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A study of the role of *TNFα-308* (G>A) gene polymorphism in recurrent pregnancy loss in random sample from Benha University Hospital

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ABSTRACT

Background: Recurrent pregnancy loss (RPL) remains a significant clinical and emotional challenge. Despite advances in reproductive medicine, the underlying causes of RPL are sometimes elusive, with genetic factors now increasingly recognized as important contributors. Among these, the single-nucleotide polymorphism (SNP) rs1800629 in the tumor necrosis factor-alpha (TNF-α) gene has emerged as a potential factor influencing susceptibility to RPL.

Aim: This case-control study intended to examine the association of *TNF-α* − 308 G > A SNP with RPL in Benha University Hospital, Egypt.

Subjects & methods: A total of 190 participants (90 women with RPL and 100 healthy controls) were involved. Genotyping of the *TNF-α* − 308 G > A SNP was performed using the restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP) technique with the *Nco*I restriction endonuclease.

Results: The frequency of GA and AA genotypes were considerably higher in the RPL females compared to controls, with the AA genotype conferring the highest risk (OR = 3.75, 95 % CI: 1.17–12.05, $p = 0.027$). The dominant model (GA + AA) also showed a strong association with RPL (OR = 2.06, 95 % CI: 1.35–3.12, $p = 0.001$). The A allele was identified as a significant risk factor (OR = 2.01, 95 % CI: 1.39–2.90, $p < 0.001$).

Conclusion: The *TNF-α* − 308 G > A polymorphism appears to be linked to increased susceptibility to RPL. Larger, multi-ethnic studies are required to further confirm these outcomes and to clarify the genetic contribution to RPL.

1. Introduction

One to 3 % of women of childbearing age worldwide suffer from repeated pregnancy loss (RPL), a complicated obstetrical state defined by the European Society of Human Reproduction and Embryology (ESHRE) as the spontaneous end of two or more consecutive pregnancies (Barrenetxea et al., 2017; Practice Committee of the American Society for Reproductive Medicine (ASRM), 2012; ESHRE Guideline Group on RPL, 2022). RPL is a reproductive disease, with chromosomal abnormalities, uterine anomalies, hormonal imbalances, and autoimmune conditions being the four major contributing factors. However, about 50 % of RPL cases remain unexplained (idiopathic) (Larsen et al., 2013; Lei et al., 2022).

Genetic factors have been increasingly recognized as important contributors to RPL, particularly those affecting immune regulation at the maternal-fetal interface. In this context, genetic polymorphisms in cytokine-related genes have received considerable attention, as they are potential to alter cytokine expression and disrupt immune homeostasis. Notably, strong associations have been reported between RPL and single nucleotide polymorphisms (SNPs) in interleukin (IL) genes such as IL-1β

Abbreviations: TNF-α, Tumor Necrosis Factor-alpha; RPL, Recurrent Pregnancy Loss; SNP, Single nucleotide polymorphism; PCR- RFLP, Restriction fragment length polymorphism-Polymerase Chain Reaction; ESHRE, European Society of Human Reproduction and Embryology; HLA, Human Leucocytic Antigen; EDTA, Ethylene diamine tetra acetic acid; BMI, Body mass index; CBC, Complete blood count; TSH, Thyroid stimulating hormone; DM, Diabetes mellitus; HWE, Hardy-Weinberg equilibrium; IL, interleukin; IFN-γ, Interferon-gamma; TGF- β1, Transforming Growth Factor-beta; VEGF-A, Vascular Endothelial Growth Factor A; G-CSF, Granulocyte Colony-Stimulating Factor.

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(-511C > T), IL-6 (-643C > G), IL-10 (−1082A>G, -592C > A) and IL-18 (−137G > C). Additional immune-related genetic variants, including polymorphisms in interferon-gamma (IFN-γ) (+874A/T), transforming growth factor-beta (TGF- β1) (+915G/C), granulocyte colony-stimulating factor (G-CSF) (+4215 T > C), vascular endothelial growth factor A (VEGF-A), lipase C-60G and miR29a rs157907A > G have also been implicated in increasing susceptibility to RPL (Stavros et al., 2024; Sekar and Veerabathiran, 2024; Eivazi et al., 2023; Salimi et al., 2021).

