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Multi-locus high-risk alleles association from interleukin's genes with female infertility and certain comorbidities

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Abstract

Objective There is evidence that cytokine genes' single nucleotide polymorphisms could be the reasons behind female infertility. This study aimed to identify the role for *Interleukin33* rs1048274 (G > A) and rs16924243 (T > C), *Interleukin22* rs1397852121 (C > T), rs1295978671 (C > T) and rs2227483 (A > T), *Interleukin17A* rs2275913 (G > A,C) and *Interleukin17F* rs763780 (T > C), *Interleukin13* 1512 (A > C) and *IL13* 2044 (G > A), and *Interleukin4* rs2243250 (C > T) and rs2070874 (C > T) gene polymorphisms in female infertility to gain a richly more detailed understanding of its genetic predisposition. Five distinct groups, each comprising 200 infertile women and 200 age-matched fertile controls, were recruited to each *Interleukins* (33, 22, 17, 13 and 4) in this case–control study and were genotyped by using an amplification refractory mutation system. Statistical analysis is conducted by SPSS software V. 22 and using Chi-square (χ^2) and logistic regression tests. Strength of association was estimated by multiple-comparison correction, population structure test and Haplotype analysis. The study was approved by the Academic Ethics Committee and each enrolled patient signed an informed consent.

Results Our statistical results revealed risk alleles in all of the substitution lines for women infertility. Current findings provided evidence that in the presence of *Interleukin33* $A_{p\text{-value rs1048274} = 0.002}$ and $C_{p\text{-value rs16924243} < 0.0001}$, *Interleukin 22* $T_{p\text{-value rs1397852121} < 0.0001}$ and $T_{p\text{-value rs2227483} = 0.000}$, *Interleukin17A* $A_{p\text{-value rs2275913} = 0.003}$ and *Interleukin17F* $C_{p\text{-value rs763780} = 0.000}$ and *Interleukin13* $C_{p\text{-value 1512} = 0.000}$ and $A_{p\text{-value 2044} = 0.003}$, *Interleukin4* $T_{p\text{-value rs2243250} = 0.001}$ and $T_{p\text{-value rs2070874} = 0.009}$ risk alleles, risk genotype also were significantly associated with increased chances of developing infertility. The relationship between risk genotypes and several well-established infertility risk factors including, polycystic ovary syndrome, premature ovarian failure, oophorectomy, diminished ovarian reserve, endometriosis, uterine fibroids, ovarian cysts, uterine polyps, fallopian tube blockage and thyroid dysfunction, also exhibited. This study suggests the significant role of interleukin gene polymorphisms in human reproductive success.

Keywords Female infertility, Polymorphisms, *Interleukin33*, *Interleukin22*, *Interleukin17*, *Interleukin13*, *Interleukin4*

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Introduction

Approximately 70% of the time infertility involves an unable female partner younger than 35 years to conceive after 12 months (or within 6 months at age older than 35 years) of regular unprotected intercourse [1, 2]. Despite recent scientific advances and the development of new assisted reproductive technologies, female infertility is relatively common which is entirely possible that is due to genetic defects including chromosomal abnormalities, submicroscopic deletions and duplications of chromosomes, and variations in DNA sequences of genes that govern a range of biological pathways critical to reproductive process [3]. At this time, there are only a handful of genes that have been shown to be strongly associated with female infertility. In other words, although numerous genes are recognized for their roles in the female reproductive system, they are often listed without a thorough evaluation of their clinical significance as contributors to reproductive disorders. However establishing a genetic factor can be a complex task that relies on a clear understanding of the gene function and structure [4, 5]. Therefore, a particular difficulty is the huge number of genes associated with infertility traits [6–8]. One approach is choosing candidate genes from a biological pathway known to be involved in infertility to search for single nucleotide polymorphism (SNP) in genes in those certain pathways. This reveals a putative marker which can be the direct cause of an infertile phenotype or may increase predisposition to this trait and even have the potential benefits for inclusion in clinical testing panels [9]. In females, up to 20% of the cases of infertility are immunological in nature [10], it is therefore important to consider the immunoregulatory molecules related signaling pathways, such as cytokines with a critical role in controlling reproductive processes [11, 12]. In other words, cytokines are mediating maternal immune tolerance alterations in cellular and humoral immune responses in the decidua which is vital for acceptance (fertility) and/or rejection of the fetus (infertility). Such an immune tolerance could break by SNPs in cytokine-encoding genes and result in infertility, as genetic variants in cytokine genes can affect the degree of cytokine production via affecting the recognition sites of the transcription factors and alteration transcriptional activity [13, 14]. Furthermore, the disturbance in production of cytokines has also been found in infertility associated reproductive disorders such as polycystic ovary syndrome (PCOS), endometriosis, tubal blockage and so forth [11]. Hence, there is an urgent need for an effective and predictive biomarker(s) useful for coexisting female infertility and associated diagnoses. To date, there are fewer studies that have targeted coexisting female infertility and associated comorbidities. As a single candidate gene is probably not correlated with all traits, here we evaluated a panel of eleven polymorphisms in five interleukin (IL) genes to gain marker(s) common for any trait.

Methods

This study was carried out in a case–control design with women experiencing fertility problems (cases for each Interleukins, n = 200) (A total of 1000 cases who provided a DNA sample were similar in overall) and women who had an ongoing pregnancy ending in a live birth and were screened for comorbidities that have been related to the cases (the ratio of controls to cases was fixed to equal), referred to Shiraz urban hospitals, Iran, from Jan 1' 2019 to June 10' 2022. Women who were diagnosed with infertility and younger than 45 years at the age range of 20–45 years were included. Demographic characteristics are shown in Table 1. We excluded females diagnosed with lower genital tract abnormalities, genitourinary infections, genital prolapse, whose partners had severe male infertility, whose partners had been diagnosed by specialists with sexual dysfunction, presence of psychiatric conditions and used drugs that affected sexual functions.

