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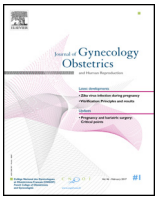

Original Article

Clinical and Genetic Investigation of Premature Ovarian Insufficiency Cases from Turkey

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ABSTRACT

Objective: Premature ovarian insufficiency is a lack of ovarian functions in patients younger than 40 years old. Genetic causes leading to accelerated follicle depletion may result in premature ovarian insufficiency. We aimed to determine genetic etiology of nonsyndromic premature ovarian insufficiency cases from Turkey.

Materials and methods: We analyzed 86 nonsyndromic premature ovarian insufficiency cases and 26 matched control female participants. Participants have been investigated in cytogenetic analysis followed by FMR1 repeat size expansions and search of variants for nine premature ovarian insufficiency-associated genes.

Results: Four cases had a structural cytogenetic abnormality. Two cases revealed with premutation size FMR1 triplet repeat expansion. Four cases carried variants in which two were very rare in FSHR and PDPK1, and three were novel in NR5A1, PDPK1, and POF1B genes. Six novel variants have been identified in NOBOX, NR5A1, POF1B, and PDPK1 in control population assigned to be benign alterations.

Conclusion: Mosaicism of sex chromosomes was responsible in 4.6% and FMR1 premutation in 2.4% of premature ovarian insufficiency cases, while the association of premature ovarian insufficiency-related genes was found very subtle. Novel variants in NR5A1, PDPK1, and POF1B may necessitate further evaluation for their association with premature ovarian insufficiency via functional studies.

Introduction

Premature ovarian insufficiency (POI) is a preferred term for premature ovarian failure (POF) [1]. POI is a lack of ovarian functions in patients younger than 40 years old and presents with oligo/amenorrhea, infertility, vasomotor symptoms or other estrogen deficiency symptoms. In other terms, POI can be defined as the development of hypergonadotropic hypogonadism before the age of 40 years [2]. The prevalence of POI is 1% per population and despite a vast amount of investigated reasons most of the cases remain with unknown etiology [3]. The incidence of family-related cases varies between 4 and 31% [4]. Surgical, medical, infectious or autoimmune ovarian damages are the other well-known reasons for POI.

Not so long ago, since the cytogenetic evaluations kept responsible few genes located at Xp21 breakpoints of balanced X-autosome translocations in 1997, and collaborative studies examining premature menopause in fragile X carriers revealed that the 16% of the premutation carriers had experienced menopause prior to the age of 40, growing number of association studies are reported since then to underlie the genetic basis of nonsyndromic POI [5,6]. Presently, at least 100 different variants in 27 different genes are found liable with the condition, either on X chromosome (*BMP15*, *COL4A6*, *PGRMC1*, *POF1B*) or on autosomes (*ADAMTS19*, *AMH*, *ARFGAP3*, *CARD11*, *CPEB1*, *DAZL*, *DMC1*, *ERCC6-PGBD3*, *FIGLA*, *FSHR*, *GDF9*, *INHA*, *MCM8*, *NOBOX*, *NR5A1*, *POU5F1*, *SCARB1*, *SOHLH2*, *STAG3*, *SYCE1*, *TGFBR3*) [7].

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We aimed here to search the genetic etiology of nonsyndromic POI in scope of chromosome aberrations, -CGG- repeat size expansions of *FMR1* gene, and variants of eight genes encoding proteins that are important in differentiation and development of gonadal cells (*BMP15*, *FIGLA*, *FSHR*, *GDF9*, *INHA*, *NOBOX*, *NR5A1*, *POF1B*), also further with a human homologous gene of *Pdpk1* (*PDPK1*).

Materials and Methods

Participitants

A case-control study was conducted with nonsyndromic non-kinship POI cases and non-kinship female control individuals at Istanbul University Cerrahpasa Medical Faculty Department of Obstetrics and Gynecology for three years. The study was reviewed and approved by the Ethical Committee of Istanbul University (diary number 2013-22547). Participants were chosen among women appointed to the outpatient clinic of Istanbul University Cerrahpasa Medical Faculty, Department of Obstetrics and Gynecology. Upon approve of informed and written consent participants were enrolled in the corresponding groups.