Tumor necrosis factor-alpha (TNF-α), an effective pro-inflammatory cytokine produced by T helper 1 cells and expressed in the placenta, is considered a critical immunomodulatory factor in early pregnancy events such as implantation, placentation and overall pregnancy outcomes. Higher-than-normal levels of TNF-α have been found in placental tissues taken from pregnancies where doctors noticed certain signs or symptoms that usually indicate a higher chance of miscarriage (Romanowska-Prochnicka et al., 2021; Liu et al., 2010; Ali et al., 2021).

During pregnancy, TNF-α influences hormone production, placental development, and embryo viability by stimulation of new vessels for successful embryo implantation. The TNF-α influences endocrine functions, particularly progesterone and human chorionic gonadotropin (hCG) levels, which are crucial for pregnancy maintenance. It can suppress progesterone production by affecting luteal cells and modulating steroidogenic enzymes in the placenta. Altered hormone levels due to TNF-α may impact the stability of the uterine lining and the success of embryo implantation (Yuan and Jin, 2014).

Additionally, TNF-α has a significant function in coagulation and endothelial function, as it is believed to activate vascular endothelial cell procoagulant activity, which in turn causes thrombotic and inflammatory events at the maternal uteroplacental blood vessels. High TNF-α levels may also inhibit trophoblast invasion and induce cell death, impairing placental function and structure. This can contribute to complications such as pre-eclampsia, intrauterine growth restriction (IUGR), or miscarriage (Laresgoiti-Servitje et al., 2010). Moreover, high TNF-α levels can harm embryo survival by promoting inflammation and cytotoxicity at the maternal-fetal interface. It can trigger oxidative stress and apoptosis in embryonic and placental cells, thereby compromising embryo viability (Agarwal et al., 2012).

Furthermore, it has been demonstrated that increased TNF-α expression hinders decidualization and inhibits embryonic stem cell differentiation, both of which impede proper embryonic development. TNF-α has been shown to inhibit blastocyst growth (Azizieh and Raghupathy, 2015; Dai et al., 2022; Kwak-Kim et al., 2025). In the presence of proinflammatory cytokines, natural killer cells differentiate into lymphokine-activated killer (LAK) cells capable of killing trophoblast cells. It was previously demonstrated that systemic levels of LAK-like cells link with high miscarriage rates (Fonseca et al., 2020).

The *TNF-α* gene is positioned on chromosome 6p21.3 within the Human Leukocyte Antigen (HLA) region. It extends across ~3.6 kb, comprising four exons, with the final exon encoding >80 % of the secreted protein (Bahmani et al., 2018). Certain polymorphisms in the regulatory regions of *the TNF-α gene* can influence gene expression and cytokine production, potentially altering immune tolerance during pregnancy (Kim et al., 2018).

There are a lot of SNPs in the promoter region of the *TNFα*-, according to the NCBI dbSNP database. Of these, *TNFα*-238G > A, *TNFα*-308G > A and *TNFα*-376G > A have been examined the most for their possible association with RPL (Stavros et al., 2021).

The *TNFα*-308 G > A SNP (ID: rs1800629) (National Center for Biotechnology Information (NCBI), n.d.) has been reported to affect the gene expression. The A allele has been shown to enhance transcriptional activity, leading to elevated mRNA expression of TNF-α compared to the G allele (Salazar-Camarena et al., 2025). The *TNFα-308 G > A* SNP has been linked to an elevated risk of RPL in multiple populations, including Iranian (Bahmani et al., 2018), Indian (Manzoor et al., 2021), and Caucasian populations (Aslebahar et al., 2019) cohorts.

Accordingly, in order to advance our knowledge of the genetic determinants underlying RPL, this study aims to assess the possible contribution of the *TNF-α* -308G > A SNP to RPL predisposition. Although the association of this variant with RPL has been studied before in other populations, we intended to investigate this variant among Egyptian women due to the genetic heterogenicity among different populations i.e. a specific variant may be prevalent in one population but not in another.

2. Subjects and methods

2.1. Study design and participants

This observational case-control study was performed at the Obstetrics and Gynecology Department and outpatient clinic in Benha University Hospital on unrelated Egyptian gravida women. The inclusion criteria consisted of gravida women having 2 or more successive pregnancy loss histories occurring prior to the 24th week of gestation and an age range of 20–35 years. Exclusion criteria included females with only one previous pregnancy loss, females who had two or more losses resulting from induced pregnancy terminations, those with two or more non-successive pregnancy losses, and females who conceived through Assisted Reproductive Technologies or In Vitro Fertilization. These criteria adhered to the European Society of Human Reproduction and Embryology (ESHRE) guidelines, with the exception of the age range requirement (ESHRE Guideline Group on RPL, 2022). Clinical records were reviewed to identify major risk factors (e.g., endocrine disorders, uterine anomalies, chronic diseases), though comprehensive investigations such as karyotyping and thrombophilia screening were not uniformly available due to the retrospective nature of the study.