Genotyping for the polymorphisms in *Interleukin* 33, 22, 17, 13 and 4 were performed after a peripheral blood sample (5 ml) collected in EDTA anticoagulated tubes was obtained from all participants by amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) which refers to amplification of specific alleles (AS-PCR). The DNA samples were extracted from peripheral blood leukocytes by salting out method using the Genomic DNA Isolation Kit (GeNet Bio, Daejeon, Korea) [15–17], and were stored at − 20 °C until use. We used a pair of control primers specific for the normal DNA sequence (A pair of

Table 1 Demographic characteristics of participants

Variables	Cases N = (200)	Controls N = (200)	p-value
Age (years)	32.23 ± 5.99	32.65 ± 6.23	0.243
Range	20–45	20–45	–
Polycystic Ovary Syndrome (PCOS) n (%)	73 (36)	–	–
Premature ovarian failure (POF) n (%)	38 (19)	–	–
Oophorectomy n (%)	21 (10.5)	–	–
Diminished ovarian reserve n (%)	10 (5)	–	–
Endometriosis n (%)	10 (5)	–	–
Uterine Fibroids n (%)	12 (7)	–	–
Ovarian Cysts n (%)	10 (5)	–	–
Fallopian tube blockage n (%)	16 (7.5)	–	–
Thyroid dysfunction n (%)	10 (5)	–	–

control primers which could not amplify mutant DNA at a given locus was used to confirm that the genomic DNA is, in principle, amplifiable) and allele-specific primers designed using Oligo7 software (version 7.54, Molecular Biology Insights Inc., Cascade, CO, USA). (The designed primer sequences reported in Table 2).

The PCR reaction mixture included 1 µL template DNA, 11 µL of 2×Master Mix Red (Ampliqon), 1 µL of each primer (10 µM), and 5 µL DNase-free water was

Table 2 Designed primers for ARMS-PCR reactions and annealing temperature

SNP	Primer sequence	Annealing temperature (°C)
Interleukin33		
rs1048274 (G > A)	F: TGAGCCTATCGTTTGGAACTG RG: GTGCTTAGCATGTGTGGAATG RA: GTGCTTAGCATGTGTGGAATA	
	F-PCR-Control: CCTCTGCACAGTTTGGAC R-PCR-Control: TCTGTCCAGCAATCCAGG	57.8 °C
rs16924243 (T > C)	F: GCAACACTCAGAGCAGATC RT: AGATTTCTGGCCTTACCATGA RC: AGATTTCTGGCCTTACCATGG	
Interleukin22		
rs1397852121(C > T)	F: AGATGAAGAGAGGTCTCTTGT RC: AGGTCATCACCTTCAATATGC RT: AGGTCATCACCTTCAATATGT	
rs1295978671(C > T)	F: GCACACATCTGAATTCTGCT RC: AGGTCATCACCTTCAATATGC RT: AGGTCATCACCTTCAATATGT	58 °C
rs2227483(A > T)	F: ATATAATATAGTGGATGAGTAAG RA: GGAAATATTATGTTGAGAATTGTGA RT: GGAAATATTATGTTGAGAATTGTGT	
	F-PCR-Control: CCTCTGCACAGTTTGGAC R-PCR-Control: TCTGTCCAGCAATCCAGG	
Interleukin17		
rs2275913 (G > A)	F: TAGAAAGGTAAGCCACTGC RG: AACAAGTAAGAATGAAAAGAGGACATGGT RA: CCCCCAATGAGGTCATAGAAGAATC	
	F-PCR-Control: CCTCTGCACAGTTTGGAC R-PCR-Control: TCTGTCCAGCAATCCAGG	58 °C
rs763780 (T > C)	F: TAGAAAGGTAAGCCACTGC RC: GCACCTCTTACTGCACAC RT: GCACCTCTTACTGCACAT	
Interleukin13		
1512 (A > C)	F: CCGCTACTTGGCCGTGTGACCGC RA: GGGGTCACACGGGCCAGTAGCGG RC: CAACCGCCGCGCCAGCGCCTTCTC	
	F-PCR-Control: CCTCTGCACAGTTTGGAC R-PCR-Control: TCTGTCCAGCAATCCAGG	60 °C
2044 (G > A)	F: TCGAAGTTTCAGTTGAACT RG: TCGAAGTTTCAGTTGAACC RA: TCGAAGTTTCAGTTGAACT	
Interleukin4		
rs2243250(C > T)	F: TGCCCATGAGCCCTTACTG RC: CTACCCCAGCACTGGAGG RT: CTACCCCAGCACTGGAGA	
	F-PCR-Control: CCTCTGCACAGTTTGGAC R-PCR-Control: TCTGTCCAGCAATCCAGG	56 °C
rs2070874(C > T)	F: TGCCCATGAGCCCTTACTG RC: GCCCCAAGTGACTGACAATC RT: TCACCTTCTGCTCTGTGTGAGG	

prepared in a final volume of 22 µL. The protocol used for Thermal cycling conditions were as follows: initial denaturation at 94 °C for 5 min and followed by 32 cycles of denaturation at 94 °C for 40 s, annealing at 56–60 °C for 40 s, and extension at 72 °C for 40 s. Final extension at 72 °C for 5 min was done to complete the PCR.

