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Correlation of TP53 (rs1625895), TP73 (rs3765730), MMP9 (rs17576), and MTHFR (rs868014) polymorphisms with low ovarian reserve

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ABSTRACT

Objective:: To investigate the influence of the Single Nucleotide Polymorphisms (SNPs) TP53 rs1625895, TP73 rs3765730, MMP9 rs17576, and MTHFR rs868014 on ovarian reserve (OR) in infertile patients.
Study design:: A prospective cross-sectional study was carried out in 145 infertile women. The patients were divided into two groups according to ovarian reserve, characterized by association between AMH levels and AFC:

- Group I: 86 patients defined as having low OR (LOR) by AMH < 1 ng/mL + AFC ≤ 9.
- Group II: 59 patients defined as having normal OR (NOR) by AMH > 2 ng/mL + AFC ≥ 15.

After patient distribution, both groups were compared (LOR X NOR) regarding the genotypes of the SNPs TP53 T/C rs1625895, TP73 G/A rs3765730, MMP9 Gln/Arg rs17576, and MTHFR A/G rs868014.

Result(s):: The frequency of the TP53-T/T genotype was greater in the LOR and the TP53-C/C genotype was more frequent in patients with NOR. This association was confirmed by the frequency of alleles, where the presence of the T allele was significantly higher in patients who exhibited LOR ($P = 0.0003$). The frequency of the TP73-G/G genotype and of the G allele was higher in the LOR group ($P = 0.01$). Considering the MMP9 gene, the frequency of the Gln/Gln genotype was higher in the LOR group. However, the Gln/Arg genotype and the Arg allele prevailed in the NOR group ($P = 0.006$). The frequency of the MTHFR-A/A genotype was higher in the LOR group, whereas that of the MTHFR-GG genotype was higher in the NOR group. The presence of allele A was significantly higher in the LOR group ($P = 0.002$). The regression analysis shows that patients who present the TP53-T/T, TP73-G/G, MMP9-Gln/Gln, and MTHFR-A/A genotypes are 3.6X, 3.1X, 3.2X, and 3.7X more likely of having LOR, respectively. In addition, the association of the TP53/TT + TP73/GG genotypes increased the chance of women being included in the LOR group in 5.7-fold.

Conclusion(s):: The genotypes TP53-T/T, TP73-G/G, MMP9-Gln/Gln, and MTHFR-A/A increase the chance of women to exhibit LOR. These polymorphisms could be useful as genetic markers of low ovarian reserve in infertile patients.

Introduction

The ovarian response (ORe) to gonadotrophin stimulation is variable [1]. It is known that the use of age alone as a predictive factor for ovarian reserve (OR), as well as ORe for IVF/ICSI cycles, is not sufficient [2]. In the vast majority, the number and quality of oocytes decrease with age, but there are women of the same age group who have totally different reproductive potentials. In addition to age, several factors have been used to predict OR and ORe [3,4], among which we highlight the levels of anti-Mullerian hormone (AMH) and antral follicle count (AFC) [5–12].

AMH, is only produced by the granulosa cells surrounding the pre-antral and small antral follicles. Additionally, AMH is independent of follicle-stimulating hormone (FSH), whereby its levels are a direct measure of the follicular pool production. Several studies

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have shown that AMH levels decrease with throughout reproductive life and have a good correlation with OR and ORe [13–16]. The AFC consists of the sum of follicles < 10 mm in both ovaries on a transvaginal ultrasound assessment during the follicular phase and has been used to predict the ovarian reserve and the patient response to ovarian stimulation. However, several factors interfere in the correct classification of the number and size of antral follicles that can induce errors in the AFC, such as patients with high body mass index (BMI), significant variations in follicle size during the menstrual cycle, variability between 2D and 3D technology, and observer-dependent information [17–20]. In addition, there is significant variation among authors in the limits used to classify antral follicles [17–20]. Although it has been observed that AMH and AFC are the best predictors of the ovarian reserve along with age [2–4,6,10,12,17,21–22], it has been reported that AMH and AFC together provide excellent effectiveness in the assessment of OR and ORe, and better than effectiveness of each parameter alone [20,23–25].