The study was extended from March 2024 to July 2024. Ethical approval was given by the Ethical Committee of the Faculty of Medicine, Benha University (Study Code: MS: 13-1-2024). Prior to enrollment, all contributors were informed of the details of the study procedures, and written consent was attained.

2.2. Anthropometric analyses

A detailed history of all patients was documented, and clinical and radiological data available were assessed—anthropometric indicators including age, height and weight. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$ According to Nuttall (2015).

2.3. Sampling process

Whole blood was collected from each participant via venipuncture under complete aseptic conditions. Two ml in ethylenediaminetetraacetic acid (EDTA) tube for molecular genetic analysis, to be stored at −80 °C. Molecular detection of *TNFα*-308 (rs1800629) SNP by PCR-RFLP technique. Complete blood count (CBC), TSH, and urine analysis were done on the participants using an Automated cell counter (Sysmex XN-550, Japan), TOSOH AIA system analyzer (Japan), PURE series, urine reagent strip (CYIS100, Germany), respectively.

2.4. Extraction of whole genomic DNA

Genomic DNA was purified from EDTA-stabilized blood samples using Biospin DNA Extraction Kit (Hangzhou Bioer Technology, China) following their recommended protocol. Subsequently, the DNA concentration was assessed with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The DNA was preserved at −80 °C until *TNF-α* genotyping.

2.5. TNF-α genotyping (PCR-RFLP)

The specific fragment of the *TNF-α* gene was amplified by the PCR

technique using designed primers (Eurofins Genomics, Europe) to identify the target polymorphism; Table 1 displays their sequences using these thermal cycling settings: initial denaturation (3 min at 95 °C, 1 cycle), followed by denaturation (30 s at 95 °C), annealing (30 s at 58 °C), extension (60 s at 72 °C) all 35 cycle and final extension (3 min at 72 °C, 1 cycle).

The PCR reaction mixture (25 μl) comprised of 12.5 μl of DreamTaq Green PCR Master Mix (Thermo Scientific, Lithuania, which contains dNTPs, Taq DNA polymerase, $MgCl_2$, and reaction buffer); 1.5 μl of 1.5 μl of both forward and reverse primers (10 pmol/μl), and 4.5 μl of nuclease-free water, and 5 μl of DNA extract (100 ng/μl). PCR amplification was conducted using a Veriti 96-Well Thermal Cycler (Applied Biosystems, Singapore).

The Amplicons generated were analyzed by 2 % agarose gel electrophoresis, then digested by the restriction enzyme *Nco*I-HF restriction enzyme (NcoI-HF Biolabs, England). The reaction mixture consisted of 0.5 μl of NcoI, 7 μl of nuclease-free water, and 6 μl of PCR product and buffer to reach 20 μl total volume. The digested products were reanalyzed by electrophoresis and a 100 bp DNA ladder was used to determine fragment sizes. The gel was visualized using an Alpha Innotech Corporation transilluminator. The NcoI enzyme cleaves the 147 bp PCR product at the C/CATGG site, producing restriction fragments of 147 bp, 127 bp, and 20 bp.

2.6. Statistical analysis

The sample size was calculated using the Quanto calculator software program (Gauderman, 2006). By using a power of 80 % and a level of significance of 0.05 in a case-control study, the minimum required sample size was 190, divided into 2 groups: 100 controls and 90 RPL cases. The data obtained was tabulated using (IBM SPSS, Armonk, NY: IBM Corp.). The numerical parameters were expressed as number (N) and standard deviation (SD) and compared using a student *t*-test. Categorical parameters were presented by percentage. Groups were compared using a chi-square (χ2) or Fisher's exact test (for a small sample size). Skewed numerical data were expressed as a range (minimum-maximum) and analyzed using Mann-Whitney and Kruskal-Wallis tests. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated by making logistic regression. The SNP in the studied groups was examined for Hardy-Weinberg equilibrium (HWE). To account for multiple comparisons across genetic models, *p*-values were adjusted using the False Discovery Rate (FDR) correction method according to Benjamini and Hochberg, which balance the control of false positives while maintaining adequate statistical power. The significance was set at $p < 0.05$ using a two-tailed test. (Kwok, 2000; Zhao et al., 2016).