Statistical analysis

Demographic data were calculated and compared between the two groups. The chi-square (χ^2) test was used for the calculation of genotypic and allelic frequency. Logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between female infertility and the presence of each polymorphism. Moreover, a Bonferroni correction for multiple testing was performed to evaluate statistical significance at an adjusted p-value threshold ($p < 0.05$). Strength of association was estimated by haplotype analysis (calculated by the Haploview v4.2 software) and calculating the level of genetic diversity in population (F-statistics; the ratio between observed and expected value of heterozygous genotypes).

Results

Hardy–Weinberg Equilibrium (HWE) analysis showed that the p value of the SNP sites (*Interleukin33* p-value rs1048274 = 0.871 and p-value rs16924243 = 0.946, *Interleukin 22* p-value rs1397852121 = 0.943, p-value rs1295978671 = 0.985 and p-value rs2227483 = 0.2, *Interleukin17A* p-value rs2275913 = 0.1 and *Interleukin17F* p-value rs763780 = 0.2, *Interleukin13* p-value 1512 = 0.1 and p-value 2044 = 0.1 and *Interleukin4* p-value rs2243250 = 0.4 and p-value rs2070874 = 0.1) were all greater than 0.05, suggesting that all were consistent with HWE.

The allele frequencies of rs1048274 ($p = 0.002$), rs16924243 ($p < 0.0001$), rs1397852121 ($p < 0.0001$), rs2227483 ($p = 0.000$), rs2275913 ($p = 0.003$), rs763780 ($p = 0.000$), 1512 ($p = 0.000$), 2044 ($p = 0.003$), rs2243250 ($p = 0.001$) and rs2070874 ($p = 0.009$) were significantly different between the cases and controls. SNP rs1295978671 was excluded from further analysis since no significant differences have observed in the rs1295978671 allele frequency between the two groups ($p = 0.879$). This may be due to race, ethnicity and/or population size and not because of poor DNA quality. Therefore, this SNP can be a good candidate for reprocessing between different ethnic groups of a larger population size.

The distribution of all SNPs risk genotypes except for rs1295978671, were higher and significantly differ in the cases group than in the control group. Moreover, after the regression analysis, the differences were still statistically significant.

On the whole, distributions of rs1048274 A allele carriers (A-) and AA and GA genotypes carriers, distributions of rs16924243 C allele carriers (C-) and CC and TC genotypes carriers, distributions of rs1397852121 T allele carriers (T-) and TT and TC carriers, distributions of rs2227483 T allele (T-) and TT and AT carriers, distributions of rs2275913 A allele and AA and GA carriers, distributions of rs763780 C allele (C-) and CC and TC carriers, distributions of 1512 C allele carriers (C-) and CC and AC carriers, distributions of 2044 A allele (A-) and AA and AG carriers, distributions of rs2243250 T allele (T-) and TT and CT carriers and distributions of rs2070874 T allele (T-) and TT and CT carriers, were all statistically significant between the cases and the control groups. Allele and genotype distribution of the all SNPs in infertile patients and controls are summarized in Table 3.

Genotypes associated with infertility comorbidities/underlying medical conditions including, Polycystic Ovary Syndrome, Premature ovarian failure (POF), Oophorectomy, Diminished ovarian reserve, Endometriosis, Uterine Fibroids, Ovarian Cysts, Uterine polyps, Fallopian tube blockage and Thyroid dysfunction were identified. Both *Interleukin33* SNPs-risk genotypes were significantly associated with all infertility comorbidities/underlying medical conditions ($p < 0.001$). Although, no relationship was observed between *Interleukin22* rs1397852121 and rs1295978671SNPs risk genotypes and infertility comorbidities, risk genotypes of rs2227483 were correlated highly with the oophorectomy ($p = 0.00$), uterine fibroids ($p = 0.00$) and thyroid dysfunction ($p = 0.00$). Moreover, no relationship was observed between *Interleukin13*-1512 (A > C) polymorphic site risk genotypes and infertility comorbidities/underlying medical conditions.

PCOS ($p = 0.000$) and fallopian tube blockage ($p = 0.01$) were positively correlated with *Interleukin17A* and *Interleukin13*-2044 (PCOS $p < 0.0001$ and fallopian tube blockage $p = 0.001$) risk genotypes. Fallopian tube blockage ($p = 0.006$) and thyroid dysfunction ($p = 0.009$) were positively correlated with *Interleukin17F*. About the *interleukin4*, risk genotypes of one SNP (rs2243250) correlated just with diminished ovarian reserve ($p = 0.03$) and another one (rs2070874) was correlated with PCOS ($p < 0.001$) and oophorectomy ($p < 0.001$) (Table 4).

In this case–control association study, population stratification issue detected by F-statistics (FST), when differ allele frequencies were observed between cases and controls. Summarized results of FST are presented in Table 5. In elaboration, the higher level of genetic diversity among loci we found for the *Interleukin22* (0.3921)

Table 3 Genotype and allele frequency distribution of polymorphisms in infertile patients and controls