European Society of Human Reproduction and Embryology Societies' guideline followed for the POI inclusion criteria, eg patients younger than 40 years old, who has at least four months of oligo/amenorrhea with double checked increased follicle stimulating hormone (FSH) level (>40 IU/l) [8]. Exclusion criteria were the history of the surgery performed at least in one of the ovaries, history of chemotherapy and/or radiotherapy, administration of agents causing the medical oophorectomy, history of any confirmed autoimmune disease. Women older than 40 years old, with a negative history for infertility and menopause occurred before age 40 were eligible for the control arm. After assessment for eligibility criteria, participants were evaluated for further clinical and hormonal findings.

Cytogenetic and molecular investigations have been performed at the Department of Medical Genetics at Istanbul University Istanbul Medical Faculty. 2 ml of peripheral blood samples were collected in heparinized tubes for cytogenetic analysis and in K_3EDTA tubes for molecular tests.

Cytogenetic Analysis

All of the POI referrals were investigated cytogenetically. Potential balanced translocations, if in control individuals, were overlooked. High-resolution G-banded chromosome analysis was performed on the peripheral blood lymphocytes following routine protocols. A minimum of 30 metaphase cells were examined for both numerical and structural chromosomal abnormalities, and four or more karyograms were created on each patient. FISH studies were performed to elucidate the further chromosomal involvements when a possible X chromosome anomaly was suspected during karyotyping.

Molecular Investigation

DNA isolation was performed by kit based automated method (MagNA Pure LC, Roche).

FMR1 premutation allele testing

Patients with confirmed normal karyotype were tested for *FMR1* gene repeat size expansion. 80 ng of genomic DNA has been used for the amplification of 5' Untranslated Region (UTR) of *FMR1* according to the recommended amplification protocol provided in the commercial PCR-based *FMR1* kit (FragilEaseTM, Perkin Elmer, USA). Amplified PCR products were purified by spin colon kit (High Pure PCR Product Purification, Roche), eluted in 20 μl of dH_2O, mixed with 9.8 μl Highly Deionized (Hi-Di) formamide (Thermo Fisher), 0.1 μl of Liz-500 bp (ABI) and run on Sequence Analyzer (ABI3500). Fragments have been analyzed by Allele Fragment Length Polymorphism (AFLP) workflow of GeneMapper software (GeneMapper v4.1, ABI).

Panel-Gene Testing

Total transcript sequences including uncoding and coding regions with up to 20 base pairs flanking to exon-intron boundaries for nine POI associated genes (*BMP15*; NM_005448.2, *FIGLA*; NM_001004311.3, *FSHR*; NM_000145.3, *GDF9*; NM_005260.4, *INHA*; NM_002191, *NOBOX*; NM_001080413.3, *NR5A1*; NM_004959, *PDPK1*; NM_002613.4, *POF1B*; NM_024921.3) were introduced to Ion Ampliseq for web-based primer design program (https://www.ampliseq.com). Total target length was 30.16 Kb, encircled by 175 primer pairs, paddled into two pools (89 and 86 primer pairs) with coverage of 96.45%. Total of 10 ng genomic DNA was used for panel-gene sequencing on Ion PGM according to the library, template, and sequencing kit instructions (Ion Torrent Thermo Scientific). The sequencing data was analyzed by using Torrent Suit Server (TSS 5.0.4) and patch plugin programs (Coverage Analysis 5.0.4.0, Torrent Variant Caller 5.0.4.0). Sequential steps followed initially excluding under qualified reads according to default filter values. Panel gene test was repeated for samples with inadequate reads. Variant annotations were reached by using the Ion Reporter Cloud Program. Patient and control population were first analyzed independently by reference sequences supported the Genome Reference Consortium human genome build 37 (GRCh37, Hg19). List of variants with deep lengths >20X, Minor Allele Frequency (MAF) value below 1% or unknown and novel occurrences according to NCBI's SNP150 data were compared between two groups [9]. *In silico* analysis programs (Mutation Taster, PolyPhen, and SIFT) were used for prediction of deleteriousness of unknown or rare variants [10,11,12]. Allele frequencies were searched from Exome Aggregation Consortium (ExAC) database [13].

Statistical Analysis

The sample distribution was assessed with the Shapiro-Wilk test and the central tendency was reported in mean with standard deviation (SD) for continuous variables. The number of cases and percentages (%) were used for nominal variables. Independent sample t-test and chi-square test were used for outcome comparison. $p < .05$ was considered statistically significant. Statistical analyses were performed with STATA 14.2 (StataCorp LP, Texas, USA) and GraphPad Prisma7.01 (GraphPad Software, California, USA).

