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Mutation analysis of the *FOXL2* and *BMP15* genes in patients with premature ovarian insufficiency

Prematür over yetersizliği olan hastalarda FOXL2 ve BMP15 genlerinin mutasyon analizi

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Abstract

Objective: Premature ovarian insufficiency (POI) is defined by irregular menstrual cycles or amenorrhea before age 40 with elevated follicle-stimulating hormone (FSH) levels. We evaluated *FOXL2* and *BMP15* variants in Turkish women with POI and assessed the distribution of the *BMP15* promoter variant c.-9C>G in a case-control setting.

Materials and Methods: Seventy-five women younger than 40 years with hypergonadotropic hypogonadism, primary/secondary amenorrhea, serum FSH ≥25 mIU/mL on two occasions at least four weeks apart, a normal 46,XX karyotype, and negative *FMR1* CGG repeat testing were included. Women with prior ovarian surgery, pelvic chemotherapy/radiotherapy, or endocrine or autoimmune disease were excluded. *FOXL2* and *BMP15* coding regions and intron-exon junctions were analyzed by Sanger sequencing. *BMP15* c.-9C>G genotype frequencies were compared with 80 ethnically matched controls with normal ovarian function. Genotype-specific analyses compared CG versus CC + GG using Fisher's exact test, with odds ratios (ORs) and 95% confidence intervals (CIs).

Results: No pathogenic POI-associated variants were detected in *FOXL2* or *BMP15*. The heterozygous *BMP15* c.-9C>G variant was identified in 34/75 patients; it occurred alone in 21, with c.308A>G in 12, and with c.352G>A in 1 patient. In the case-control comparison, the CG genotype was more frequent in POI than in controls (34/75, 45.3% vs. 15/80, 18.8%) and was associated with increased POI risk (OR=3.59, 95% CI: 1.74-7.40; p=0.0005).

Conclusion: No pathogenic *BMP15* or *FOXL2* variant was identified. The *BMP15* c.-9C>G variant may be associated with susceptibility to POI in this Turkish cohort, but this finding requires confirmation in larger, unrelated, well-matched populations and functional studies.

Keywords: *BMP15*, *FOXL2*, premature ovarian insufficiency

Öz

Amaç: Prematür over yetersizliği (POY), 40 yaşından önce düzensiz menstrüel sikluslar veya amenore ile birlikte yüksek folikül stimülan hormon (FSH) düzeyleri ile tanımlanır. Bu çalışmada, POY tanılı Türk kadınlarda *FOXL2* ve *BMP15* varyantları değerlendirildi ve *BMP15* promotör varyantı c.-9C>G'nin dağılımı olgu-kontrol düzeninde araştırıldı.

PRECIS: *FOXL2* and *BMP15* sequencing in 75 Turkish women with premature ovarian insufficiency (POI) revealed no pathogenic variants; the *BMP15* c.-9C>G CG genotype was common and associated with increased POI risk.

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Gereç ve Yöntemler: Hipergonadotropik hipogonadizm, primer/sekonder amenore, en az dört hafta arayla yapılan iki ölçümde serum FSH düzeyinin ≥25 mIU/mL olması, normal 46,XX karyotip ve negatif *FMR1* CGG tekrar testi bulunan 40 yaş altındaki 75 kadın çalışmaya dahil edildi. Daha önce over cerrahisi geçirmiş, pelvik kemoterapi/radyoterapi almış veya endokrin ya da otoimmün hastalığı bulunan kadınlar dışlandı. *FOXL2* ve *BMP15* genlerinin kodlayan bölgeleri ile intron-ekzon sınırları Sanger dizileme yöntemiyle analiz edildi. *BMP15* c.-9C>G genotip frekansları, normal over fonksiyonuna sahip etnik olarak eşleştirilmiş 80 kontrol ile karşılaştırıldı. Genotipe özgü analizlerde CG genotipi, CC + GG genotipi ile Fisher'in kesin testi kullanılarak karşılaştırıldı; olasılık oranları (OR) ve %95 güven aralıkları (GA) hesaplandı.

Bulgular: *FOXL2* veya *BMP15* genlerinde POY ile ilişkili patojenik varyant saptanmadı. Heterozigot *BMP15* c.-9C>G varyantı 34/75 hastada belirlendi; bu varyant 21 hastada tek başına, 12 hastada c.308A>G ile birlikte ve 1 hastada c.352G>A ile birlikte bulundu. Olgu-kontrol karşılaştırmasında, CG genotipi POY grubunda kontrollere göre daha sık bulundu (34/75, %45,3; 15/80, %18,8) ve artmış POY riski ile ilişkiliydi (OR=3,59; %95 GA: 1,74-7,40; p=0,0005).