3. Results

3.1. Characteristics of the study group

No statistical difference was detected among the studied groups concerning age, smoking status, family history of thrombosis, and hypertension. However, RPL cases showed significantly higher gravidity, paternal age, BMI and diabetes mellitus (DM) prevalence ($p < 0.01$) (Table 2). Ultrasonographic findings revealed strong associations between RPL and both fibroids and ectopic pregnancies, while no significant correlations were observed with low hemoglobin levels, TSH, or urine analysis (Table 3).

Table 1
Primer sequences applied in amplification:

Forward primer:	-5 GAGGCAATAGGTTTTGAGGGCCAT 3′
Reverse primer:	-5 GGGACACACAAGCATCAAG 3′

Table 2
Demographics and clinical data across the study groups.

Variable		RPL ($n = 90$)	Control ($n = 100$)	*p*-value
Age (years) median (range)		27.0 (18.0–35.0)	25.0 (22.0–35.0)	**0.235**
Gravidity median (range)		4.0 (2.0–12.0)	3.0 (2.0–5.0)	**<0.001***
Smoking	No	88 (97.8 %)	100 (100.0 %)	**FE 0.223**
	Yes	2 (2.2 %)	0 (0.0 %)	
Family history of thrombosis	No	88 (97.8 %)	100 (100.0 %)	**FE 0.223**
	Yes	2 (2.2 %)	0 (0.0 %)	
Paternal age (years) median (range)		32.0 (22.0–45.0)	30.0 (20.0–42.0)	**0.034***
DM	No	81 (90.0 %)	98 (98.0 %)	**0.018***
	Yes	9 (10.0 %)	2 (2.0 %)	
Hypertension (mmHg)	No	88 (97.8 %)	100 (100.0 %)	**FE 0.223**
	Yes	2 (2.2 %)	0 (0.0 %)	
BMI (kg/m^2) Mean ± SD		27.03 ± 2.87	25.55 ± 2.64	**<0.001***

U: Mann Whitney test. χ^2, FE: Fisher Exact, BMI: body mass index.

3.2. TNFα-308 G>A genotyping

Genotyping of the TNFα-308 SNP was performed for 190 samples (90 patients and 100 controls). The amplification yielded a 147 bp product which, when digested with the NcoI, generated distinct fragments representing the three genotypes: GG (wild-type): 127 bp and 20 bp, GA (heterozygous): 147 bp, 127 bp and 20 bp, AA (homozygous variant): 147 bp. However, because of its small size, the 20 bp fragment was invisible in the gel (Fig. 1).

The genotypic and allelic frequencies distribution of TNF-α in the studied groups is presented in (Table 4). All were in accordance with HWE. In the RPL group, 62.2 % of patients had (GG) genotypes, while the (GA) and (AA) genotypes were observed in 31.1 % and 6.7 % of patients, respectively. In the control group, the genotype frequencies for GG, GA, and AA were 84 %, 15 %, and 1 %, respectively. The frequency of the G allele was 77.8 % in patients and 91.5 % in controls, whereas the A allele frequency was 22.2 % in patients and 8.5 % in controls. The distribution of genotypes GG, GA, and AA differs significantly between the two groups, with *p*-values indicating significance for GA ($p = 0.004$), AA ($p = 0.027$), dominant model ($p = 0.001$), recessive model ($p = 0.049$), and alleles ($p < 0.001$).

To evaluate the genetic association of the *TNFα*-308 SNP with RPL risk, various inheritance models were applied. The analysis demonstrated a substantial relation between the *TNFα*-308 SNP and elevated RPL risk under multiple models: heterozygous (GG vs. GA; OR = 1.90, p = 0.004), dominant (GG vs. GA + AA; OR = 2.06, p = 0.001) and recessive (GG + GA vs. AA; OR = 3.22, p = 0.049).

As demonstrated in (Table 5), the AA genotype group demonstrated a notably higher median gravidity than other groups (p = 0.001). Nevertheless, there was no substantial relation to age.