On the other hand, as complex clinical phenomenon the OR and ORe is influenced also by environmental and genetic variables. In fact, single-nucleotide polymorphisms (SNPs) in several genes have been studied to assess whether genetic markers can predict OR and /or ORe [26–29]. Vagnini *et al.* [30] demonstrated that in the Brazilian population, the polymorphism (rs4648551, A > G) in the Tumor Protein p73 (TP73) gene are associated with decreased OR. The Tumor Protein p53 (TP53) gene is involved in the embryonic implantation process and the maintenance of germ cell integrity, but there are still no studies showing its influence on ovarian function. The TP73 gene, in turn, controls meiotic spindle assembly and is involved in the cellular response to stress and development. Although animal studies show that changes in this gene can lead to a reduction in the follicular pool and an increase in oocytes with defected spindle assembly [31], there are few studies in humans demonstrating such correlation [32]. The matrix metalloproteinase (MMP) system regulates the changes that occur in ovarian and uterine extracellular architecture. This system controls the remodeling processes of connective tissue and is composed of a proteolytic component, the MMPs, and a regulatory component, the metalloproteinase inhibitors [33]. The Matrix Metallopeptidase 9 (MMP9) gene is expressed only in granulosa cells, and studies indicate that it is involved in different stages of female reproduction, such as the menstrual cycle, ovulation, implantation, and delivery [34]. Despite its role in female reproduction, little is known regarding its influence on OR. The enzyme methylenetetrahydrofolate reductase (MTHFR) is present in human oocytes and embryos in the pre-implantation stage. Evidence shows that polymorphisms in the MTHFR gene are associated with high baseline levels of FSH and may be a determinant of OR and ORe, suggesting that such polymorphism could be the modulator of folliculogenesis [35].

Due to the relevance of these genes in human reproduction, the investigation of possible SNPs capable of predicting OR plays a crucial role in the search for markers that can ensure the efficacy and safety in IVF/ICSI treatments. In preliminary analysis, using next-generation sequencing, we identified four polymorphisms that were somehow related to ovarian reserve: TP53 rs1625895, TP73 rs3765730, MMP9 rs17576, and MTHFR rs868014. The objective of the present study was to investigate the influence of these polymorphisms on OR of infertile patients evaluated by the association of the levels of AMH and AFC.

Materials and methods

Study population

This cross-sectional study was conducted between 2018 and 2019, with 145 Brazilian women undergoing IVF/ICSI treatment at the following two centres: Department of Gynecology and Obstetrics of the São José do Rio Preto Medical School (FAMERP) and the Human Reproduction Centre – Prof. Franco Jr. (CRH). All women attending these centres for infertility treatment approached and invited to participate in the study. The AMH measurements and SNP genotyping were carried out at the Paulista Centre for Diagnosis, Research, and Training (CPDP). The AFC was performed in the above-mentioned centres.

Inclusion criteria and data collection

The inclusion criteria comprised age $\leq$ 37 years, regular menstrual cycle, presence of both ovaries assessed by ultrasound, no history of ovarian surgery, hydrosalpinx, infections, or endocrine problems. The patients were divided into two groups according to ovarian reserve, characterized by association between AMH levels and AFC:

- Group I: 86 patients defined as having low OR (LOR) by AMH < 1 ng/mL + AFC $\leq$ 9 [2,3,10,20].
- Group II: 59 patients defined as having normal OR (NOR) by AMH > 2 ng/mL + AFC $\geq$ 15 [2,3,10,20].

After patient distribution, both groups were compared (LOR X - NOR) regarding the genotypes of each polymorphism.

All women recruited for this study were Brazilian, from all over the country. Despite the high rate of interracial marriages in the Brazilian population, which prevents ethnic classification, all study participants described their skin color as ‘‘white”.

Ultrasound evaluation

The patients underwent transvaginal ultrasound during the follicular phase in cycles before IVF/ICSI. The ultrasound marker used in this study was the AFC. The total number of antral follicles measuring 2 to 9 mm in both ovaries was used to classify these patients.