Results

Overall, 112 participants, 86 in POI and 26 in control groups, were enrolled in the study. Missing values for demographic findings of two patients were not imputed for data analysis. Demographic and clinical findings of all participants were summarized and presented in Tables 1 and 2. Hormone profile of POI cases was presented in Table 3.

Four POI cases (Case-83, -84, -85, -86) had cytogenetic abnormalities (46,XX/47,XXX [47/3]; 45,X/46,X,idic(X)(q22)[26/12].ish(DXZ1 × 1/DYZ3 × 0)/(DXZ1 × 3/DYZ3 × 0)[33/14]; 45,X/46,XX [12/18]; 45,X/46,XX [7/18]). Eighty-two POI cases and 26 control individuals with normal karyotypes have been tested for *FMR1* gene repeat size expansion. Two POI cases (Case-5, -6) presented

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Table 1
Demographic, Clinical and Reproductive findings of patients in the POI and control groups*.

Characteristics	POI group (number (%), n = 86)	Control group (number (%), n = 24)	*P value*[a]
Parity			
0	42 (48.8)	2 (8.3)	<**.001**
≥1	44 (51.2)	22 (91.7)	
Menarche			
Spontaneous	79 (91.9)	24 (100)	0.15
Medical	7 (8.7)	0	
Drug inducing menstruation			
None	14 (16.3)	24 (100)	<**.001**
using gestagen	22 (25.6)	0	
using HRT	49 (57.0)	0	
using OCP	1 (1.1)	0	
Comorbidity			
Absent	71 (82.6)	18 (75)	0.40
Present	15 (17.4)	6 (25)	
Smoking			
Absent	56 (65.1)	14 (58.3)	0.54
Present	30 (34.9)	10 (41.7)	
Family history of POI			
Absent	70 (81.4)	24 (100)	0.63
Present	16 (18.6)	0	
History of OCP use			
Absent	63 (73.3)	14 (58.3)	0.16
Present	23 (26.7)	10 (41.7)	
Secondary sex characteristics			
Normal	78 (90.7)	24 (100)	0.66
Tanner stage I	3 (3.5)	0	
Tanner stage II	1 (1.2)	0	
Tanner stage III	3 (3.5)	0	
Tanner stage IV	1 (1.2)	0	
Vasomotor symptoms			
Absent	42 (48.8)	24 (100)	<**.001**
Present	44 (51.2)	0	
Consanguineous marriage			
Absent	62 (72.1)	21 (87.5)	0.12
Present	24 (27.9)	3 (12.5)	
Infertility			
Absent	37 (43)	24 (100)	<**.001**
Primary	18 (20.9)	0	
Secondary	10 (11.6)	0	
Virgin	21 (24.4)	0	

Abbreviations: POI premature ovarian insufficiency; HRT hormon replacement therapy; OCP oral contraceptive pills.
[a] Chi-square test was applied.
* Missing values for demographic findings of two patients were not imputed for data analysis.

Table 2
Fertility outcomes of participants in the POI and control groups.

Characteristics	POI group (mean ± SD, n = 86)	Control group (mean ± SD, n = 24)	*P value*[a]
Age (years)	32.1 ± 7.2	50.7 ± 12.0	<**.001**
Menarche age (years)	13.6 ± 1.6	13.2 ± 1.2	0.24
Maximum amenorrhea duration (months)	8.9 ± 8.6	0	<**.001**
Infertility duration (months)	1.1 ± 2.3	0	<**.001**
Gravidity	1.2 ± 1.3	2.6 ± 1.8	<**.001**
Parity	0.9 ± 1.0	2.0 ± 1.2	<**.001**
Abortus	0.3 ± 0.6	0.6 ± 1.1	0.24

Abbreviations: POI = premature ovarian insufficiency
[a] Independent sample t-test was used.

premutation level expansions in the *FMR1* gene, 94 and 71 repeat sizes, respectively that revealed to be 2.4 % in our cohort. All of the control individuals had normal level of repeat sizes below 50.

Investigation of POI cases (n = 86) by panel-gene test resulted in five alleles with distinct variants unique in four POI cases (Table 4 and Fig. 1 for Sanger sequence electropherogram results).