The RPL diagnosis was predicted using a logistic regression analysis in (Table 6), which identified paternal age, DM, BMI, and the *TNF-α* − 308 (GA + AA) genotype as significant independent predictors of RPL. Each one-year increase in paternal age was associated with a 3.6 % rise in RPL risk (OR = 1.039, $p = 0.020$), while DM nearly tripled the risk (OR = 2.795, $p = 0.022$). Similarly, each unit increase in BMI was related to a 24 % elevation in the odds of RPL (OR = 1.25, $p = 0.0002$). Furthermore, the *TNF-α* − 308 (GA + AA) polymorphism doubled the risk (OR = 2.057, $p = 0.001$). These associations continued to be significant after multivariate adjustment for confounders, confirming that paternal age (OR = 1.036, $p = 0.038$), DM (OR = 2.669, $p = 0.042$), BMI (OR = 1.24, $p = 0.0005$), and *TNF-α* − 308 (OR = 2.041, p = 0.001) independently contribute to RPL susceptibility.

4. Discussion

The complicated physiological process of pregnancy is dependent on

Table 3
Radiological and laboratory data across study groups.

Variable			RPL (n = 90)	Control (n = 100)	p-value
Ultrasound findings	Free				
			64 (71.1 %)	70 (70.0 %)	0.867
	Positive		26 (28.9 %)	30 (30.0 %)	
	Fibroid		4 (4.4 %)	0 (0.0 %)	**0.049***
	Ectopic pregnancy		6 (6.7 %)	0 (0.0 %)	**0.010***
Hemoglobin (g/dL) median (range)			10 0.90 (8.0–15.0(	10.25 (8.60–13.20)	**0.103**
TSH (mIU/L) Mean ± SD			2.71 ± 1.26	2.94 ± 0.87	0.222
Urine analysis findings	Pus (cells/HPF) Mean ± SD		6.22 ± 15.13	10.40 ± 15.39	0.111
	Crystals (semi-quantitatively):	Free	58 (64.4 %)	50 (50.0 %)	**MC 0.145**
		Urate	32 (35.6 %)	45 (45.0 %)	
		Phosphate	0 (0 %)	5 (5 %)	

MC: Monte Carlo, FE: Fisher Exact. *: Significant when $p < 0.05$, HPF: high power field.

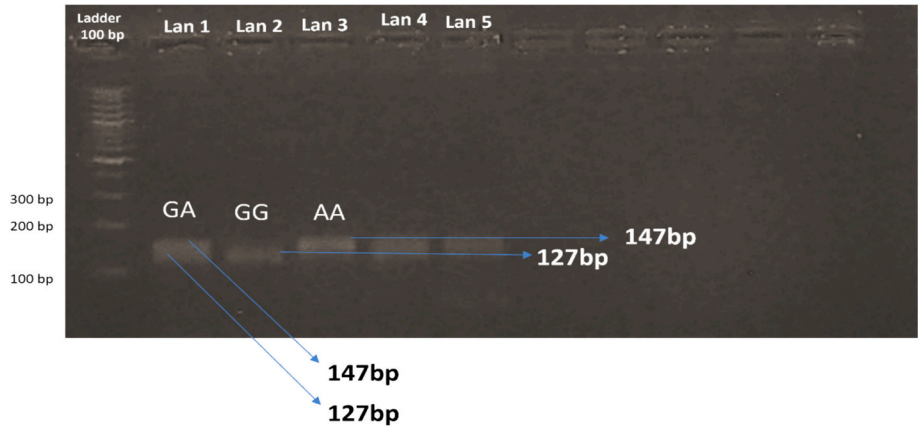

Fig. 1. *TNFα*-308 restriction products. Digestion with *Nco*I yield fragments of PCR product. (GG) wild-type (127 + 20 bp); (AA) Homozygous variant (147 bp) and (GA) heterozygous variant (147 + 127 + 20 bp). A DNA ladder 100 bp was used.

Table 4
Genotypic and allelic frequency distribution of *TNF-α* in the studied groups.