Enzymatic assay

The AMH measurements were performed on peripheral blood using the Gen II ELISA kit (Beckman Coulter Inc., ref. A73818), following the manufacturer’s instructions. The detection sensitivity of this kit is 0.01 ng/mL, and the coefficients of variation within and between assays were 3.3% and 6.5%, respectively.

DNA samples and genotyping

Genomic DNA for the entire studied population was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer’s instructions. Nucleotide changes were evaluated in duplicate by real-time polymerase chain reaction (PCR) using individual Taq-Man SNP genotyping assays (Thermo Fisher) for each SNP TP53 (rs1625895), TP73 (rs3765730), MMP9 (rs17576), and MTHFR (rs868014) and Taq-Path ProAmp Master Mix, following the manufacturer’s instructions, on a StepOne™ Real-Time PCR System. The PCR conditions were as follows: 60 °C for 30 s (pre-read); 95 °C for 5 min (initial denaturation, enzyme activation), 40 cycles of 95 °C for 15 s (denaturation), and 60 °C for 1 min (annealing/extension). The genotyping results were validated and confirmed with an automatic sequencer (XL 3500 Genetic Analyzer, Applied Biosystems) using 20 samples of each genotype from each polymorphism (normal homozygous, heterozygous, and mutated homozygous), selected at random. In order to determine the minor allele frequency of each polymorphism, all genotypes were sequenced.

Sample size

Sample size was calculated by performing a comparison between two proportions. A sample size of 50 subjects in each group has 80% power to detect an increase of 30% with a significance level of 0.05 (two-tailed).

Statistical analysis

All data were analyzed using the StatsDirect statistical software, version 2.7.9, and the Hardy-Weinberg equilibrium was applied using an online calculator.

Differences in the frequencies of the SNP genotypes, alleles, or both, in the LOR and NOR groups, were evaluated. In order to compare the means of continuous variables, the nonparametric Mann-Whitney test was used when the continuous variables were not normally distributed, and Student's *t*-test and one-way analysis of variance were performed when the continuous variables were normally distributed. The results were expressed as the arithmetic mean ± SD. For categorical variables, Fisher's exact test was used, and the results were expressed as percentages.

Logistic regression analysis was conducted to determine a significant association between all polymorphisms as a tool in predicting LOR or NOR. LOR and NOR, are the two categories of a binary variable and was used as the dependent variable. All genotypes of the polymorphisms (TP53 T/T, TP53 T/C, TP53 C/C; TP73 G/G, TP73 G/A, TP73 A/A; MMP9 Gln/Gln, MMP9 Gln/Arg, MMP9 Arg/Arg, and MTHFR A/A, MTHFR A/G, MTHFR G/G) was included in this model as separate binary variables. In addition, the association of combination of these genotypes (two or more) from different genes with LOR or NOR were also analyzed. Each combination of genotypes was included in this model as separate binary variables. Odds Ratio was used to determine the prediction power. Age was included as a variant (potential confounder) in all logistic regression calculation.

All statistical tests were considered significant at $P < 0.05$.

Ethical approval

The study was authorized by the FAMERP Ethics Committee in Research (CAAE 60245216.0.0000.5415). Written informed consent was obtained from all recruited subjects.

Results

All invited patients accepted to participate and were included in the study. The characteristics of the 145 patients involved in the present study are shown in Table 1. There were no significant differences between the variables age, duration, and causes of infertility. Meanwhile, the parameters that characterize OR showed a significant difference between the two population groups (LOR and NOR).

The majority genotypic frequencies in both the LOR and the NOR groups observed during this study were consistent with the Hardy-Weinberg equilibrium. However, genotype distribution of TP53 rs1625895 and MMP9 rs17576 was not under Hardy–Weinberg equilibrium.

SNP genotyping and association with OR

Table 2 shows the minor allele frequency of SNP in the General Population, LOR and NOR Groups. The frequency distribution of the genotypes and alleles of each analyzed polymorphism is shown in Table 3.