Genotypes and alleles	Patient group n (%)	Control group n (%)	OR (95% CI)	Uncorrectedp	Corrected p
Interleukin33-rs1048274 (G > A)					
GG	63 (31.5)	122 (61)	0.6 (0.3–1)	0.087	0.061
AA	34 (17)	22 (11)	1.2 (0.6–2.2)	0.005	0.009
GA	103 (51.5)	55 (27.5)	3.4 (2.2–5.2)	< 0.001	< 0.005
G	229 (57.3)	299 (75.1)	0.6 (0.5–0.7)	0.000	0.004
A	171 (42.8)	99 (24.9)	1.4 (1.2–1.6)	0.001	0.002
Interleukin33-rs16924243 (T > C)					
CC	134 (67)	44 (22)	1.6 (1.06–2.5)	0.002	0.003
TT	22 (11)	25 (12.5)	0.40 (0.22–0.70)	0.001	0.002
TC	152 (76)	20 (10)	2.5 (1.1–5.4)	0.002	0.005
C	222 (55.5)	192 (48.7)	1.02 (0.46–2.26)	0.009	< 0.0001
T	178 (44.5)	202 (31.3)	0.9 (0.45–1.7)	0.077	0.093
Interleukin22-rs1397852121 (C > T)					
CC	95 (47.5)	121 (60.5)	0.5 (0.3–0.8)	0.009	< 0.0001
TT	18 (9)	11 (5.5)	1.6 (0.7–3.6)	0.001	0.003
TC	87 (43.5)	68 (34)	1.4 (0.99–2.2)	0.005	0.007
C	277 (69.5)	310 (77.5)	0.6 (0.40–0.93)	0.021	0.001
T	123 (30.8)	90 (22.5)	1.8 (0.02–1.06)	0.006	< 0.0001
Interleukin22-rs1295978671(C > T)					
CC	95 (47.5)	91 (45.5)	–	0.819	0.867
TT	19 (9.5)	17 (8.5)	–		
TC	86 (43)	92 (46)	–		
C	276 (69)	274 (68.5)	–	0.879	0.090
T	124 (31)	126 (31.5)	–		
Interleukin22-rs2227483(A > T)					
AA	80 (53)	120 (60)	1.9 (1–2.3)	0.046	0.050
TT	17 (8.5)	9 (4.9)	1.1 (0.2–1.4)	< 0.001	< 0.002
AT	103 (38.5)	71 (35.1)	1.7 (0.9–1.9)	0.001	0.001
A	97 (254.6)	303 (75.7)	0.8 (1.4–2.2)	0.000	0.001
T	103 (74.4)	97 (24.3)	0.5 (1.3–2.6)	0.000	0.000
Interleukin17A-rs2275913 (G > A)					
GG	80 (40)	121 (60.5)	0.4 (0.2–0.6)	0.001	0.002
AA	19 (9.5)	5 (2.5)	5.7 (2.06–16.01)	< 0.001	< 0.004
GA	101 (51)	74 (37)	2.06 (1.3–3.1)	0.001	0.001
G	261 (45.2)	316 (54.8)	0.3 (0.2–0.6)	0.000	0.0001
A	139 (62.3)	84 (37.7)	2 (1.4–2.7)	0.000	0.003
Interleukin17F-rs763780 (T > C)					
CC	37 (18.5)	13 (6.5)	8.1 (3.9–16.5)	< 0.001	0.000
TT	47 (23.5)	134 (67)	1.2 (0.5–3)	0.063	0.056
TC	116 (58)	53 (26.5)	6.2 (3.9–9.9)	< 0.001	0.000
C	190 (70.6)	79 (29.4)	3.6 (2.6–5.03)	< 0.001	0.000
T	210 (39.5)	321 (60.5)	0.3 (0.2–0.5)	0.000	0.003
Interleukin13-1512 (A > C)					
AA	74 (42.5)	100 (57.5)	0.8 (0.6–1.1)	0.043	0.035
CC	19 (67.9)	9 (32.1)	1.3 (0.15–0.8)	0.01	0.02
AC	57 (58.2)	41 (41.8)	1.5 (0.3–0.8)	0.01	0.01
C	95 (61.7)	59 (38.3)	1 (0.3–0.7)	0.001	0.000
A	205 (46)	241 (54)	0.3 (0.2–05)	0.000	0.004

Table 3 (continued)

Genotypes and alleles	Patient group n (%)	Control group n (%)	OR (95% CI)	Uncorrectedp	Corrected p
Interleukin13-2044 (G > A)					
GG	6 (35.3)	11 (64.7)	0.3 (0.1–0.9)	0.037	0.043
AA	93 (61.6)	58 (38.4)	1.8 (1.05–1.09)	0.006	< 0.0001
AG	81 (61.4)	51 (38.6)	1.3 (0.2–0.6)	0.000	0.001
A	237 (54.6)	197 (45.4)	1.2 (0.4–1.4)	0.000	0.003
G	63 (38)	103 (62)	0.5 (0.3–0.7)	0.000	0.005
Interleukin4-rs2243250(C > T)					
CC	45 (22.5)	102 (51)	3.2 (1.9–4.8)	0.000	0.003
TT	37 (18.5)	25 (12.5)	3.3 (1.8–6.2)	0.000	0.004
CT	118 (59)	73 (36.5)	3.6 (2.3–5.7)	0.000	0.001
C	208 (42.9)	277 (57.1)	2 (1.5–2.7)	0.000	0.000
T	192 (61)	123 (39)	1.8 (1.1–1.9)	0.000	0.001
Interleukin4-rs2070874(C > T)					
CC	83 (41.5)	110 (55)	1 (1.1–1.4)	0.000	0.006
TT	17 (8.5)	8 (4)	0.3 (0.1–0.8)	0.01	0.03
CT	100 (50)	82 (41)	0.6 (0.4–0.9)	0.02	0.03
C	266 (46.8)	302 (53.2)	1.2 (1.6–2.9)	0.000	0.005
T	134 (57.8)	98 (42.2)	0.6 (04–0.8)	0.005	0.009

Corrected p-values were calculated by using Bonferroni's correction

and the lower one—for the *Interleukin17* (0.2124). The mean FST value over all loci was 0.2640 which showed a 26% overall genetic diversity.

In a further step, haplotype analysis showed that IL 4 CTTT and CTTC as well as IL 13 ACGG haplotypes were associated with a roughly 2 and threefold increased risk of female infertility, respectively. The haplotype analysis did not yield additional information from other haplotypes of rest interleukins. In other words, they had a protective role in the development of female infertility (Table 6).