		RPL n = 90		Control *n* = 100		p-value	OR (95 % CI)	FDR-adjusted p-value
		N°	%	N°	%			
Genotypes	**GG**	56	62.2	84	84.0		Reference	Reference
	GA	28	31.1	15	15.0	0.004*	1.90 (1.23–2.95)	0.020*
	AA	6	6.7	1	1.0	0.027*	3.75 (1.17–12.05)	0.034*
Alleles	**G**	140	77.8	183	91.5		Reference	Reference
	A	40	22.2	17	8.5	<0.001*	2.01 (1.39–2.90)	<0.01*
Dominant model	**GG**	56	62.2	84	84.0		Reference	Reference
	GA + AA	34	37.8	16	16.0	0.001*	2.06 (1.35–3.12)	0.01*
Recessive model	**GG + GA**	84	93.3	99	99.0		Reference	Reference
	AA	6	6.7	1	1.0	0.049*	3.22 (1.01–10.32)	0.049*

Reference, according to NCBI database; G, Guanine; A, alanine; OR<1, protective; OR>1, risky; *: Significant when $p < 0.05$. P-values were adjusted for multiple comparisons using the False Discovery Rate (FDR) correction method.

the mother's ability to tolerate the semi-allogenic fetus that carries paternal antigens. RPL, one of the most unfavorable pregnancy outcomes, is characterized by ≥2 pregnancy losses prior to 20 gestation weeks (Dirisipam et al., 2025), whereas ESHRE Guideline Group on RPL (2022) extends the definition to include loss after 24 weeks.

Recurrent pregnancy loss affects approximately 1–5 % of women of reproductive age globally, with some studies suggesting higher rates in the MENA region, including Egypt, due to a combination of genetic, infectious, consanguineous, and environmental factors. In Egypt and many MENA countries, cultural emphasis on fertility and childbearing increases the psychological burden of RPL, often leading couples to seek care early but through fragmented pathways (Elhady et al., 2020).

Clinical management of RPL in the region typically involves basic investigations (ultrasound, antiphospholipid antibodies, hormonal profiling) and advanced testing (less consistently applied): genetic karyotyping, thrombophilia panels, immunological markers like TNF-α polymorphisms, uterine abnormalities via 3D ultrasound or hysteroscopy. However, variability in practice exists due to unequal access to diagnostic technologies, limited clinician awareness or training on international RPL guidelines (e.g., ESHRE or ASRM), and out-of-pocket costs in private sector clinics that dominate reproductive care (El-Hefnawy et al., 2021).

Common in rural Egypt and across the MENA region, contributing to the increased prevalence of hereditary thrombophilias and genetic abnormalities associated with RPL. However, premarital or genetic counseling is still not widely practiced or culturally normalized. Egypt and most MENA countries lack standardized national protocols for RPL evaluation or care. Data on prevalence, outcomes, and etiologies are

Table 5
Association between *TNF-α* − 308 G > A SNP and patients' characteristics.

	TNF-α − 308 G > A SNP			p1	Pairwise
	GG *N* = 56	GA *N* = 28	AA *N* = 6		
Age (years) Mean ± SD.	27.57 ± 5.30	27.50 ± 4.79	27.67 ± 6.12	0.980	
Gravidity Median (range)	4.0 (2.0–12.0)	4.0 (2.0–6.0)	7.0 (6.0–8.0)	0.001*	p2 = 0.170 p3 = 0.002* p4 < 0.001*

SD. Standard deviation, p1: Comparing the different *TNF-α* − 308 categories, p2: Comparing GG and GA, p3: Comparing GG and AA, p4: Comparing GA and AA, *: Significant when $p < 0.05$.

often underreported or regionally scattered. Public health authorities do not currently treat RPL as a specific reproductive health priority. Infertility and pregnancy loss are often stigmatized, pushing couples toward private, unregulated reproductive centers. This creates risks of overtreatment, misinformation, and delay in appropriate care (Issa et al., 2021).

Several genetic, physiological, clinical, and environmental variables affect gene expression Genetic polymorphisms are a major cause of this diversity. Our community is an ideal genetic model for this type of research because to its relative genetic homogeneity and lack of inter-ethnic differences. Evolution refers to the process by which populations of individuals change over many generations. These modifications are the result of genetic variants (Gregory, 2009) We focused on the TNF-α-308G > A variant allele because to its high frequency and known effect on TNF-α production and function. Future research will include a wider range of SNPs.