The frequency of the TP53-T/T genotype was significantly greater in the LOR group than in the NOR group. On the other hand, the TP53-C/C genotype was significantly more frequent in patients with NOR. This association was confirmed by the frequency of alleles, where the presence of the T allele was significantly higher in patients who exhibited LOR ($P = 0.0003$). The frequency of the TP73-G/G genotype was more evident in the LOR group. Although the frequency of the G allele was significantly higher in the LOR group ($P = 0.01$), the presence of the TP73-AA genotype was not significantly higher in patients with NOR. Considering the MMP9 gene, the frequency of the Gln/Gln genotype was higher in the LOR group. Although the presence of the Arg/Arg genotype did not show a significant difference between groups, the Gln/Arg genotype prevailed in the NOR group, indicating that the presence of the Arg allele is significantly higher in NOR ($P = 0.006$). The frequency of the MTHFR-A/A genotype was significantly higher in the LOR group, whereas that of the MTHFR-GG genotype was significantly higher in the NOR group, evidencing an association in homozygosis since the presence of the MTHFR-AG genotype did not show a significant difference. The presence of allele A was significantly higher in the LOR group ($P = 0.002$).

Odds ratio analysis of LOR identification

According to Table 4, patients who present the TP53-T/T, TP73-G/G, MMP9-Gln/Gln, and MTHFR-A/A genotypes are 3.6X, 3.1X, 3.2X, and 3.7X more likely of having LOR, respectively. On the other hand, women who present the TP53-C/C, MMP9-Gln/Arg, and MTHFR-G/G genotypes are 58%, 68%, and 56% more likely of not being included in the LOR group, respectively.

Polymorphism combination analysis in LOR prediction

The association of the TP53/TT + TP73/GG genotypes increased the chance of women being included in the LOR group in 5.7-fold when compared with those in the NOR group (Table 5). Other different combinations of genotypes among the four polymorphisms studied did not show statistical significance.

Discussion

The members of the p53 family (TP53, TP63, and TP73) are involved in cell cycle regulation, transactivation, and apoptosis in response to DNA damage. Studies indicate that TP73 ensures normal mitosis during blastocyst development, and TP53 regulates embryo implantation through leukemia inhibitory factor (LIF) [36]. Moreover, members of the p53 family have also been described as regulators of human reproduction processes, maintaining germ cell integrity [37,38].

Studies have shown that variations in the TP63 and TP73 genes are essential in maintaining the size of the follicular pool, in genomic stability by checking the spindle assembly checkpoint (SAC), and in the quality of the oocyte [31,32]. Although small variations in the genes of the p53 family are expected to influence ovarian function, little is known concerning the action of polymorphisms in these genes and their influence on the OR of infertile women.

In the present study, a significant association of LOR with two polymorphisms of the p53 family (TP53-rs1625895 and TP73-rs3765730) was observed. The TP53/TT and TP73/GG genotypes are associated with LOR. Although the TP73-rs3765730 polymorphism has not been described so far, TP53-rs1625895 has been studied in different populations with ovarian cancer diseases, but no work has depicted the evaluation of this polymorphism regarding OR [39].

Table 1
Population characterization.

		Total	Normal Ovarian Reserve	Low Ovarian Reserve
n		145	59	86
Age (years)*		33.3 ± 2.6 (25–37)	32.9 ± 2.8 (26–37)	33.6 ± 2.4 (25–37)
AMH (ng/mL)*		2.3 ± 3.2 (0.01–20.8)	4.9 ± 3.8 (2.1 ± 20.8)	0.4 ± 0.29 (0.01–0.99)
Antral Follicle Count (n)*		13.8 ± 10.8 (0–66)	24.6 ± 8.8 (15–66)	6.3 ± 2.3 (0–9)
Duration of infertility (years)*		3.8 ± 2.7 (1–13)	3.5 ± 2.7 (1–12)	4.0 ± 2.8 (1–13)
Etiology	Male	46.9% (68/145)	47.4% (28/59)	46.5% (40/86)
	Idiopathic	40.7% (59/145)	35.6% (21/59)	44.2% (38/86)
	Tuboperitoneal	9.0% (13/145)	11.9% (7/59)	7.0% (6/86)
	Male + Tuboperitoneal	3.4% (5/145)	5.1% (3/59)	2.3% (2/86)
Infertility	Primary	83.4% (121/145)	86.4% (51/59)	81.4% (70/86)
	Secondary	16.6% (24/145)	13.6% (8/59)	18.6% (16/86)

*Values represented as mean ± standard deviation (minimum and maximum value).