Table 3
Hormone profile of the patients in the POI group.

Characteristics	Mean values ± SD (min-max)
FSH (IU/L) (n = 86)	76.3 ± 28.4
LH (IU/L) (n = 86)	39.3 ± 17.1
Estradiol (pg/mL) (n = 86)	14.6 ± 16.6
Prolactin (μg/L) (n = 71)	13.3 ± 7.3
AMH (ng/mL) (n = 62)	0.5 ± 0.6 (0.01-3.6)
TSH (IU/mL) (n = 75)	4.0 ± 8.6 (0.04-75.0)
Free T3 (pg/mL) (n = 47)	3.2 ± 0.6
Free T4 (ng/dL) (n = 52)	1.4 ± 1.2

Abbreviations: POI = premature ovarian insufficiency; FSH = follicle stimulating hormone; LH = luteinizing hormone; TSH = thyroid stimulating hormone; AMH = anti-mullerian hormone; T3 = triiodothyronine; T4 = thyroxine.

Two of these variants were identified in the same patient (Case-34); in Follicle Stimulating Hormone Receptor *(FSHR)* (c.1664C > T) and in *NR5A1* (c.1233C > T) genes. The variant identified in *FSHR* was altering threonine to isoleucine (p.Thr555Ile), via striking '7 transmembrane receptors' domain (7tm_1) of FSHR protein (UniProtKB no: P23945) [14] previously listed in NCBI-SNP database with reference SNP number of rs200144377 shown only in one individual with unknown gender and phenotype in heterozygous form out of 121.412 ExAC alleles. The other variant was novel and predicted to cause synonymous alteration (p. His410=) in NR5A1 protein. Analysis of this variant with *in silico* program (Mutation Taster) predicted to be harmful due to creating a novel pattern for splicing of the transcript.

Two different variants are identified in *PDPK1* gene, in case-1 and in case-13. The variant in case-1 was novel, located at the 3' UTR region (c.*5177C > T), predicted to be harmful (Mutation Taster). A variant in case-13 (c.745 G > A) was resulting to missense alteration (p.Val249Ile) that was located in the protein kinase domain of the encoded protein (UniProtKB no: O15530). This alteration reported to be very rare in different populations and predicted to be damaging by two (Mutation taster and SIFT) and benign by one (PolyPhen2) *in silico* online analysis programs.

One novel heterozygous variant (c.439-2A > G) in intron 4 of *POF1B* gene located in X chromosome revealed in case-56, alters evolutionally conserved splicing acceptor site and predicted to be harmful.

No other potentially pathogenic variants are observed in *BMP15*, *FIGLA*, *GDF9*, *INHA* and *NOBOX* in our POI cases from Turkey.

Six novel variants identified in our control individuals are considered benign rare alterations of our population (Table 5).

The full list of genetic test results is presented in Supporting Information, Table S1.

Discussion

Menopause before the age of 40 years defined as POI [15]. It approximately affects 1% of women and 0.1% of them are below the age of 30 years [16]. Premature ovarian insufficiency is not only affecting the hormonal balance and menopause period but also affects the fertility period of women. POI can be represented as either primary (autoimmune and/or genetic causes) or secondary to many factors including surgery, chemo/radiotherapy and infections [17–20]. Haller-Kikkatalo et al. [21] analyzed the POI women regarding their clinical and demographic characteristics and found that the age of menarche, the number of pregnancies or live births, smoking or alcohol consumption, the use of oral contraceptives for some time during their life, and the presence of irregular menstrual cycles were determinants on the age of menopause. In our cohort, differences were observed only in obstetric history, in the presence of vasomotor symptoms and infertility, in the duration of amenorrhea and infertility between

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Table 4
Variants identified either below 1% MAF or novel, identified in nonsyndromic POI group but not in the control group.