Our results elucidate several significant risk determinants for RPL, including Gravidity, Paternal age, DM, high BMI and ultrasound-detected abnormalities. Sekhavat and Tabatabaie (2024) implicated advanced paternal age as a factor in the heightened risk of pregnancy loss, attributing this to a greater occurrence of chromosomal abnormalities in the sperm. The role of DM in RPL is also substantiated by Cheng et al. (2024), who found a significant increase in pregnancy loss risk (HR = 1.407, $p = 0.007$), plausibly due to enhancing sympathetic activity while reducing parasympathetic tone. This imbalance enhances pro-inflammatory pathways while weakening anti-inflammatory defenses, resulting in endothelial dysfunction, disrupted placental development, and impaired immune tolerance at the maternal-fetal interface. Nielsen et al. (2024) proved that obesity (BMI ≥30 kg/m^2) is significantly related to an amplified risk of RPL; the study postulated that chronic inflammation, insulin resistance and hormonal disturbances associated with excess adiposity may compromise endometrial receptivity and implantation success. Lastly, Smith and Doe (2025) emphasized that structural uterine abnormalities such as fibroids can create a suboptimal intrauterine environment, hindering embryo implantation and placental function.

Given TNF-α's critical role in pregnancy maintenance, genetic variations in its regulatory regions may influence susceptibility to RPL. The -308G > A SNP, located in the promoter region of the TNF-α gene, has been widely studied for its potential impact on TNF-α expression. The A allele is associated with increased transcriptional activity, resulting in elevated TNF-α levels and an intensified pro-inflammatory response (Begum et al., 2021). This study, sharing similar objectives, investigates the association of the TNF-α-308G > A SNP with RPL risk to understand its role in pregnancy loss better. The serum TNF-α levels couldn't be measured in this study due to financial limitations as this study is self-funded.

This study on Egyptian patients revealed a substantial correlation between the *TNF-α* − 308 G > A SNP and RPL, with the A allele ($p < 0.001$) and AA genotype ($p = 0.027$) conferring an elevated risk. The dominant model (GA + AA) further supported the role of this polymorphism in RPL susceptibility ($p = 0.001$).

Our findings align with early investigations that informed that the *TNF-α* − 308 G > A polymorphism contributes to increased inflammatory response, potentially resulting in pregnancy complications. In this regard, Manzoor et al. (2021) investigated this polymorphism in Kashmiri women from North India. They found an important link between the AA genotype and recurrent miscarriage, with its frequency being notably higher in cases (2.5 %) compared to controls (0.4 %) ($p < 0.05$). Additionally, the A allele was dominant in the RPL group (32 %) than in controls (24 %), with an associated 1.5-fold increased risk ($p < 0.05$).

Similarly, Aboutorabi et al. (2018) studied the Iranian population and reported genotype distributions of 69 % GG, 18 % GA, and 12 % AA in RPL patients, compared to 85 % GG, 15 % GA, and 0 % AA in the control group. Their findings suggested a protective effect of the G allele against spontaneous abortion, reinforcing the potential immersion of the *TNF-α* − 308 G > A SNP in RPL.

Conversely, certain studies could not establish a significant relation between *TNF-α* polymorphism and RPL. For instance, Xue et al. (2023) conducted a study in a Chinese population and reported that the A allele did not show a significant correlation with RPL ($p = 0.659$). Additionally, the distribution of GG, GA, and AA genotypes exhibited no considerable variance between cases and controls.

Similarly, Stavros et al. (2021) stated that the *TNF-α* 308 variant was identified in heterozygosity (GA) both in RPL 45.16 % and control groups 36.73 %, displaying no substantial association ($p = 0.374$). The AA genotype existed in 11.29 % and 12.24 % of the patient and control groups, respectively ($p = 0.901$). Hence, *TNF-α* 308 variants don't relate to the RPL risk in Greek women.

The divergent observations across studies may be owing to a combination of genetic, methodological, and environmental factors. Stavros et al. (2024) explained that historical and geographic factors shape distinct allele frequencies and gene-environment dynamics among ethnic groups, resulting in population-specific effects of this polymorphism. Variations in study design, sample size, and statistical power also contribute to these inconsistencies, as smaller studies may lack the ability to detect significant associations. Furthermore, differences in the inclusion or exclusion of risk factors—such as lifestyle behaviors, environmental exposures, and comorbidities—can further impact study outcomes. Ng et al. (2021) similarly emphasized that these factors may influence TNF-α expression and, consequently, reproductive outcomes.

Our analysis revealed a significant difference in median gravidity

Table 6
Logistic regression analysis to predict the risk of risk of RPL.