Table 2
Minor allele frequency of SNPs in the General Population, LOR and NOR Groups.

Gene	rs	Functional Consequence	Location (GRCh38.p12)	MINOR ALLELE FREQUENCY (MAF)		
				LOR Group	NOR Group	Global*
TP53	rs1625895	Intron Variant	chr17:7674797	T = 0.52	T = 0.30	T = 0.1663
TP73	rs3765730	Intron Variant	chr1:3690956	A = 0.23	A = 0.37	A = 0.2368
MMP9	rs17576	Missense	chr20:46011586	Arg (G) = 0.19	Arg (G) = 0.34	G = 0.4555
MTHFR	rs868014	3 Prime UTR Variant	chr1:11789390	A = 0.51	A = 0.31	A = 0.0671

*1000 Genomes Project. https://www.internationalgenome.org.

Table 3
Frequency distribution of the genotypes and alleles in groups LOR and NOR.

			Age	*P*	AMH	*P*	AFC	*P*	LOR (n = 86)	NOR (n = 59)	*P*
TP53 (rs1625895)	Genotype	TT	33.6 ± 3.0	0.51	1.2 ± 1.6[a]	[a]0.002	9.5 ± 6.2[b,c]	[b]0.02	36 (41.8%)	10 (17.0%)	0.005
		TC	33.3 ± 2.7		2.5 ± 4.0		14.2 ± 10.4[b]	[c]0.001	17 (19.8%)	15 (25.4%)	
		CC	33.4 ± 2.8		2.8 ± 3.6[a]		16.5 ± 12.4[c]		33 (38.4%)	34 (57.6%)	
	Allele	T	33.7 ± 2.9	0.50	1.8 ± 2.9	0.05	11.4 ± 8.5	0.005	89 (51.7%)	35 (29.7%)	0.0003
		C	33.4 ± 2.8		2.7 ± 3.7		15.8 ± 11.8		83 (48.3%)	83 (70.3%)	
TP73 (rs3765730)	Genotype	GG	33.4 ± 3.1	0.71	1.9 ± 3.3	0.09	11.6 ± 9.1[a]	[a]0.03	54 (62.8%)	24 (40.7%)	0.04
		GA	33.8 ± 2.5		2.7 ± 3.4		15.8 ± 11.8[a]		24 (27.9%)	26 (44.1%)	
		AA	33.6 ± 3.0		2.7 ± 2.8		17.6 ± 12.8		08 (9.3%)	09 (15.2%)	
	Allele	G	33.6 ± 2.9	0.62	2.2 ± 3.3	0.13	13.2 ± 10.4	0.08	132 (76.7%)	74 (62.7%)	0.01
		A	33.8 ± 2.6		2.7 ± 3.3		16.2 ± 12.0		40 (23.3%)	44 (37.3%)	
*MMP9 (rs17576)	Genotype	Gln/Gln	33.7 ± 2.7	0.76	1.9 ± 3.3[a]	[a]0.03	11.6 ± 9.8[b]	[b]0.001	60 (70.6%)	26 (44.1%)	0.005
		Gln/Arg	33.3 ± 3.0		2.5 ± 2.2[a]		16.2 ± 8.7[b]		18 (21.2%)	26 (44.1%)	
		Arg/Arg	33.3 ± 3.4		3.2 ± 5.4		19.5 ± 17.7		07 (8.2%)	07 (11.8%)	
	Allele	Gln	33.6 ± 2.8	0.61	2.2 ± 3.0	0.20	13.2 ± 9.7	0.01	138 (81.2%)	78 (66.1%)	0.006
		Arg	33.3 ± 3.1		2.7 ± 3.2		17.0 ± 11.4		32 (18.8%)	40 (33.9%)	
MTHFR (rs868014)	Genotype	AA	33.8 ± 3.0	0.65	1.2 ± 1.6[a,b]	[a]0.02	8.7 ± 5.6[c,d]	[c]0.0009	25 (29.1%)	06 (10.2%)	0.008
		AG	33.7 ± 2.9		2.6 ± 3.8[a]	[b]0.03	14.4 ± 10.3[c]	[d]0.0005	37 (43.0%)	25 (42.4%)	
		GG	33.3 ± 2.9		2.4 ± 3.3[b]		16.0 ± 12.6[d]		24 (27.9%)	28 (47.4%)	
	Allele	A	33.7 ± 2.9	0.58	2.1 ± 3.3	0.42	12.5 ± 9.4	0.06	87 (50.6%)	37 (31.4%)	0.002
		G	33.5 ± 2.9		2.5 ± 3.6		15.1 ± 11.4		85 (49.4%)	81 (68.6%)	