Discussion

Understanding the genetic factors contributing to complex biology of female infertility and diseases influencing female fertility are of broad general interest because female infertility continues to increase in prevalence annually. To date, much less is known about the genetic contribution to this complex fertility trait and associated comorbidities [18, 19]. Our research comprehensively shows genetic contribution to female infertility with identifying multi polymorphic loci associated with infertility and associated diagnoses.

Our findings show that the genotype (AA/GA) and the A risk allele of *Interleukin33* —rs1048274 were significantly associated with female infertility, especially in women with diagnoses such as polycystic ovary syndrome, premature ovarian failure, oophorectomy, diminished ovarian reserve, endometriosis, uterine fibroids, ovarian cysts, uterine polyps, fallopian tube blockage and thyroid dysfunction. Similarly, the results suggested that the genotype (CC/TC) and the C risk allele of *Interleukin33*—rs16924243 are significantly associated with female infertility, once more especially in women with diagnoses such as polycystic ovary syndrome, premature ovarian failure, oophorectomy, diminished ovarian reserve, endometriosis, uterine fibroids, ovarian cysts, uterine polyps, fallopian tube blockage and thyroid dysfunction. Recent investigations have revealed a noteworthy yet insufficiently understood link between the *IL-33*, and pregnancy-related disorders in women. Genome-wide association studies have pinpointed SNPs in the *IL33* that are associated with recurrent or idiopathic pregnancy loss [20–22]. Furthermore, other research teams have found abnormal levels of *IL-33* or its receptor ST2 in the serum or uterine tissue of women suffering from conditions such as preeclampsia [23–25], placenta previa accrete [26], gestational diabetes mellitus [27], and recurrent or impending pregnancy loss [28, 29]. It is significant to note that both low and high expressions of *IL-33* have been reported across various pregnancy disorders, indicating that the level and timing of *IL-33* signaling may critically influence its specific biological effects on pregnancy outcomes [30, 31]. In this study *Interleukin22* -rs1295978671 was excluded from further analysis since no significant differences have observed in

Table 4 Association of polymorphisms and the infertility comorbidities/underlying medical conditions

	Interleukin33 **rs1048274 (G > A)**			***Interleukin22*** **rs1397852121 (C > T)**			***Interleukin17A*** **rs2275913 (G > A)**			***Interleukin13*** **1512 (A > C)**		
	GG	**GA**	**AA**	**CC**	**TC**	**TT**	**GG**	**GA**	**AA**	**AA**	**AC**	**CC**
Polycystic Ovary Syndrome (PCOS)	5 (11.9)	27 (8.2)	5 (18.5)	5 (17.2)	15 (9.7)	18 (4.8)	4 (21.1)	26 (25.7)	8 (10)	10 (52.6)	36 (63.2)	48 (64.9)
Uncorrected p	0.00			0.472			0.02			0.6		
Corrected p	< 0.001			0.279			0.000			0.4		
Premature ovarian failure (POF)	0 (0)	2 (0.6)	2 (4.8)	0 (0)	3 (1.9)	2 (0.9)	0(0)	1(1)	4(5)	19(100)	57(100)	71(95.9)
Uncorrected p	0.00			0.472			0.1			0.2		
Corrected p	< 0.001			0.279			0.1			0.203		
Oophorectomy	0 (0)	2 (0.6)	0 (0)	0 (0)	1 (0.6)	1 (0.5)	0 (0)	2 (2)	0 (0)	19 (100)	56 (98.2)	73 (98.3)
Uncorrected p	0.00			0.472			0.3			0.8		
Corrected p	< 0.001			0.279			0.2			0.9		
Diminished ovarian reserve	0 (0)	9 (2.7)	1 (2.4)	0 (0)	6 (3.9)	4 (1.9)	1 (5.3)	6 (5.9)	3 (3.8)	18 (94.7)	55 (96.5)	71 (95.9)
Uncorrected p	0.00			0.472			0.7			0.9		
Corrected p	< 0.001			0.279			0.1			0.5		
Endometriosis	1 (2.4)	6 (1.8)	3 (11.1)	0 (0)	5 (3.2)	5 (2.3)	0 (0)	5 (5)	5 (6.3)	19 (100)	54 (94.7)	68 (91.9)
Uncorrected p	0.00			0.472			0.5			0.3		
Corrected p	< 0.001			0.279			0.3			0.1		
Uterine Fibroids	0 (0)	2 (0.6)	0.0	0 (0)	1 (0.6)	1 (0.5)	1 (5.3)	0 (0)	1 (1.3)	19 (100)	57 (100)	73 (98.6)
Uncorrected p	0.00			0.472			0.1			0.5		
Corrected p	< 0.001			0.279			0.4			0.8		
Ovarian Cysts	1 (2.4)	8 (2.4)	1 (3.7)	0 (0)	3 (1.9)	7 (3.3)	1 (5.3)	4 (4)	5 (6.3)	18 (94.7)	55 (96.5)	71 (95.9)
Uncorrected p	0.00			0.472			0.7			0.9		
Corrected p	< 0.001			0.279			0.3			0.8		
Uterine polyps	1 (2.4)	2 (0.6)	1 (1.3)	0 (0)	2 (1.3)	2 (0.9)	1 (5.3)	1 (1)	2 (2.5)	19 (100)	55 (96.5)	73 (98.6)
Uncorrected p	0.00			0.472			0.4			0.5		
Corrected p	< 0.001			0.279			0.7			0.1		
Fallopian tube blockage	0 (0)	8 (2.4)	4 (14.8)	0 (0)	5 (3.2)	5 (2.3)	0 (0)	3 (3)	9 (11.3)	17 (89.5)	55 (96.5)	68 (91.9)
Uncorrected p	0.00			0.472			0.03			0.4		
Corrected p	< 0.001			0.279			0.01			0.1		
Thyroid dysfunction	0 (0)	1 (0.3)	0 (0)	0 (0)	1 (0.6)	0 (0)	0 (0)	0 (0)	1 (1.3)	19 (100)	55 (96.5)	72 (97.3)
Uncorrected p	0.00			0.472			0.4			0.7		
Corrected p	< 0.001			0.279			0.1			0.6		