	Univariate			Multivariate		
	P	OR	95 % CI	P	OR	95 % CI
Paternal age	0.020*	1.039	1.006–1.073	0.038*	1.036	1.002–1.070
DM	0.022*	2.795	1.156–6.755	0.042*	2.669	1.035–6.881
BMI	0.0002*	1.25	1.11–1.40	0.0005*	1.24	1.10–1.39
Abnormal imaging findings	0.867	0.967	0.654–1.430			
TNF-α − 308 (GA + AA)	0.001*	2.057	1.354–3.124	0.001*	2.041	1.333–3.125

OR < 1, protective; OR > 1, risky; *: Significant when p < 0.05.

across genotypes, with the AA genotype group demonstrating a notably higher median, suggesting that the *TNF-α − 308 G > A* SNP may influence the number of pregnancies among women with RPL. These findings imply that the A allele may be correlated with higher gravidity prior to experiencing recurrent losses, indicating a potential predisposition to multiple pregnancy attempts before pregnancy loss occurs. Wilcox et al. (2020) attributed the higher gravidity detected in patients with RPL to the greater variety of pregnancy outcomes, which may reflect the complex nature of recurrent losses. It is also plausible that women with the AA genotype exhibit a heightened reproductive drive driven by a strong desire to achieve a successful pregnancy despite prior losses.

The Study by Manzoor et al. (2021) stated a significant relationship between the GA and AA genotypes of the *TNF-α* gene and RPL (particularly among females under 30 years of age). The GA genotype presents in 75.4 % of cases and AA in 2.8 % of cases. Our study did not reproduce these findings. In our case-control Study, no statistically significant difference was observed regarding these genotypes when stratified by age.

Based on our awareness, this is a unique work in Egypt that explores the correlation between the *TNF-α − 308* (rs1800629) SNP and the risk of RPL. The study met some restrictions, such as examining a single variant. In contrast, many SNPs are located within the *TNF-α* gene, the inability to evaluate the serum TNF-α concentrations to determine if the studied variant affects gene expression, the statistical sample size and the use of RFLP method for genotyping was another technical limitation. The PCR-RFLP technique, while accessible and widely used, has inherent technical constraints such as gel fragility and primer limitations, which posed challenges during optimization. Nevertheless, these issues did not substantially affect the validity or interpretation of the results. Sequencing, which could have provided higher accuracy and validation, was not feasible due to financial constraints, as this is a self-funded study. Despite that, our findings open the door for additional research to examine the roles these variables play in RPL pathogenesis, especially in populations having a high rate of consanguineous marriage, which could result in the creation of tailored treatments or prophylactic measures. Additionally, our results illuminate the necessity of studying TNF-α inhibitors and their potential application in managing RPL, aligning with current trends in targeted immunotherapy.

The study's primary constraint is the comparatively small sample size of the patient and control groups. Even though the findings might have therapeutic implications, more instances in the study are needed to validate our data. To validate our results and determine the prevalence of this SNP in other populations while taking ethnic heterogeneity into account, more research is advised. Furthermore, to fully comprehend the potential of the TNF-α candidate gene for recurrent pregnancy loss susceptibility, additional research, including large sample sizes, gene-gene and gene-environment interactions, is needed.

5. Conclusion

In conclusion, the present investigation reveals a significant relationship between the *TNF-α − 308* (rs1800629) SNP, particularly the GA and AA genotypes and the A allele, and an increased risk of RPL, with the AA genotype demonstrating the strongest association. These results highlight the potential involvement of this polymorphism in disease susceptibility and predisposition. Moreover, this work stresses the importance of integrating demographic and clinical parameters to achieve a more comprehensive assessment of RPL.

CRediT authorship contribution statement

Osama Saad Al Shaer: Writing – review & editing, Validation, Supervision. **Eman Ramadan Abd El Gwad:** Writing – review & editing, Validation, Supervision. **Dalia Mohamed Abd E.L. Hassib:** Supervision, Methodology, Formal analysis. **Omar Khaled Naser:** Software, Formal analysis, Data curation. **Walaa Afifi Nasr Afifi:** Writing – original draft, Methodology, Data curation, Conceptualization. **Amira Osama Abd El-Ghaffar:** Writing – original draft, Investigation.

Informed consent

Every participant gave their informed consent for this study.

Ethical acceptance

Every procedure used in this study, including the use of human participants, complied with the 1964 Helsinki Declaration and its amendments as well as the institutional research committee's ethical guidelines.

Declaration of competing interest

The authors disclose that they have no financial ties, funding, or potential conflicts of interest related to the study's topic.

Data availability

Data will be made available on request.

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