Sonuç: *BMP15* veya *FOXL2* genlerinde patojenik varyant saptanmadı. *BMP15* c.-9C>G varyantı bu Türk kohortunda POY yatkınlığı ile ilişkili olabilir; ancak bu bulgunun daha büyük, akraba olmayan, iyi eşleştirilmiş popülasyonlarda ve fonksiyonel çalışmalarla doğrulanması gerekmektedir.

Anahtar Kelimeler: *BMP15*, *FOXL2*, prematür over yetersizliği

Introduction

Premature ovarian insufficiency (POI) is defined as irregular menstrual cycles or amenorrhea for at least four months occurring before the age of 40. This diagnosis is established when two or more follicle-stimulating hormone (FSH) measurements obtained at least four weeks apart demonstrate levels ≥25 mIU/mL[1]. POI affects approximately 1% of women and commonly presents with infertility and hypoestrogenic symptoms[1,2]. In addition to infertility, women with POI are at an increased risk of developing osteoporosis, cardio-cerebrovascular, neurodegenerative, and metabolic diseases[3]. The pathogenesis is heterogeneous and may involve a reduced primordial follicle pool, accelerated follicular atresia, or impaired follicular growth, with genetic, autoimmune, metabolic, infectious, iatrogenic (e.g., gonadotoxic cancer treatments), and environmental contributors[4,5]. Genetic factors account for 25-30% of cases of POI and up to ~50% in familial POI, and more than 100 genes have been implicated[6,7]. In this context, we focused on *FOXL2* and *BMP15*, key regulators of oocyte-granulosa cell communication. *FOXL2* is critical for granulosa-cell differentiation and identity, and loss-of-function variants cause blepharophimosis-ptosis-epicanthus inversus syndrome, which may be associated with POI[8,9]. *BMP15* is an oocyte-derived transforming growth factor β superfamily ligand that modulates granulosa-cell proliferation, steroidogenesis, and FSH responsiveness; pathogenic *BMP15* variants have been linked to ovarian dysgenesis/POI through a dosage-sensitive mechanism[10-12]. This study aimed to investigate *FOXL2* and *BMP15* variants in Turkish women with POI and to assess the association of the *BMP15* c.-9C>G promoter variant with POI in a case-control setting.

Materials and Methods

A total of 75 unrelated patients were included in the study: 50 who presented to the Ankara University Faculty of Medicine, Department of Medical Genetics clinic with a preliminary diagnosis of POI and 25 who presented to the Zekai Tahir Burak Women's Health Education and Research Hospital, Clinic of Medical Genetics. The Ankara University Clinical Research Ethics Committee approved the study (approval number: 05-187-13, date: 25.03.2013), and 75 patients were included after obtaining their informed consent. The inclusion criteria of the study were being under 40 years of age, serum FSH ≥25 mIU/mL on two occasions at least four weeks apart, presence of primary or secondary amenorrhea, normal karyotype: 46,XX, and negative *FMR1* CGG repeat testing. The exclusion criteria were a history of ovarian surgery, pelvic chemotherapy, pelvic radiation exposure, or endocrine or autoimmune disease. Eighty women who presented to our clinic and were assessed as having normal ovarian function were recruited as the control group. Controls were ethnically matched women who had regular menstrual cycles, no history of primary or secondary amenorrhea, and no prior diagnosis of POI. Women with a personal history suggestive of POI or amenorrhea were excluded. Genotype distributions of *BMP15* c.-9C>G were compared in POI cases and controls using Fisher's exact test. In addition, exploratory inheritance-model analyses were performed, and effect sizes were reported as odds ratios (ORs) with 95% confidence intervals (CIs). Departure from Hardy-Weinberg equilibrium for *BMP15* c.-9C>G was assessed in the control group using an exact test. All statistical analyses were performed using SPSS Statistics, version 31.0.1.0.

Lymphocyte Cell Culture from Peripheral Blood

Peripheral blood lymphocytes were cultured using standard cytogenetic procedures. Heparinized peripheral blood was incubated in culture medium at 37 °C for 72 hours. Colchicine treatment, hypotonic incubation, and fixation were subsequently performed, and metaphase chromosome analysis was carried out on the prepared samples.