Table 4 (continued)

	Interleukin33 **rs16924243 (T > C)**			***Interleukin22*** **rs1295978671 (C > T)**			***Interleukin17F*** **rs763780 (T > C)**			***Interleukin13*** **2044 (G > A)**		
	TT	**TC**	**CC**	**CC**	**TC**	**TT**	**TT**	**TC**	**CC**	**GG**	**GA**	**AA**
Polycystic Ovary Syndrome (PCOS)	7 (26.9)	52 (16.1)	14 (27.5)	10 (27.8)	32 (18)	31 (16.7)	14 (37.8)	43 (37.1)	16 (34)	3 (27.3)	8 (9.9)	18 (31)
Uncorrected p	0.00			0.324			0.9			0.006		
Corrected p	< 0.001			0.471			0.5			< 0.0001		
Premature ovarian failure (POF)	0 (0)	4 (1.2)	0 (0)	2 (5.6)	0 (0)	3 (1.6)	0 (0)	4 (3.4)	1 (2.1)	0 (0)	2 (2.5)	1 (1.7)
Uncorrected p	0.00			0.324			0.4			0.8		
Corrected p	< 0.001			0.471			0.4			0.1		
Oophorectomy	0 (0)	1 (0.3)	1 (2)	1 (2.8)	0 (0)	1 (0.5)	1 (2.7)	0 (0)	1 (2.1)	0 (0)	2 (2.5)	0 (0)
Uncorrected p	0.00			0.324			0.2			0.4		
Corrected p	< 0.001			0.471			0.5			0.8		
Diminished ovarian reserve	2 (7.7)	16 (5)	3 (5.9)	1 (2.8)	4 (2.2)	5 (2.7)	2 (4.3)	4 (3.4)	4 (10.8)	0 (0)	2 (2.5)	0 (0)
Uncorrected p	0.00			0.324			0.1			0.4		
Corrected p	< 0.001			0.471			0.3			0.9		
Endometriosis	2 (3.9)	5 (1.6)	3 (11.5)	1 (2.8)	5 (2.8)	4 (2.2)	1 (2.7)	7 (6)	2 (4.3)	0 (0)	5 (6.2)	4 (6.9)
Uncorrected p	0.00			0.324			0.6			0.6		
Corrected p	< 0.001			0.471			0.2			0.3		
Uterine Fibroids	0 (0)	2 (0.6)	0 (0)	1 (0.5)	0 (0)	1 (2.8)	0 (0)	1 (0.9)	1 (2.1)	0 (0)	1 (1.2)	0 (0)
Uncorrected p	0.00			0.324			0.6			0.6		
Corrected p	< 0.001			0.471			0.4			0.7		
Ovarian Cysts	1 (2)	7 (2.2)	2 (7.7)	0 (0)	5 (2.8)	5 (2.7)	2 (5.4)	6 (5.2)	2 (4.3)	0 (0)	5 (6.2)	1 (1.7)
Uncorrected p	0.00			0.324			0.9			0.3		
Corrected p	< 0.001			0.471								
Uterine polyps	0 (0)	3 (0.9)	1 (3.8)	1 (2.8)	2 (1.1)	1 (0.5)	0 (0)	3 (2.6)	1 (2.7)	0 (0)	1 (1.2)	2 (3.4)
Uncorrected p	0.00			0.324			0.5			0.5		
Corrected p	< 0.001			0.471			0.1			0.6		
Fallopian tube blockage	0 (0)	11 (3.4)	1 (2)	0 (0)	6 (3.4)	6 (3.2)	0 (0)	11 (9.5)	1 (2.7)	0 (0)	9 (11.1)	1 (1.7)
Uncorrected p	0.00			0.324			0.04			0.06		
Corrected p	< 0.001			0.471			0.006			0.001		
Thyroid dysfunction	0 (0)	1 (0.3)	0 (0)	0 (0)	1 (0.6)	0 (0)	2 (5.4)	1 (0.9)	4 (8.5)	0 (0)	2 (2.5)	2 (3.4)
Uncorrected p	0.00			0.324			0.04			0.7		
Corrected p	< 0.001			0.471			0.009			0.1		

Table 4 (continued)