Gene name Transcript ID Protein ID	Case No	Zygosity	Variants				Location	Frequency in POI group (n = 86)	Number of reads of reference/ minor (Deep)	*In silico* analysis		
			Reference SNP no	Genotype frequency / DataBase	Nucleotide	Protein				Mutation Taster	PolyPhen2	SIFT
FSHR NM_000145.3 NP_000136	34	heterozygous	rs200144377	0.000008 / ExAC	c.1664C > T	p. Thr555Ile	Exon 10	1.20%	C = 242, T = 197 (439)	Disease Causing	Disease Causing	Disease Causing
NR5A1 NM_004959 NP_004950	34	heterozygous	none	This study	c.1233C > T	p. His410=	Exon 7	1.20%	G = 82/94, A = 82/112 (370)	Disease Causing	NA	NA
PDPK1 NM_002613 NP_002604	13	heterozygous	rs370069297	0.00003 / ExAC 0.0004 / 1000 Genome 0.00008 / GO-ESP	c.745 G > A:	p. Val249Ile	Exon 7	1.20%	G = 30/88, A = 14/61 (193)	Disease Causing	Benign	Disease Causing
PDPK1 NM_002613 NP_002604	1	heterozygous	none	This study	c. *5177C > T	?	3'UTR	1.20%	C = 32/43, T = 29/60 (174)	Disease Causing	NA	NA
POF1B NM_024921 NP_079197	56	heterozygous	none	This study	c.439-2A > G	?	Intron 4	1.20%	T = 10/17, C = 15/17 (59)	Disease Causing	NA	NA

Abbreviations: MAF = Minor Allele Frequency; POI = Premature Ovarian Insufficiency; SNP - Single Nucleotide Polymorphism; SIFT - Sorting Intolerant from Tolerant; ExAC - Exome Aggregation Consortium; NA = not applicable; Go-ESP - "Grand Opportunity" Exome Sequencing Project; UTR – Untranslated Region.

the POI group and the control groups, what might be explained by the clinical nature of the disease.

It is estimated that up to 40% of POI cases can result from genetic causes [22]. Genetic analyses of POI cases identified sex chromosomal abnormalities, mutations in X chromosome and autosomal genes [23]. A recent study on Turkish women with POI concluded that there was a genetic cause involving chromosomal abnormalities, Xq and Xp deletions, translocations, and numerical aberrations, fragile X premutation carriers in 52% of these patients [24]. Another study aiming to determine the frequency of fragile X associated POI among Turkish fragile X premutation carriers found that POI could be manifested in up to 34.2% of these women [25]. Geckinli et al. [26] emphasized the importance of routinely assessing chromosomal studies in patients with primary amenorrhea and POI because of the high prevalence of chromosomal abnormalities. In our study population, we investigated whether there is an association between nonsyndromic POI and chromosome aberrations, -CGG- repeat size expansions of the *FMR1* gene, and variants of eight genes encoding proteins that are important in differentiation and development of gonadal cells (*BMP15*, *FIGLA*, *FSHR*, *GDF9*, *INHA*, *NOBOX*, *NR5A1*, *POF1B*), also further with a human homologous gene of *Pdpk1* (*PDPK1*) previously demonstrated to cause premature ovarian failure in mice with encoding phosphoinositide 3-kinase enzyme deficient oocytes [27]. We