Values within rows with the same superscript letter were significantly different.
*n=85 in LOR group.

The proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiological processes, including embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMPs are secreted as inactive proproteins that are activated when cleaved by extracellular proteinases. The enzyme encoded by this gene degrades collagens type IV and V. Studies suggest that the expression of matrix metalloproteinase-9 (MMP-9) is involved in different stages of female reproduction, such as menstrual cycle, ovulation, implantation, delivery, and mammary gland involution after lactation [40,41].

The role of extracellular MMPs in remodeling ovarian tissue during the life span of the follicle has been documented in numerous studies. The expression of MMP-1, −2, −3, and −9 has been demonstrated in mammalian ovaries [42,43] and that of MMP-2 and MMP-9 in human granulosa cells [44]. Luddi *et al.* [34] showed that MMP-9 is expressed only in granulosa cells. Although it is widely documented that MMP-9 expression is important since it generates the necessary proteolytic activity at the time of ovulation, little is known about the relationship between MMP-9 gene polymorphisms and OR. While our findings evidence an association between the MMP9-Gln/Gln genotype and LOR, other studies have not identified such relationship. Kim *et al.* [45] analyzed the association between matrix metalloproteinase polymorphisms and the risk of primary ovarian failure. They concluded that MMP-2 gene (rs243865) polymorphisms might contribute to the increase of primary ovarian failure in the studied population, but found no correlation with MMP-9 rs17576. Barišić *et al.* [46] published a review on several MMP polymorphisms correlated with infertility and complications during

Table 4
Genotypes × odds ratio of presenting LOR.

SNPs	Genotype	*Odds Ratio*	CI 95%	*P*
Age (years)		*1.20*	0.95	1.46
TP53 (rs1625895)	TT	3.65	1.54–8.60	0.003
	TC	0.79	0.34–1.81	0.57
	CC	0.42	0.21–0.87	0.02
TP73 (rs3765730)	GG	3.11	1.46–6.6	0.003
	GA	0.41	0.19–1.00	0.05
	AA	0.53	0.18–1.57	0.25
MMP9 (rs17576)	Gln/Gln	3.20	1.51–6.70	0.002
	Gln/Arg	0.32	0.15–0.71	0.004
	Arg/Arg	0.67	0.21–2.17	0.50
MTHFR (rs868014)	AA	3.74	1.35–10.35	0.01
	AG	0.98	0.48–2.01	0.96
	GG	0.44	0.21–0.93	0.03

CI: confidence interval.
OBS: Age was included as a variant in the calculations.

Table 5
Association between the polymorphisms TP53 (rs1625895) and TP73 (rs3765730) in the prediction of ovarian response.

	TP53-T/T + TP73-G/G (n = 26)	Other genotype combinations (n = 119)	*P*	*Odds ratio*	CI 95%
LOR (n = 86)	22 (84.6%)	64 (53.8%)	0.006	5.73	1.67–19.85
NOR (n = 59)	04 (15.4%)	55 (46.2%)			

CI: confidence interval.
OBS: Age was included as a variant in the calculations.

pregnancy. The authors concluded that further research in the field is required due to the modest associations of these polymorphisms and conflicting results between the studies analyzed.