Fragment Analysis

Genomic deoxyribonucleic acid was isolated from peripheral blood collected in ethylenediamine tetraacetic acid tubes, using the Magna Pure LC Instrument (Roche Applied Science). Fragment analysis was performed using fluorescently labeled polymerase chain reaction (PCR) primers and capillary electrophoresis with the ROX 1000 size standard. Fragment sizes were analyzed using GeneMapper software (Applied Biosystems).

Sanger Sequencing

FOXL2 and *BMP15* were analyzed by bidirectional Sanger sequencing. The single exon of *FOXL2* and the two exons of *BMP15*, including exon-intron junctions, were amplified by PCR using gene-specific primers. Amplification was verified by agarose gel electrophoresis, purified, and then sequenced on a 3130 Genetic Analyzer (Applied Biosystems). Sequence data were analyzed using SeqScape version 2.7 and Sequencing Analysis version 5.1 software. NM_023067.4 and NM_005448.2 were used as the reference transcripts for *FOXL2* and *BMP15*, respectively. Variants were described and classified according to American College of Medical Genetics and Genomics (ACMG) guidelines[13]. Further details of the laboratory protocols are available from the corresponding author upon reasonable request.

Results

The ages at diagnosis ranged from 11 to 39 years, with a mean of 29.93±6.86 years. Of the 75 patients included in the study, 11 (14.67%) were diagnosed with primary amenorrhea and 64 (85.33%) with secondary amenorrhea. Hormonal analysis revealed a mean FSH level of 70.69±29.58 mIU/mL and a mean luteinizing hormone level of 29.76±15.54 mIU/mL. In the patients, chromosomal analysis and *FMR1* CGG repeat testing were normal. The clinical and laboratory characteristics of the patients are summarized in Table 1.

FOXL2 sequencing was performed in three fragments in 67 of the 75 patients; in the remaining eight patients, only the first two fragments could be analyzed because of technical difficulties. Variants in the *FOXL2* gene were detected in four of the 75 patients. Three patients carried both the c.501C>T (rs61750361) and c.536C>G (rs7432551) variants, and one patient carried the c.672A>T variant. The *FOXL2* variants c.501C>T and c.536C>G were classified as benign, whereas the c.672A>T variant was classified as likely benign. *BMP15* variants were detected in 36 of the 75 patients. The c.-9C>G variant was homozygous in two patients (cases 6 and 20) and heterozygous in 34 patients. In this heterozygous group, the c.-9C>G (rs3810682) promoter variant was present alone in 21 patients, in combination with c.308A>G (rs41308602) in 12 patients, and in combination with c.352G>A (rs142156356) in one patient. The *BMP15* variants c.-9C>G and c.308A>G were classified as benign, whereas the c.352G>A variant was classified as likely benign. Detailed information on *FOXL2* and *BMP15* variants is presented in Table 2.

A control group of 80 women (ethnically matched women with normal ovarian function) was analyzed to determine the frequency of the *BMP15* c.-9C>G variant in the Turkish population. In the control group, 15 women were heterozygous, and 9 were homozygous for this variant. The distribution of *BMP15* c.-9C>G genotypes in the patient and control groups is shown in Table 3.

In the control group, the *BMP15* c.-9C>G genotype distribution deviated from the Hardy-Weinberg equilibrium (exact p<0.01). Formal haplotype analysis was not performed in our study. In the patient group, the c.308A>G variant was observed only in individuals carrying the c.-9G allele (CG or GG genotypes), which may suggest linkage between these two variants. However, the same pattern was observed in the control group, and the distribution of c.308A>G among c.-9C>G carriers was similar in patients and controls. Therefore, our current data do not support a clear disease-specific effect attributable to a c.-9C>G/c.308A>G haplotype. The observed association appears more likely attributable to c.-9C>G heterozygosity, although this interpretation remains preliminary in the absence of formal haplotype-based analysis. In the case-control comparison, the genotype distribution for *BMP15* c.-9C>G differed significantly between POI cases and controls (2×3 Fisher's exact test, p=0.0006). The association signal was mainly driven by enrichment of the heterozygous CG genotype in POI cases (34/75, 45.3%) compared with controls (15/80, 18.8%). In exploratory inheritance-model analyses, the overdominant model (CG vs. CC + GG) provided the best fit, and showed that heterozygous carriage was associated with increased risk of POI (OR=3.59, 95% CI 1.74-7.40; Fisher's exact test, p=0.0005). By contrast, the GG genotype was not significantly associated with case status. The distribution of the c.-9C>G and c.308A>G variants in the patient and control groups is shown in Table 4.