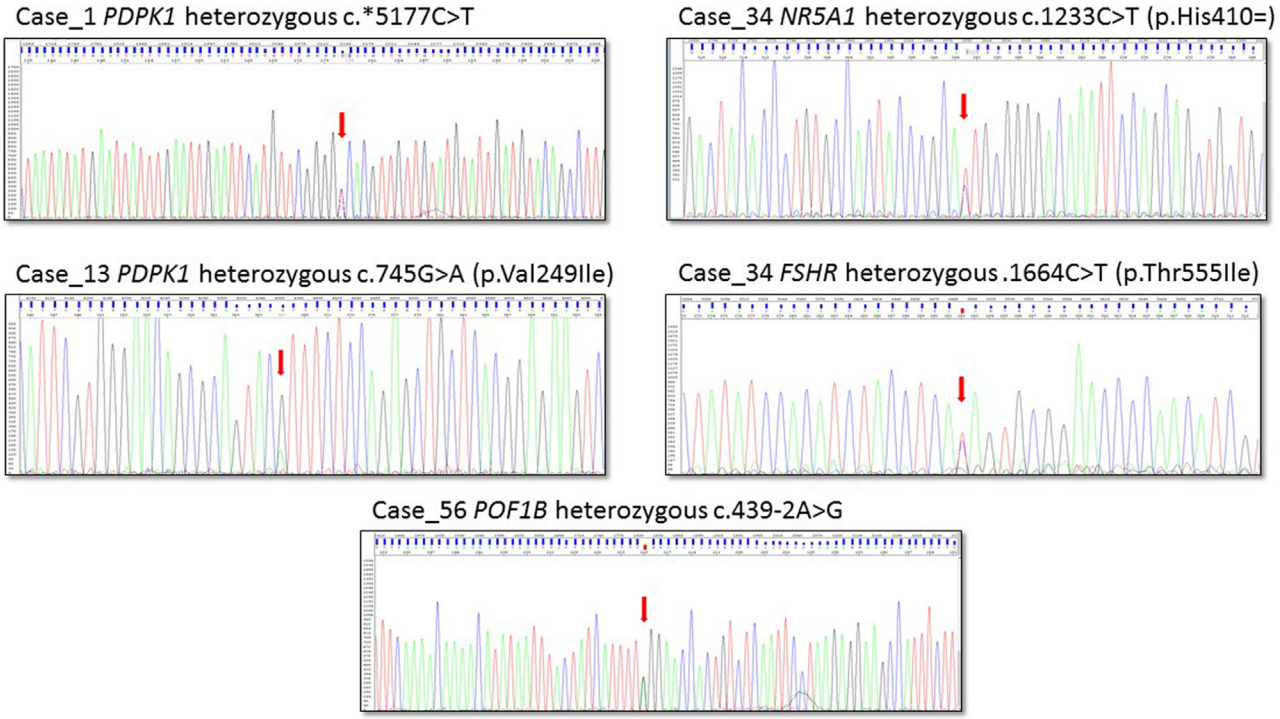

Fig. 1. Electropherograms of four nonsyndromic POI cases (Case #1, #13, #34, #56) revealing novel and rare variants predicted pathogenic via at least one *in silico* programs.

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Table 5
Novel variants identified in the control group

Gene Transcript Protein	Zygosity	Variants		Location	Frequency in POI control group (n = 26)
		Nucleotide	Protein		
POF1B NM_024921 NP_079197	heterozygous	c.1566 + 16G > T	?	Intron 14	3.33%
POF1B NM_024921 NP_079197	heterozygous	c.-136G > A	-	5'UTR	3.33%
NOBOX NM_001080413 NP_001073882	heterozygous	c.281 A > G	p.Gln179Arg	Exon 4	3.33%
PDPK1 NM_002613 NP_002604	heterozygous	c.*1205T > C	?	3'UTR	3.33%
NR5A1 NM_004959 NP_004950	heterozygous	c.*170A > G	?	3'UTR	3.33%
NR5A1 NM_004959 NP_004950	heterozygous	c.*918C > T	?	3'UTR	3.33%

Abbreviations: POI = premature ovarian insufficiency; UTR - Untranslated Region.

identified five alleles with distinct variants with being novel in three genes, *NR5A1, PDK1,* and *POF1B*.

Two of these variants in *FSHR* and in *NR5A1* gene were identified in case-34. The DNA sample of her mother with normal ovarian function was not available for segregation analysis that may otherwise have helped us to further evaluate if these two variants were coincidental occurrences or play role in POI. *FSHR* gene encodes a receptor for follicle stimulating hormone and mutations in this gene alter follicle development and hormonal signaling. Inactivating mutations of *FSHR* were found to be responsible for autosomal recessive POI cases (OMIM#136435) [28].

NR5A1 gene, also known as steroidogenic factor-1 (SF-1), located on chromosome 9q33.3, encodes a nuclear receptor protein that regulates the transcription of genes involved in the hypothalamic-pituitary-steroidogenic axis (OMIM#184757). *NR5A1* is expressed in fetal and adult adrenal cortex, testis and ovaries during fetal development, postnatal and prepubertal growth, and at maturity [29,30]. Newborn mice that are deficient in *NR5A1* lack both gonads and adrenal glands [31]. Dominant mutations of *NR5A1* have been related with a spectrum of phenotypes associated with the development of gonads in males and females encompassing from disorders of sex development to adrenocortical insufficiency, premature ovarian failure to spermatogenic failure [32–38].