	***Interleukin*22 rs2227483 (A > T)**			***Interleukin*4 rs2243250 (C > T)**			***Interleukin*4 rs2070874 (C > T)**		
	AA	**AT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Polycystic Ovary Syndrome (PCOS)	2 (5.6)	14 (7.9)	22 (11.8)	4 (10.8)	22 (18.6)	12 (26.7)	2 (11.8)	26 (26)	10 (12)
Uncorrected p	0.9			0.1			0.04		
Corrected p	0.1			0.2			< 0.001		
Premature ovarian failure (POF)	0 (0)	2 (5.6)	3 (1.6)	1 (2.7)	2 (1.7)	2 (4.4)	0 (0)	4 (4)	1 (1.2)
Uncorrected p	0.1			0.6			0.3		
Corrected p	0.7			0.8			0.1		
Oophorectomy	0 (0)	1 (2.8)	1 (0.5)	0 (0)	2 (1.7)	0 (0)	0 (0)	1 (5.9)	1 (1)
Uncorrected p	0.00			0.4			0.03		
Corrected p	< 0.001			0.5			< 0.001		
Diminished ovarian reserve	1 (2.8)	5 (2.7)	4 (2.2)	0 (0)	6 (5.1)	4 (10.8)	2 (11.8)	4 (4.8)	4 (4)
Uncorrected p	0.2			0.03			0.3		
Corrected p	0.1			0.005			0.6		
Endometriosis	1 (2.8)	5 (2.8)	4 (2.2)	1 (1.2)	4 (3.4)	3 (8.1)	1 (5.9)	6 (7.2)	3 (3)
Uncorrected p	0.2			0.4			0.5		
Corrected p	0.1			0.6			0.1		
Uterine Fibroids	0 (0)	1 (2.8)	1 (0.5)	0 (0)	1 (8)	1 (2.2)	0 (0)	2 (2)	1 (3)
Uncorrected p	0.00			0.5			0.3		
Corrected p	< 0.001			0.1			0.1		
Ovarian Cysts	0 (0)	5 (2.8)	5 (2.7)	1 (2.7)	7 (5.9)	2 (4.4)	0 (0)	6 (7.2)	4 (4)
Uncorrected p	0.2			0.7			0.3		
Corrected p	0.25			0.7			0.6		
Uterine polyps	1 (0.5)	2 (1.1)	1 (2.8)	1 (2.7)	2 (1.7)	1 (2.2)	0 (0)	2 (2.4)	2 (2)
Uncorrected p	0.1			0.9			0.8		
Corrected p	0.1			0.4			0.9		
Fallopian tube blockage	0 (0)	6 (3.4)	6 (3.2)	2 (4.4)	5 (13.5)	5 (4.2)	2 (11.8)	5 (6)	5 (5)
Uncorrected p	0.3			0.1			0.5		
Corrected p	0.5			0.1			0.6		
Thyroid dysfunction	0 (0)	1 (0.6)	0 (0)	0 (0)	1 (2.7)	0 (0)	0 (0)	1 (1)	0 (0)
Uncorrected p	0.00			0.1			0.6		
Corrected p	0.003			0.3			0.4		

Corrected p-values were calculated by using Bonferroni's correction

Table 5 F-statistics for all polymorphic loci

Locus	FST
Interleukin33	0.2744
Interleukin22	0.3921
Interleukin17	0.2124
Interleukin13	0.2176
Interleukin4	0.2238
Mean	0.2640

FST: F-statistics

the rs1295978671 allele and genotype frequency between the two groups (p = 0.879), However, the genotype (TT/TC) and the T risk allele of *Interleukin22*-rs1397852121 and genotype (TT/AT) and the T risk allele of rs2227483 were found significantly associated with female infertility, nonetheless the association between rs1397852121 and female infertility comorbidities/ underlying medical condition was not established. We discovered that infertile rs2227483 carrier-women may be at higher risk to develop oophorectomy, uterine fibroids and thyroid dysfunction comorbidities. *Interleukin-22* has become a prominent subject of research concerning various systemic inflammatory diseases; however, its implications for reproductive health are not yet fully elucidated. In a study published in 2013, Wang et al. found that fetal trophoblasts express the IL-22 receptor (IL-22R1) and exhibit increased proliferation in the presence of *IL-22*. Additionally, their results indicated that specimens from spontaneous abortions had significantly lower levels of IL-22R1 protein expression compared to those from normal pregnancies. At present, there is a notable absence of research quantifying the gene and protein expression of *IL-22* in normal pregnancies or in cases of unexplained recurrent pregnancy loss within the decidua. However, observations suggest that the normal function of *IL-22* may be crucial for maintaining decidual homeostasis and regulating the inflammation commonly observed in early pregnancy [32, 33]. Further findings show that the genotype (AA/GA) and the risk A allele of *Interleukin17A*-rs2275913 were significantly associated with female infertility, especially in women with PCOS and fallopian tube blockage diagnosis. Besides, the genotype (CC/TC) and the risk C allele of *Interleukin17F*-rs763780 were significantly associated with female infertility, especially in women with fallopian tube blockage and thyroid dysfunction diagnoses. Recent research involving eutherian mammals has revealed that endometrial IL-17 at the fetal–maternal interface is suppressed due to the ancestral development of decidual stromal cells and placentation. This suppression is believed to have played a crucial role in the evolution of embryo implantation and the maintenance of a viable fetal–maternal interface. In line with these observations, higher concentrations of IL-17 have been reported in the serum and plasma of women suffering from recurrent pregnancy loss and pre-eclampsia [34–36]. Evidence suggests that women suffering from endometriosis exhibit elevated serum concentrations of IL-17 and various other inflammatory cytokines, including IL-1A and IL-6. Furthermore, it is commonly recognized that endometriosis is the primary diagnosis found in women with unexplained infertility when assessed through laparoscopy [37, 38]. Moreover, although the genotype (CC/AC) and the risk

Table 6 Haplotype analysis of Interleukin 33, 22, 17, 13 and 4 genes between infertile patients and healthy controls

IL 33 haplotypes	Control/Patient freq	p	OR (95% CI)	IL 22 haplotypes	Control/Patient freq	p	OR (95% CI)
GATC	–	–	Ref.	CATT	–	–	Ref.
GACC	11/ 7	0.02	0.6 (0.2–1.6)	CTTT	87/68	0.05	1.4 (0.9–2.2)
AATC	19/17	0.08	0.9 (0.4–1.7)	TTAT	18/11	0.01	1.6 (0.7–3.6)
IL 17 haplotypes	**Control/Patient freq**	**p**	**OR (95% CI)**	**IL 13 haplotypes**	**Control/Patient freq**	**p**	**OR (95% CI)**
GATC	–	–	Ref.	AACG	–	–	Ref.
GACC	20/12	0.1	1.7 (0.8–3.7)	ACGG	9/4	0.00	3 (1.9–4.7)
AATC	17/6	0.1	3 (1.8–7.9)	GACC	13/13	0.1	1 (0.4–2)
IL 4 haplotypes	**Control /Patient freq**	**p**	**OR (95% CI)**				
CCTT	–	–	Ref.				
CTTT	13/6	0.04	2.2 (0.8–6.1)				
CTTC	26/13	0.02	2.1 (1–4.3)				