The enzyme MTHFR plays a central role in many biological processes considered important for embryonic division and development. This enzyme regulates the transfer of carbon units between DNA synthesis and methylation reactions, the process responsible for the irreversible conversion of 5,10 methylenetetrahydrofolate into 5-methyltetrahydrofolate, a co-substrate for homocysteine re-methylation to methionine [47]. Mehahed and Taher [48] analyzed the levels of folate and homocysteine in pregnancy and identified that a polymorphism in the MTHFR C677T gene (rs1801133) that causes the substitution of alanine for valine promotes changes in the methylation reactions that are essential for embryonic growth and the regulation of gene expression, as well as high levels of homocysteine, which could harm folliculogenesis by increasing oxidative stress.

In 2005, Ferrara *et al.* [49] reported a case of ovarian hyperstimulation syndrome associated with two polymorphisms in the MTHFR gene: C677T and A1298C. Meanwhile, Rosen *et al.* [35] showed that the MTHFR enzyme is present in human oocytes and embryos in the pre-implantation stage, and that only the MTHFR A1298C polymorphism, but not the C677T polymorphism, is associated with high baseline FSH levels and may be a determinant of the response to ovarian stimulation, suggesting that MTHFR A1298C alone would be the modulator of folliculogenesis. In addition to being controversial, only few studies show the influence of polymorphisms on the MTHFR gene in IVF cycles, and, to date, no research has analyzed the MTHFR polymorphism (rs868014) in infertile patients.

In 2017, He *et al.* [50] first identified this polymorphism and concluded that the polymorphism in MTHFR (rs868014) is associated with an increased risk of developing ischemic stroke. In addition to such association, the authors found, through the analysis of linkage disequilibrium, that this SNP (rs868014) is strongly linked to the MTHFR A1298C polymorphism, which has an important role in the variability of ovarian follicular activity after ovarian stimulation.

Our study revealed that the presence of the genotypes TP53-T/T, TP73-G/G, MMP9-Gln/Gln, and MTHFR-A/A increases the chance of a patient having LOR by 3.6, 3.1, 3.2 and 3.7-fold, respectively. On the other hand, women who present the genotypes TP53-C/C, MMP9-Gln/Arg, and MTHFR-G/G are 58%, 68%, and 56% more likely of not being included in the LOR group, respectively. In addition, we demonstrated that the presence of the combination of the TP53-T/T + TP73-G/G genotypes increases the chance of women having LOR by 5.7-fold.

However, some points should be considered. As the genotype distribution of TP53 rs1625895 and MMP9 rs17576 was not under Hardy-Weinberg equilibrium, it can be speculated that the results have some kind of bias. Increasing the number of women analyzed is likely to solve this issue. On the other hand, the results show that women who are homozygous for the major allele of the variants in TP73 rs3765730 (GG) and MMP 9 rs17576 (Gln/Gln) have a threefold increased risk of having low ovarian reserve. It would be expected that any alleles that lead to significantly impaired reproductive outcome such as low ovarian reserve, would be under strong negative selection. The findings of major alleles/genotypes having a high disease risk seems contradicting that evolutionary tendency. It rather looks like the minor alleles TP73 rs3765730 (AA) and MMP 9 rs17576 (Arg/Arg) tend to have a protective effect against low ovarian reserve although statistically not significant. However, it should be noted that LOR is not directly associated with the possibility or not of pregnancy. As long as there is availability of oocytes, even in small numbers, pregnancy could occur. Furthermore, this result expresses a specific population of women from couples undergoing infertility evaluation/treatment. Again, analyzing a larger population, including the general population as well (and not just infertile women) will help to elucidate these findings.

Conclusions

Based on the present findings, it can be concluded that the genotypes TP53-T/T, TP73-G/G, MMP9-Gln/Gln, and MTHFR-A/A increase the chance of women to exhibit LOR. These polymorphisms could be useful as genetic markers of low ovarian reserve in infertile patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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