Lourenço D et al. [35] tested the hypothesis that mutations in *NR5A1* are associated with disorders of ovarian development and function by sequencing the gene in affected subjects from four families with medical history of 46,XY disorders of sex development and 46,XX ovarian insufficiency, and 25 women with 46,XX sporadic ovarian insufficiency, in addition using 1465 subjects of various ancestral origins who did not carry *NR5A1* mutations as control. There was no clinical evidence of adrenal insufficiency among any of the affected patients. Distinct alterations predicted to be pathogenically revealed in members of each of the four families and two of the 25 subjects with isolated ovarian insufficiency and none in control individuals Functional effects of these variants were tested on transformed immortal human kidney cells (tsA201) and revealed measurable reduction in the transactivation of Cytochrome P450C11A1 enzyme that converts cholesterol to pregnenolone, encoded by *CYP11A1*, and aromatase enzyme that converts androgen to estrogen, encoded by *CYP19A1*. These findings indicate that mutations in *NR5A1* are associated with a number of ovarian anomalies characterized by loss of ovarian reproductive capacity. All of these studies show that the role of the *NR5A1* gene in reproductive biology is clear and any pathogenic alteration in this gene might require further counseling for couples.

We also identified two variants of *PDPK1* gene in two different POI cases. Human *PDPK1* gene is located at chromosome 16p13.3, encoding 3-phosphoinositide-dependent protein kinase 1 enzyme. PDPK1 activates a group of protein kinases belonging to the AGC [PKA (protein kinase A)/PKG (protein kinase G)/PKC (protein kinase C)]-kinase family that play important roles in mediating diverse biological processes [39]. One of the most important signaling pathways involving PDPK1 is PI3K/PTEN/Akt signaling which affects the developmental steps of the primordial follicles including their activation, survival, and death [40]. PDPK1 signaling in oocytes appears to be crucial for maintaining the survival of primordial follicles, which in turn determines the duration of female fertility. The *PDPK1* gene-deficient mice ovaries showed POI and infertility. The defect in the signaling of PDPK1 pathway resulted in clearance of primordial follicles from their dormant state and reproductive aging [41]. No human phenotypes have been associated with this gene as of today. Our two distinct variants may suggest that *PDPK1* gene variants might have a role in the molecular pathogenesis of POI in humans and future studies may further explore our results.

Furthermore, we identified one novel intronic variant in *POF1B* gene which alters evolutionally conserved splicing acceptor site sequences and was found to be harmful. *POF1B* is a gene located on Xq13.3, where balanced X chromosome and autosome translocation occurred in a patient with POI [42].

Linkage analysis and sequencing of *POF1B* gene at the concomitance region revealed a homozygous point mutation resulting to a missense change of p.Arg329Gln in five sisters afflicted with POI in a Lebanese family. Furthermore, actin binding assay in nonmuscle cells showed that the capacity of the binding of mutant (p.Arg329Gln) POF1B to actin filaments decreased four fold in comparison to wild POF1B [43].

Recently, a reciprocal translocation between chromosomes X and 3 [46,X,t(X;3)(q21.1;q21.3)] and an additional heterozygous missense alteration same as in Lebanese family (c.986 G > A; p.Arg329Gln) is detected in a POI case reported from Germany [44].

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Our POI cases did not present any pathogenic variants in any of the other panel-genes including *BMP15, FIGLA, GDF9, INHA* and *NOBOX* [45]. This does not mean that these genes have no significant associations with POI cases from Turkey. Screening of a higher number of cases may alter this acknowledgment in ovarian failure genes.

Advancement of sequencing and functional technologies in genetics increase the expectations for revealing novel alterations in previously linked genes and novel genes associated with pathways leading to premature ovarian follicle depletion and subsequent loss of ovarian function that in turn further enlighten our knowledge in the etiology of POI.

Conclusion

According to our findings, mosaicism of sex chromosomes is responsible in 4.6% and *FMR1* premutation in 2.4% of premature ovarian insufficiency cases, while the association of POI-related genes was found minor. As a conclusion, although *FMR1* mutation and mosaicism of sex chromosomes are mainly responsible, we propose five alleles in NR5A1, PDK1, and POF1B genes may be associated with POI in our cohort.

Contributors

Engin Oral participated in the design of the study, writing and reviewing the article for submission.

Guven Toksoy participated in the design of the study, writing and reviewing the article for submission.

Nigar Sofiyeva participated in the design of the study, writing and reviewing the article for submission.

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Conflict of Interests

The authors have stated explicitly that there are no conflicts of interest in connection with this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jogoh.2019.04.007.

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