Discussion

The etiology of POI is the result of the interplay of several genes, and *FOXL2* and *BMP15* represent functionally important genes that have a role in this process[3]. In the present study, we analyzed *FOXL2* and *BMP15* in patients with POI to determine the spectrum and frequency of variants and to assess their pathogenicity.

We did not identify any *FOXL2* or *BMP15* pathogenic variants in the analyzed regions of our cohort. Because *FOXL2* sequencing was incomplete in eight patients, rare variants in the unanalyzed *FOXL2* region cannot be fully excluded. Although the *BMP15* promoter variant c.-9C>G is classified as benign according to ACMG criteria, its relatively high frequency in our cohort and the case-control comparison suggested a possible association between the CG genotype at position -9 and POI susceptibility. This finding should not be interpreted as evidence of pathogenicity, but rather as a preliminary association signal that requires cautious interpretation and confirmation in larger independent cohorts.

Functional *in vitro* studies have demonstrated that the *BMP15* c.-9C>G promoter variant results in a significant increase in *BMP15* expression and has been proposed to contribute to the pathogenesis of POI[14]. In the study by Fonseca et al.[14],

Table 1. The clinical and laboratory characteristics of the patients

No	Age	Diagnosis	FSH	LH	E2
Case 1	24	SA	54	12	-
Case 2	24	SA	115	66	<20
Case 3	32	SA	75	25	62
Case 4	38	SA	60	32	<20
Case 5	35	SA	48	-	-
Case 6	29	SA	95	74	11
Case 7	38	SA	182	50	<20
Case 8	18	PA	68	16	<20
Case 9	39	SA	49	18	<20
Case 10	33	SA	45	16	22
Case 11	31	SA	42	22	<20
Case 12	29	SA	48	20	<20
Case 13	33	SA	121	49	34
Case 14	32	SA	48	11	<20
Case 15	39	SA	66	27	32
Case 16	32	SA	177	13	10
Case 17	39	SA	63	37	<20
Case 18	28	SA	60	17	25
Case 19	11	PA	68	14	<20
Case 20	24	SA	75	17	<11
Case 21	35	SA	106	37	-
Case 22	25	SA	53	15	28
Case 23	34	PA	48	25	6
Case 24	29	SA	52	19	5
Case 25	29	PA	83	29	<20
Case 26	33	SA	90	28	21
Case 27	39	SA	54	21	<20
Case 28	26	PA	57	16	<20
Case 29	36	SA	73	29	48
Case 30	22	SA	94	48	<20
Case 31	28	SA	103	34	33
Case 32	32	SA	88	49	<20
Case 33	38	SA	102	51	<20
Case 34	27	SA	99	46	34
Case 35	37	SA	42	13	<20
Case 36	28	SA	111	49	21
Case 37	38	SA	66	-	35
Case 38	30	SA	41	15	<20
Case 39	39	SA	45	7	<20
Case 40	39	SA	44	31	<20
Case 41	38	SA	130	56	<20
Case 42	19	SA	52	45	42
Case 43	26	SA	41	5	35
Case 44	25	SA	88	37	21
Case 45	29	SA	62	55	128
Case 46	31	SA	40	17	<20
Case 47	29	PA	40	23	25
Case 48	39	SA	116	41	<20
Case 49	25	SA	40	11	<20
Case 50	30	SA	46	13	<20
Case 51	26	SA	40	12	<11.8
Case 52	32	SA	71	28	<20
Case 53	18	PA	61	15	<11.8
Case 54	15	SA	94	48	17
Case 55	22	PA	57	35	60
Case 56	38	SA	48	21	64
Case 57	39	SA	74	55	42
Case 58	20	SA	75	30	26
Case 59	23	SA	87	29	<20
Case 60	19	SA	67	41	<20
Case 61	26	SA	45	25	31
Case 62	37	SA	40	-	-
Case 63	38	SA	45	-	-
Case 64	17	PA	101	30	16
Case 65	39	PA	46	38	<20
Case 66	33	SA	81	36	<20
Case 67	28	SA	100	55	33
Case 68	29	SA	85	10	19
Case 69	21	SA	70	47	12
Case 70	28	SA	47	19	19
Case 71	39	PA	81	39	<20
Case 72	33	SA	46	27	<20
Case 73	28	SA	49	32	87
Case 74	29	SA	65	8	10
Case 75	25	SA	62	32	48