Haplotypes with a frequency of < 1% were excluded in the analysis

C allele of *Interleukin13*-1512 were significantly associated with female infertility, the association between *Interleukin13*-1512 and female infertility comorbidities/underlying medical condition was not established. Our findings show that the genotype (AA/AG) and the risk A allele of *Interleukin13*-2044 were significantly associated with female infertility, especially in women with PCOS and fallopian tube blockage diagnosis. There is evidence to suggest that, in normal pregnancies, there is a notable production of IL-13, first by the placenta and later by the fetus. Therefore, normal function of *IL-13* is a requirement for healthy pregnancy [39]. Finally, about the *interleukin4*, risk T allele of rs2243250 and risk T allele of rs2070874 were significantly associated with female infertility as well as risk genotypes of rs2243250 SNP correlated just with diminished ovarian reserve and another one (rs2070874) was correlated with PCOS and oophorectomy. The relationship between the absence of fetal tolerance and T helper 1 cells is well established. IL-4 facilitates the conversion of naïve T helper cells, upon antigen stimulation, into T helper 2 effector cells and further promotes T helper 2 responses by binding to its receptor, IL-4Rα, thereby activating the *STAT6* signaling pathway. *STAT6*, by inducing the zinc-finger transcription factor GATA3, may effectively suppress the development of T helper 1 cells through the downregulation of *IFNγ* expression [40].

Taken together, SNPs are the most common type of genetic variations in humans. Understanding the functions of SNPs can greatly help us perceive the complex genetic basis of female infertility, because risk genotypes of SNPs by influencing promoter activity (gene expression), messenger RNA (mRNA) conformation (stability), and translational efficiency may be responsible for genetic susceptibility of women to infertility or may also play a direct role with or without other factors in the phenotypic expression of infertility [41].

Some literature also show immunological abnormalities have been implicated in female reproductive failure, for example, abnormal IL-1β by indicating impairment of folliculogenesis might contribute to the infertility of women [42]. A positive correlation has been reported between the low level of IL-18 and infertility in women [43, 44]. A majority of the findings have indicated that IL-12 is associated with infertility [45–47]. In addition, higher level of IL-23 is known to participate in infertility [48]. It is worth noting that it wasn't possible to do much more comparison of findings due to the limited number of literatures. In this study, we identified a number of SNPs in interleukin genes, which may be suitable for genetic studies of female infertility. Prior knowledge to the presence of these polymorphisms could be used to stratify risk of infertility on an individual basis. Therefore, in routine clinical practice, screening for these SNPs could form part of the clinical work-up on admission.

Limitations

To overcome the present study limitations and enhance the quality of our research, authors suggest investigating the functional consequences of the identified SNPs, exploring gene-environment interactions, or conducting studies in a more diverse population. Addressing these limitations can help structure the study better.

Conclusion

In particular, the search to find a panel of genetic biomarkers which can predict female infertility could be a significant strategic step for achieving such a goal, since a single gene is probably not sufficient to constitute an effective, predictive biomarker useful for all patients. Additionally, testing multiple SNPs simultaneously with a multilocus model can capture the underlying architecture of complex quantitative traits better. Taken together, in a multilocus model, potentially associated markers are considered and panel study is led to many clinically significant findings. Studying how gene–gene interaction and genetic predispositions come together with environmental factors (gene-environment interaction) which would improve disease prediction and facilitate prevention and broadly speaking has great potential for advancing the risk-prediction models, is suggested. To ensure the credibility of the observed genotype–phenotype association, it is recommended to replicate these results in diverse populations, thereby confirming that the association is not a chance occurrence or an artifact of uncontrolled biases.

Abbreviations

SNP	Single nucleotide polymorphism
IL	Interleukin
PCOS	Polycystic ovary syndrome
ARMS-PCR	Amplification refractory mutation system–polymerase chain reaction
AS-PCR	Amplification of specific alleles
HWE	Hardy–Weinberg Equilibrium
POF	Premature ovarian failure
OR	Odds ratio
CIs	Confidence intervals
FST	F-statistics

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Author contributions

N.S. designed the study and critically reviewed the manuscript. K.V.K, K.V.S, B.N, D.M, S.M, K.M and N.R. performed formal analysis. N.S. administrated project. K.V.K wrote the manuscript. The final manuscript has been approved by all authors.

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Data availability
The datasets generated and/or analyzed during the current study are available in the [dbSNP] repository [http://www.ncbi.nlm.nih.gov/SNP]" and SNPs can be searched for using the dbSNP ID (rs1048274, rs16924243, rs1397852121, rs1295978671, rs2227483, rs2275913, rs763780, rs1881457, rs20541, rs2243250 and rs2070874).

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the approved institutional guidelines of the Islamic Azad University-Kazerun Branch in Iran. All participants signed the informed consent form and their data were anonymized prior to analysis. The Ethics Committee of the Islamic Azad University-Kazerun Branch in Iran approved this study and all experimental protocols (IR.IAU.KAU.REC.1398.065, IR.IAU.KAU.REC.1398.072, IR.IAU.KAU.REC.1398.010 and IR.IAU.KAU.REC.1398.031).

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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