $(CAG)_n$, and $(GGN)_n$ repeat lengths and POI in Serbian women. We did assess whether the combined presence of microsatellite variants had an impact on the risk of POI, but could not identify any contribution by particular combinations of variants in each of the genes that were tested, with and without adjustment for age.

POI is now considered a multifactorial disease, where the phenotype is most probably the result of sequence variation in more than one gene. The current study is based on a clinically well-defined cohort with homogeneous racial background, and confirmation of these observations could be obtained by demonstration of co-inheritance of variants and POI in families. The effect of gonadal steroid receptor gene polymorphisms on the follicular pool size or rate of follicular depletion and the role of germline genetic variants in the etiology of POI remain to be further clarified and explored. The analysis of microsatellite length variability in this study, and of single nucleotide polymorphisms in other studies, can help in further understanding regulatory mechanisms that determine oocyte reserves and their depletion. Such data can be used in constructing variant-testing strategies that can help in screening women with menstrual irregularities suggestive of a transition to premature ovarian insufficiency.

Authors' roles J.L. contributed to the study design, data analysis and manuscript preparation; R.D. contributed to the study design and edited the manuscript; S.V., S.D-D., M.I., M.I. and M.T. contributed to the clinical sample collection, biochemical analyses and assembly of data; J.T. contributed to statistical analysis of the data; F.A-A. contributed to the study design and participated in the critical discussion.

Conflict of interest No potential conflict of interest was reported by the authors.

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References

1. Goswami D, Conway GS. Premature ovarian failure. *Hum Reprod Update* 2005;11:391–410
2. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986;67:604–6
3. Beck-Peccoz P, Persani L. Premature ovarian failure. *Orphanet J Rare Dis* 2006;1:9–14
4. Kokcu A. Premature ovarian failure from current perspective. *Gynecol Endocrinol* 2010;26:555–62
5. Simpson JL. Genetic and phenotypic heterogeneity in ovarian failure: overview of selected candidate genes. *Ann N Y Acad Sci* 2008;1135:146–54
6. Bione S, Rizzolio F, Sala C, *et al.* Mutation analysis of two candidate genes for premature ovarian failure, DACH2 and POF1B. *Hum Reprod* 2004;19:2759–66
7. Dixit H, Rao LK, Padmalatha VV, *et al.* Missense mutations in the *BMP15* gene are associated with ovarian failure. *Hum Genet* 2006;119:408–15
8. Kovanci E, Rohozinski J, Simpson JL, *et al.* Growth differentiating factor-9 mutations may be associated with premature ovarian failure. *Fertil Steril* 2007;87:143–6
9. Wang B, Wen Q, Ni F, *et al.* Analyses of growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) mutation in Chinese women with premature ovarian failure. *Clin Endocrinol (Oxf)* 2010;72:135–6
10. Gemayel R, Vinces MD, Legendre M, *et al.* Variable tandem repeats accelerate evolution of coding and regulatory sequences. *Annu Rev Genet* 2010;44:445–77
11. Yoon SH, Choi YM, Hong MA, *et al.* Estrogen receptor α gene polymorphisms in patients with idiopathic premature ovarian failure. *Hum Reprod* 2010;25:283–7
12. Ascenzi P, Bocedi A, Marino M. Structure-function relationship of estrogen receptor α and β: impact on human health. *Mol Aspects Med* 2006;27:299–402
13. Tsezou A, Tzetis M, Gennatas C, *et al.* Association of repeat polymorphisms in the estrogen receptors alpha, beta (*ESR1*, *ESR2*) and androgen receptor (*AR*) genes with the occurrence of breast cancer. *Breast* 2008;17:159–66
14. Kolibianakis EM, Papanikolaou EG, Fatemi HM, *et al.* Estrogen and folliculogenesis: is one necessary for the other? *Curr Opin Obstet Gynecol* 2005;17:249–53
15. Syrrou M, Georgiou I, Patsalis PC, *et al.* Fragile X premutations and (TA)n estrogen receptor polymorphism in women with ovarian dysfunction. *Am J Med Genet* 1999;84:306–8
16. Bretherick KL, Hanna CW, Currie LM, *et al.* Estrogen receptor α gene polymorphisms are associated with idiopathic premature ovarian failure. *Fertil Steril* 2008;89:318–24
17. Tetsuka M, Whitelaw PF, Bremner WJ, *et al.* Developmental regulation of androgen receptor in rat ovary. *J Endocrinol* 1995;145:535–43
18. Ehrmann DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev* 1995;16:322–53
19. Shiina H, Matsumoto T, Sato T, *et al.* Premature ovarian failure in androgen receptor-deficient mice. *Proc Natl Acad Sci USA* 2006;103:224–9
20. Chatterjee S, Singh R, Kadam S, *et al.* Longer CAG repeat length in the androgen receptor gene is associated with premature ovarian failure. *Hum Reprod* 2009;24:3230–5
21. Sugawa F, Wada Y, Maruyama T, *et al.* Premature ovarian failure and androgen receptor gene CAG repeat lengths weighted by X chromosome inactivation patterns. *Fertil Steril* 2009;91:649–52
22. Panda B, Rao L, Tosh D, *et al.* Germline study of *AR* gene of Indian women with ovarian failure. *Gynecol Endocrinol* 2011;27:572–8
23. Westberg L, Baghaei F, Rosmond R, *et al.* Polymorphisms of the androgen receptor gene and the estrogen receptor β gene are associated with androgen levels in women. *J Clin Endocrinol Metab* 2001;86:2562–8
24. Ayvaz ÖÜ, Ekmekci A, Baltacı V, *et al.* Evaluation of in vitro fertilization parameters and estrogen receptor alpha gene polymorphisms for women with unexplained infertility. *J Assist Reprod Genet* 2009;26:503–10
25. Gennari L, Merlotti D, De Paola V, *et al.* Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. *Am J Epidemiol* 2005;161:307–20
26. Tsukamoto K, Inoue S, Hosoi T, *et al.* Isolation and radiation hybrid mapping of dinucleotide repeat polymorphism at the human estrogen receptor β locus. *J Hum Genet* 1998;43:73–4
27. Nicolás Díaz-Chico B, Germán Rodríguez F, González A, *et al.* Androgens and androgen receptors in breast cancer. *J Steroid Biochem Mol Biol* 2007;105:1–15