FSH and LH values in the table are presented in mIU/mL, and E2 values are presented in pg/mL. PA: Primary amenorrhea, SA: Secondary amenorrhea, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, E2: Estradiol

Table 2. Pathogenicity and other characteristics of the identified variants

Gene variants	Protein position	Variant type	GnomAD AF	TGP AF	ACMG criteria	ACMG classification
FOXL2 c.501C>T	p.Phe167=	S	0.040	0.039	BA1, BS2, BP7, BP6	Benign
FOXL2 c.536C>G	p.Ala179Gly	M	0.042	0.039	PP2, BA1, BS2, BP6	Benign
FOXL2 c.672A>T	p.Ala224=	S	-	-	PM2, BP4, BP7	Likely benign
BMP15 c.-9C>G	-	-	0.210	-	BA1, BS2, BP7, BP6	Benign
BMP15 c.308A>G	p.Asn103Ser	M	0.071	-	BA1, BS2, BP4, BP6	Benign
BMP15 c.352G>A	p.Gly118Ser	M	0.000020	-	PM2, BP4	Likely benign

M: Missense, S: Synonymous, AF: Allele frequency, GnomAD: Genome aggregation database, TGP: Turkish Genome Project, ACMG: American College of Medical Genetics and Genomics

Table 3. Genotype distribution of the *BMP15* c.-9C>G variant in patient and control groups

	CC	CG	GG	Total
Patients	39	34	2	75
Controls	56	15	9	80
Total	95	49	12	155

the *BMP15* c.-9G allele was associated with POI. It has been established that *BMP15* is co-expressed with the transcription factor *PITX1* (pituitary homeobox 1), which binds to the *BMP15* promoter between positions -14 and -8. *PITX1* can transactivate the *BMP15* promoter carrying either the C or the G allele at position -9; however, the c.-9C>G substitution modifies the *PITX1*-binding site and results in enhanced *BMP15* transcription, which has been implicated in POI[14]. It has been reported that *BMP15* expression increases significantly in the presence of the c.-9G allele, leading to altered granulosa cell proliferation. Moreover, high ovarian *BMP15* levels have been shown to further reduce *FSHR* messenger RNA expression. Consistent with these findings, a transgenic mouse model with high *BMP15* expression exhibited an increased number of primary follicles but a reduced number of secondary follicles, and increased granulosa-cell mitosis was associated with accumulation of primary follicles and atresia of secondary follicles[15]. Collectively, these data provide biological plausibility for a possible contributory role of the *BMP15* c.-9G allele in ovarian dysfunction; however, they do not establish this common variant as a pathogenic cause of POI.

Several studies have shown that the *BMP15* c.-9C>G variant is associated with POI[16-18]. In a study of 202 Indian patients with POI, Dixit et al.[16] demonstrated that the *BMP15* c.-9G allele forms part of a haplotype associated with the disease. They performed haplotype analysis of three variants that are commonly observed in POI patients (c.-9C>G, c.308A>G, and c.852C>T) and found a significant association between the G-G-C haplotype (c.-9G, c.308G, c.852C) and POI[16]. Although c.308A>G was observed only in carriers of the c.-9G allele in our cohort, a similar pattern was present in controls, and formal haplotype analysis was not performed. Therefore, our data do not allow firm conclusions regarding a disease-specific *BMP15* haplotype. Morón et al.[17] investigated the association between *BMP15* alleles and ovarian hyperstimulation syndrome. The study included 307 women undergoing in vitro fertilization treatment, of whom 35 had a high ovarian response to stimulation. A significant association was found between carriage of the *BMP15* c.-9G allele and a high ovarian response to ovarian stimulation in this cohort[17]. In another study, Hanevik et al.[18] examined the association between *BMP15* variants and the ovarian response to stimulation. They found a significant association

Table 4. Genotype distribution of *BMP15* c.-9C>G genotypes (CC, CG, GG) and carriage of c.308A>G (AG) in POI patients and controls

Patient (n=75)						Control (n=80)					
CC (n=39)		CG (n=34)		GG (n=2)		CC (n=56)		CG (n=15)		GG (n=9)	
CC	CC + AG	CG	CG + AG	GG	GG + AG	CC	CC + AG	CG	CG + AG	GG	GG + AG
39	0	22*	12	1	1	56	0	10	5	5	4

* One POI patient carrying c.-9C>G together with c.352G>A (rs142156356), without c.308A>G, was included in the CG (without AG) cell. POI: Premature ovarian insufficiency

between a high ovarian response and carriage of the *BMP15* c.-9G allele. Taken together, these studies suggest that the *BMP15* c.-9G allele is associated with an increased ovarian response to stimulation, perhaps by increasing the number of primary follicles at the early stages of folliculogenesis[18].

Although functional and association studies support a potential link between the *BMP15* promoter variant c.-9C>G and reproductive phenotypes, the published evidence remains inconsistent across populations. Peluso et al.[19] analyzed 186 infertile Brazilian women undergoing their first assisted reproduction cycle and reported higher serum AMH levels among women homozygous for c.-9C>G; however, they found no significant association with ovarian stimulation parameters or assisted reproduction outcomes. Mehdizadeh et al.[20] screened *BMP15* exon 1 in 70 Iranian women with polycystic ovary syndrome and detected c.-9C>G in 31.4% of patients (28.6% heterozygous and 2.9% homozygous), suggesting a possible contributory role in disease susceptibility rather than a primary causal effect. In contrast, several case-control studies in POI reported no significant association between c.-9C>G and POI risk, including cohorts from Brazil (74 women with POI and 88 controls)[21] and the Chinese Hui population (63 women with POI and 58 controls)[22]; similarly, no significant difference for *BMP15* rs3810682 was observed in a Korean recurrent implantation failure cohort (133 patients and 317 controls)[23]. In a single-case report of POI coexisting with blepharophimosis-ptosis-epicanthus inversus syndrome, Settas et al.[24] identified a *de novo FOXL2* mutation alongside *BMP15* variants and did not consider c.-9C>G to be causally related to the POI phenotype in that patient.

Study Limitations

An important limitation of this study is incomplete sequencing of *FOXL2* in eight patients. Although *FOXL2* sequencing was successfully completed for all three fragments in 67 of the 75 patients, only the first two fragments could be analyzed in the remaining eight patients because of technical difficulties. Therefore, rare *FOXL2* variants located in the unanalyzed region cannot be excluded from this subset. This limitation is important for the interpretation of our negative *FOXL2* findings, which should therefore be interpreted with caution, particularly in these partially analyzed cases.

A significant limitation of this study is the deviation from the Hardy-Weinberg equilibrium observed for the *BMP15* c.-9C>G variant in the control group. This pattern, characterized by a deficit of heterozygotes and an excess of homozygotes, may be attributable to sampling variation related to the modest sample size, subtle population stratification, or, less likely, technical/genotyping issues. Although genotyping was performed by bidirectional Sanger sequencing, which reduces the likelihood of systematic misclassification, systematic misclassification, this possibility cannot be completely excluded. Because deviation from Hardy-Weinberg equilibrium may bias estimates of genotype-specific associations, particularly for the CG genotype, the observed association between *BMP15* c.-9C>G and POI should be interpreted with caution. Therefore, our findings should be considered preliminary and require validation in larger, independent, well-matched cohorts. Because *BMP15* c.-9C>G is classified as a benign variant under ACMG criteria, the observed case-control association should be interpreted not as evidence of pathogenicity but as a hypothesis-generating finding.

Formal haplotype analysis of *BMP15* variants was not performed. Therefore, the possible combined contribution of c.-9C>G and c.308A>G to POI susceptibility could not be fully assessed in this cohort.

Conclusion

In our study, no pathogenic variant associated with POI was detected in *BMP15* or in the analyzed regions of *FOXL2*. Because *FOXL2* sequencing was incomplete in eight patients, rare variants in the unanalyzed region cannot be entirely excluded. Although the *BMP15* c.-9C>G variant was relatively frequent in our Turkish POI cohort and showed a genotype-based association in the case-control comparison, it is classified as benign according to ACMG criteria and should not be interpreted as a pathogenic cause of POI. Rather, it may represent a common variant associated with disease susceptibility in this cohort. These findings should be interpreted cautiously and confirmed in larger, unrelated, well-matched populations, ideally supported by functional and haplotype-based analyses.

Ethics

Ethics Committee Approval: The Ankara University Clinical Research Ethics Committee approved the study (approval number: 05-187-13, date: 25.03.2013).

Informed Consent: Written informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Concept: M.B.M., H.G.K., Design: M.B.M., H.G.K., Data Collection or Processing: M.B.M., V.T., Ş.E.Ç., Y.Ü., A.B., C.S.A., H.I.R., H.G.K., Analysis or Interpretation: M.B.M., V.T., A.B., H.I.R., H.G.K., Literature Search: M.B.M., H.I.R., H.G.K., Writing: M.B.M., H.G.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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